Contaminantes emergentes

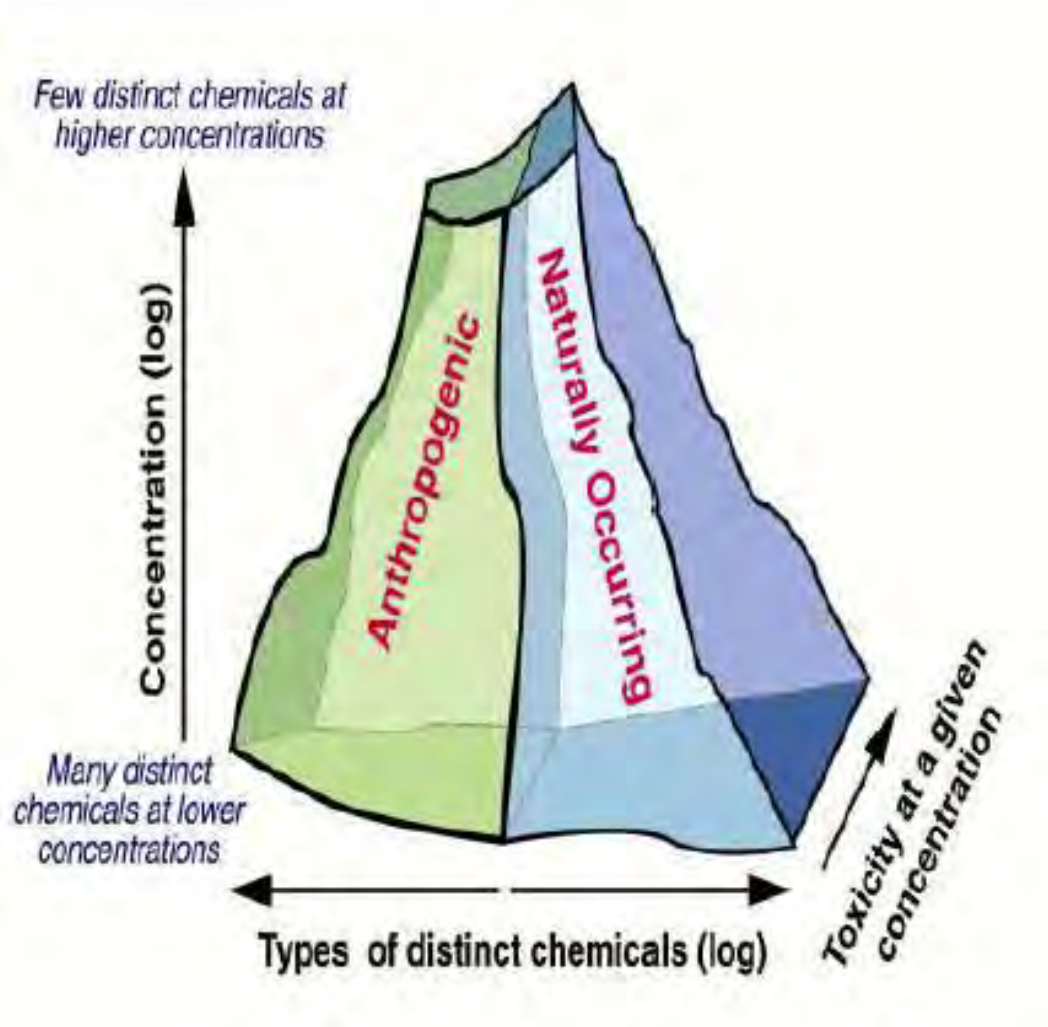
Horacio Heinzen

GACT

NANO

IS IT BOY OR A GIRL?
BOTH, ACTUALLY, THANKS TO ALL THE CHEMICALS IN THE WATER THESE DAYS.

El universo quimico

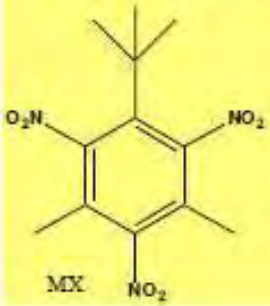


Origin, Transport & Fate of Synthetic Musk Compounds in the Las Vegas Basin

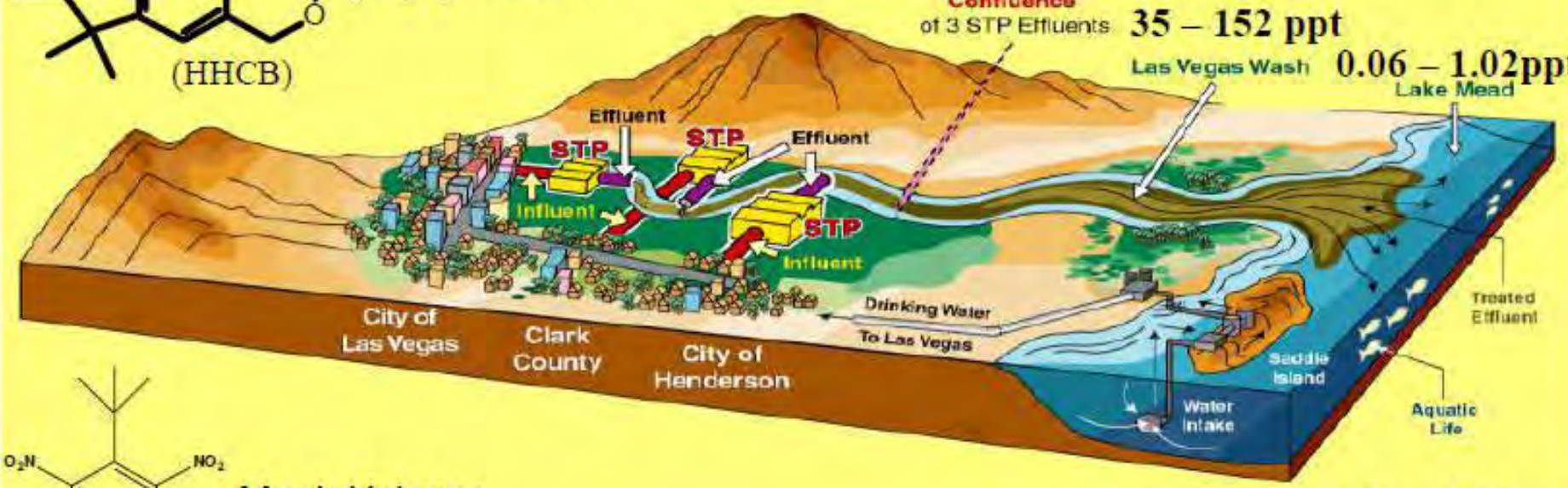
see Osemwengie et al.:
http://epa.gov/nerlesd1/chemistry/pharma/musks.htm

Galaxolide:
polycyclic class

(HHCB)



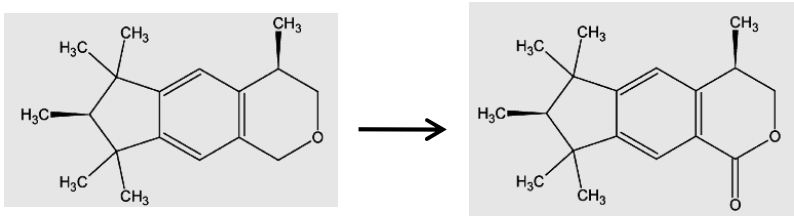
Musk Xylene:
Nitro musk class

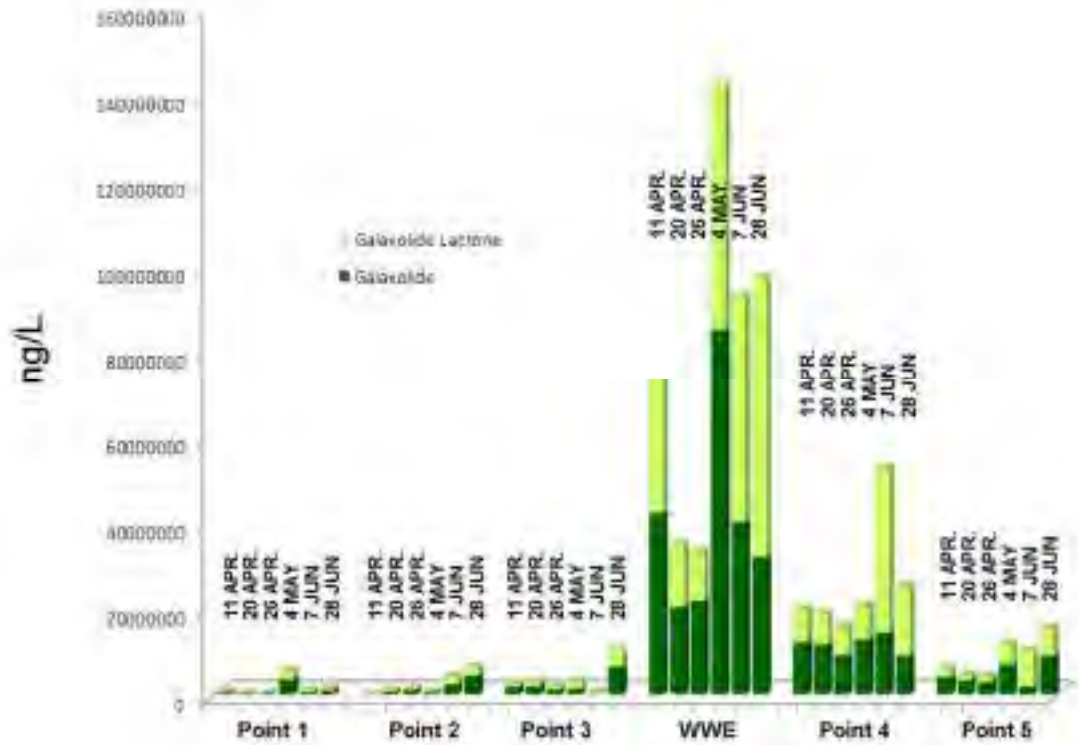


1400-4500 ppt

Slide courtesy of Dr. Lantis Osemwengie

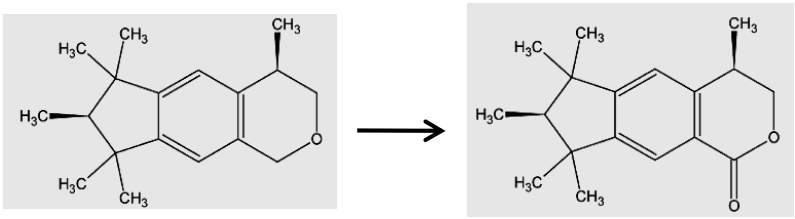
Galaxolide en el Rio Henares, España





Traces of galaxolide were detected in 97% of breast milk samples in mothers from Massachusetts in a 2004 study

Galaxolide



Galaxolide **Galaxolide-lactone**

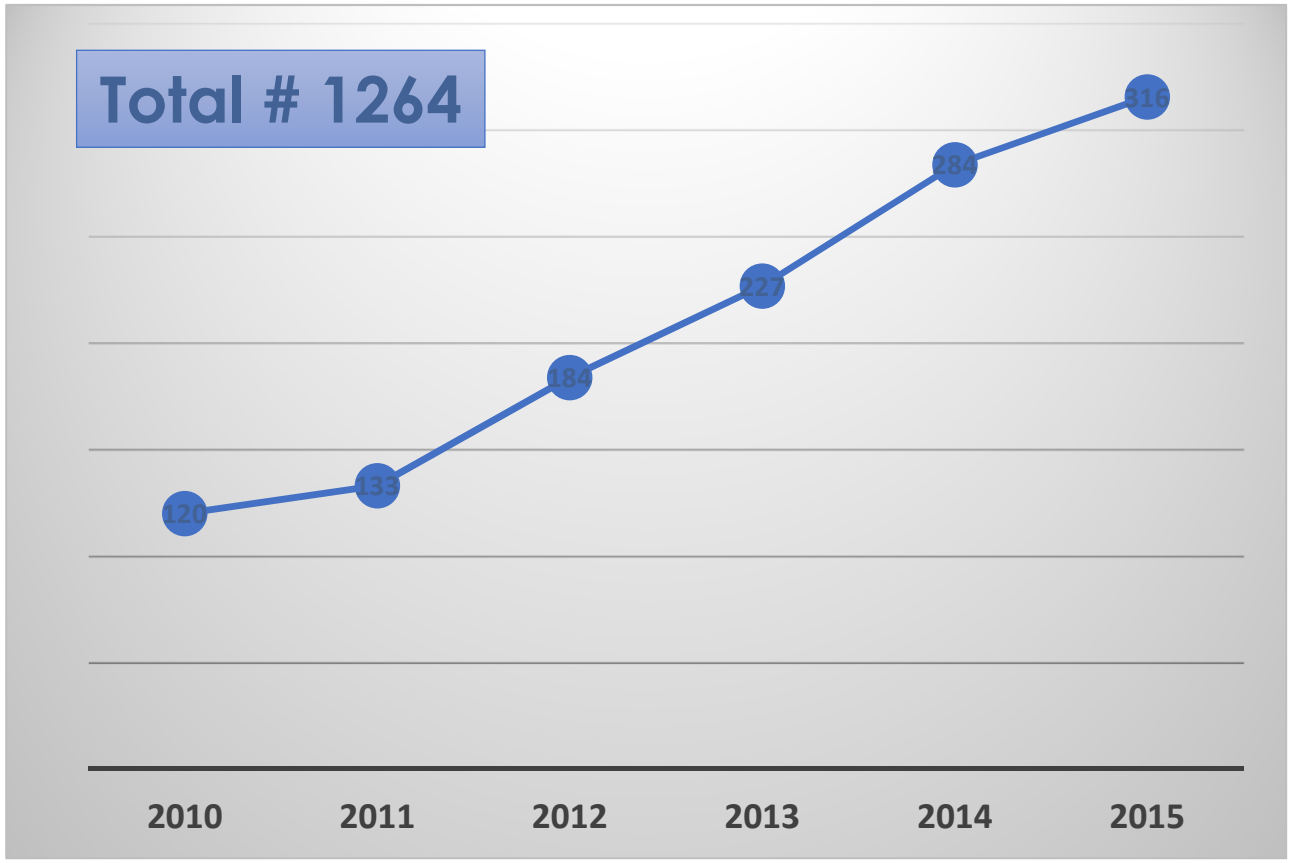
EU: no es un PBT: Persistent, Bioaccumulative and Toxic to the environment (PBT)

EPA: El perfil toxicológico excede el criterio de EPA para un PBT

Traces of galaxolide were detected in 97% of breast milk samples in mothers from Massachusetts in a 2004 study

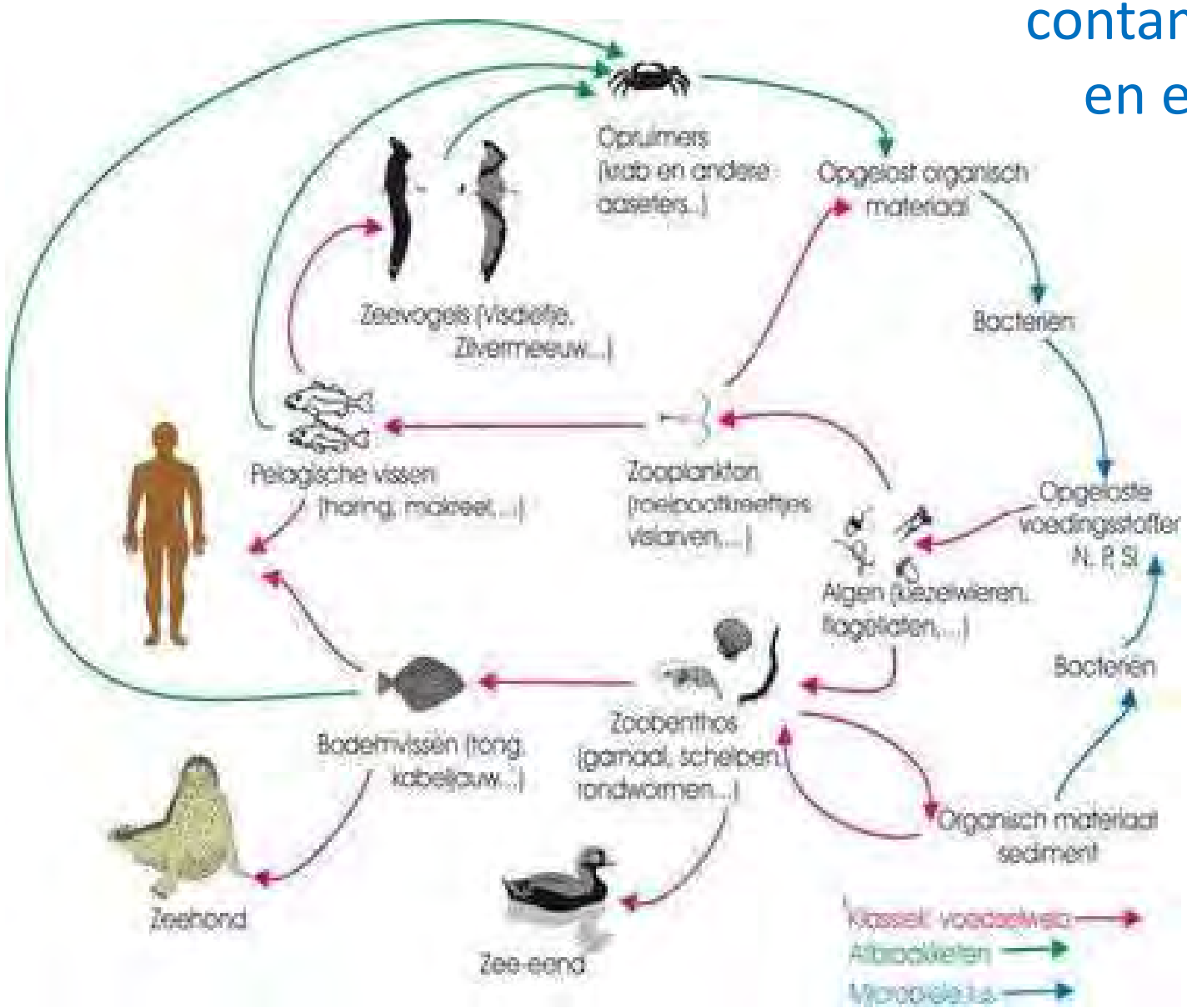
Emerging contaminants & water

Documents/year



Source: Scopus http://www.scopus.com/

Ciclo de contaminantes en el agua

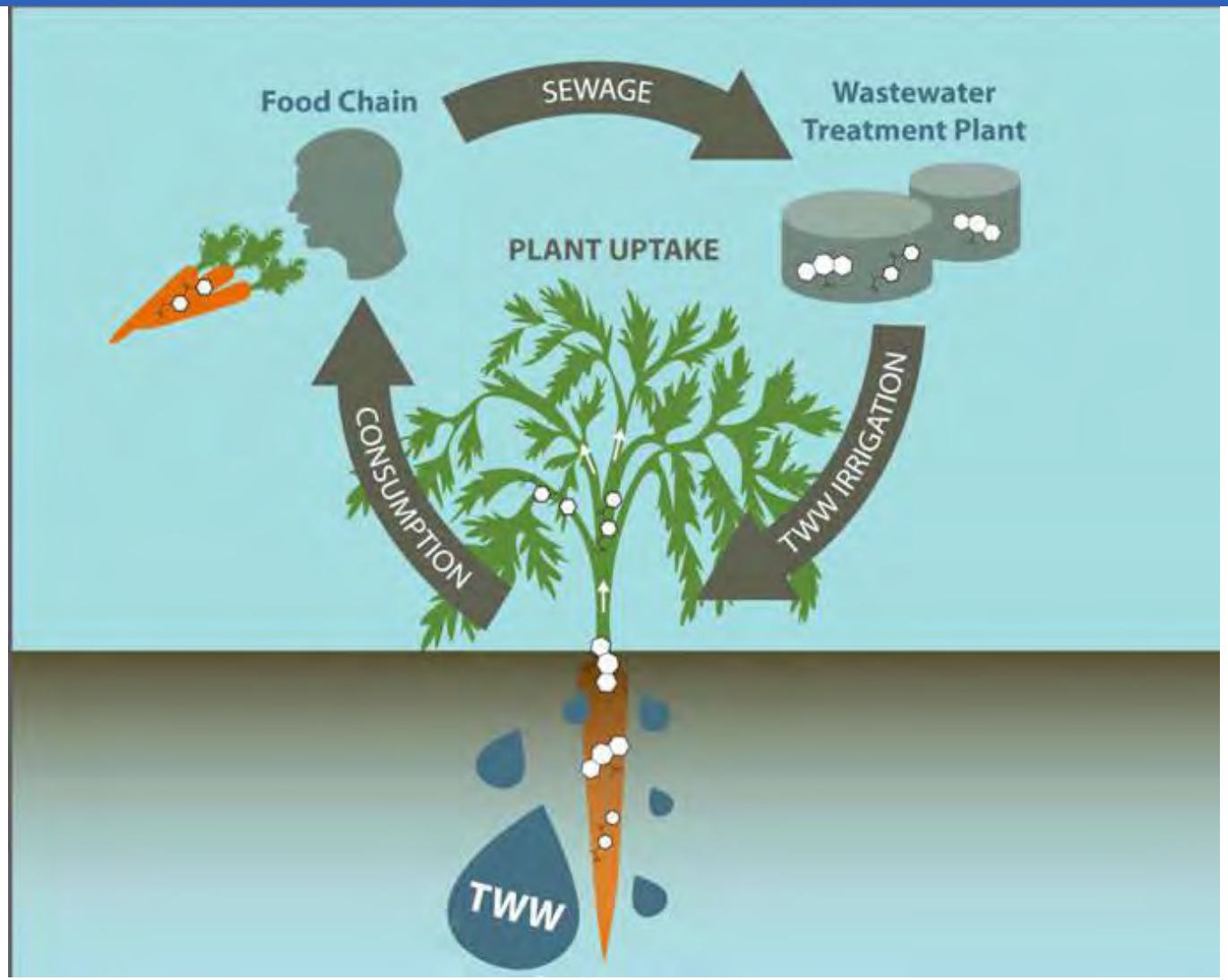


Hallazgos de contaminantes emergentes en distintas regiones del globo

Compounds	Range in concentration (ng/l)						Lowest PNEC (ng/l)	Percentage of parent compound excreted
	North America		Europe		Asia and Australia			
	Effluent, WWTP/STP	Freshwater-rivers, canals	Effluent, WWTP/STP	Freshwater-rivers, canals	Effluent, WWTP/STP	Freshwater-rivers, canals		
Antibiotics								
Trimethoprim	<0.5–7900	2–212	99–1264	0–78.2	58–321	4–150	1000	≥70
Ciprofloxacin	110–1100	–	40–3353	–	42–720	23–1300	20	≥70
Sulfamethoxazole	5–2800	7–211	91–794	<0.5–4	3.8–1400	1.7–2000	20,000	6–39
Analgesics and anti-inflammatory								
Naproxen	<1–5100	0–135.2	450–1840	<0.3–146	128–548	11–181	37,000	–
Ibuprofen	220–3600	0–34.0	134–7100	14–44	65–1758	28–360	5000	≤5
Ketoprofen	12–110	–	225–954	<0.5–14	–	<0.4–79.6	15.6×10^6	–
Diclofenac	<0.5–177.1	11–82	460–3300	21–41	8.8–127	1.1–6.8	10,000	6–39
Salicylic acid	47.2–180	70–121	40–190	<0.3–302	9–2098	–	–	6–39
Mefenamic acid	–	–	1–554	<0.3–169	4.45–396	<0.1–65.1	–	–
Acetaminophen	–	24.7–65.2	59–220	12–777	1.8–19	4.1–73	9200	≤5
Antiepileptics								
Carbamazepine	111.2–187	2.7–113.7	130–290	9–157	152–226	25–34.7	25,000	≤5
Beta-blockers								
Propranolol	–	–	30–44	20	50	–	500	<0.5
Atenolol	879	–	1720	314	–	–	10×10^6	50–90
Blood lipid regulators								
Clofibric acid	ND–33	3.2–26.7	27–120	1–14	154	22–248	12,000	–
Gemfibrozil	9–300	5.4–16	2–28,571	–	3.9–17	1.8–9.1	100,000	–
Bezafibrate	ND–260	–	233–340	16–363	–	–	100,000	40–69

ND—not detected; dashed line—not reported.

Contaminacion del cultivo por aguas residuales



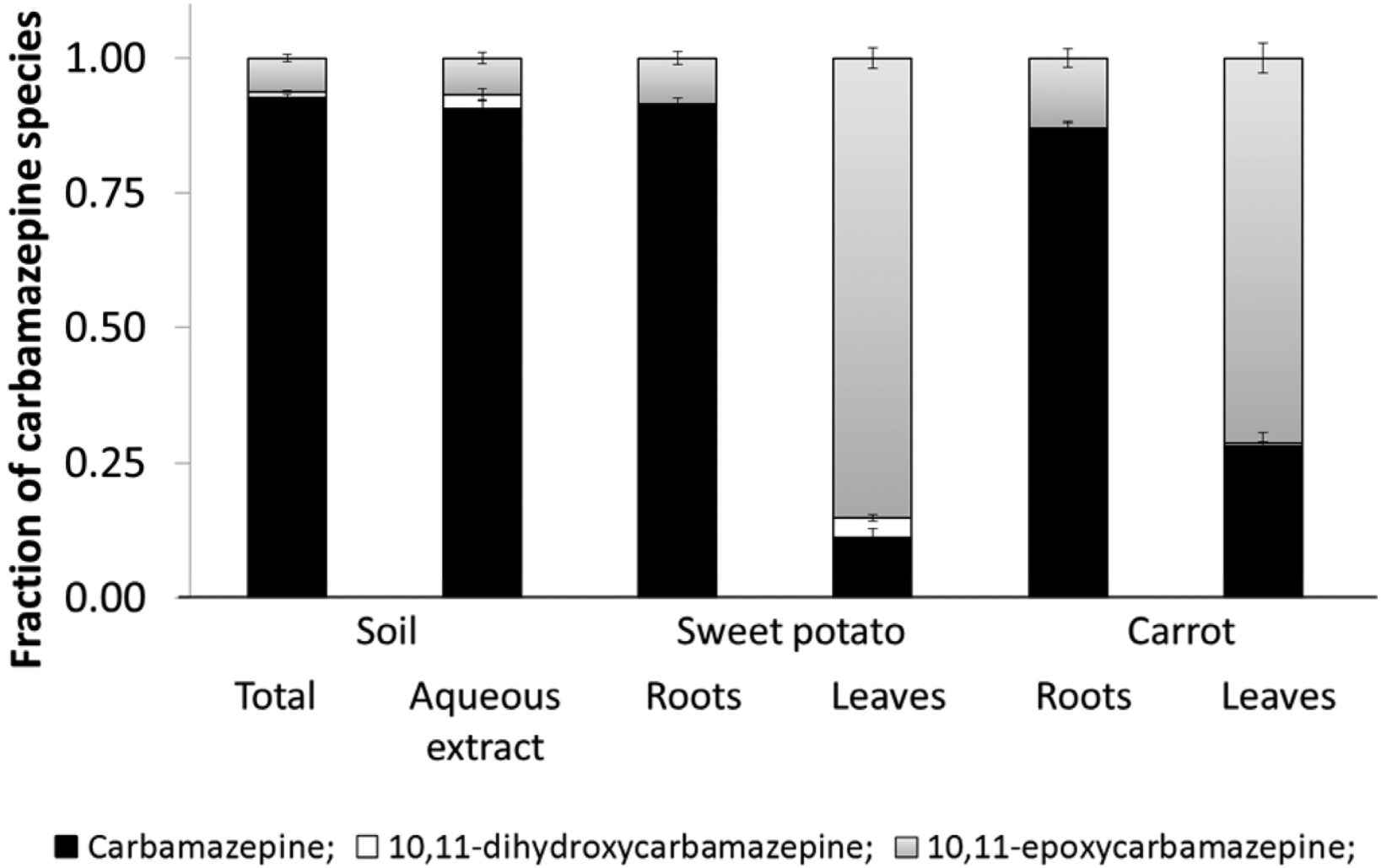
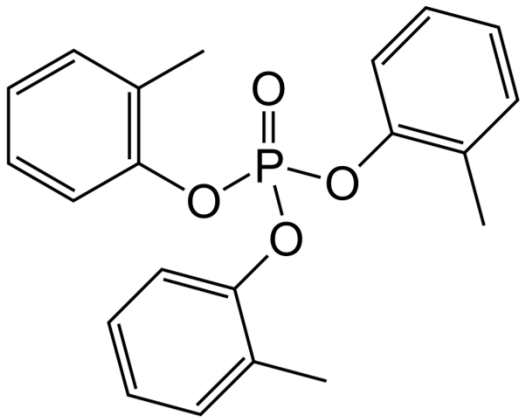
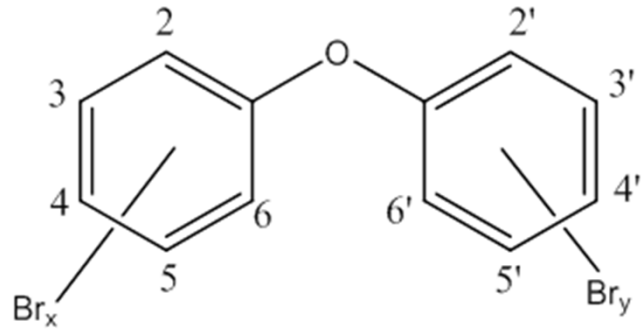


Figure 3. Distribution of carbamazepine and its metabolites, 10,11 epoxycarbamazepine and 10,11-dihydroxycarbamazepine, in the bulk soils, soil aqueous extracts, roots, and leaves of carrots and sweet potatoes. Mean values and standard errors are presented (n = 6).

Algunas familias de contaminantes de riesgo

flame retardants: Organo phosphates & PBDES





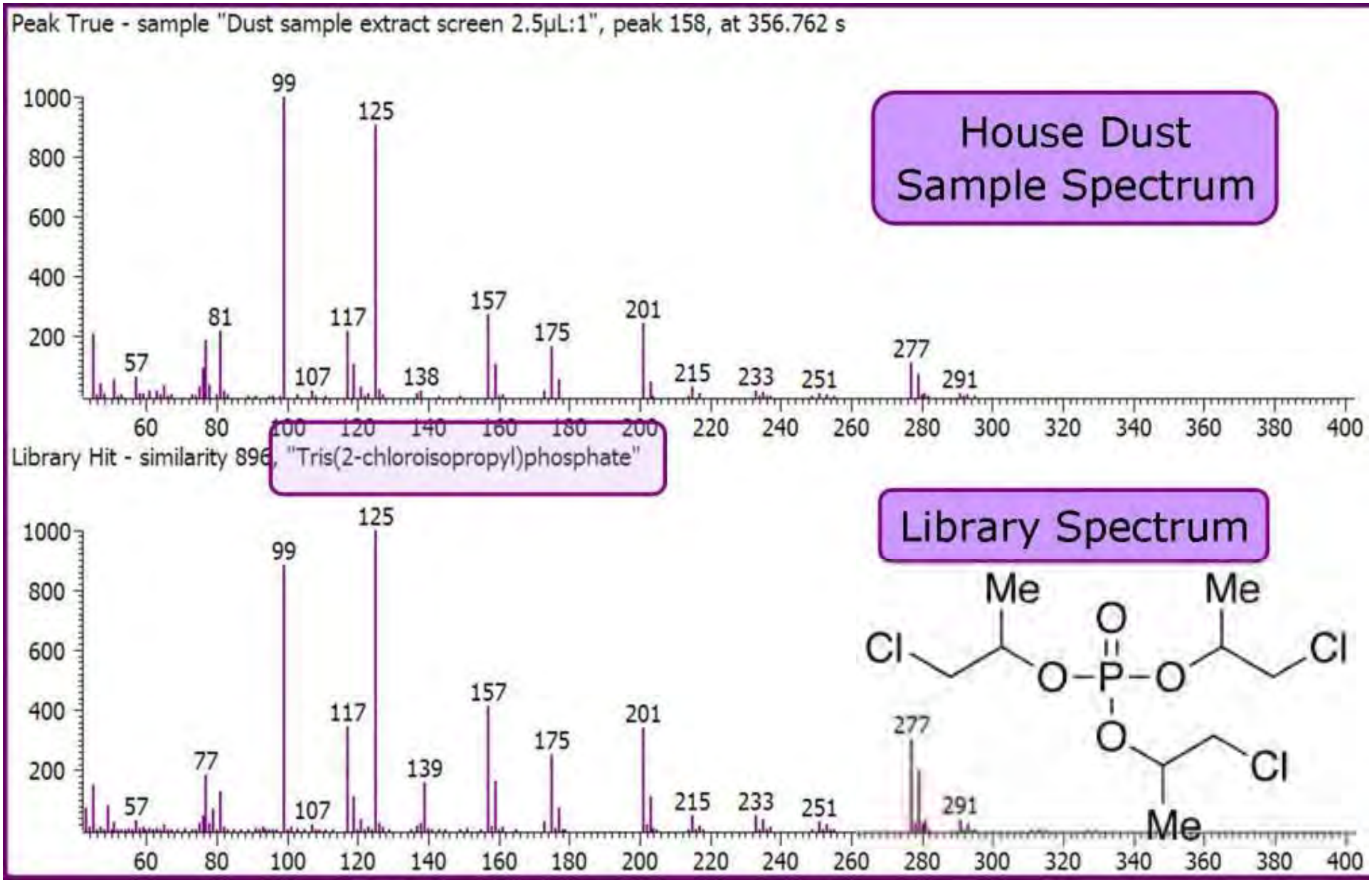
PBDEs

Table 1. Market demand of PBDEs in 1999 (BSEF 2003)

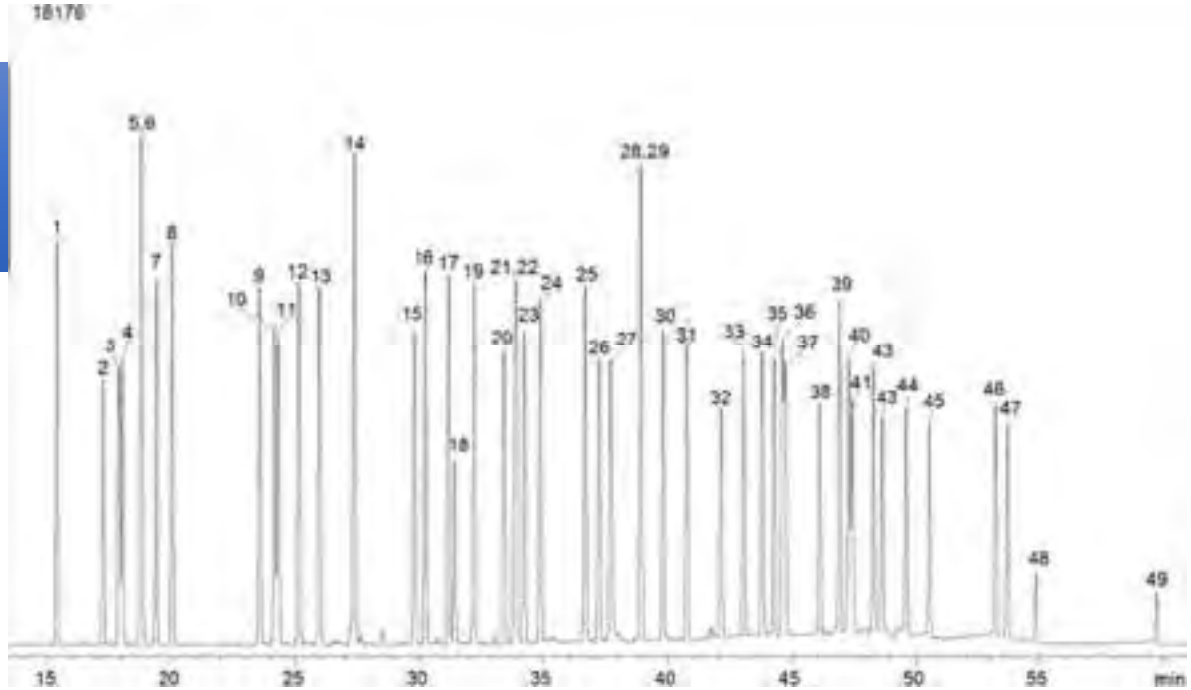
Commercial product	Americas[a]		Europe[b]		Asia[c]	
	Market demand	Estimated consumption (tonnes)	Market demand	Estimated consumption (tonnes)	Market demand	Estimated consumption (tonnes)
DBDE	44%	24 500	13%	7 600	43%	24 050
OBDE	40%	1 500	16%	610	44%	1 680
PeBDE	95%	7 100	2%	150	3%	250

[a] All countries in North, South and Central America were included.
[b] All countries in Eastern and Western Europe were included.
[c] Australia, New Zealand and the Indian subcontinent were included.

Organo phosphates flame retardants



PBDEs


15
20
25
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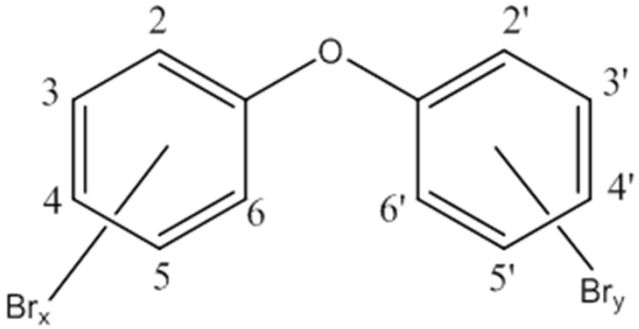
2
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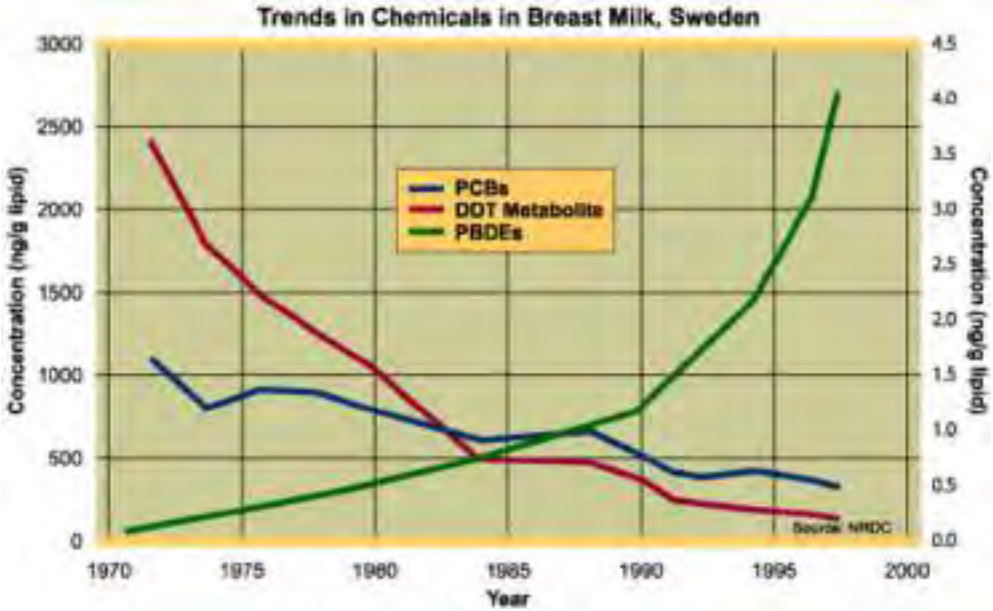
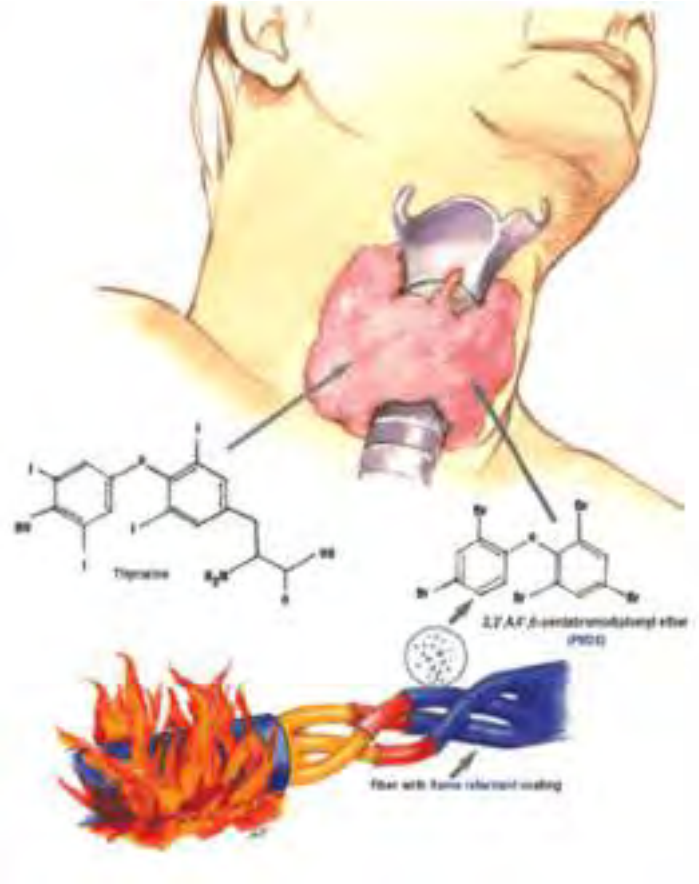
O
Br_x
Br_y

PBDEs


Trends in Chemicals in Breast Milk, Sweden
PCBs
DDT Metabolite
PBDEs
Concentration (ng/g lipid)
Year
Source: NRDC



Polybrominated diphenyl ether (PBDE) flame retardants, widely used in polyurethane foams, electronics and other consumer items, diffuse from the polymer matrix into the air and bioaccumulate in the food chain. Human uptake is through inhalation, dermal absorption and consumption of contaminated foods. PBDEs are structural analogues of thyroid hormones and have been associated with tumors, neurodevelopmental toxicity and thyroid hormone imbalance. PBDE levels in North American women's breast adipose tissue are the highest recorded in the world (forty times the levels in Swedish women).

Table 2. Selected physical and chemical properties of commercial PBDEs and their constituents

Property	PeBDE	OBDE	DBDE
Molecular weight	485.8 (tetraBDE) 564.7 (pentaBDE) (WHO 1994)	643.6 (hexaBDE) 722.3 (heptaBDE) 801.4 (octaBDE) (WHO 1994)	880.4 (nonaBDE) 959.2 (decaBDE) (WHO 1994)
Physical state (20°C; 101.325 kPa)	viscous liquid or semi-solid, white crystalline solid (pure isomers of pentaBDE) (European Communities 2000)	powder or flaked material (European Communities 2002b)	crystalline powder (European Communities 2002a)
Vapour pressure (21°C; Pa)	4.69×10^{-5} (Stenzel and Nixon 1997 in European Communities 2000)	6.59×10^{-6} (CMABFRIP 1997a) 1.58×10^{-6} - 4.68×10^{-7} (hexa – heptaBDEs, 25°C; Tittlemier et al. 2002)	4.63×10^{-6} (CMABFRIP 1997e) 2.95×10^{-9} (estimated for decaBDE; Wania and Dugani 2003)
Water solubility (25°C; µg/L)	13.3 10.9 (tetraBDE) 2.4 (pentaBDE) (Stenzel and Markley 1997)	0.5 (CMABFRIP 1997b)	<0.1 (CMABFRIP 1997f)
Log K_{ow}	6.57 (MacGregor and Nixon 1997)	6.29 (CMABFRIP 1997c) 8.35-8.90 (Watanabe and Tatsukawa 1990 in European Communities 2002b)	6.27 (CMABFRIP 1997g) 9.97 (Watanabe and Tatsukawa 1990 in European Communities 2002a)
Log K_{oa}	10.53- 11.31 (tetra- and pentaBDEs) (Harner and Shoeib 2001)	12.78- 13.61 (hepta- and octaBDEs) (Tittlemeier et al. 2002)	14.44 - 15.27 (estimated for nona- and decaBDE) (Tittlemier et al. 2002)
Henry's law constant (25°C; Pa·m^3/mol)	11 (European Communities 2000)	10.6 (estimated) (European Communities 2002b)	>44 (estimated) (European Communities 2002a)

Hidrocarburos Perfluorados

High lipophyllicity

C’-F bond stable, chemically and biologically inert non inflamability

Surface active, (the acids)

Bioaccumulable compounds

Many of them come from TEFLON disrupting and degradation

Still used in packaging material

Hidrocarburos Perfluorados

- Limited toxicological information
- PFOS &PFOA toxicidad moderada a roedores
- Vida media de 3.5 años en el plasma
- Disminuye la tasa de nacimiento
- Riesgo de mortalidad postnatal
- Disruptores endocrinos sexuales
- Alto riesgo de provocar cancer (higado, pulmones, vegiga)

Hidrocarburos Perfluorados

- EFSA
- Tollerable Daily Intake: 2000ng/Kg BW
- PFOS clasificado como PBT

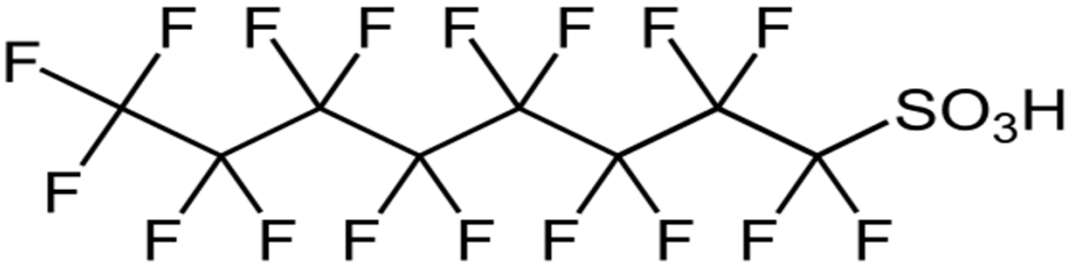
  (PERSISTENT BIOACCUMABLE TOXIC)

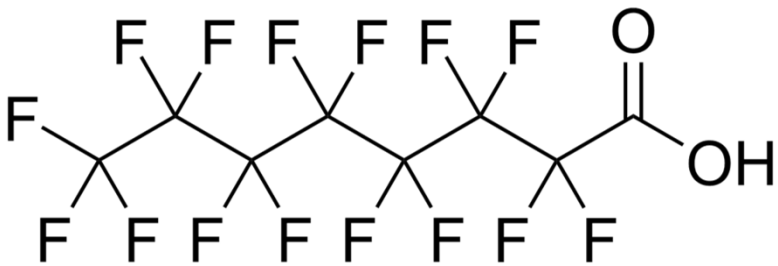
Hidrocarburos Perfluorados

Table 4
Analysis of microwave popcorn packaging before and after microwave process.

Concentration ± SD (ng/g)[a]		PFBA	PFPeA	PFHxA	PFHpA	Total PFCAs[b]
Salty A (ST-A)	1	256 ± 8	36.8 ± 1.0	405 ± 13	7.5 ± 1.3	705 ± 15
	2	208 ± 3	37 ± 3	497 ± 32	7.6 ± 1.0	750 ± 32
Butter A (B-A)	1	280 ± 27	37 ± 3	272 ± 35	3.32 ± 0.17	592 ± 44
	2	236 ± 8	43 ± 4	453 ± 32	4.06 ± 0.16	736 ± 33
Sweet A (SW-A)	1	n.d.	n.d.	2.24 ± 0.06	1.26 ± 0.03	3.50 ± 0.07
	2	n.d.	n.d.	3.4 ± 0.3	1.18 ± 0.18	4.6 ± 0.3
Salty B (ST-B)	1	158 ± 6	26.6 ± 2.3	142 ± 4	2.74 ± 0.20	329 ± 8
	2	158 ± 21	30.3 ± 1.2	303 ± 21	2.18 ± 0.16	493 ± 30
No added fats B (NF-B)	1	n.d.	n.d.	3.79 ± 0.17	1.47 ± 0.13	5.26 ± 0.21
	2	<LOQ	n.d.	8.0 ± 0.5	1.7 ± 0.3	9.7 ± 0.6
Salty C (ST-C)	1	n.d.	n.d.	7.4 ± 0.6	1.91 ± 0.15	9.3 ± 0.6
	2	n.d.	n.d.	7.6 ± 0.7	2.4 ± 0.3	10.0 ± 0.8

1: Microwave popcorn bags before cooking.
2: Microwave popcorn bags after cooking.
n.d.: not detected. Concentration below the detection limit. PFOA, PFNA, PFDA, PFUnA, PFDoA and PFOS were not detected in any sample.
<LOQ: concentration below the quantification limit. Detected but not quantifiable.
[a] Standard deviation ($N = 3$).
[b] Total PFCAs calculated by the sum of the selected PFCAs.





Extraction procedure for the analysis of perfluorinated compounds in popcorn packages

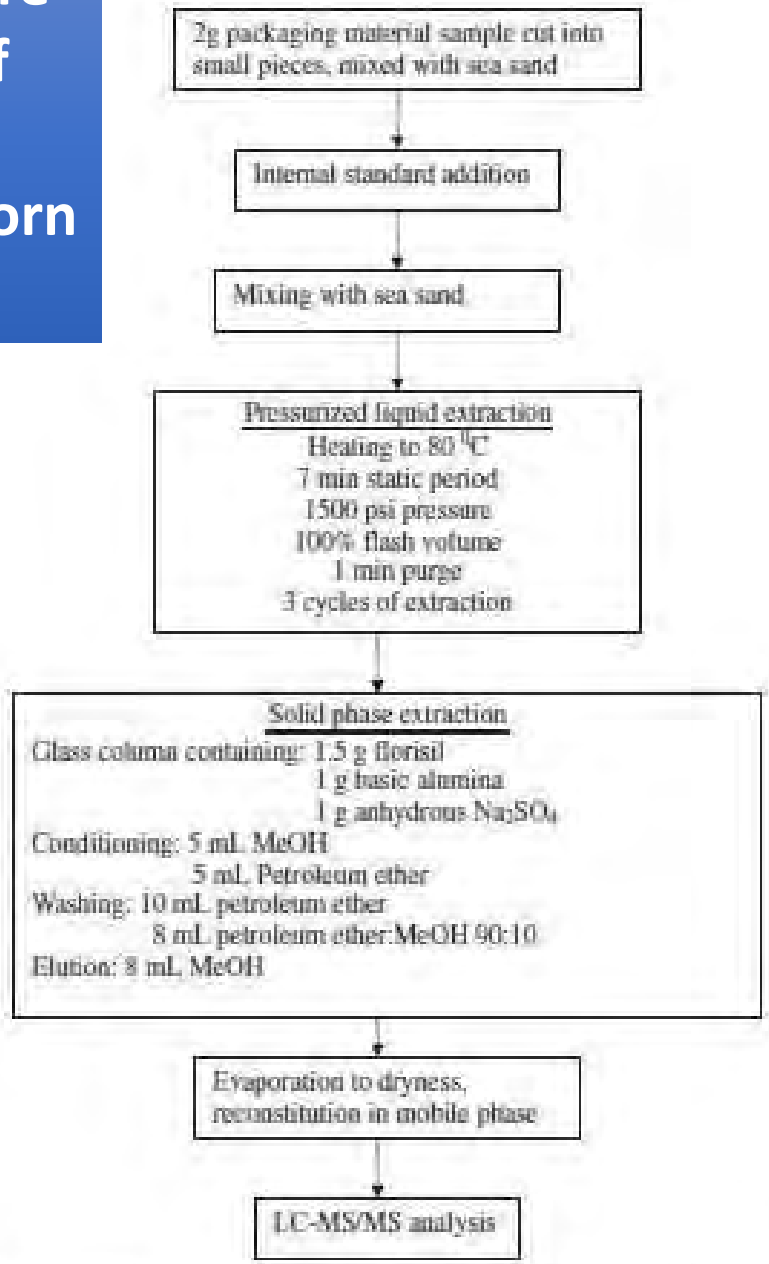
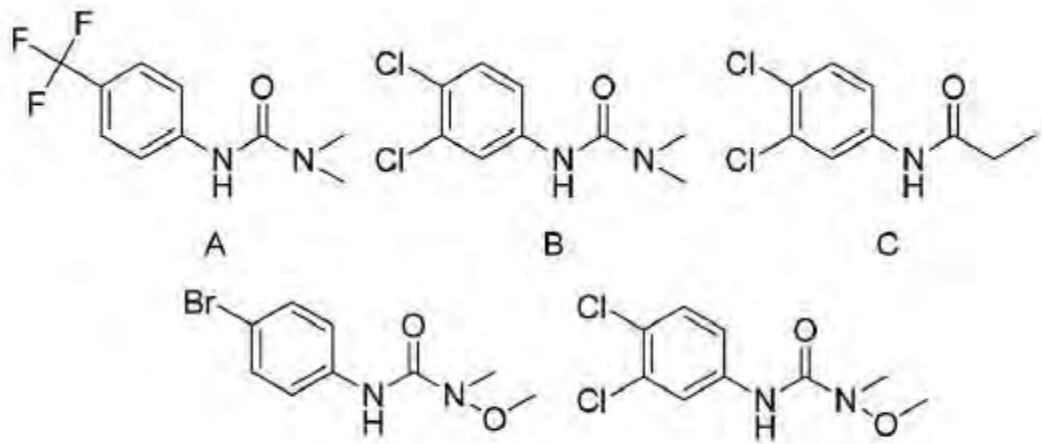
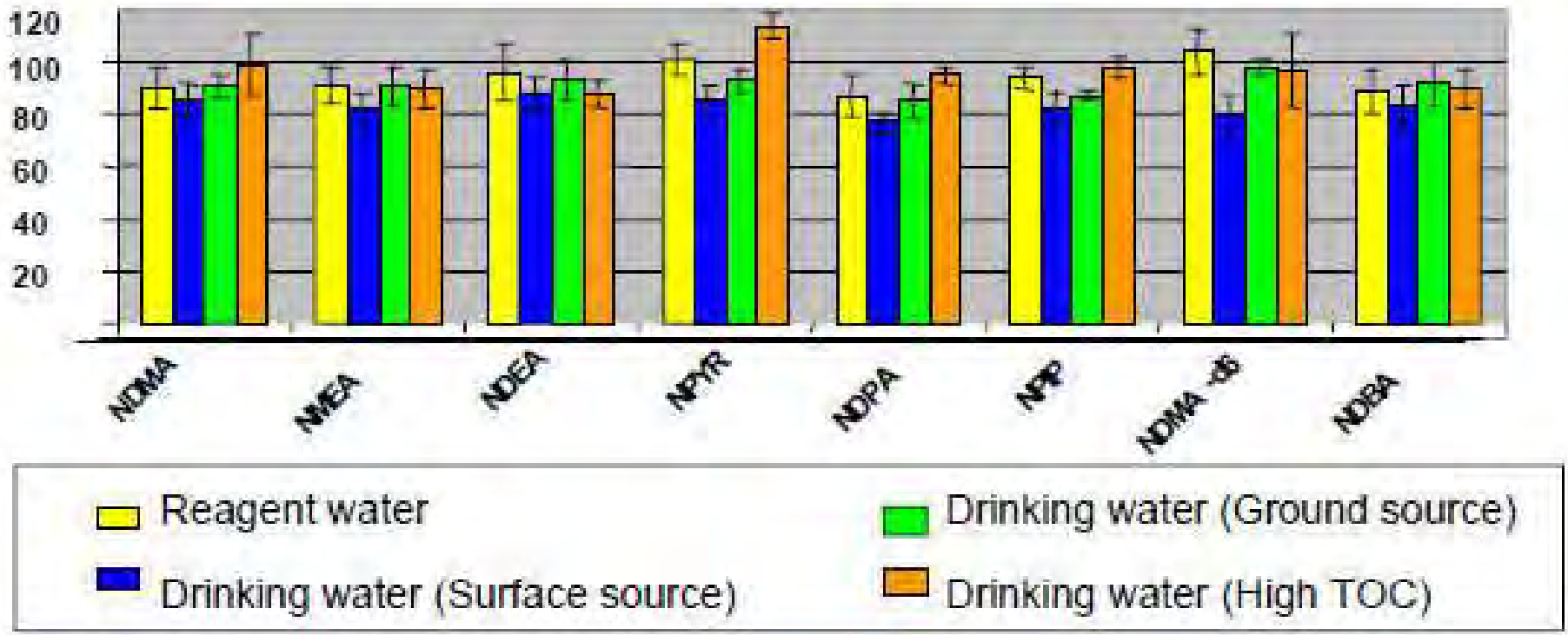


Fig. 1. Schematic presentation of the analytical protocol for PFC analysis in food packaging materials.



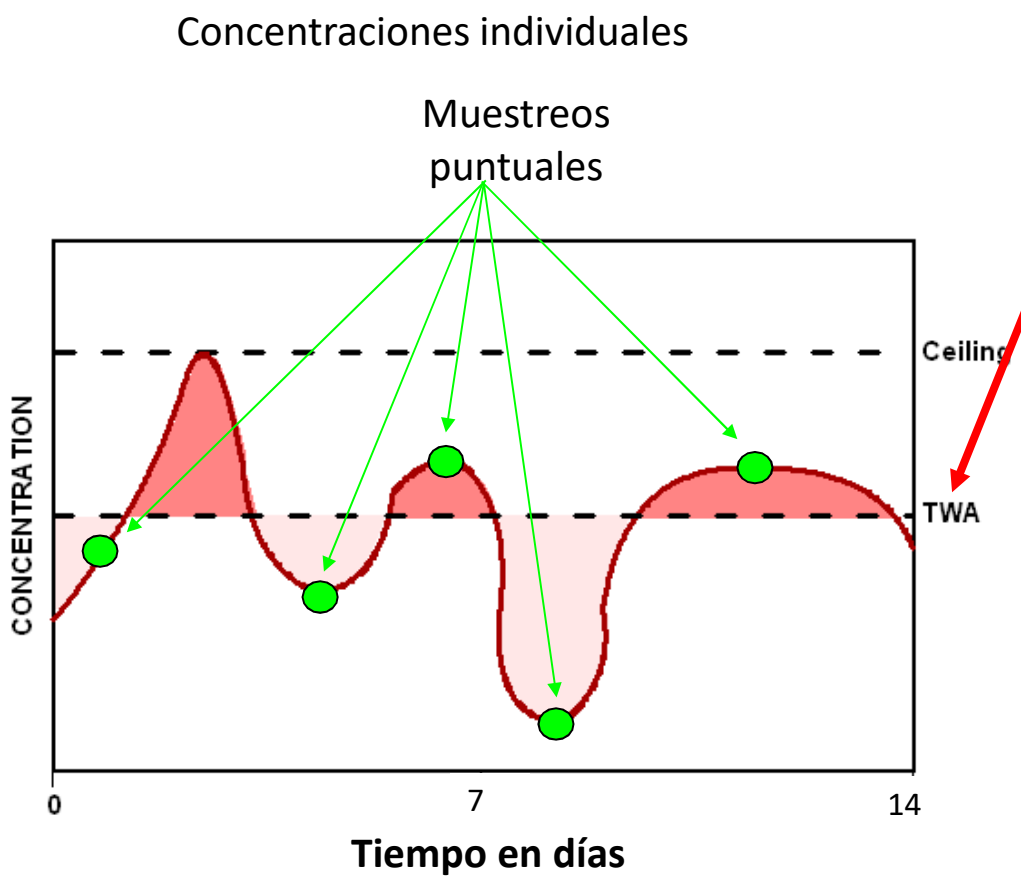
$$NH^{+}_{4} + Cl_{2} \longrightarrow NH_{2}Cl$$

Lowest Concentration Minimum Reporting Level (LCMRL) range 1.2 -2.1 ng/L



Muestreo

Variaciones temporales



TWA = Promedio ponderado temporal
(Temporal weighted avarage)

Variaciones espaciales

Tipos de muestreo

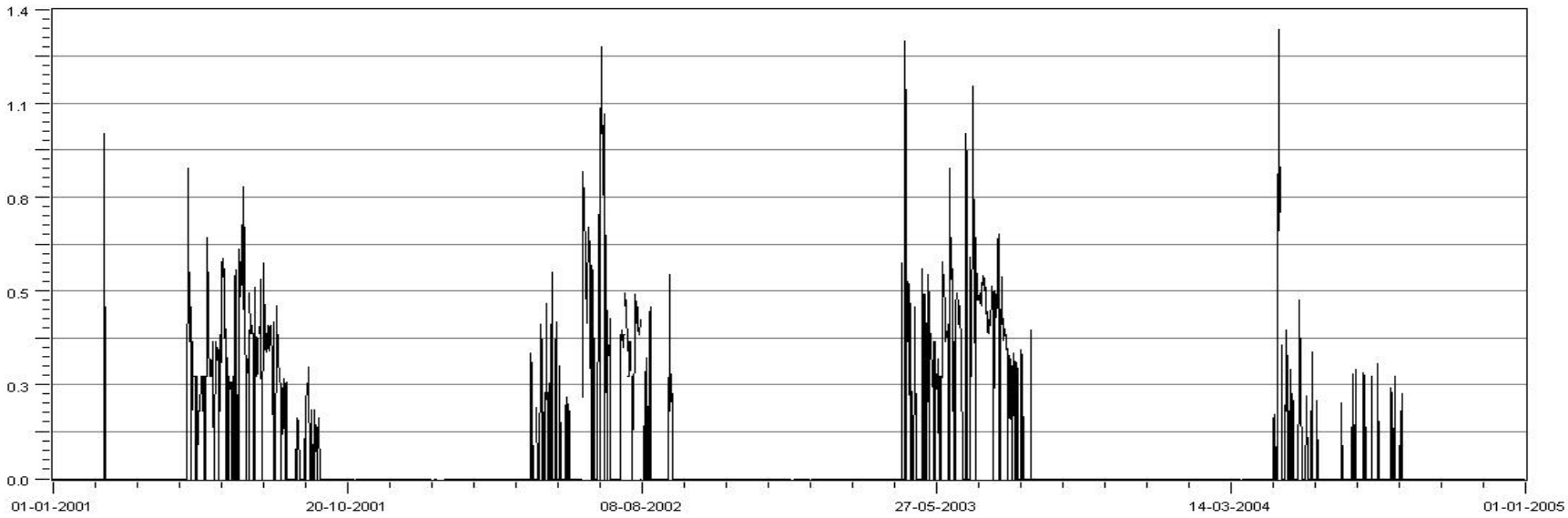
DISCONTINUO

- Muestras puntuales (snap shot)
- Muestras compuestas (espaciales y temporales)

CONTINUO

- Muestra integrativa (activo y pasivo)

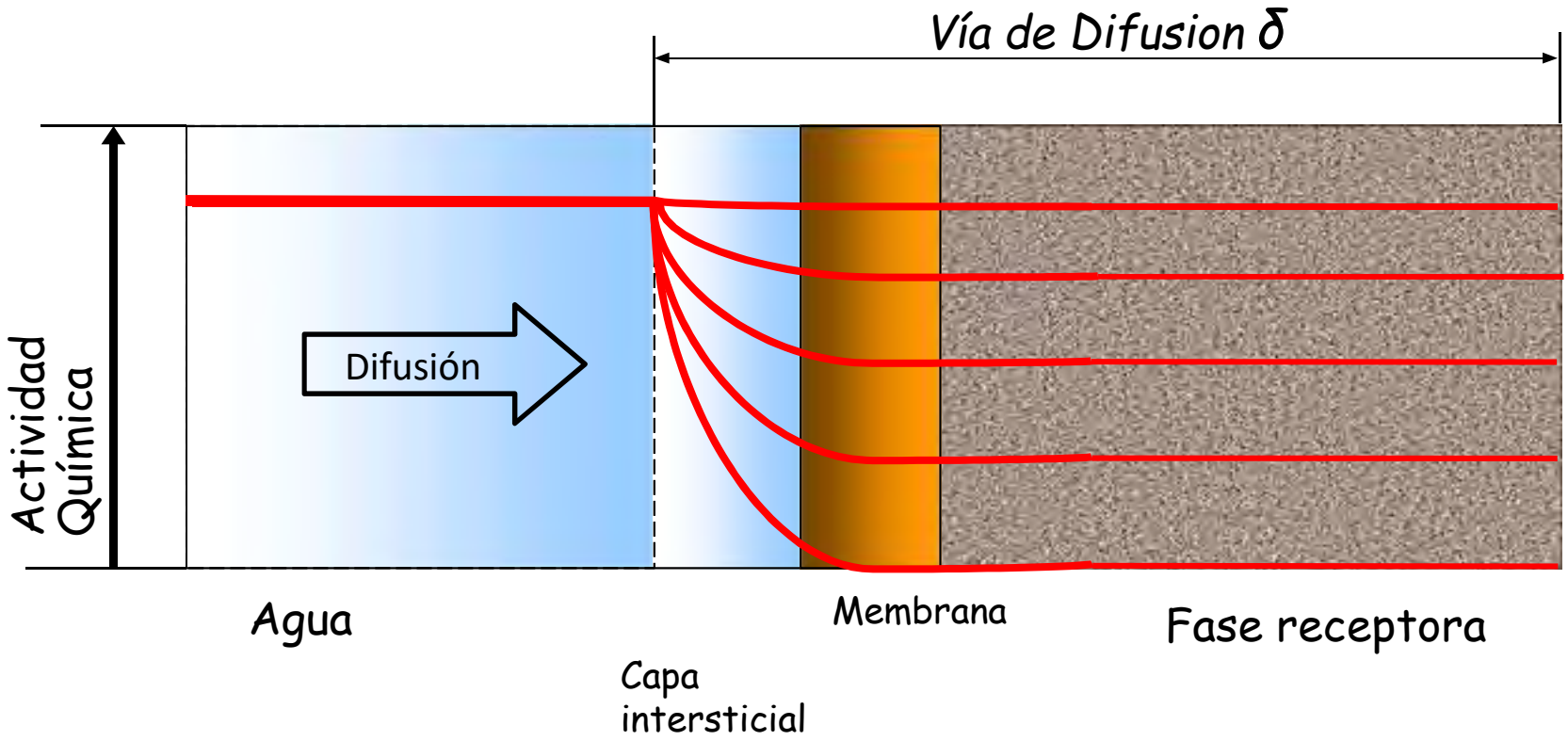
Variaciones temporales



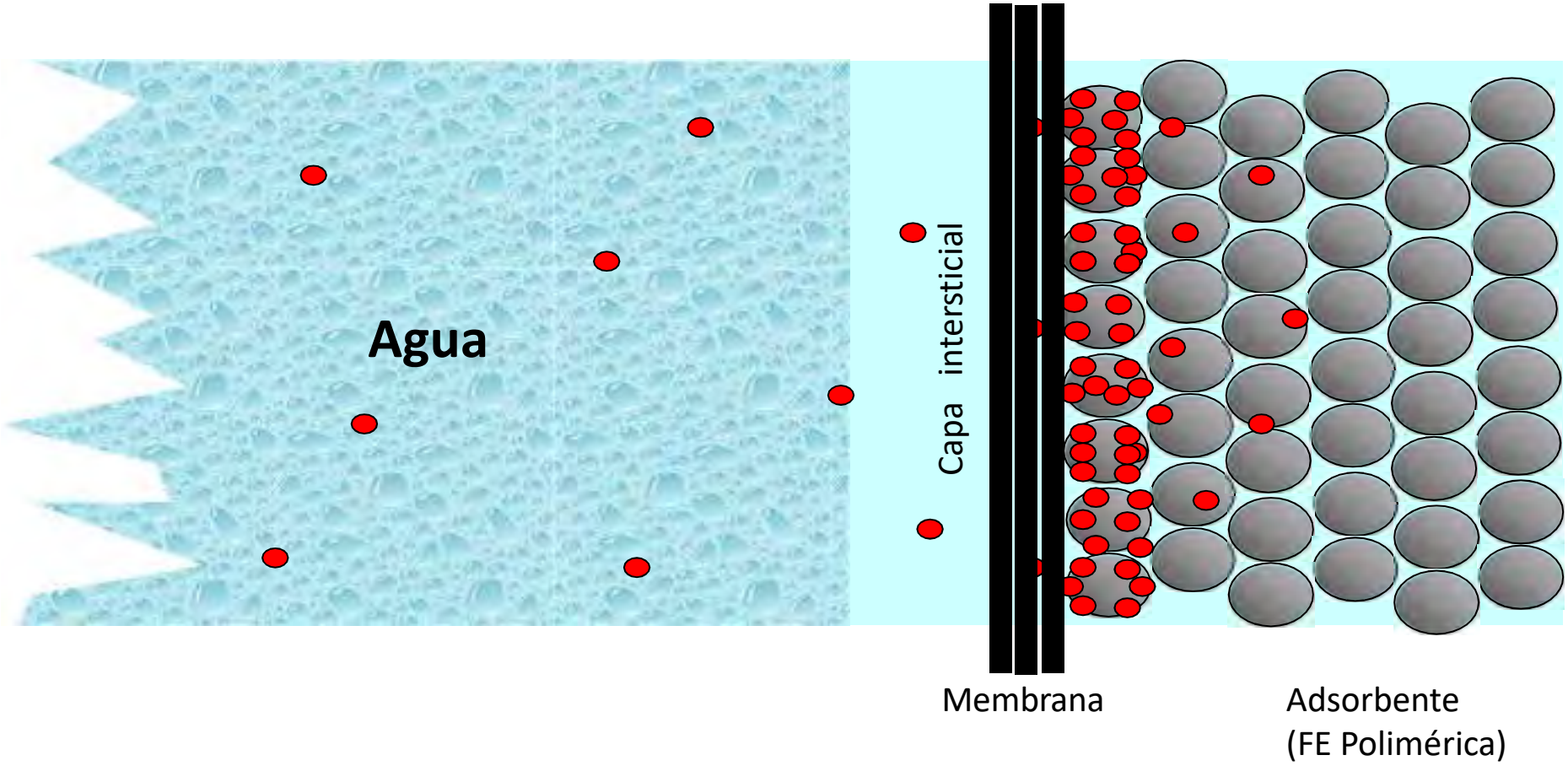
Diuron concentration (µg/ L) continuously monitored in the Meuse River - RIZA's Eijsden station (2001-2005)

Graham Mills Emerging contaminants in waters and soils, practical considerations: Sampling, analysis and consequences Sheffield, 4th March, 2015

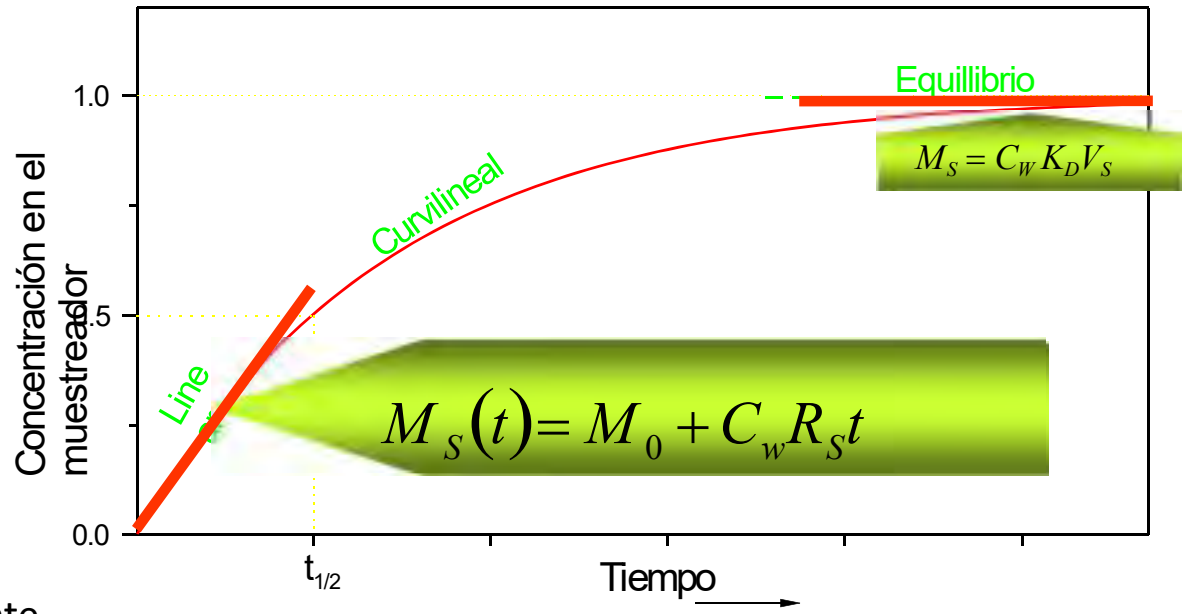
Principios de muestreo pasivo



Ejemplo: muestrador para compuestos polares



Cinética del muestreo pasivo



C_W = constante

R_S = Tasa de muestreo especifica del compuesto [L d^{-1}]

Tasa de muestreo R_S [L d^{-1}] – volumen equivalente de agua "limpiada" del analito por unidad de tiempo

$$Rs = \frac{C_s V_s}{C_W t}$$

C_W = C agua

Métodos Multirresiduos(MRMs)

Metodos analíticos de trazas que intentan cubrir la mayor cantidad de analitos, pesticidas en nuestro caso, realizando un único protocolo de preparación de muestra

Contaminantes Emergentes

US EPA

- Dorgas farmaceuticas
- Disruptores Endocrinos (EDCs)
- Difenil eteres Polibromadas (PBDEs)
- Toxinas algales
- *Cryptosporidium* & *Giardia*
- PCBs
- Metyl-*tert*-butil eter (MTBE)
- DBPs (including NDMA)
- Perchlorate
- Arsenic

EU Framework

- Difenil eteres Polibromados (PBDEs).
- Disruptores Endocrinos (EDCs).
- Alkilfenoles- detergentes.
- Ftalatos

- Prioridades(Candidatos a Monitorear)
  - PPCPs (productos farmaceuticos y de cuidado personal) Diclofenac,Ibuprofeno, EDCs
  - Drogas veterinarias y de uso para alimentacion animal
  - MTBE y compuestos relacionados

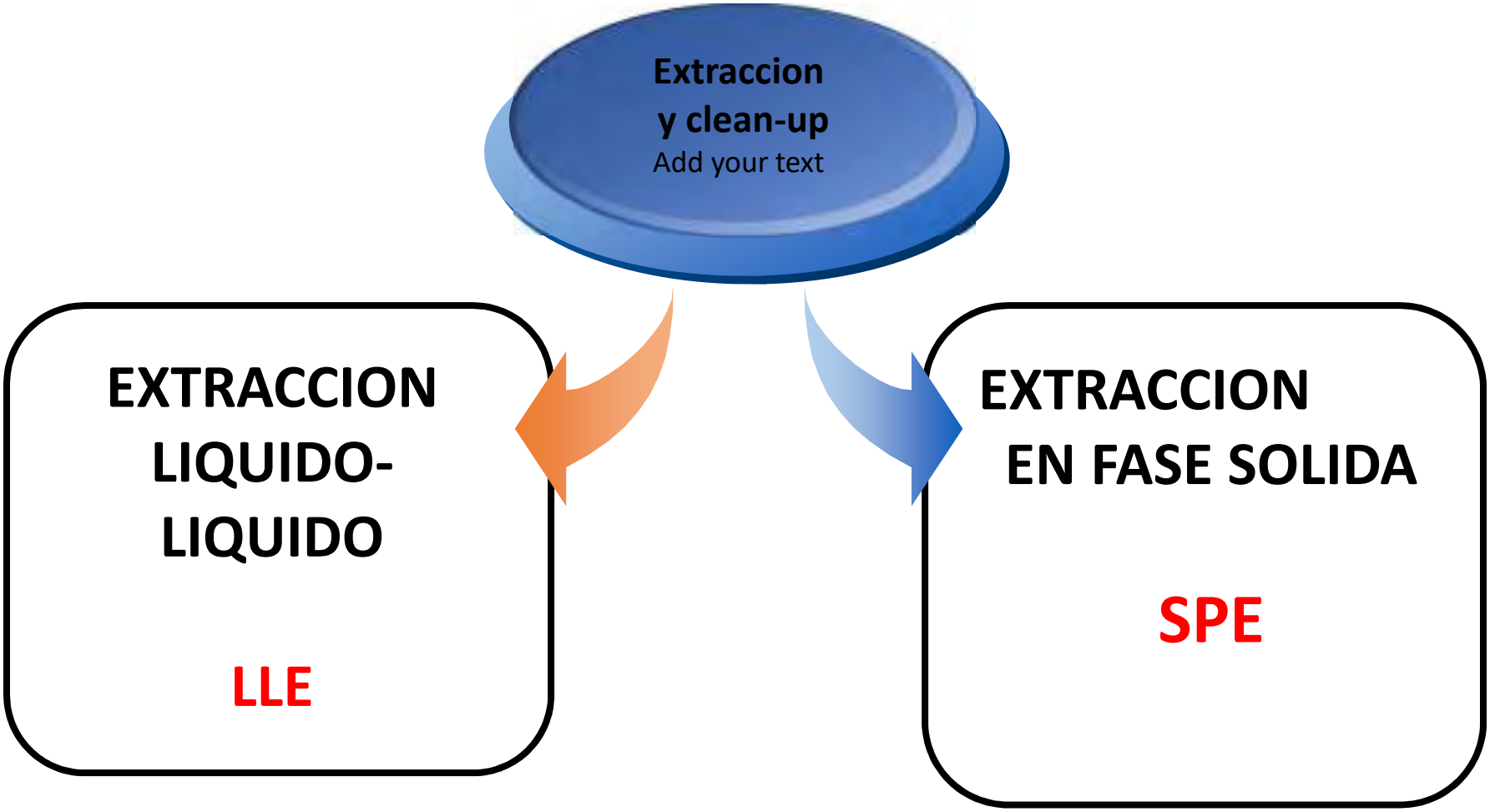
Etapas del análisis

La etapa de preparación de muestra es uno de lo pasos críticos dentro del proceso analítico global

LC/GC Magazine 2002

Tratamiento de muestra: es un proceso por el cual se debe asegurar que la muestra mantiene sus características fisicas y quimicas que la hacen representativa del objeto del estudio

La extraccion y clean up de los analitos target



Otras metodologias usadas para preparacion de muestra

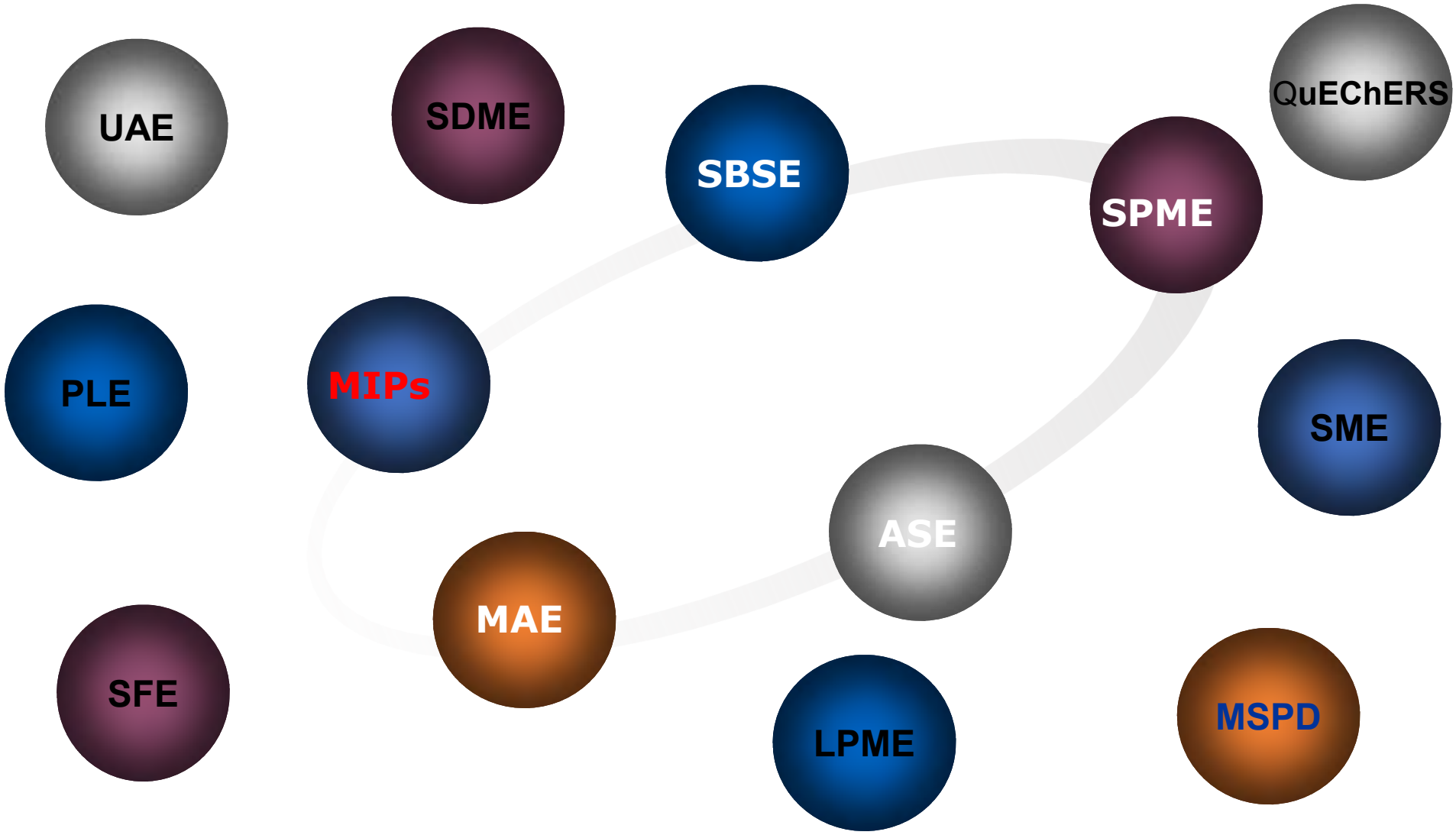
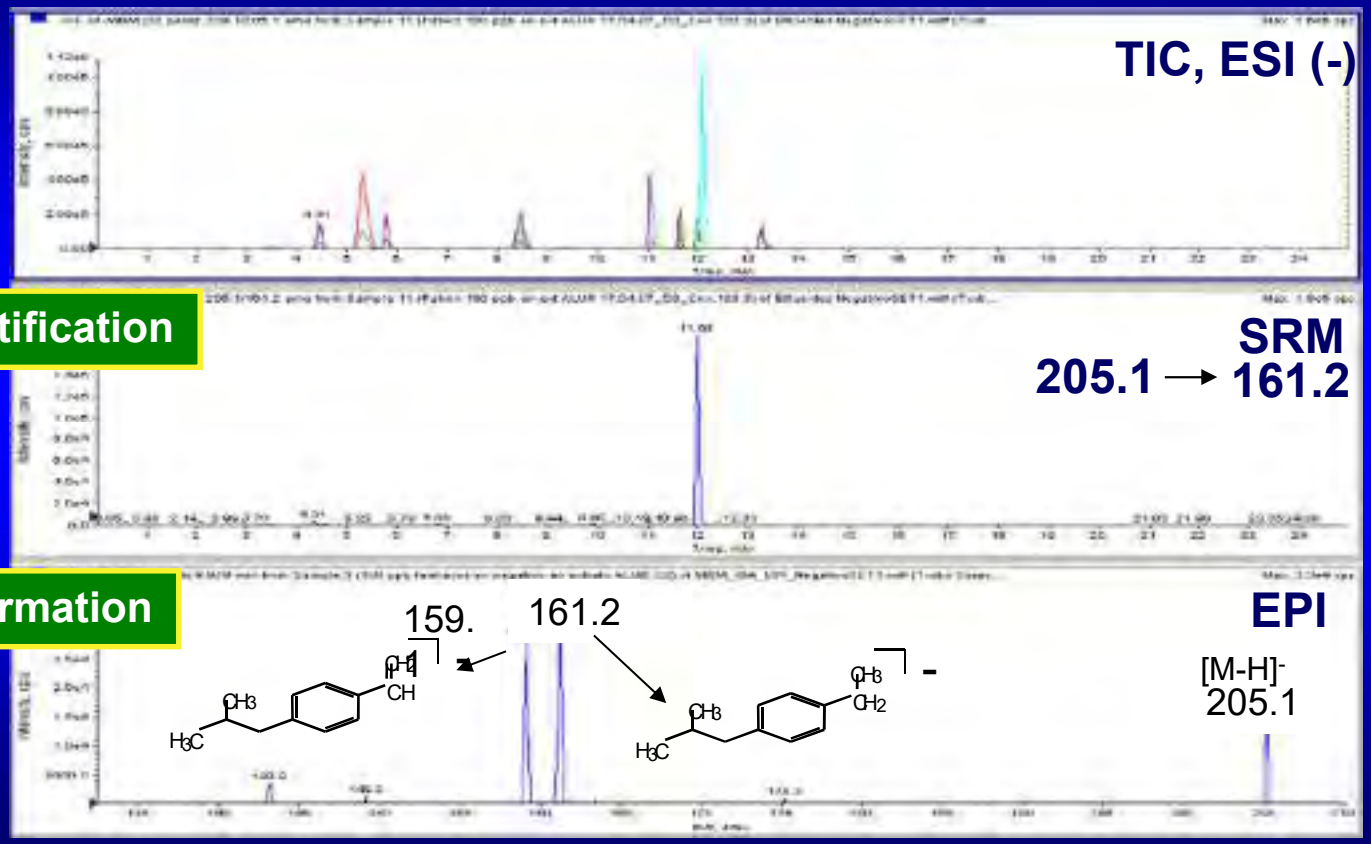


Table 1
Analytical methods reported for benzotriazoles (BTRs), benzothiazoles (BTs) and benzenesulfonamides (BSAs) in environmental samples

Compounds	Matrix	Extraction technique	Instrumental analysis	LODs (ng/L, ng/g)
BT, MeSBT, OHBT, BTR, 4TTR, 5TTR, MepTSA, pTSA, EtpTSA, EtmTSA, EtoTSA	River water and sewage	SPE; Strata-X	GC x GC-HRMS	5–112
BTR, 4TTR, 5TTR, XTR, ClBTR, BT, MeBT, NH_2BT, OHBT, SHBT, MeSBT, BSA, pTSA, EtpTSA	River water and sewage	SPME; polyacrylate	GC-MS/MS	0.1–7 500
BT, MeBT	Influent sewage	SBSE; PA Twister	TD/GC-MS	256
BT, NH_2BT, MeSBT, SO_3BT, SHBT, OHBT	Sewage	SPE; Oasis HLB	LC-MS/MS	25–420 (LOQ)
BTR, 4TTR, 5TTR, OHBTR, ClBTR, BT, NH_2BT, OHBT, MeSBT	River water and effluent sewage	SPE; Oasis HLB	LC-HRMS	1–10.0
BTR, 4TTR, 5TTR	River water and sewage	Direct injection or SPE, Oasis HLB	LC-MS/MS	10–25 (LOQ)
BTR, 4TTR, 5TTR	River water and effluent sewage	SPE; Oasis HLB	LC-MS/MS	0.2 (LOQ)
BTR, 4TTR, 5TTR	River water	SPE, C_{18}	GC-MS	–
BTR, 4TTR, 5TTR	Soil	USAE, DCM	GC-MS	–
BTR, 4TTR, 5TTR	Soil	LSE, ACN/water (1:1)	LC-MS/MS	2.2–3.1
BTR, TTR	River water	SPE; Oasis HLB	LC-MS/MS	3.0–8.0
NH_2BT, BT, MeBT, TCMTBT, MeSBT, SO_3BT, SHBT, OHBT	Sewage	Direct injection	LC-MS/MS	10.0-2 500
BT, MeSHBT, BTR, OHBT, 5TTR, XTR, SHBT	Sewage	SPE; Strata-X	GC-MS	25–562
BTR, TTR, OHBT, NH_2BT, XTR, SHBT, BT, MeBT, MeSBT	River water and sewage	SPE; Oasis MAX	LC-MS/MS	2–286 (LOQ)
BTR, 5TTR, ClBTR, XTR	River water and sewage	SPE; Oasis HLB	GC-MS/MS	14–47
	Sludge and sediment	PLE; MeOH/DCM (50:50)	GC-MS/MS	3.0–14.0
BTR, OHBTR, TTR, XTR, BT, OHBT, MeSBT, NH_2BT	Sewage	SPE; Strata-X	LC-MS/MS	0.08–16.70
	Sludge	USAE and SPE; MeOH/water (1:1)	LC-MS/MS	0.04–12.50
BT, MeSBT, SO_3BT, OHBT, MorBT	River water and sewage	SPE; HLB	LC-MS/MS	0.5–200 (LOQ)
	Sludge	PLE and SPE; MeOH/water (1:1)	LC-MS/MS	2.5–100 (LOQ)
BTR, 5TTR	Sludge and sediment	LSE and SPE; MeOH and Oasis HLB	LC-MS/MS	0.07
OHBTR, BTR, 4TTR, 5TTR, ClBTR, XTR, NH_2BT, OHBT, SHBT, BT, MeSBT	Influent sewage	DLLME; TBP	LC-Flu-UV	40-2 600
			LC-MS/MS	–
BSA, pTSA, oTSA	River water and sewage	SPE; SDB	LC-MS/MS	10–20 (LOQ)
BTR, TTR	River water and sewage	SPE; Oasis HLB	LC-MS/MS	3.0–8.0
BTR, MeBTR, 4TTR, 5TTR	Effluent sewage and sludge	SPE; Oasis HLB	GC/IRMS	1 900-6 000
BTR, 4TTR, 5TTR, XTR, ClBTR, BT, NH_2BT, OHBT, MeSBT, BSA, pTSA, oTSA, MepTSA, EtpTSA	River water and sewage	SPE; Oasis HLB and Florisil	LC-MS/MS	1.0–20.0
BTR, 4TTR, 5TTR, XTR, ClBTR, BT, NH_2BT, OHBT, MeSBT, BSA, pTSA, oTSA, MepTSA, EtpTSA	Sludge	PHWE and SPE; Water and Oasis HLB and Florisil	LC-HRMS	0.25–25
BTR, 4TTR, 5TTR, XTR, ClBTR, BT, NH2BT, OHBT, MeSBT, BSA, pTSA, oTSA, MepTSA, EtpTSA	Sludge	QuEChERS; ACN and Z-sep+	LC-HRMS	0.5–10
BTR	Sewage	Derivatization and LLE; Toluene	GC-MS	10 000 (LOQ)

LC-QLIT-MS/MS

IBUPROFEN



Estrategias analiticas

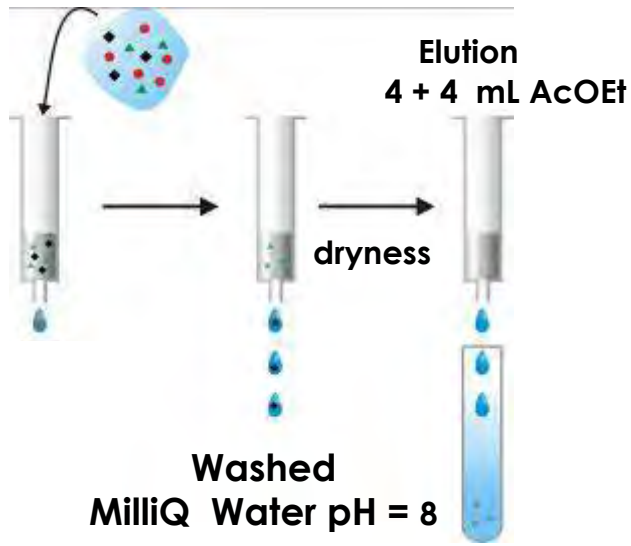
- Naturaleza química de los compuestos a estudiar
- Que metodología analítica separativa tengo disponible?

HPLC DAD, GC y detectores convencionales, GC MS, GCMS/MS, LC MS/MS

Sample Preparation

pH8
Filtration
0.8 µm prefilter
0.45 µm filter

OASIS HLB®
200 mL sewage water pH = 8
400 mL surface water



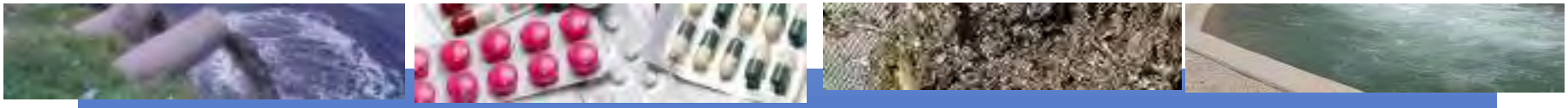
SPE

Evaporation
N_2

GC-MS

1mL TPP internal std
0,5 µg/mL

Gómez MJ *et al.* (2007) *Chemosphere* 66: 993-1002



Analysis GC-MS (SIM mode)

EXPERIMENTAL

Matrix match calibration (synthetic sewage water)

Confirmation:

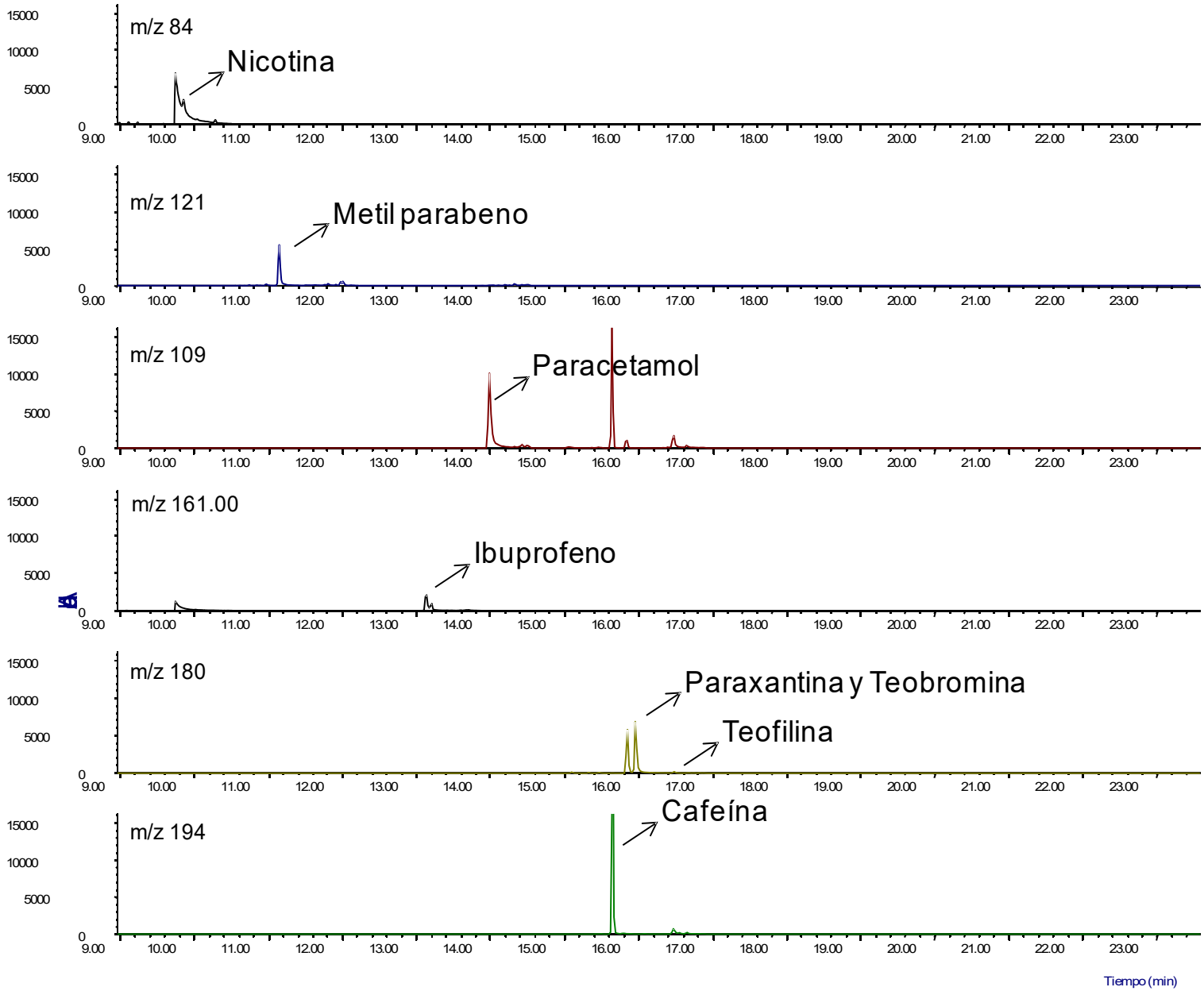
- Retention time
- 3 specific ions(1 Quant + 2 Qual)
- Ion ratio (criterio DG-SANCO/12571/2013)

OECD (2001) Monograph 303 A: Activated Sludge Units *OECD guideline for the testing of chemicals* p.6

Evaluated parameters

- **Linearity (LOQ – 1000 µg/L)**
- **Detection and Quantification Limits (LODs & LOQs)**
- **Accuracy: Recoveries (two concentration levels, 1 y 10 µg/L)**
- **Precision (RSD %)**

RESULTS


m/z 84
Nicotina
m/z 121
Metil parabeno
m/z 109
Paracetamol
m/z 161.00
Ibuprofeno
m/z 180
Paraxantina y Teobromina
Teofilina
m/z 194
Cafeína
15000
10000
5000
0
9.00
10.00
11.00
12.00
13.00
14.00
15.00
16.00
17.00
18.00
19.00
20.00
21.00
22.00
23.00
Tiempo (min)

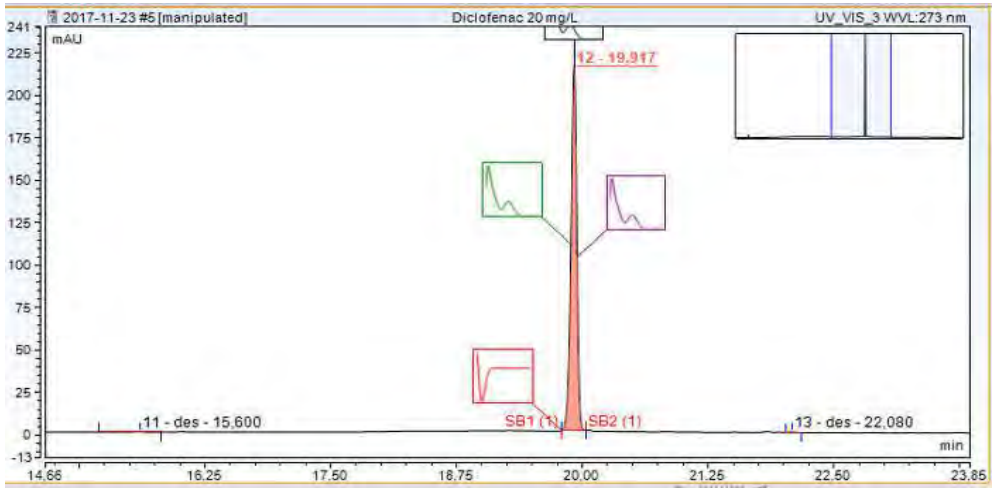
Contaminantes emergentes por HPLC-DAD

#	Compuesto	Longitud de onda característica (nm)
1	Cafeína	273
2	Ibuprofeno	225
3	Paracetamol	241
4	Diclofenac	275
5	Ketoprofeno	254
6	Carbamazapina	215
7	Ácido salicílico	241

Método EC-1	
Fase estacionaria	Hypersil Gold (Thermo Scientific)
	C18 (5µm, 150 × 4.6mm)
Fase móvil	Agua 0.1 % Ácido fórmico / MeOH / MeCN
Flujo de columna	1.0 mL. min^{-1}
Temperatura columna	35 °C
Duración de la corrida	35 minutos
Longitudes de onda monitoreadas (nm)	220 / 241 / 273 / 254
Vinyección	20 µL
Modo de inyección	Full loop
Observaciones	Método adaptado según Baranowska et al, 2011.

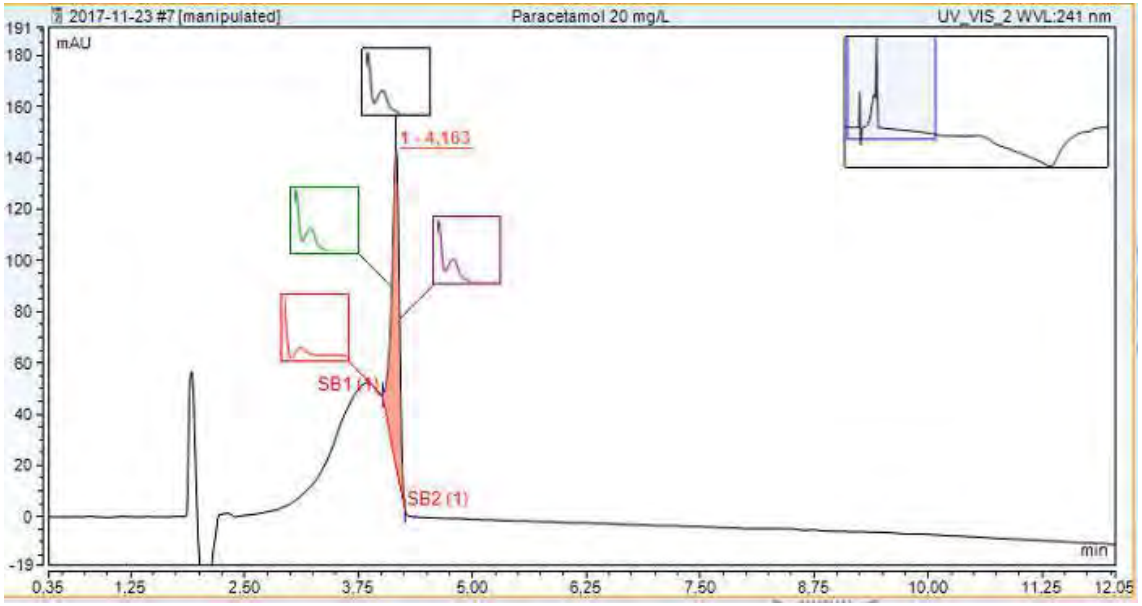
Gradiente de fase móvil

Tiempo (min)	Solvente			Flujo ($mL.min^{-1}$)
	A (%)	B (%)	C (%)	
0.0	89	10	1	1.0
10.0	50	30	20	1.0
15.0	50	30	20	1.0
17.0	30	20	50	1.0
25.0	5	5	90	1.0
30.0	89	10	1	1.0



ibuprofeno

paracetamol

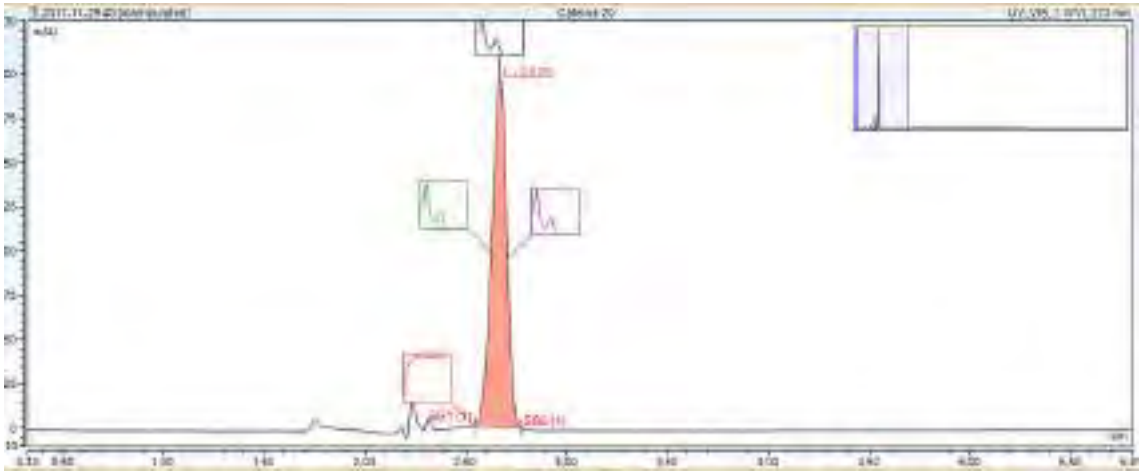


Fase estacionaria	Hypersil Gold (Thermo Scientific) C18 (5µm, 150 × 4.6mm)
Fase móvil	Agua / MeCN
Flujo de columna	1.0 mL. min^{-1}
Temperatura columna	35 °C
Duración de la corrida	30 minutos
Longitudes de onda monitoreadas (nm)	220 / 241 / 273 / 254
Vinyección	20 µL
Modo de inyección	Full loop
Observaciones	Método adaptado, Natalia G.

Gradiente de fase móvil

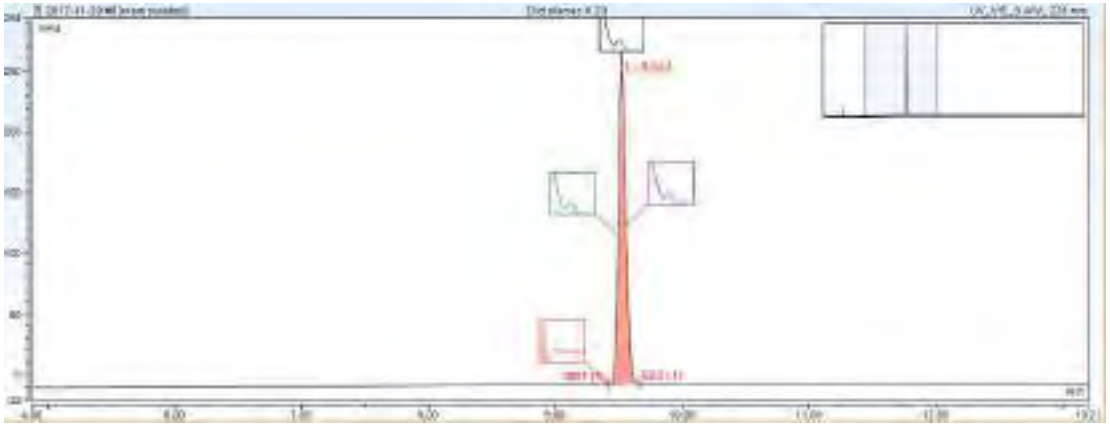
Tiempo (min)	Solvente		Flujo (mL.min^{-1})
	A (%)	C (%)	
0.0	80	20	1.0
5.0	50	50	1.0
6.0	40	60	1.0
8.0	30	70	1.0
12.0	20	80	1.0
16.0	15	85	1.0
20.0	0	100	1.0

cafeina

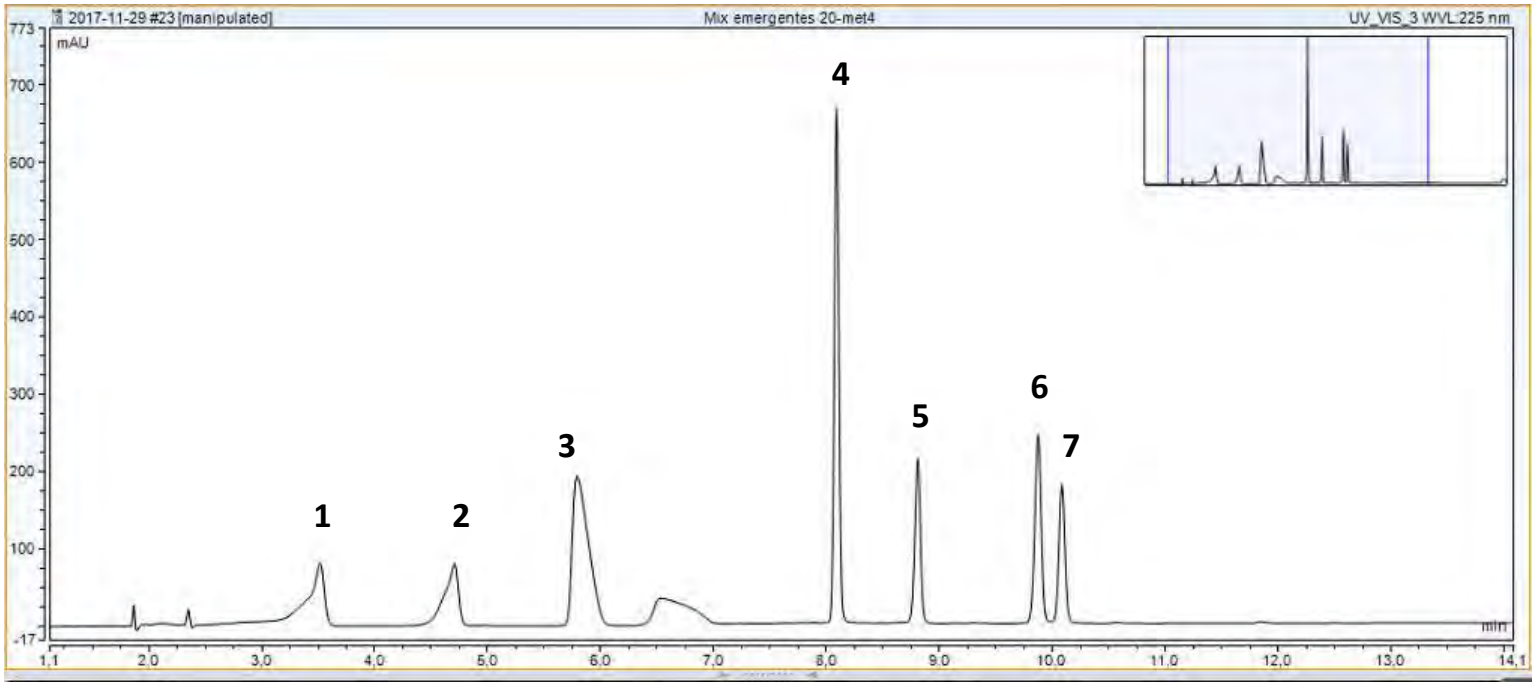


ibuprofeno

diclofenac



Cromatograma HPLC DAD de los distintos contaminantes emergentes



1- Paracetamol,2- Cafeína,3- Ácido salicílico, 4- Carbamazepina
5- Ketoprofeno, 6- Diclofenac, 7- Ibuprofeno

Ensayos interlaboratorio:DEFINICIÓN

Determinación de la performance analítica de un laboratorio a través de la comparación de sus resultados con los de otros laboratorios

ISO/IEC Guia 43/1997

Ensayos interlaboratorios PTs

OBJETIVOS

- **Determinar el desempeño de laboratorios y su seguimiento**
- **Identificar problemas en los laboratorios**
- **Iniciar acciones correctivas: por ejemplo el desempeño del personal o con la calibración del instrumental**
- **Establecer la efectividad y el grado de comparación de nuevos métodos y el seguimiento de los métodos**
- **Proveer confianza adicional a los clientes de los laboratorios;**
- **Identificar diferencias interlaboratorios.**
- **Determinar las características de desempeño de un método, a través de parámetros como repetibilidad, reproducibilidad e incertidumbre.**

Ensayos interlaboratorios PTs

Para dar un sustento técnico a la necesaria demostración de comparabilidad entre las mediciones que se realizan en diferentes laboratorios y cumplir con la normativa ISO/IEC 17 043

Ensayos interlaboratorios PTs

Por que son necesarios?

- **Es un requisito de la norma ISO/IEC 17 025**
- **Los organismos de Acreditación como EA 03/04 lo postulan como una herramienta para la acreditación del análisis**
- **Es una recomendación de documentos oficiales para garantizar la confiabilidad de los resultados**

Los Proficiency Tests se ven como una herramienta poderosa para demostrar competencia frente a organismos de acreditación o cualquier otro tercero

“It is clear that the primary aim of proficiency testing has a significant educational element”

EA-03/04 3.2

Herramienta útil para aquellos laboratorios que no están acreditados de demostrar competencia

- TRAZABILIDAD
- TRANSPARENCIA
- CAPACIDAD TECNICA

PORQUE DE LA IMPORTANCIA DE RESULTADOS CONFIABLES

PT en contaminantes emergentes RLA7019

Organizador

Universidad de Almeria, Prof. Amadeo R. Fernandez Alba

Fecha de distribución de muestras: diciembre 2017

Entrega de resultados 31 de enero 2018

Tipo de ensayo

Ring Test

todos los analitos están presetnes, en distintas concentraciones

Analitos

IBUPROFENO **IMIDACLOPRID**

CAFEINA **TEBUCONAZOL**

DICLOFENAC **CARBARILO**

PARACETAMOL **CLORPIRIFOS**

ATRAZINA

reflexiones

El universo de los contaminantes emergentes es muy vasto

Su principal amenaza es a las fuentes de aguas, por lo tanto algunos pesticidas son considerados dentro de este grupo (atrazina, diuron, clorpirifos)

Paulatinamente se van incluyendo en las normativas para preservación del recurso hídrico

Es necesario conocer las características de los compuestos para ajustar las condiciones de análisis

Si bien la cromatografia acoplada a LC o GC (MS/MS) es la técnica de elección, los detectores convencionales pueden ser una guía para estudiarlos.

La participacion en proficiency tests es una forma útil para los laboratorios para comenzar a incursionar en la temática.

Agradecimientos
Natalia Gerez
Estudios por LC-DAD

Muchas gracias!!!!