Stable isotope ratios of POPs
–
a tracer that can lead to the origins of pollutions

Walter Vetter[1], Wolfgang Armbruster[1], Simon Gaul[1], Gerd Gleixner[2], Jürgen Schleucher[3], Tatiana Beston[3], Katja Lehnert[1]

[1] **Universität Hohenheim, Institut für Lebensmittelchemie**
  D-70593 Stuttgart, Germany

[2] **Max-Planck-Institut für Biogeochemie**
  D-07701 Jena, Germany

[3] **Medical Biochemistry and Biophysics, Umeå University,**
  SE-901 87 Umeå, Sweden

Background

- research on halogenated pollutants is still an urgent task

- understanding of transport and fate is on a high scientific level

    $\Rightarrow$ important models for the distribution and partitioning in environmental compartments developed (e. g. Wania and Mackay)

however:

- to date no successful tracing back of contaminations in a sample to the real source (i. e. a specific product or producer) achieved

    $\Rightarrow$ isotopic data may provide such information

The polluter pays ?

left-over pesticide residues (photos 2006)

- chemical analysis will provide kind of product

- question that remains: who was the producer ?

photos: Bernstorff

Masses and ratios of stable isotopes

- many elements possess stable (non-radioactive) isotopes

m a s s e s   **i n v a r i a b l y**   **c o n s t a n t**

^{1}H	1.0078	^{2}H	2.0140	^{12}C	12.0000	^{13}C	13.0034

ratios of stable isotopes vary slightly due to fractionating processes

H = (0.99985)*(1.007825) + (0.00015)*(2.0140) = 1.007**9**7 g/mol

adundance *   mass   +   adundance   * mass   = atomic mass

- the same compound can have slightly different ^{13}C (^{2}H) content
- deviations occur in the forth digit after the period
- slight variations in the permil range (‰) are of great relevance

Processes that *can* lead to isotope fractionation

- evaporation/condensation

- chromatography

- biochemical processes

- chemical reactions

- exchange with surrounding

Exact determination of stable isotopes

- due to fractionation processes slight enrichment/depletion of the more heavy isotopes

- technique used for authenticy determinations for food

    $\Rightarrow$ sugar, water, ethanol, flavor compounds

- technique established in different fields of natural sciences (geochemistry, ecology, food chemistry)

- little isotopic data exist for halogenated compounds

IRMS technique

- **measurements not possible with standard MS**
  - ⇒ **high precision gas analysis isotope MS required**

for carbon analysis: organic material is combusted to CO_2	**for hydrogen analysis: sample is pyrolyzed to H_2**

- isotopic composition of the gas is measured

- direct inlet or GC inlet available

- samples weighed in tin/silver capsules (mg´s)

- >50 ng per compound required
- compounds must not co-elute

Introduction of the δ-notation ($\delta^{13}C$ values [‰])

- absolute determinations suffer from bad reproducibility
  $\Rightarrow$ relative measurements possible with high precision
  $\Rightarrow$ international conventions: use of "V_{PDB}-standard"

$$\delta^{13}C \text{ [‰]} = \frac{[^{13}C/^{12}C]_{sample} - [^{13}C/^{12}C]_{standard}}{[^{13}C/^{12}C]_{standard}} \times 1000$$

values in permil

- smaller $^{13}C/^{12}C$ ratio in sample compared to standard leads to negative $\delta^{13}C$ values

$\Rightarrow$ the poorer a sample is in ^{13}C, the more negative the $\delta^{13}C$ value is

Stable isotope analysis – where useful

- synthetic compounds (petrol chemistry) not considered to vary significantly in stable isotope composition

- **Example 1: toxaphene:**

  $\Rightarrow$ synthesis starts from a natural product
      (unique for chloropesticides!)
  $\Rightarrow$ the monoterpenes camphene or α-pinene
  $\Rightarrow$ monoterpenes are known to vary in their content of
      stable isotopes in dependence of the origin

- isotopic differences also found for toxaphene products from different sources ?

Toxaphene

- chloropesticide with broad range of applications

- exhaustive photochlorination of camphene/α-pinene
  $\Rightarrow$ starting material is waste of the wood industry

- degree of chlorination 67 - 69 %

- mixtures of several hundred compounds

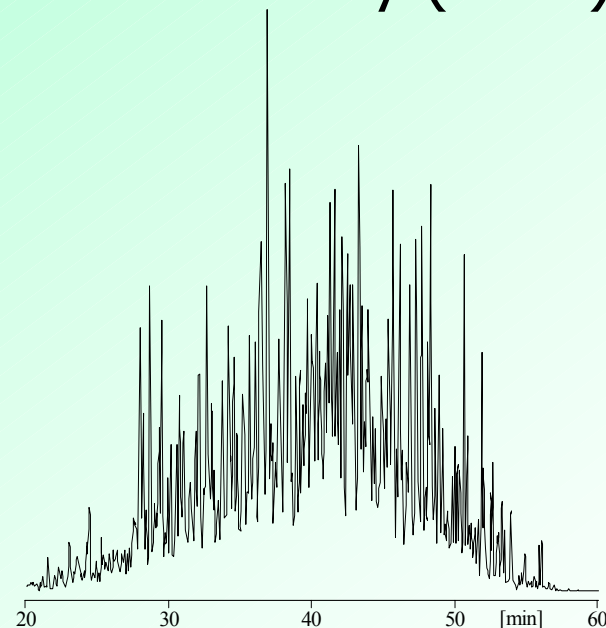- only ~10% of the constituents structurally known

Ref.:   M. A. Saleh, *Rev. Environ. Contam. Toxicol*. 118 (1991) 1-85
          W. Vetter, M. Oehme, Handbook of Environmental Chemistry, Springer, 2000

Toxaphene products investigated

Toxaphene®
USA, 1978
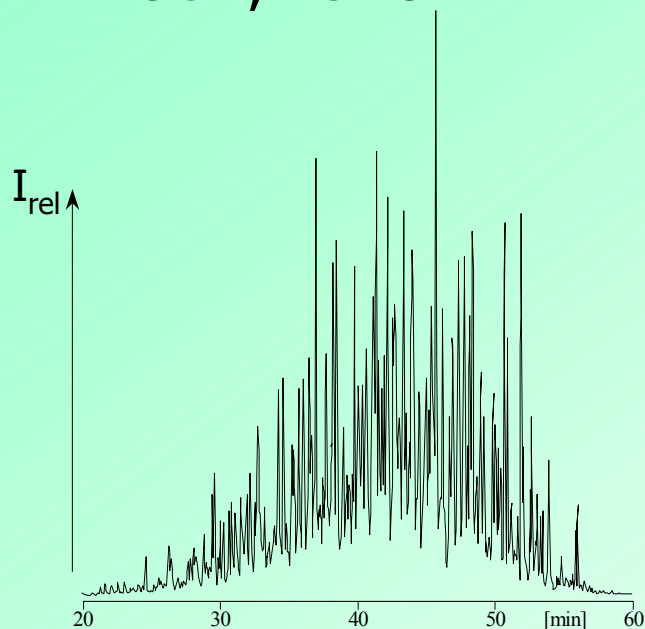
Melipax®
Eastern-Germany (1979)

Cl content:
~ 67-69%

I_{rel}

20 30 40 50 [min] 60

20 30 40 50 [min] 60

GC/ECD chromatograms of the investigated products

- very complex mixture, all peaks are interfered

  $\Rightarrow$ direct GC/IRMS analysis impossible

  $\Rightarrow$ EA/IRMS

Contribution (weight-%) of individual CTTs to Melipax® and Toxaphene®

CTT	Toxaphene	Melipax
B6-923 (Hx-Sed)	0.06	0.38
B7-1001 (Hp-Sed)	0.74	0.06
B8-1413 (P-26)	0.45	0.44
B8-2229 (P-44)	0.67	0.51
B8-806/809 (P-42)	2.36	0.91
B9-1679 (P-50)	0.76	0.58
B9-1025 (P-62)	1.84	2.62
Σ**25 CTTs**	**21.2 %**	**16.8 %**

<u>Ref</u>.: W. Vetter, G. Gleixner, W. Armbruster, S. Ruppe, G. A. Stern, E. Braekevelt, *Chemosphere* 58 (2005) 235-241

IRMS setup

- Delta^{Plus}XP (Hohenheim)
- Delta^{Plus}XL (Jena)

  (ThermoFinnigan)

- EA 1110 element analyzer, tin capsules
- oxidation/reduction furnaces 1020/650 °C

- sample weigth: ~0.5 mg
- internal standards used for validation

$\delta^{13}C$ values [‰] of Melipax and Toxaphene

SAMPLE	#1	#2	#3	Mean	STD
Melipax®	-27.70	-27.45	-27.59	**-27.58**	0.13
Toxaphene®	-28.23	-28.43	-28.41	**-28.36**	0.11

* $\delta^{13}C$ [‰]= ($[^{13}C/^{12}C]_{sample}$ / $[^{13}C/^{12}C]_{VPDB}$ -1) x 1000

- good reproducibility of measurements
- $\Delta\delta^{13}C_{(Toxaphene®-Melipax®)}$ values ~ 10 fold larger than STD
- values in the typical range of C_3-plants (-18 to -35 ‰)
- Toxaphene® significantly lighter in ^{13}C than Melipax®

Results

- both investigated products (Melipax®, Toxaphene®) could be distinguished by their $\delta^{13}C$ contents

  $\Rightarrow$ Toxaphene® lighter in ^{13}C than Melipax®

What about single compounds ?

- isolation of a key-congener from both technical mixtures and from an environmental sample
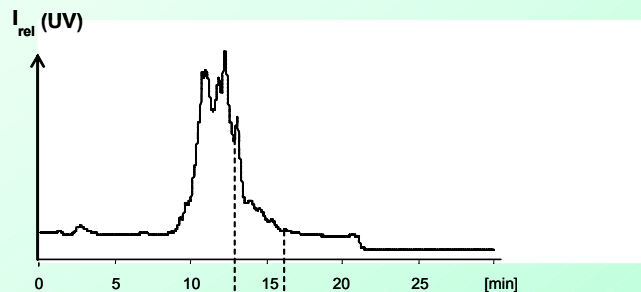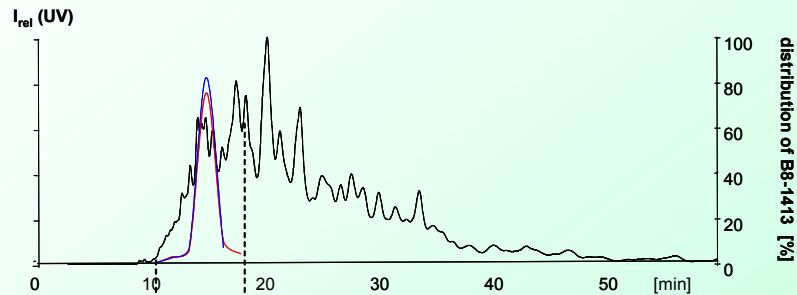
- analysis by GC-IRMS

GC-IRMS determination of $\delta^{13}C$ values

Quantitative HPLC-Isolation of B8-1413

1st step

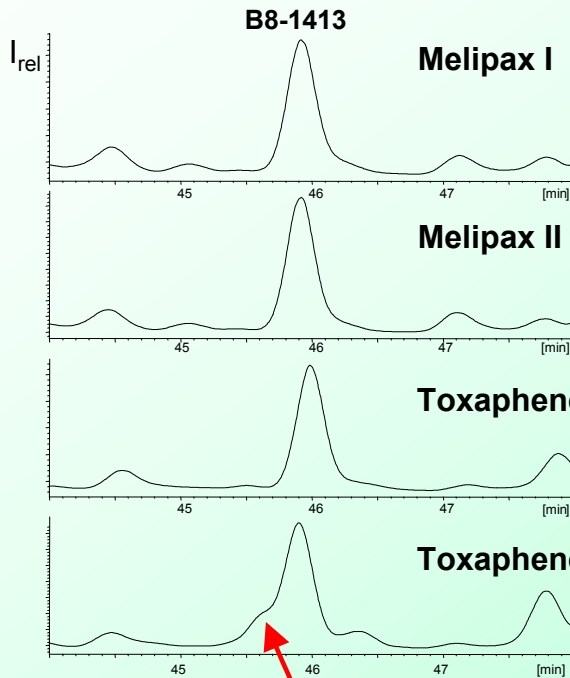NP-HPLC, three serially coupled columns (75 cm)

mobile phase: *n*-hexane

2nd step

RP-HPLC, three serially coupled columns (70 cm)

mobile phase: acetonitril

Ref.: W. Vetter, J. Schlatterer, G. Gleixner, *J. Chromatogr.* A 1110 (2006) 165-170

GC-IRMS determination of $\delta^{13}C$ values
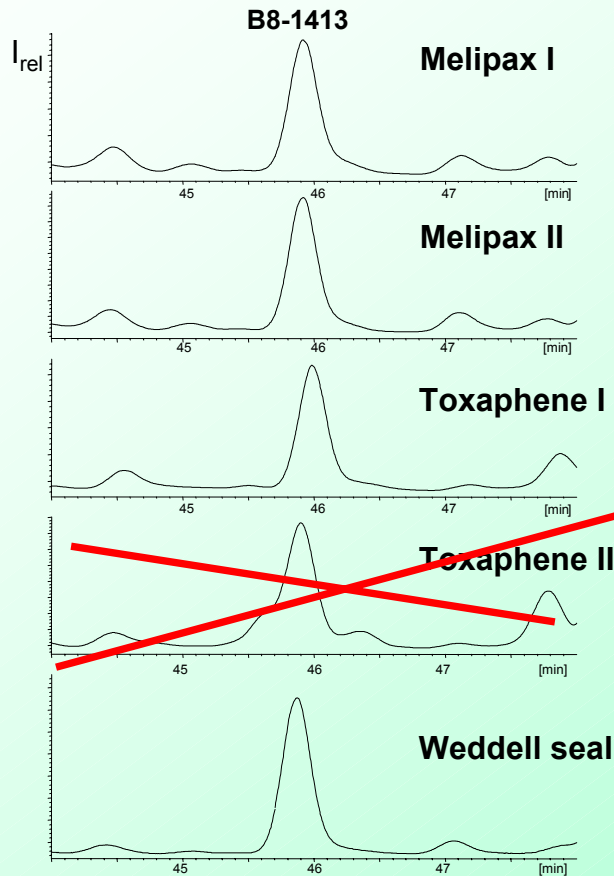
Samples for GC-IRMS

-24.6±1.0 ‰

-26.5±1.3 ‰

-27.3±1.1 ‰

not suitable due to interference

- relatively high deviation between Melipax duplicates

- High standard deviation in all cases (increases with decreasing amounts available)

- B8-1413 in toxaphene seems to be more depleted in ^{13}C

(no statistic proof)

Ref.: W. Vetter, J. Schlatterer, G. Gleixner, *J. Chromatogr*. A 1110 (2006) 165-170

GC-IRMS determination of $\delta^{13}C$ values

Samples for GC-IRMS

B8-1413

Melipax I   -24.6±1.0 ‰

Melipax II   -26.5±1.3 ‰

Toxaphene I   -27.3±1.1 ‰

Toxaphene II   not suitable due to interference

Weddell seal   -26.2 ‰

- HPLC procedure repeated for Antarctic Weddell seal

- $\delta^{13}C$ value in range of both products

Ref.: W. Vetter, J. Schlatterer, G. Gleixner, *J. Chromatogr*. A 1110 (2006) 165-170

δ^{13}C values of B8-1413 in technical products and Antarctic Weddell seal

- **assignment to a specific product not (yet) achieved**

However:

- first δ^{13}C value in a POP in samples from polar regions presented

- δ^{13}C value in Antarctic seal not significantly different from that of technical products

  $\Rightarrow$ long-range transport and food-chain enrichment did not significantly alter the δ^{13}C value

  $\Rightarrow$ better data may indeed lead to the polluting product

Ref.: W. Vetter, J. Schlatterer, G. Gleixner, *J. Chromatogr*. A 1110 (2006) 165-170

GC-IRMS determination of the halogenated natural product Q1
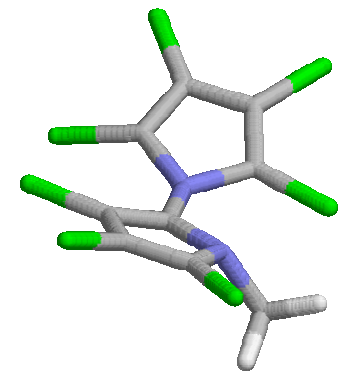
Q1	synthesized standard	cetacean (Australia)	skua liver (Antarctic)
$\delta^{13}C$ value (‰)	-34.20 ±0.27	-22.80 ±0.33	-21.47 ±1.42

- man-made product varies from natural product in isotopic composition

- two species from two continents showed narrow range of $\delta^{13}C$ value

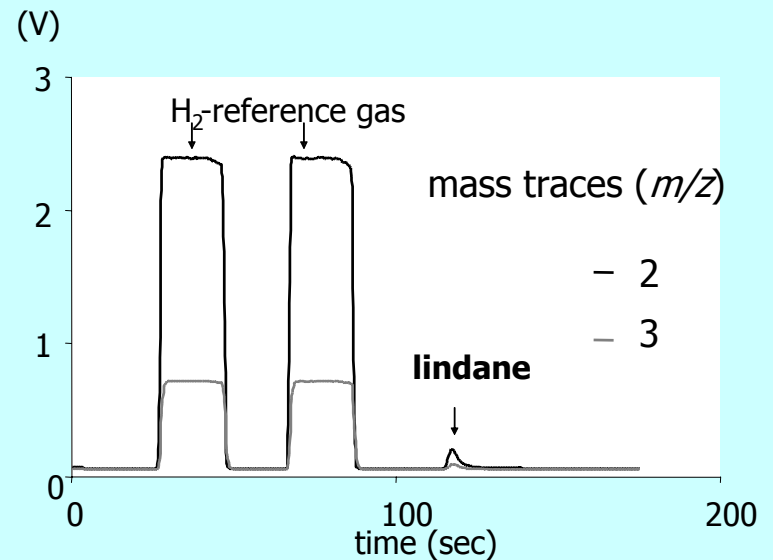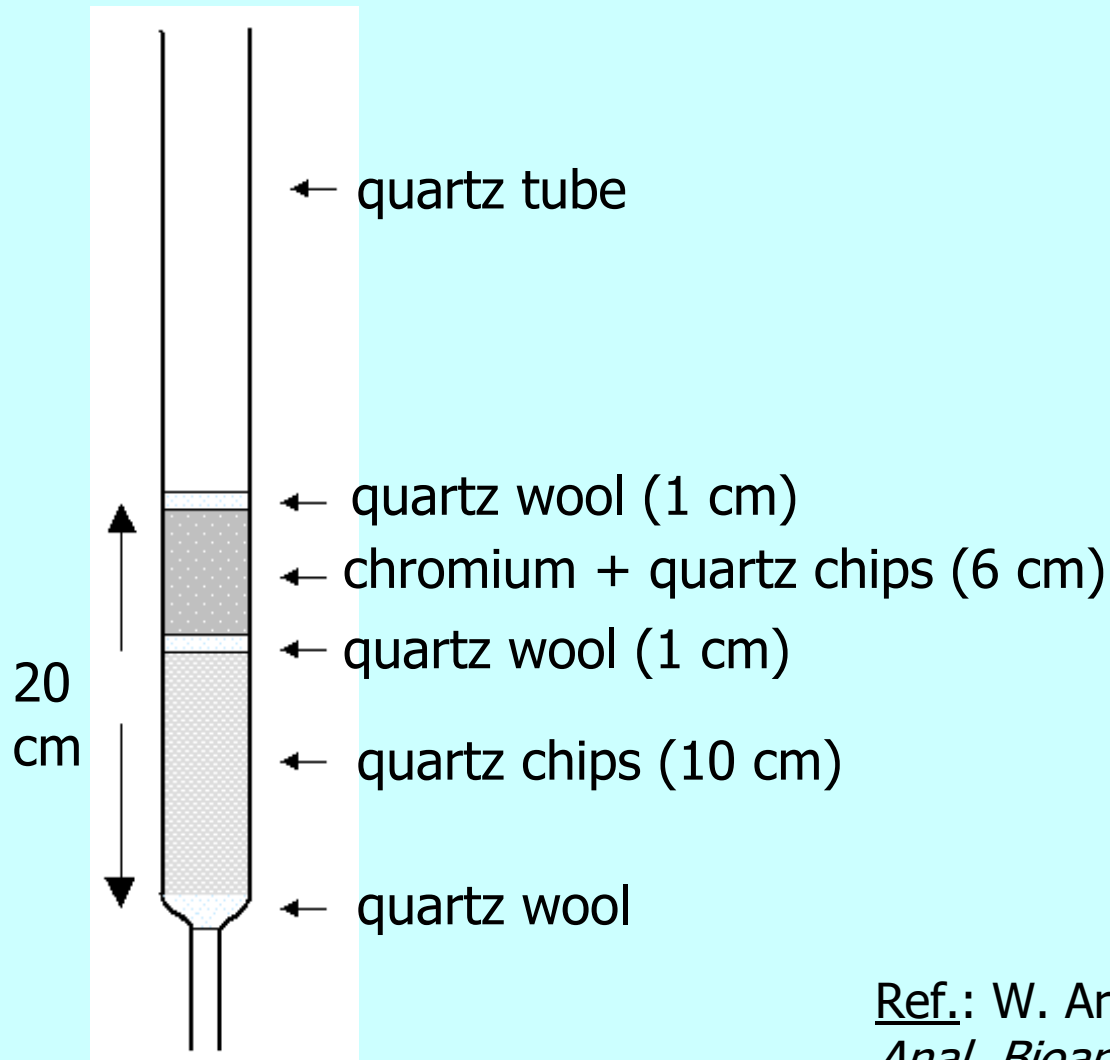Ref.: W. Vetter, G. Gleixner, in preparation for "Dioxin 2006"

Improvement of method

- additional isotopic data expected to be of great value

  $\Rightarrow \delta^2 H$ values

Problem:

- unlike other organic molecules, polyhalogenated compounds do not give sufficient signal for hydrogen isotopes
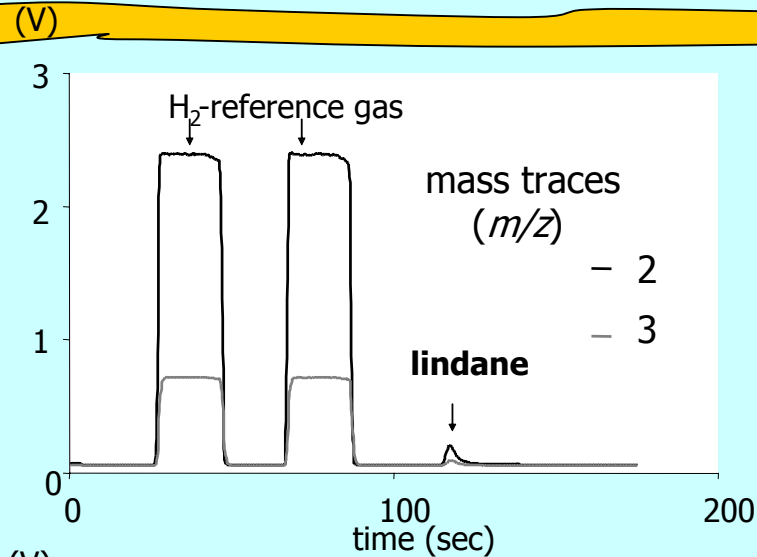
  $\Rightarrow$ most likely due to formation of HCl

(V)

H_2-reference gas

mass traces (*m/z*)

— 2

— 3

lindane

time (sec)

Development of a method using a chromium reactor

quartz tube

quartz wool (1 cm)

chromium + quartz chips (6 cm)

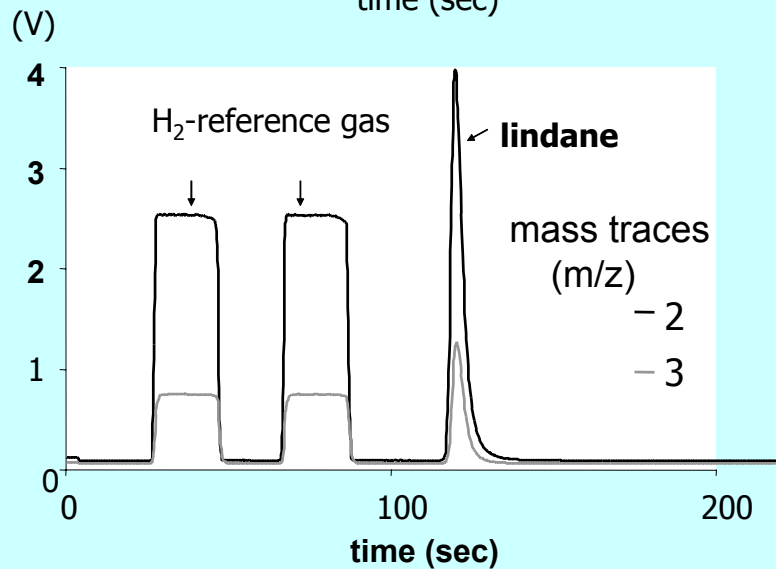quartz wool (1 cm)

quartz chips (10 cm)

20 cm

quartz wool

Ref.: W. Armbruster, K. Lehnert, W. Vetter, *Anal. Bioanal. Chem*. 384 (2006) 237-243

Improvement of δ^2H method

without chromium reactor

$\Rightarrow$ no significant signal for lindane

with chromium reactor

$\Rightarrow \delta^2H$ values can be established

δ^2H values of Melipax® and Toxaphene®

SAMPLE	#1	#2	#3	mean
Melipax®	-178.7	186.4	-181.6	**-182.2**
Toxaphene®	-98.8	-100.2	-102.2	**-100.4**

!

- first published δ^2H values for POPs

- Melipax® significantly lighter in ^{2}H than Toxaphene®

Ref.: W. Armbruster, K. Lehnert, W. Vetter, *Anal. Bioanal. Chem*. 384 (2006) 237-243

δ^2H values of lindane from different sources

SAMPLE	n	δ^2H	δ^{13}C
lindane (Aldrich)	11	**-109.1**	**-26.2**
lindane (Riedel)	8	**-70.1**	**-25.9**

- lindane is gathered from technical HCH by extraction and/or re-crystallisation

  $\Rightarrow$ processes known to cause isotope fractionation

* δ^2H [‰]= ([^{2}H/^{1}H]$_{sample}$ / [^{2}H/^{1}H]$_{standard}$ -1) x 1000

δ^2H values of bromophenols and -anisoles

| SAMPLE	δ^2H	$\delta^{13}C$
2,4,6-tribromoanisole	-15.56	
2,4-dibromoanisole	-91.14	-30.32
2,4-dibromophenol	52.50	-27.04
2,6-dibromophenol	-92.82	-28.07
3,5-dibromophenol	-62.68	-31.31
2,4,6-tribromophenol	52.71	-28.54
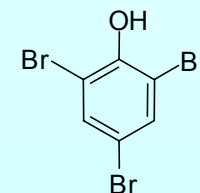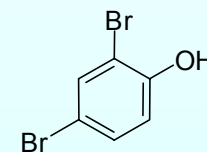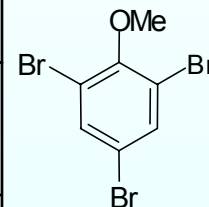
- different δ^2H values suggest different sources
  (that means different way of production/purification)

Bromophenols and bromoanisoles

- IRMS determination requires previous combustion or pyrolysis of the molecule under investigation

- the δ-values ($\delta^{13}C$, δ^2H) represent the mean isotope distribution within a molecule

    $\Rightarrow$ information on atom-specific isotope distribution gets lost

    $\Rightarrow$ such information can be provided by ^2H-NMR

        quantification of isotopomers

    $\Rightarrow$ however: about 100 mg required (only pure standards)

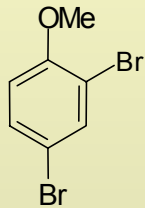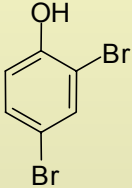# ^{2}H-NMR quantification of isotopomers of 2,4-dibromoanisole*
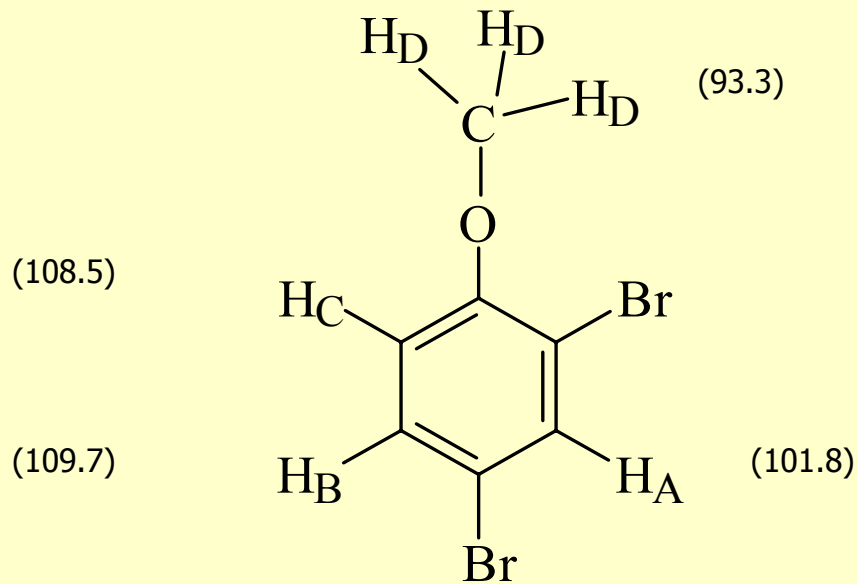
- multiplication of values with δ^2H value divided by 100 yields absolute atom-specific isotope distribution

* ^{2}H abundance of each isotopomer, relative to the average deuterium abundance of the whole molecule (= 100)

Cooperation with J. Schleucher, UMU, Sweden

Relative abundance of deuterium in the aromatic C-H positions of 2,4-dibromoanisole and -phenol*

Position	Anisole		Phenol	
	mean	standard error (n = 8)	mean	standard error (n = 6)
3 (*meta*)	95.4	1.7	87.8	0.9
5 (*meta*)	102.8	1.2	90.3	0.8
6 (*ortho*)	101.7	1.4	122.0	0.6

- **different isotope pattern proves different synthesis procedures**

* ^2H abundance relative to the average ^2H abundance of all aromatic C-H, which was arbitrarily set to 100

Summary

- $\delta^{13}C$ values of POPs can be determined with routine equipment

  $\Rightarrow$ first data indicate only slight alteration of $\delta^{13}C$ values by

  long-range transport and food-chain bioaccumulation

- EA-IRMS method for determination of δ^2H values established

  $\Rightarrow$ first δ^2H values of organochlorine compounds presented

  $\Rightarrow$ significant differences for toxaphene, lindane, and

  bromophenols from different sources

- ^{2}H-NMR quantification of deuterium isotopomers gives
  information with regard to the synthesis pathway

<u>Future</u>

- stable isotope measurements may bring light into the following problems:

- differentiation of naturally produced and anthropogenic POPs

- differentiation of POPs such as lindane/toxaphene that stem from different sources

- these goals have not yet been reached

    $\Rightarrow$ results are promising but method still a great challenge

**Simon Gaul,
Wolfgang Armbruster**

**Jürgen
Schleucher**

**Tatjana
Betson**

SNIF NMR
(Umeå)

Katja Lehnert

EA-IRMS, GC-IRMS,
δ^2H (Hohenheim)

**Gerd
Gleixner**

**Steffen
Rühlow**

GC-IRMS (Jena)

thank you

for your attention !