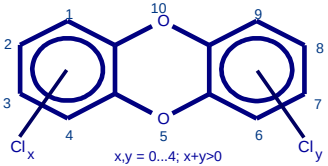
Hydrogen as a GC carrier gas for PCDD/F-analysis with *Agilent 7010* MS-MS systems: Implementation and Evaluation



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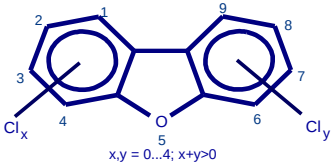


(1) Introduction

(2) Detector comparison — He 7010A vs. 7000C (routine column)

(3) First experiences with H_2 — H_2 7010A (routine column)

(4) Final method — H_2 7010A (improved GC column)

(5) Validation on real samples

(6) Long term experience

(7) adverse effects of H_2

(8) Conclusions

(9) About risks?

(1) Exploration of Hydrogen as Carrier Gas

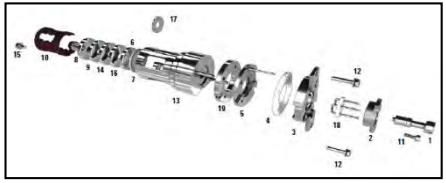
Expectations

- Advantage: reduction in analysis time
  - Different properties of H_2; Van Deemter-Curve (interaction of density, diffusion coefficient and viscosity, higher average linear velocity: above 40cm/sec)
- Advantage: reduction in costs
  - Independence from future helium shortages and raising He price
- Disadvantages: adverse effects of H_2
  - expected reduction of instrument sensitivity
  - Reactions due to Hydrogen in GC and MS system?
  - Safety issues

(1) The idea

Use of Agilent 7010 Triple Quadrupole Ion Source

- new source design/electronics → lower iDLs / higher sensitivity
- → Possibility to compensate for hydrogen-related decrease in system performance in order to maintain LOQs needed for analyzing dioxins (and many other parameters…) in food/feed samples



- ***Remark:*** EU food/feed regulations allow for use of GC-MS/MS (TripleQuads)

COMMISSION REGULATION (EU) 2017/644; COMMISSION REGULATION (EU) 2017/771

1.3. 'Confirmatory methods' means methods that provide full or complementary information enabling the PCDD/Fs and dioxin-like PCBs to be identified and quantified unequivocally at the maximum or, in case of need, at the action level. Such methods utilise gas chromatography/high resolution mass spectrometry (GC-HRMS) or gas chromatography/tandem mass spectrometry (GC-MS/MS).

(1) PCDD/F-analysis: Scope

Analysis of PCDD/F in food/feed samples

- 17 PCDD/F congeners; method for supervision of food/feed acc. to EU regulatory limits

- *Example:* pork meat >2% fat: maximum level (limit value) for sum of dioxins and furans (WHO(2005)-PCDD/F TEQ (upper-bound)) 1.0 pg WHO-TEQ / g fat.

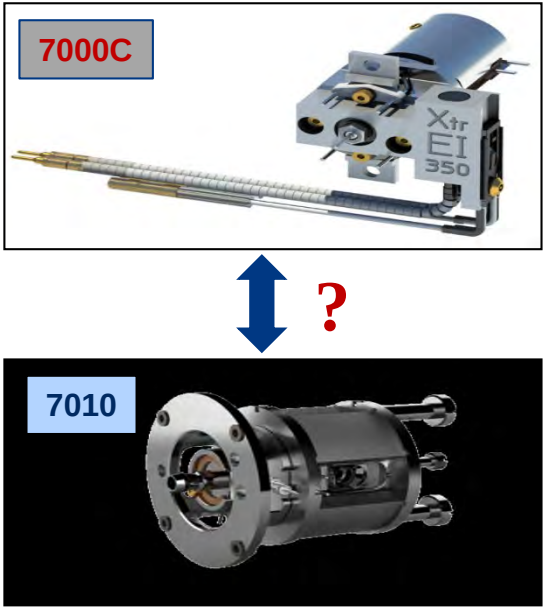
- *Theoretical calculation example:*

sample intake	5	g	
assumption: conc. = at limit value	5	pg TEQ abs.	WHO-TEQ
assumption: 1/10 of TEQ	0,5	pg TEQ abs.	2378-TCDD
TEF value =1 -->	0,5	pg abs.	2378-TCDD
target: monitoring 1/5 limit value	0,1	pg abs.	2378-TCDD
injected aliquot	50	%	e.g. 10 of 20 µL
--> necessary LOQ	**0,05**	**pg abs.**	**= 50 fg o.c.**

Order of magnitude

(2) Detector comparison 7010 vs. 7000C

- **Starting point**
  - Routine PCDD/F method
  - Isotope dilution, 16 internal ^{13}C-standards
  - VF-Xms column 60m * 0.25 mm i.D * 0.25 µm df; He
  - Agilent 7000C detector
- ***Remarks***
  - *On the following slides, focus is set on 2,3,7,8-TCDD being as well a typical example for the results as one of the most important PCDD/Fs*
  - *Limit of Quantifications (LOQs) are discussed as iLOQs, based on Signal to Noise Ratios (SNR)*



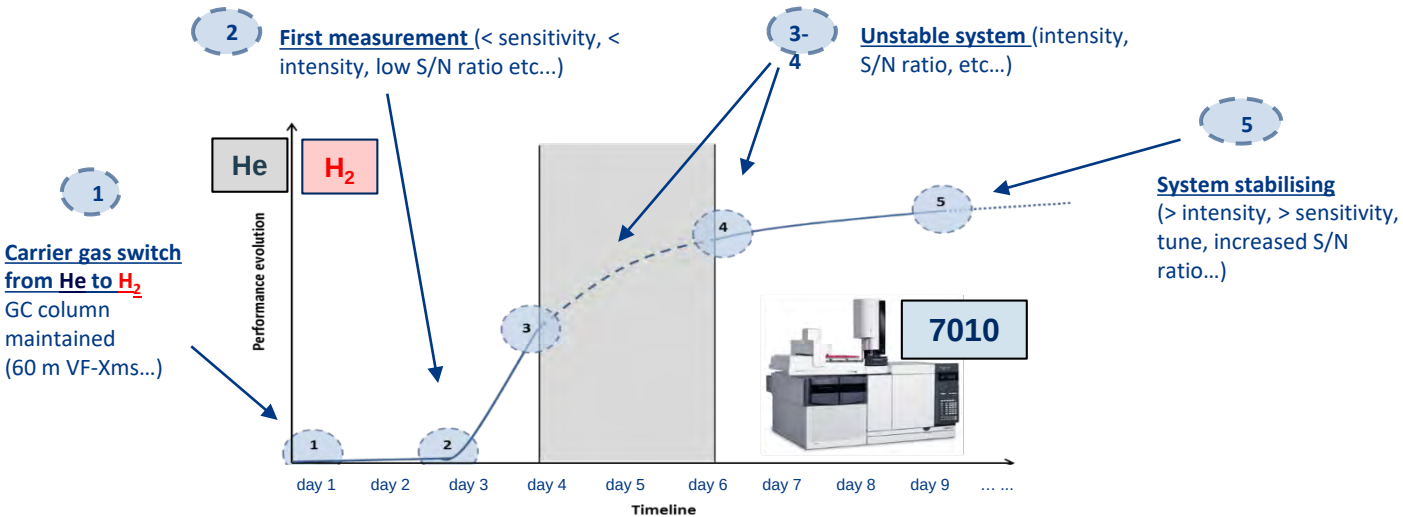
(2) 7010 Detector qualification

(SNR 10:1); n=10	**7000C**	**7010**	
2,3,7,8-TetraCDD (pg/inj.)	0.025 +-26%	0.006 +-11%	better for PCDD/F
Remark:	*7000C acceptable; used e.g. for PCBs, also as backup for PCDD/F*		
Range (pg µL^{-1}) ;)	0.007- 40	0.002 – 40	could be expected

(*) approx. 20 times larger peak areas; much better signal quality at low concentrations below ≈ 0.008 pg µL^{-1}

Performance of 7010 ion source
approximately by F ≈ 3 - 4 better
compared to the 7000C ion source

(3) Change to H_2: instrument stabilisation



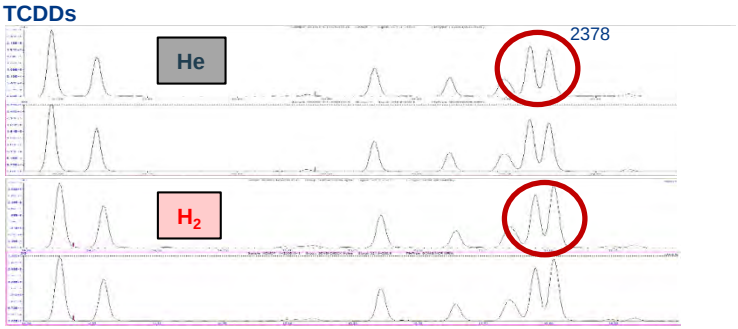
Stability (intensities, spectra) ≈ 5 days after carrier gas switch; final stability after 2-4 weeks

→ Stabilisation phase recommended

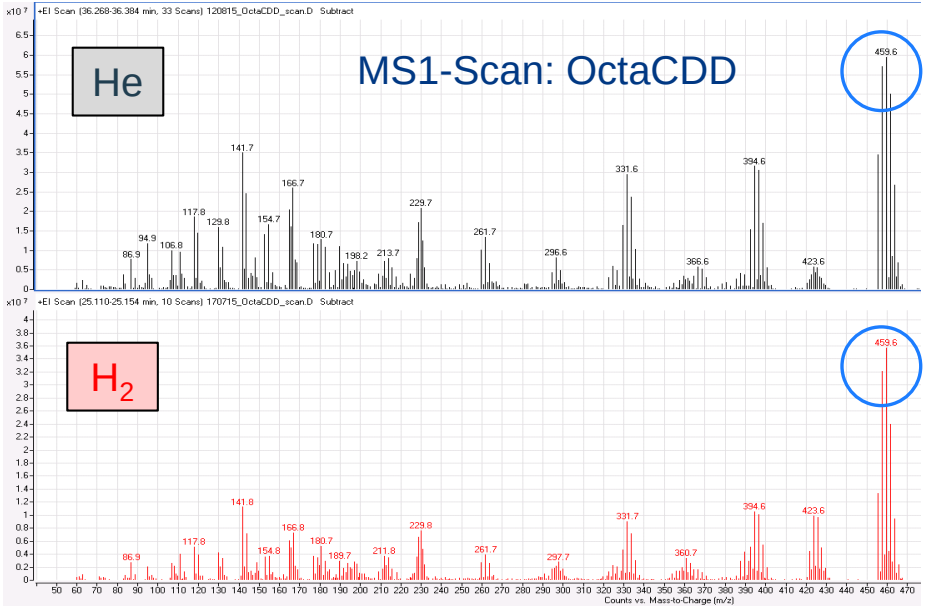
(3) Performance under H_2

- iLOQ of 2,3,7,8-TCDD (SNR 10:1)
  - 7000C, He: 0.025
  - 7010A, He: 0.006
  - 7010A, H2, first trial: 0.010 … 0.012 — first impression: as expected about 1/2
- Retention: 16 min vs. 23 min — considerably faster elution
- Peak separation: (see below) — equivalent

Promising!



(3) MS1 Mass spectra



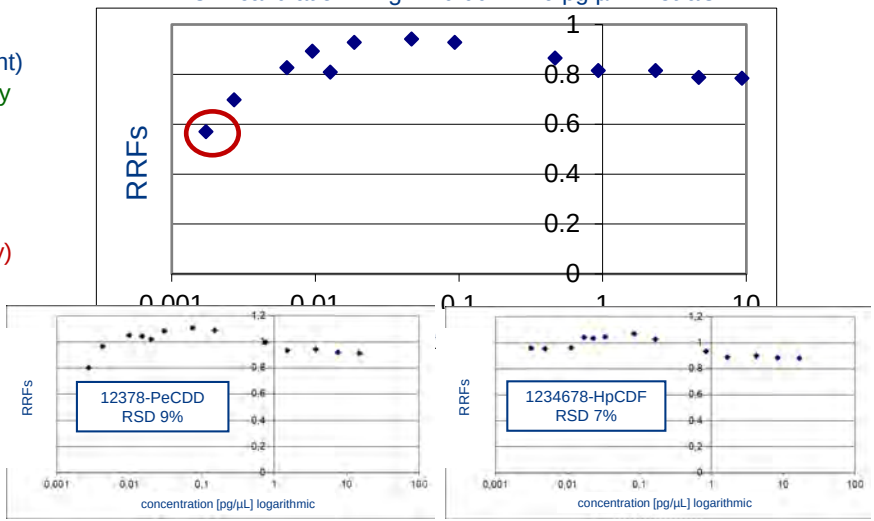
- Similar mass spectra
- Ion ratios mainly the same
- Fragmentation pattern different only to a minor degree

(4) Next step: 40m GC column & validation

VF-Xms 60m * 0.25 mm i.D * 0.25 µm df → 40m * 0.18mm * 0.18 µm(*)

- **iLOQ** 2378-TetraCDD (SNR)
  - ≈ 0.010 pg on column (lowest cal.point)
    critical / still about the same as initially
  - (lowest cal. Point = SNR 10…15:1)
- **Linearity** (RRF):
  - critical at lowest cal.point (Tetras only)
    RSD (2378-TetraCDD) 12.5%(**)

RRFs in calibration range ≈ 0.002 – 10 pg µL^{-1} TetraCDD



(*) Agilent method translator
(**) highest RSD of all compounds

(4) GC separation efficiency

GC separation test solution containing critical TetraCDD/F congeners

carrier gas		He	H2
column		VF-Xms 60*0.25*0.25	VF-Xms 40*0.18*0.18
Analyte (n=8)		separation (% valley)	separation (% valley)
TetraCDD	1237/1238 and 1379	10%	36%
	1379 and 2378	**20%**	**15%**
TetraCDF	1249/2347 and 1279/2348	15%	18%
	1279/2348 and 2378	20%	38%
HexaCDD	123478 and 123678	10%	2%
	123467 and 123789	24%	11%
HexaCDF	123478 and 123678	3%	0,4%

US EPA1613B: ≤ 25%

COMMISSION REGULATION (EU) 017/644: ≤ 25%

(4) LOQ improvement and Productivity

- **Reduction in *method* LOQ on average for all congeners**
  - (as compared to original routine LOQs)
  - **- 35%**

- **Productivity**

	acquisition time				productivity		
	min	hours	%	reduction	runs/d	capacity %	increase
He	53.0	0.88	100		27	100%	
H2	34.5	0.58	65	**-35%**	41	152%	**52%**

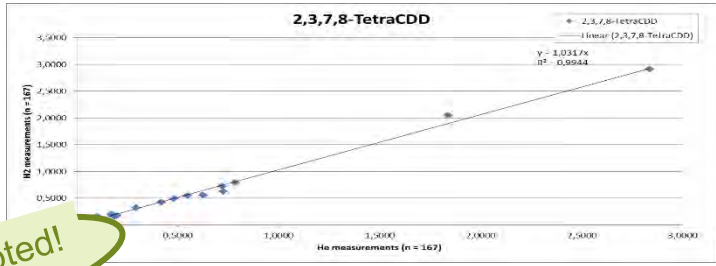
(5) Validation: comparison of real sample results

- **PCDD/F-measurements: He (7000C) = routine and H_2 (7010A)**
  - 167 Food/feed samples (reflecting various matrices; randomly chosen)
    - Eggs, egg powder, spices, milk, yoghurt, milk powder infants, fat (powder and oils from plant or animal), feed (oils and powders, plant or animal, urea), mineral feed (copper sulphate, trace element powder), meat meal, different kind of fish and seafood, poultry (fat, meat, muscle), whey powder, feather meal
- **Evaluation**
  - Comparison of results for both measurements = data pairs.
  - H2: more positive results → different number of data pairs per congener and sample
  - Evaluation only of data sets with positive results for both systems (He / 7000C and H2 / 7010)
- **Criterion**
  - correlation of results for all analytes between measurements in He and H_2-systems demonstrating equivalency

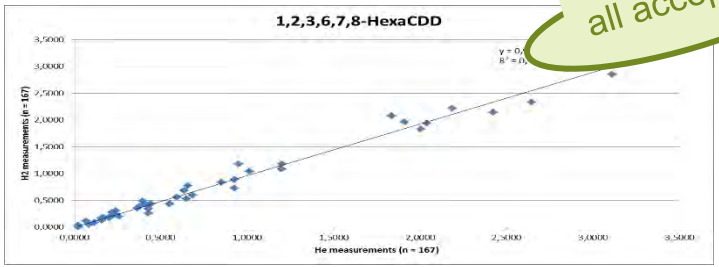
    → ***y (slope) = 0.9 x – 1.1 x*** at reasonable correlation coefficient (R^2 = 0.8 – 1)

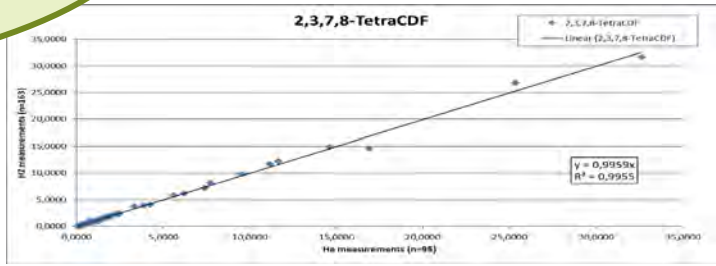
(5) Real samples: correlations H_2 vs. He

- n = 24 - 95 positive data pairs
- y = 0.956 x … y = 1.075 x, R^2 = 0.937 - 0.998
- → implies comparable results and linear relation between He and H_2
- (except: 123789-HexaCDF: only two positive data pairs)



all accepted!

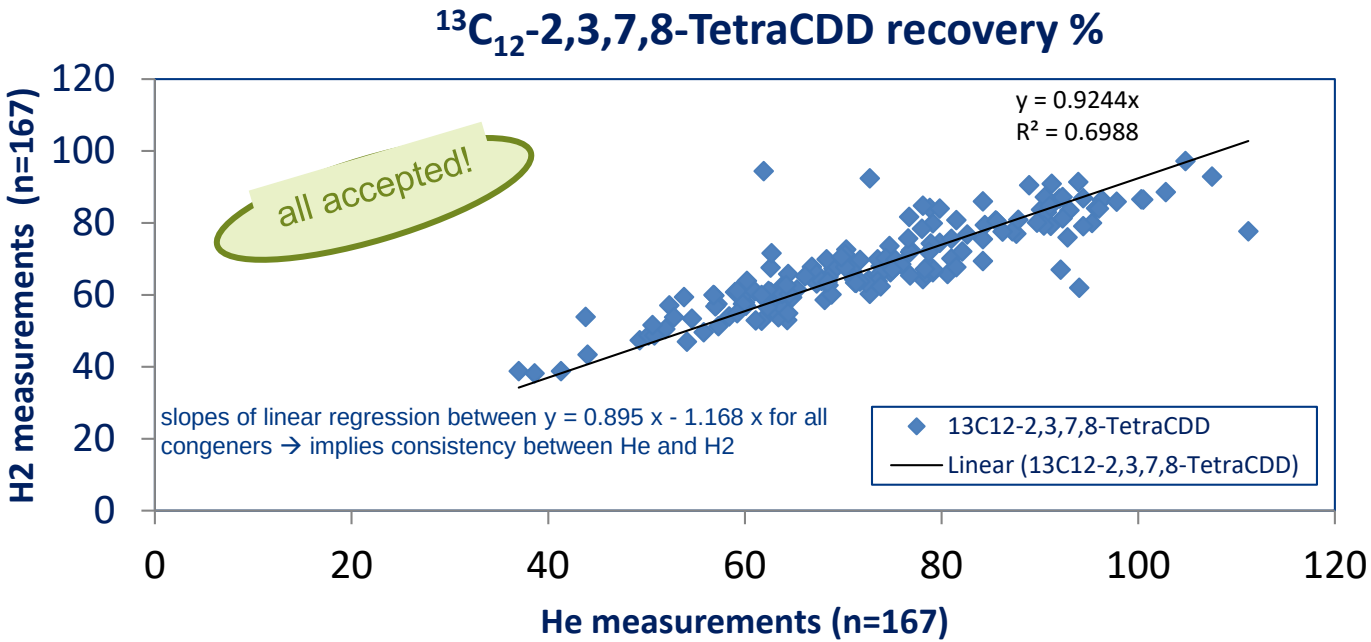




All data pg/sample; only data sets from data pairs with positive results for both systems (He and H_2)

(5) Real samples: ^{13}C-standard recovery rates

Correlation of RR between the He- and the H_2-system



(6) Review: after 5 months

Review after 5 months and approx. 3000 injections

Stability and robustness

- Precision constant; Linearity stable;
- RRFs stable (n = 150); RRFs H_2 constant and equivalent to He
- Reference samples: accuracy OK; Blanks OK (… more detects)
- GC separation stable (2378-TetraCDD overlap to other congeners 15 % →17.5 %)

iLOQ (2,3,7,8,TetraCDD): 0,012 pg → 0,016 pg still good performance

(6) Long term experience

after 5 years and thousands of routine samples

- More instruments, all kind of samples run under routine workload
- Stable and without specific technical issues
- Performance and maintenance comparable to Helium machines

  (more maintenance due to more throughput)

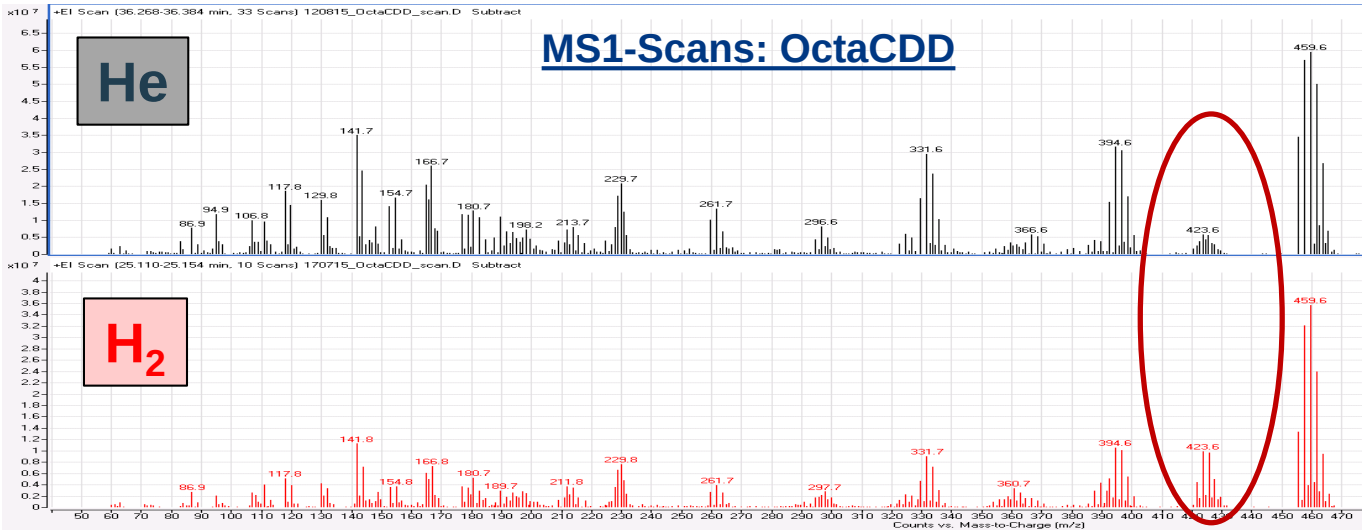
- Present iLOQs (2,3,7,8-TCDD) calculated from signal/noise-ratio:
  - H_2 0,023 pg; He 0,006 pg
  - Sensitivity settled, but fit for purpose and technically acceptable

Regarding costs:

- Gas price for He appears to be ≈ ≥ 5 times higher than for H2

(7) Caveat: *ion source* dechlorination

- Distortion of spectra present but only to some degree
- Results only in intensity loss due to registration of less target analyte molecules



(7) Caveat: *injector* dechlorination

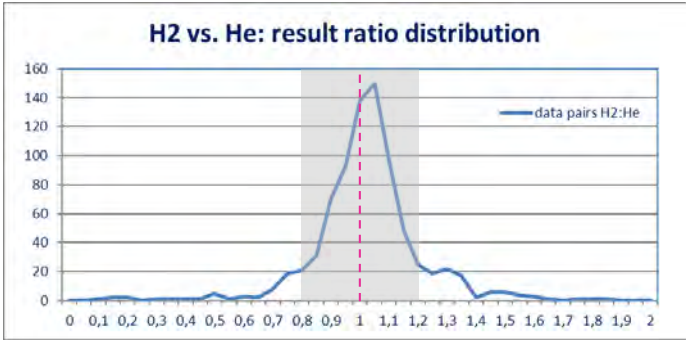
- **Dechlorination products formed in the injector?**
  - Formation of PCDD/Fs of lower chlorination degrees
  - Indistinguishable from original analytes

- → **Dechlorination experiment:** Worst case = native OctaCDF and OctaCDD
  - PCDD/Fs of the highest chlorination degree may most likely form lower chlorinated PCDD/Fs
  - Mainly **Non-2,3,7,8**-congeners observed
  - 2,3,7,8-congeners formed by dechlorination of OcCDD/F (*) :
    - Te-HxCDD/F: < 0.5 % (moderately negligible)
    - HpCDF: 1234678-HeptaCDF: 3.3 %; 1234789-HeptaCDF: 2.7 %
    - HpCDD: 1234678-HeptaCDD: 4.0 %;

(*) also true for ^{13}C-OcCDD/F

(7) *injector* dechlorination (2)

- Might prove to be important especially for pattern recognition and congener group analysis
- For TEQ calculations: no significant influence observed on sample data H2 vs. He *Still: be careful! It cannot be excluded that high relative Hexa- and HeptaCDD/F concentrations may result in overestimation of results due to Tetra-/Penta-formation → check for dechlorination in samples near / above limit values*



(8) Conclusions

- GC Performance equivalent to classical analytical systems; same results for real samples under H_2 and He
- Sensitivity losses under H_2 approximately compensated by use of the 7010 ion source; performance (validation; new quipment) stable; performance reduction under routine conditions and after 5 years acceptable
- In combination with different column run time substantially decreased; +52 % productivity;
- Carrier gas cost saving vs. He
- Frequent carrier gas alternation not recommended
- Dechlorination, but assumably not relevant for majority of cases

- **Potentially explosive (4 % to 75 % by vol. in air at atmospheric pressure)**
  - 4 % will hardly be reached in air ←high diffusion coefficient (0.61 cm^2/s in air)

- **Is there a risk in using Hydrogen?**
  - **NO** … **IF** you invest in
    - H_2 *oven* **sensor** and *room* sensor, **connection** of vent lines to air exhaust, **removal** of loose covers (for safety reasons)
    - **Time** … considering that you might not want to start up your MS source whilst having H_2/atmosphere mixture within the vacuum system
- **… otherwise …**


KABLOOIE!!

Thank you for your attention!

This work would not have been possible without all those people

Eurofins:

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