Benzo[a]pyrene Method Development

Dr Michael Intorp
CORESTA
Cooperation Center for Scientific Research Relative to Tobacco

FDA Center for Tobacco Products
Scientific Workshop – Tobacco Product Analysis

30 – 31 July 2013

CORESTA
Special Analytes Sub-Group

- **Creation**
  - Set up in 1999 initially as a Task Force
- **Background**
  - Growing regulatory interest
  - Lack of standardised methods
  - Measurement reproducibility not established
- **Objective**
  - Propose practical and robust recommended methods for compounds in cigarette mainstream smoke (CORESTA Recommended Methods, CRMs)
- **Work Program**
  - One of the priority compounds was identified as benzo[a]pyrene

CORESTA Special Analytes Sub-Group
Development of Recommended Methods

- **Approach used for the development of robust methods**
  - ✓ **Should involve a relatively large number of laboratories**
    - CORESTA well represents global expertise in this type of measurement. There are more than 20 active laboratories from 12 countries
  - ✓ **Should involve a wide range of product and design styles**
    - Range of blend styles
    - Range of tar yields
    - Manufactured in various global regions
  - ✓ **Consensual and unanimous decisions on "standardisation"**

B[a]P method development between 1999 and 2003

- **Review of existing methods for cigarette smoke**
  - Two types of methods were identified (HPLC-FLD and GC-MS)
  - Both types required matrix reduction (clean up)
- **Joint experiments using HPLC-FLD methods**
  - Decision was based on most widely used method (HPLC-FLD)
  - However, reduction of initially observed variability appeared technically difficult (poor separation from other components)
- **Investigation of GC-MS methods as alternative**
  - Collaborative study results demonstrated lower between laboratory variability compared to HPLC-FLD

B[a]P method comparison: HPLC-FLD versus GC-MS

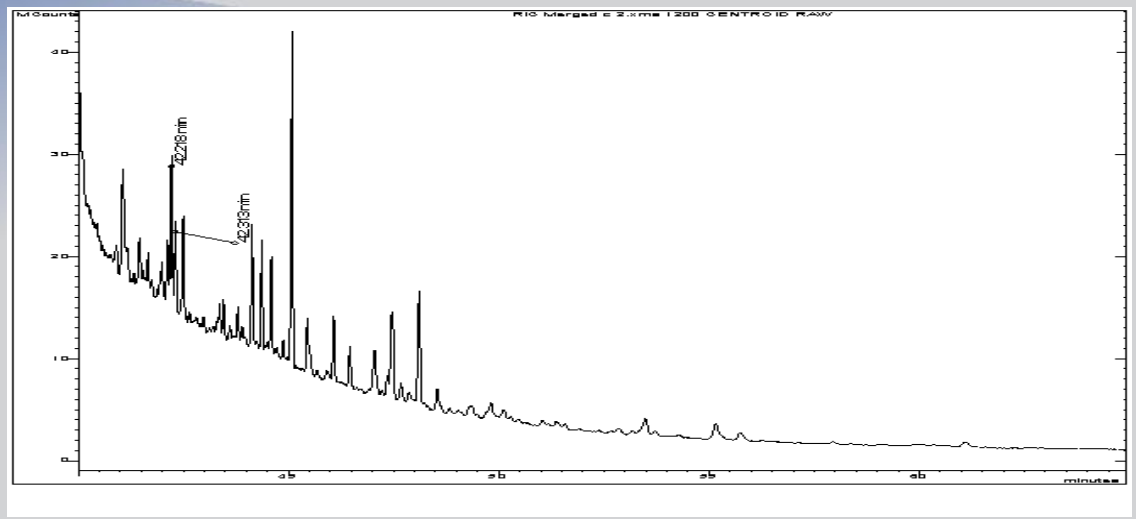
Data from 12 laboratories on the 2R4F reference cigarette

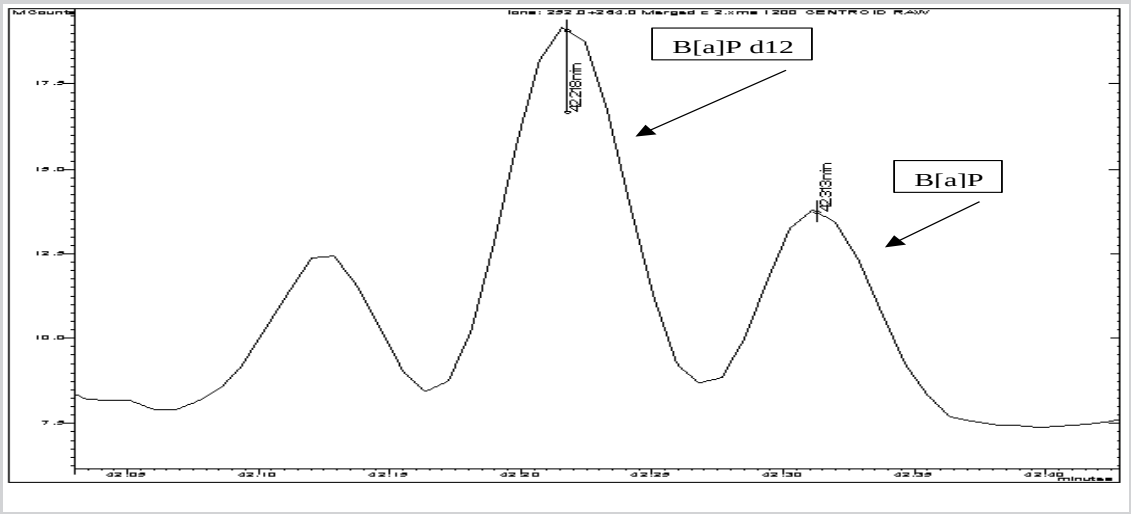
Laboratory	Benzo[a]pyrene (ng/cig) under ISO smoking regime						Statistical Difference (ISO 5725-2) HPLC v GS-MS
	HPLC			GC-MS			
	Mean	SD	RSD (%)	Mean	SD	RSD (%)	
1	5.10	0.60	11.6	7.50	0.50	6.2	yes
2	4.98	0.74	14.9	6.80	0.44	6.5	yes
3	3.20	0.72	27.4	5.60	0.33	5.8	yes
4	2.69	0.53	19.8	5.25	0.20	3.9	yes
5	5.12	0.54	10.5	5.72	0.10	1.7	no
6	2.36	0.50	21.2	5.49	0.80	14.6	n/a
7	4.92	0.60	12.2	-	-	-	-
8	5.87	0.20	3.3	4.60	0.18	3.8	yes
9	6.08	0.91	14.9	7.28	1.15	15.7	no
10	4.95	0.35	7.1	4.97	0.16	3.3	no
11	5.28	0.46	8.7	5.54	0.39	7.0	no
12	4.07	0.27	6.7	4.71	0.30	6.5	yes
Mean across all data	4.55	1.21	26.5	5.77	0.99	17.3	

Benzo[a]pyrene GC-MS method
Analytical details

- Conditioning and machine smoking according to ISO 3402 and ISO 3308.
- Methanol extraction of the total particulate matter collected on the glass-fibre filter pad and dilution with water.
- Clean up through solid phase extraction (CH), followed by the elution of B[a]P with cyclohexane.
- Analytical determination using single ion monitoring (SIM) detection mode.
- Ion traces for quantification and confirmation:
  - B[a]P: m/z 252 (quantification) and 250 (confirmation)
  - B[a]P-d12: m/z 264 (quantification) and 260 (confirmation)

Benzo[a]pyrene GC-MS method Analytical details





30 m capillary column with a methylphenyl (5 %) polysiloxane stationary phase, 0.25 mm id and 0.25 µm film.

Retention times of the B[a]P and B[a]P-d12 peaks are between 40 and 45 minutes.

The upper chromatogram shows the portion of chromatogram located between 40 and 65 min and the lower one is a zoom around the B[a]P and B[a]P-d12 peaks

Benzo[a]pyrene GC-MS method Collaborative Study 2003

- 13 laboratories (8 countries) – 7 test cigarettes – ISO 3308 smoking
- Repeatability (r) and Reproducibility (R) estimates by ISO 5725-2

Cigarette	*Mean (ng/cig)*	*r (ng/cig)*	*R (ng/cig)*	*R%*
2R4F	7.3	*1.3*	*2.5*	35
A	1.8	*0.5*	*1.0*	56
B	5.3	*1.1*	*2.5*	48
C	6.5	*1.1*	*2.2*	34
D	7.8	*1.5*	*2.9*	37
E	8.7	*1.4*	*2.7*	31
F	14.1	*2.3*	*5.9*	42

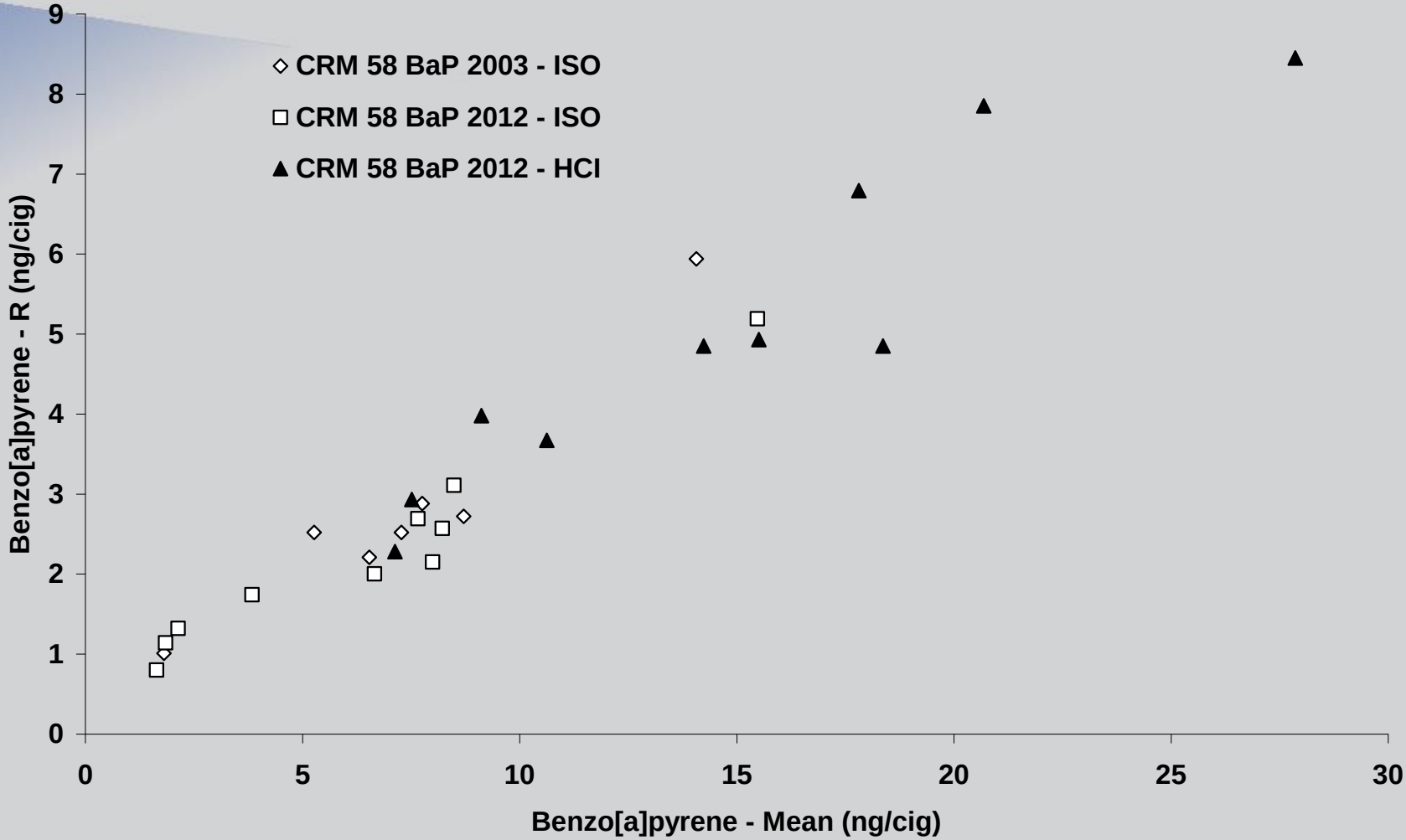
Benzo[a]pyrene GC-MS method Collaborative Study 2011

- Statistical Data obtained from Collaborative Study 2011 – outliers removed
- 12 laboratories (maximum) / 10 test cigarettes

Cigarette Sample	Benzo[a]pyrene (ng/cig)									
	ISO 3308 regime					Health Canada T-115 regime				
	N	Mean	r	R	R%	N	Mean	r	R	R%
CM6	10	15.48	2.48	5.19	33.5	11	27.86	4.65	8.45	30.3
1R5F	10	1.64	0.41	0.80	48.8	12	7.13	1.25	2.28	32.0
3R4F	11	6.66	0.85	2.00	30.0	12	15.51	1.68	4.93	31.8
1	10	8.49	2.00	3.11	36.6	9	17.81	3.06	6.79	38.1
2	10	8.00	1.26	2.15	26.9	9	18.37	1.88	4.85	26.4
3	9	8.22	1.24	2.57	31.3	10	20.69	4.88	7.85	37.9
4	8	3.84	0.69	1.74	45.3	9	10.63	1.97	3.67	34.5
5	8	2.14	0.55	1.32	61.7	10	7.52	1.67	2.93	39.0
6	9	7.66	0.99	2.69	35.1	9	14.24	2.28	4.85	34.1
7	8	1.85	0.58	1.14	61.6	10	9.12	1.83	3.98	43.6

General increase in R and decrease in R% with increasing BaP yields for both regimes

Benzo[a]pyrene
R versus Mean yield from CRM 58



Conclusions & Achievements

- CRM 58 was developed for BaP in cigarette mainstream smoke and is on the CORESTA website.
- Some observations on data variability is being made publicly available in a Beiträge publication.
- Round-table discussions during development provide valuable insight into causes and ways to reduce inter-laboratory data variability
- Inter-laboratory variability from collaborative studies is still higher compared to TNCO even when working to a CRM.

Benzo[a]pyrene Method Development
Summary

- 2003 Collaborative Study - GC-MS method - 13 labs – ISO 3308
- 2004 Optimised method published as CRM 58
- 2008 Published as international standard (ISO 22634)
- 2011 Collaborative study (11 labs) using ISO 3308 and HC T-115
- 2013 Revision of CRM 58 including HCl data on CORESTA website
- 2013 Publication submitted to Beiträge

Outlook

- **CORESTA Special Analytes Sub-Group provides a valuable forum for method development, in this example for BaP in cigarette mainstream smoke.**
- **Bringing together the wide ranging global expertise represented by the tobacco manufacturers, contract laboratories, governmental and other laboratories has led to:**
  - Open discussions and the readiness of members to modify their internal methodologies
  - Advantages for participating laboratories to make use of shared knowledge
  - Significant optimisation of many aspects of the methodology
  - Participation in collaborative studies has helped with aspects of laboratory accreditation under ISO 17025 or equivalent.
  - Learnings are incorporated in the final Recommended Methods and are also recorded via papers published by the Sub-Group

Appendix - Publications

- **Purkis, S., Intorp, M., Hauleithner, A.,**
  Updates of CORESTA Recommended Methods after further collaborative studies carried out under both ISO and Health Canada Intense smoking regimes;
  Beitr. TabakForsch. Int. 2013 submitted to Journal.

Questions?