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A REVIEW OF SOME RESEARCH PRESENTED  
AT THE TWENTIETH TOBACCO CHEMISTS'  
RESEARCH CONFERENCE: PART 2.  
COMPLEXED CYANIDE IN COLLECTED  
SMOKE – IS IT STILL VIABLE AT AGE FORTY?

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# Outline for presentation

- Background
- Objectives
  1. Review experimental results from 1966
  2. Value of results in 1966 context
  3. Value of results in 2006 context
- Conclusions

# Background

- The presence of hydrogen cyanide (HCN) in tobacco smoke had been known for decades (Vogel, 1858, as cited by Neurath, 1969)
- In early 1960s, HCN in mainstream cigarette smoke (MSS) reported to reduce ciliary activity in mammals (Kensler & Battista, 1963)
- Most believed HCN to be only in gas-vapor phase (GVP) on account of its volatility
  - Boiling point: 25-26°C
  - Vapor pressure: 742 mm Hg @ 25°C

# Objective 1 – Review 1966 results

- Work by Nall & Rosene at Brown & Williamson
- Work was part of methods development
  - Reproducibility problems as had been noted by others within BAT group (Scherbak & de Souza)
  - Unexpected changes in response with pH
  - Had to resolve problems to find suitable method
- Compared results from smoking directly into 9/1 MeOH/H<sub>2</sub>O trap @-80°C with those obtained by Cambridge filter followed by 9/1 MeOH/H<sub>2</sub>O trap @-80°C
- Smoke fractions analyzed after distillation

## HCN retention of Cambridge filters ( $\mu\text{g}/\text{cig}$ )

Particulate HCN	Vapor HCN	Sum	Whole Smoke
130	149	279	
141	130	271	282 (3 replicates)
130	148	278	

# HCN recovery from trapping systems

Absorber	Added ( $\mu\text{g}$ )	Found ( $\mu\text{g}$ )	% Recovery
Carbon	630	20	4
9/1 MeOH/H <sub>2</sub> O	630	630	100

## Added HCN recovery from carbon + smoke

Sample	Added ( $\mu\text{g}$ )	Expected ( $\mu\text{g}$ )	Found ( $\mu\text{g}$ )
Smoke (1 cig)	0	250-275	261
Smoke (1 cig) + HCN	103	350-375	257

Something in the smoke had reacted with the added HCN

# Free and total HCN on carbon from GVP

Free ( $\mu\text{g}/\text{cig}$ )	Total ( $\mu\text{g}/\text{cig}$ )	% Free
18	164	11
17	153	11

Free measured under acidic conditions, Total under basic

## Free HCN from whole smoke on traps

Carbon trap (%)	MeOH/H <sub>2</sub> O trap (%)
30	103
27	103
28	101
Mean = 28	Mean = 102

These results indicated complexed cyanide an artifact

# Free and complexed HCN on Cambridge filters

Free ( $\mu\text{g}/\text{cig}$ )	Complexed ( $\mu\text{g}/\text{cig}$ )	% Complexed
63	103	62
43	80	65
52	99	66
		Mean = 64

Cyanohydrins isolated from eluates of GVP on carbon

# Isolation and identification of cyanohydrins

- Work by Johnson & Alford at B&W
- Cyanohydrins extracted from carbon used to trap GVP from over 10,000 cigarettes
- Isolation and identification procedure
  - Carbon extracted with MeOH
  - Extract concentrated and trimethylsilylated
  - TMS derivatives concentrated by distillation
  - TMS derivatives of cyanohydrins isolated by preparative gas chromatography (GC)
  - <sup>1</sup>H NMR and IR obtained on GC fractions and compared spectra of authentic samples
  - Lactonitrile and other cyanohydrins identified

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## More on cyanohydrins in MSS

- Johnson & Alford found the cyanohydrins of acetaldehyde (lactonitrile), propionaldehyde, and isobutyraldehyde
  - Compounds found in 5:2:1 ratio
  - Cyanohydrin of acrolein not found
- Dube & Green (1982 TCRC) found same cyanohydrins in water-trapped smoke
- Schumacher et al. (1977) reported acetone cyanohydrin in water-soluble portion of smoke

## Objective 2 – Value of results in 1966 context

- Was work reported by Nall significant?
  - Cited in several journal articles on compounds found in smoke
  - Method developers had to ensure that methods for cyanide in smoke converted complexed cyanide to cyanide to prevent under reporting of HCN
- Was there significance for bioassays?
  - Bioassays of the time included
    - In vitro ciliotoxicity bioassay
    - In vitro cytotoxicity assay with KB cells in culture
  - Kensler of A. D. Little recognized that cyanohydrins might be more toxic than starting materials

# Cytotoxicity of smoke compounds for KB cells

Compound	μg/puff	ID <sub>50</sub> (μg/mL)	ID <sub>50</sub> /Cigarette
Methanol	11-27	>10,000	<0.1
Formaldehyde	2.5-4.1	2.5	10-16
Butanedione	4.3-20	4	10-50
Crotonaldehyde	1.6-2.0	1.5	11-13
Acetaldehyde	81-202	19	43-106
Acetone	39-57	>10,000	<6
Acrolein	6.5-14	4	16-35
Hydrogen cyanide	22-50	2.7	80-185
Acetaldehyde cyanohydrin	130-320	4	325-800
Acetone cyanohydrin	57-83	1.8	315-460
Acrolein cyanohydrin	10-20	2	50-100

Source: Letter of February 5, 1969, from C. J. Kensler of A. D. Little to T. J. Grady of Federal Trade Commission

## Objective 3 – Value of results in 2006 context

- Toxicological assessment of MSS is common
  - Chemical and biological assessments
  - Assessment with several smoking protocols
  - Chemical results versus biological results
- Those demanding more intensive smoking regimens assume “tar” up, toxicity up
- Does HCN and HCN-related toxicity follow that assumption?
- Extensive data on HCN deliveries reported by Counts *et al.* in 2005

# HCN ratios by smoking regimen for KY1R4F

ISO	ISO	ISO	ISO	MDPH	MDPH	MDPH	MDPH	HCI	HCI	HCI	HCI
HCN <sub>vap</sub> / TPM	HCN <sub>vap</sub> / Tar	HCN <sub>pad</sub> / TPM	HCN <sub>pad</sub> / Tar	HCN <sub>vap</sub> / TPM	HCN <sub>vap</sub> / Tar	HCN <sub>pad</sub> / TPM	HCN <sub>pad</sub> / Tar	HCN <sub>vap</sub> / TPM	HCN <sub>vap</sub> / Tar	HCN <sub>pad</sub> / TPM	HCN <sub>pad</sub> / Tar
6.92	8.07	5.06	5.90	7.31	23.5	3.91	12.5	6.76	11.1	2.88	4.72

ISO (International Organization for Standardization) = 35/2/60 with no vent-blocking

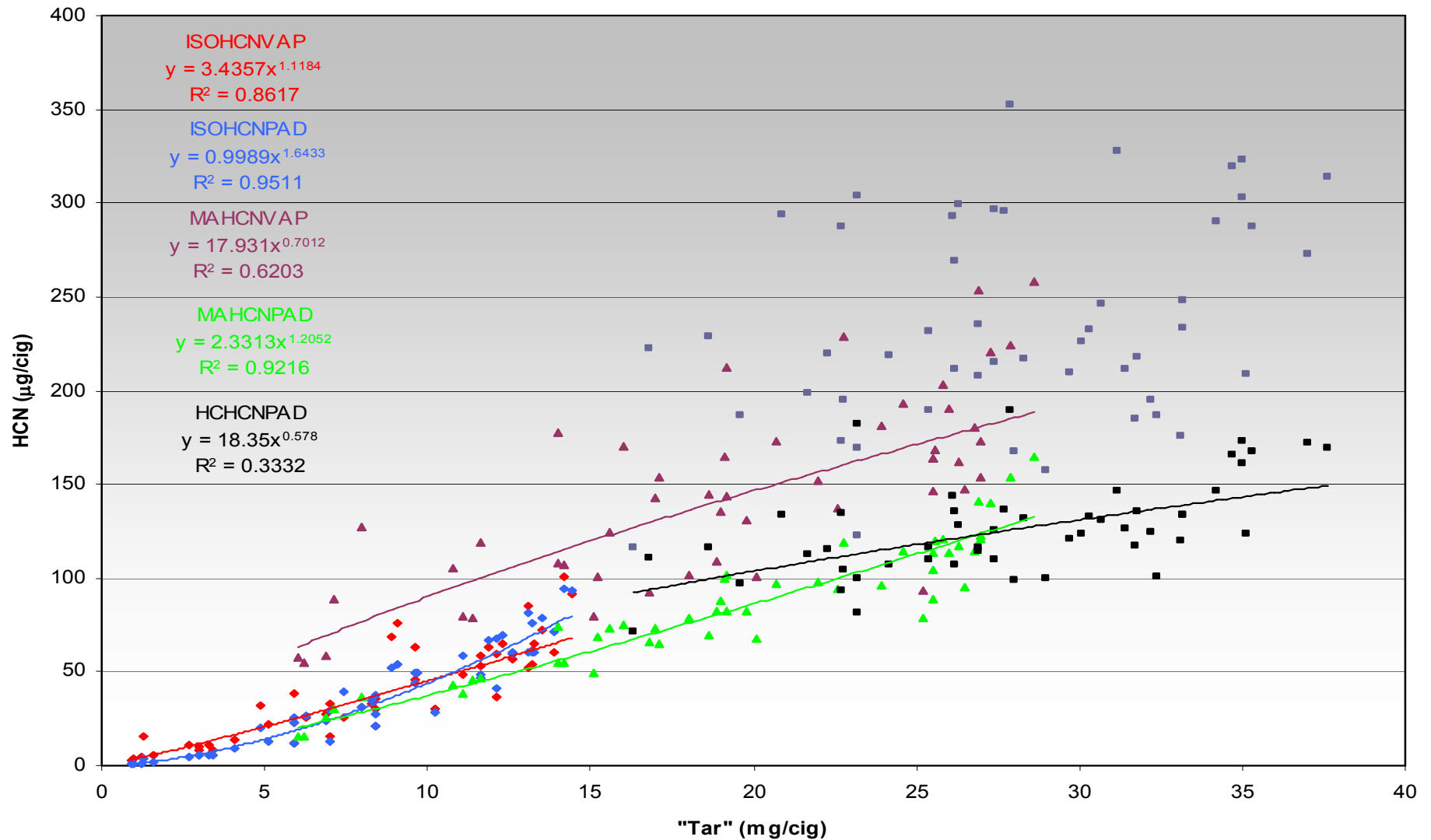
MDPH (Massachusetts Department of Public Health) = 45/2/30 with 50% vent-blocking

HCI (Health Canada Intensive) = 55/2/30 with 100% vent blocking

# Changes in cytotoxicity with smoking regimens

- In 2003, Rickert *et al.* reported Neutral Red Uptake (NRU) cytotoxicity data for ISO and HCl conditions
  - Little change in TPM cytotoxicity on going from ISO to HCl conditions when results put on “tar” basis
  - Significant increase in cytotoxicity for gas-vapor phase on going from ISO to HCl conditions
- HCN concentrations support GVP results, but not necessarily TPM results
  - Do findings support cyanohydrins?
  - Are other species involved?

# HCN versus “tar” – Counts *et al.*



# HCN deliveries versus “tar” explanation

- With ISO smoking, both  $\text{HCN}_{\text{pad}}$  and  $\text{HCN}_{\text{vap}}$  tend to increase proportionately more than “tar”
- With MADPH conditions,  $\text{HCN}_{\text{pad}}$  tends to increase proportionately more than “tar” while at higher deliveries,  $\text{HCN}_{\text{vap}}$  tends to increase proportionately less than “tar”, but  $\text{HCN}_{\text{vap}}$  always greater than  $\text{HCN}_{\text{pad}}$
- With HCl smoking, there is no significant trend between  $\text{HCN}_{\text{vap}}$  and “tar” as filter ventilation is completely blocked

# Differences in mainstream smoke collection

- When MSS is collected for NRU cytotoxicity, conditions different than for HCN determination
  - TPM dissolved in DMSO instead of 0.1N NaOH
  - GVP collected in PBS instead of 0.1N NaOH
  - Thus, very toxic cyanohydrins are probably NOT destroyed in the NRU cytotoxicity assay but are converted to cyanide for the HCN determination
- Studies to understand compounds causing smoke cytotoxicity should also include cyanohydrins and other cytotoxic agents that are not included in Hoffmann analytes

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# Conclusions

- Nall's presentation on complexed cyanide is a relevant today as it was forty years ago
- Complexed cyanides such as acetone cyanohydrin may have higher toxicity than acetone and HCN
- Differences in smoke collection systems make correlations between smoke chemistry and smoke cytotoxicity difficult
- Research is needed to identify the cytotoxic agents in smoke fractions used for NRU assay

# References

## ■ Internet documents

- Johnson, R. R., B&W report 67-1, “Summary of 20<sup>th</sup> tobacco chemists’ conference,” January 6, 1967, Bates numbers 402441154-402441420
- Johnson, R. R. and Alford, E. D., B&W report 66-30R, “Cyanohydrins in carbon-trapped vapor phase of cigarette smoke,” October 10, 1966, Bates numbers 650104782-650104796
- Kensler, C. J., letter to T. J. Grady of FTC, February 5, 1969, Bates numbers 01132303-01132316
- Nall, J. F. and Rosene, C. J., B&W report 66-2, “Complexed hydrogen cyanide in smoke,” March 11, 1966, Bates numbers 650334980-650335002

# References

- Journal articles and meeting abstracts
  - Counts, M. E. *et al.*, ***Regul. Toxicol. Pharmacol.*** 2005;41(3):185-227
  - Dube, M. F. and Green, C. R., ***Rec. Adv. Tob. Sci.*** 1982;8:42-102
  - Kensler, C. J. and Battista, S. P., ***N. Engl. J. Med.*** 1963;269:1161-1166
  - Neurath, G., ***Beitr. Tabakforsch.*** 1969;5(3):115-133
  - Rickert, B. *et al.*, ***Tob. Sci. Res. Conf.***, 2003, 57, p. 27., abstr. 15.
  - Schumacher, J. N. *et al.*, ***J. Agric. Food Chem.*** 1977;25(2):310-320