

RESEARCH & DEVELOPMENT

# Three Waves of Tobacco Science: Analysis, Biomarkers and Beyond – Where is the Science Heading?

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# Outline

- Introduction
- Tobacco and smoke analysis & fractionation – the **first wave**
- Exposure biomarkers – the **second wave**
- Effects biomarkers – the **third wave**
- Risk biomarkers and beyond – the **next wave**
- Concluding remarks

# Introduction

- **First Wave – smoke analysis & fractionation**
  - Tars, CO, nicotine, particulates first
  - Hoffmann list
- **Second Wave – exposure biomarkers**
  - Nicotine, cotinine, TSNA metabolites
  - Blood, urine, other tissues / fluids
  - Clinical
- **Third Wave – biomarkers of effect**
  - Biological consequences of exposure
  - Clinical
  - Epidemiology

# Introduction

- Risk / harm conclusions drawn from any one wave can be erroneous – unsupportable
- Weight of evidence creates the next wave – biomarkers of risk or harm
- Risk framework
  - Chemical / constituent characterization
  - Exposure assessment
  - Effects assessment
  - Risk characterization

# Smoke Analysis & Fractionation – The First Wave

- Nicotine isolated from tobacco 1828
  - Posselt & Reimann
- Chemical analysis of tobacco and smoke compounds
  - Kosak 1954 50
  - Stedman 1968 (USDA) 1200+
  - Baker 2002 (BAT) 5000+
  - Rodgman & Perfetti 2009 (RJR) ~ 8700

# First Wave

- Smoke chemistry generally characterizes burley, flue-cured and oriental tobacco
- **Mainstream**, sidestream and environmental tobacco smoke
- CORESTA analytical methods
  - Cooperation Centre for Scientific Research Relative to Tobacco
- ISO 3308 standard analytical smoking method
- Constituents produced – **vapor** ----- **particulate** phases
  - mg **CO** **nicotine**
  - µg **NO** **phenols**
  - ng **hydrazine** **NNK**
  - pg **----** **4-aminobiphenyl**

# First Wave

- Analysis / identification of constituents outpaces association with specific exposure & disease states (effects)

## Major Constituent Classes of Hoffmann Analytes – toxicity and carcinogenicity (Hoffmann & Hoffmann 1998)

### Chemical Class

Aromatic Amines

PAH

Volatile Carbonyls

Trace Metals

TSNA

Phenols

Semi-volatiles

Miscellaneous

### Examples

4-aminobiphenyl (4-ABP)

benzo[a]pyrene (BaP)

formaldehyde

cadmium, lead

NNK, NNN

hydroquinone, phenol

nicotine

hydrogen cyanide, NO<sub>x</sub>

# First Wave

- WHO Study Group on Tobacco Products Regulation (TobReg) advisory opinion on recommended regulatory limits for selected cigarette smoke constituents (2007 & 2008)

## Initial list of priority toxicants (2008)

Acetaldehyde	Benzo[a]pyrene	Formaldehyde
Acrolein	1,3-Butadiene	HCN
Acrylonitrile	Cadmium	Hydroquinone
4-Aminobiphenyl	CO	NO <sub>x</sub>
2-Aminonaphthalene	Catechol	NNN
Benzene	Crotonaldehyde	NNK

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- Wynder and others in the 50's and 60's published on tar and mouse skin painting – producing skin tumors
    - Begin to branch into exposure and even effects-based testing early on in mid 20<sup>th</sup> Century



# Exposure Biomarkers – The Second Wave

- Non-invasive or invasive biomarkers
  - Breath, saliva, urine, hair – CO, cotinine, NNAL
  - Blood, tissues – CO, hydroxypyrene, modified proteins
- In vitro, ex vivo, in vivo
  - Cells, cellular constructs
  - Excised lung, blood vessels, trachea
  - Actual organism – rodent, dog, primate, human
- Filter butts – mouth level exposure (MLE)
- Clinical studies

# Second Wave

- Nitration of proteins (Zweier et al. 2009; Peluffo et al. 2009)
- Heme adducts from 1,3-BD (Boysen et al. 2007); from 4-ABP (Scherer 2005)
- DNA adducts in lung & lymphocytes from PAH & carbonyls (Arif et al. 2006)
- Urinary NNAL<sub>Total</sub> & 'nicotine + 5' (Carmella et al. 2009)
  - 17 days after smoking cessation, 81-91% decrease in urinary biomarkers

# Second Wave

- Urinary hydroxypyrene & NNAL similar among smokers of regular, lights and ultralights (Hecht et al. 2005)
- Switching from regular to light or ultralights produced substantial reductions in urinary biomarkers (Mendes et al. 2008)
- Similar COHb and urinary nicotine<sub>Total</sub> & NNAL<sub>Total</sub> for smokers of matched menthol & nonmenthol cigarettes (Heck 2009)
- Tar yield was significantly associated with exposure biomarkers in urine and blood (Mendes et al. 2009)

# Second Wave

- Mouth level exposure (MLE) compared to urinary biomarkers correlated well for nicotine & cotinine (St.Charles et al. 2006)
- Shepperd et al. (2006; 2009) used MLE or butt analyses
  - Excellent correlations compared to machine calibrated analysis and urinary levels
  - Lower yielding cigarettes result in lower exposure biomarkers
- CDC MLE estimates machine measured mainstream nicotine and TSNA using solanesol in butts (Polzin et al. 2009)
- Roethig et al. (2009) total exposure study demonstrated
  - Young adult < older smoker levels
  - Female < male smoker levels

# Effects Biomarkers – The Third Wave

- IoM (Stratton et al. 2001) defines a biomarker of harm / effect with respect to tobacco:
  - Significant, objective, measurable, alteration in a biological sample after smoking a tobacco product
    - Predictive of pathologic change
  - Altered in a proportion of smokers and is reversible on cessation of smoking
  - Development of predictive markers of disease risk insufficient to support risk-reduction judgments
- Currently no formally validated biomarkers for tobacco effects and no consensus regarding their selection and potential use (Gregg et al. 2006; Hatsukami et al. 2006)
  - Some biomarkers have advanced to preliminary testing

# Third Wave

- Life Sciences Research Office (LSRO 2008)  
more optimistic:
  - Weight-of evidence judgment with presently-available, comprehensive array of testing and evaluation tools
  - Supports a scientifically-defensible judgment in regard to potential reduced-risk tobacco products

# Third Wave

- In vitro in bacterial, mammalian, human cell lines
  - NRU
  - Ames
  - Micronucleus
- Ex vivo
  - Pig trachea
  - Mouse vascular epithelium
- In vivo in mice, rats, humans (clinical)
  - Proteomics – nitration of proteins & C reactive proteins
  - Vascular endothelium – dysfunction & NO generation
  - Cholesterol and triglycerides
  - Physiological changes – BP, cardiac wall & lung volume

# Third Wave

- Patskan et al. (2008) completed a toxicological comparison of three types of cigarettes to the reference 1R4F cigarette in vitro and in vivo in rats
  - Cigarette constructs similar -- no meaningful toxicological differences on TPM and tar normalized basis
  - Tars were equipotent – on a per cigarette basis lower tar = lower effect
- Roemer et al. (2009) combined strengths of in vitro mutagenesis and cytotoxicity assessments with mouse skin tumor bioassay
  - More intensive smoking conditions associated with lower in vitro & in vivo activity / mg TPM



# Third Wave

- Lowe et al. (2009) looked at candidate biomarkers of both exposure and harm in smokers, former smokers and never smokers (n = 80)
  - Exposure biomarkers discriminatory
  - Effect biomarkers inconsistent and variable in ability to discriminate among the 3 smoking categories
- Such studies explore the possibility that discrimination among different cigarettes in terms of effect (risk) might be achieved with application of existing methodologies
- Validated biomarker data will add substantially to smoke chemistry, in vitro and in vivo toxicology studies on tobacco products, better informing the risks associated with their use

- Last step in formal risk assessment -- putative determination of risks associated with exposure to and effects of a substance
- Weights or lines of evidence can be compiled that inform the risk assessment from many different perspectives
- Is the effect negligible or substantial?  
Adaptive?
- Allow the smoker to make risk management decisions to reduce their risk

# Next Wave

- Epidemiology - study of factors affecting the health and illness of human populations
  - Relative risks and odds ratios may be derived that place the exposure, effects and product in perspective
- SGR (2004) determinations of disease causation are primarily based on epidemiology studies
  - Controlled laboratory studies are weighted less
- Biomarkers of effect and ultimately risk are the ‘holy grail’ for academic, industry and regulatory scientists
  - Intelligent & strategic design of reduced exposure and ultimately reduced risk products is essential
- We will have succeeded when well-designed, complete, controlled experimental & clinical studies inform the risk of smoking and equally complement epi studies

# Concluding Remarks

- Tobacco and its combustion truly generate unique experiences, challenges and opportunities
- Focus on addressing what is in tobacco and what is produced during combustion & pyrolysis
- Develop various biomarkers of tobacco smoke exposure
- Couple components and exposures in biomarkers of effects informing the risk assessment in the use of this many faceted product of both nature and technology

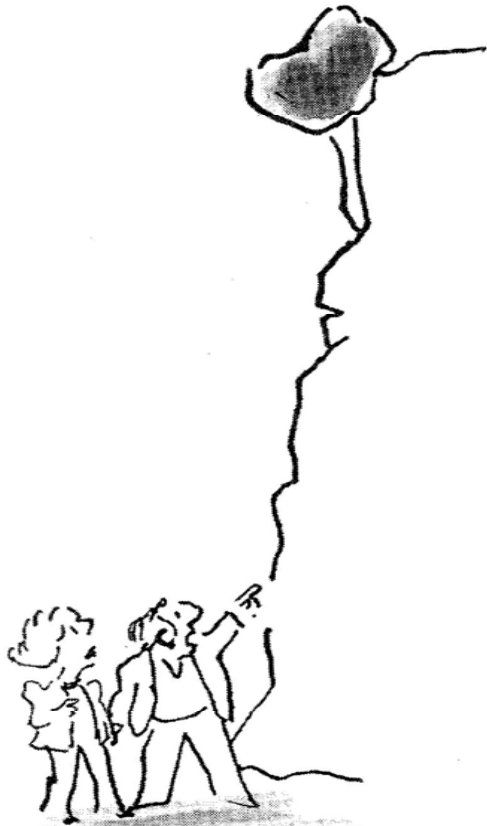
# Concluding Remarks

- Use of controlled experimental approaches takes its proper place as an important and valued component to evaluate chronic human disease risks from smoking
- Recent events – FDA, WHO FCTC, etc. will help to chart the future direction of tobacco science
- We can only hope that regulatory oversight will be grounded in the best science – we have an obligation to enable this to happen!

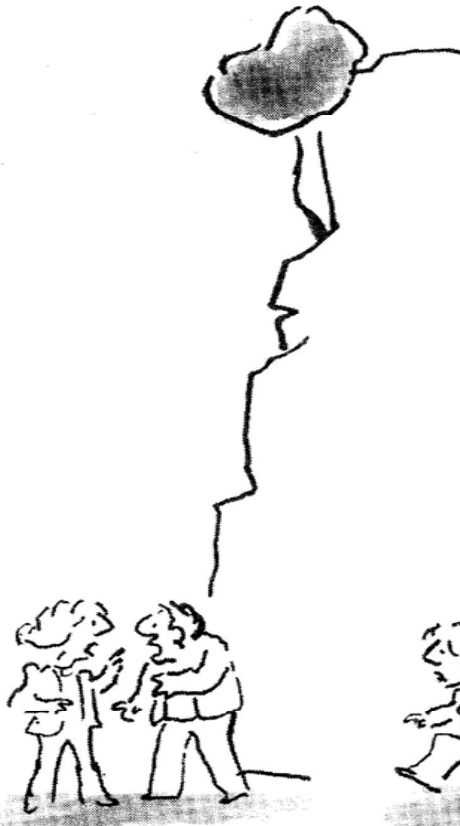
# Our Thanks To:

- Organizers of this year's and past year's TSRC conferences for their continued interest in bringing tobacco scientists together for this global exchange of ideas
- Analytical chemists, exposure scientists, toxicologists, risk assessment, regulatory and human health professionals who have toiled long and hard to inform tobacco science

RISK PERCEPTION



RISK ASSESSMENT



RISK MANAGEMENT

