Lorillard TOBACCO COMPANY

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	Examples
Aromatic Amines	4-aminobiphenyl (4-ABP)
PAH	benzo[a]pyrene (BaP)
Volatile Carbonyls	formaldehyde
Trace Metals	cadmium, lead
TSNA	NNK, NNN
Phenols	hydroquinone, phenol
Semi-volatiles	nicotine
Miscellaneous	hydrogen cyanide, NO_x

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_x
2-Aminonaphthalene	Catechol	NNN
Benzene	Crontonaldehyde	NNK

- 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 20th 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 $NNAL_{Total}$ & ‘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 $nicotine_{Total}$ & $NNAL_{Total}$ 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

Risk Biomarkers and Beyond – The Next Wave

- 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

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