Cigarette Acceptability & Harm Reduction Testing Approaches

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Figure 1
Process for Reduced-Harm Product Use and Claims

Pre-Market Research

Acceptability Tests
- Smoke Chemistry
- Genotoxicity
- Cytotoxicity
- Sub-chronic Inhalation

Subjectives Testing
- Smoke Panels

Market Place

Post Market Research

Provisional Product Claim
Special Harm Reduction Tests, if needed
- e.g. Organ/System Testing

Post

Human Exposure Measurement
- Biomarkers of exposure

12-36 Months

12-36 Months

Long Term Human Health Effects Studies/Epidemiology
- Harm Reduction Monitoring

Time for testing, not development

6-12 Months

12-36 Months

12-36 Months

5-20 Years

Discussion Document Prepared by Philip Morris USA
for the LSRO Meeting - October 29 & 30, 2001
Toxicology Testing Approach

• Purposes
  – Evaluate Acceptability of Product Changes
  – Evaluate Harm Reduction Efforts
Acceptability Testing vs. Harm Reduction Testing

• Acceptability
  – No significant increase in existing activity
  – No significant introduction of new activity

• Harm Reduction
  – Decrease existing activities
Testing Approach

• Relative comparison of activity
  – Test versus an appropriate cigarette control

• Selection of control
  – Single change relative to control (e.g. ingredient on tobacco)
  – Use of a standard reference cigarette (e.g. novel cigarette design)
Assay Selection

• Responsive to smoke
• Able to differentiate among different cigarettes
• Meet typical regulatory standards
  – OECD (Organization for Economic Development and Cooperation)
  – ICH (International Congress on Harmonization)
  – GLP (Good Laboratory Practices)
• Covers a spectrum of endpoints
  – Complementary battery of tests
Smoke Generation & Collection

• Standard conditions
  – FTC (Federal Trade Commission)
  – ISO (International Standards Organization)
• Relative comparison of cigarettes
  – Not meant to mimic human smoking
  – Consistency is important for appropriate comparisons
<table>
<thead>
<tr>
<th>Puffing Parameters Applied to Cigarette</th>
<th>Puff Volume (ml)</th>
<th>Puff Frequency (puffs/min.)</th>
<th>Ventilation Holes Blocked (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1R4F, 1R5F</td>
<td>35</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>EHC</td>
<td>35</td>
<td>1</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>2</td>
<td>---</td>
</tr>
</tbody>
</table>

Remarks: For all regimens, the puff duration was 2 seconds.

(Rustemeier et al, 2000)
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0 50 100 150 200 250 300 350
Ratio (%)

35/1/0 55/2/0 55/2/100 35/1/- 55/2/-
Cigarette and Puffing Regimen

1R4F 1R5F EHC

TPM (mg/cig.)

tpm "tar" nicotine carbon monoxide acrolein benzo(a)pyrene catechol
Alternate Smoke Machine Profiles

• Conclusions
  – Different smoking conditions give different total yields
  – The relative proportions of smoke constituents appear relatively unchanged

• Plans
  – Conduct biological assays under alternate smoking machine conditions
Electrically Heated Cigarette Smoking System (EHCSS)

- Objective
- Technology
- Toxicology testing
EHCSS: Objective

• Reduce biological activity
• Reduce yield of toxic smoke constituents
• Reduce environmental tobacco smoke
• Low ignition propensity
• No ashes
• Low odor
EHCSS: Technology

• Goal to reduce burn temperature
• Controlled electric heating of tobacco
  – Energy
  – Duration
• Special cigarette design
• Fixed number of puffs
Selection of Control

- 1R4F
  - University of Kentucky reference
  - 9 mg tar, 0.8 mg. nicotine delivery
  - Representative of the middle of the US market
  - Commonly used in cigarette research
Role of Experimental Results in Harm Reduction Evaluations

• Possible basis for comparison
  – Cigarette
  – TPM
  – CO
  – Nicotine
  – Other

• Link to human exposure
Smoke Chemistry

• List developed from chemicals identified by the CPSC (Consumer Product Safety Commission) and IARC (International Agency for Research on Cancer)
• Analysis of both gas/vapor and particulate phases
• Covers many chemical classes
Smoke Chemistry
(EHCSS yield per cigarette relative to 1R4F)

TPM

Percent

Terpstra et al., 1998
Smoke Chemistry
(EHCSS yield per cigarette relative to 1R4F)
Smoke Chemistry
(EHCSS yield per cigarette relative to 1R4F)
Smoke Chemistry

• Not quantifiable in either EHC or 1R4F
  – $N$-nitrosodimethylamine
  – $N$-nitrosodiethylamine
  – $N$-nitroso-n-propylamine
  – $N$-nitroso-n-butylamine
  – $N$-nitrosopiperidine
  – Dibenz(a,j)acridine
  – Dibenz(-)anthracenes
  – 5-methylchrysene
  – Chromium
  – Nickel

• Validated methods not currently available for all items on the chemistry list
Genotoxicity

• *Salmonella* reverse mutation (Ames Test)
  – +/- S9
  – Test material: particulate phase

• TK Assay in mammalian cells
  – Test material: particulate phase
  – Under validation

• Rat micronucleus
  – Test material: diluted smoke
  – Under validation
Ames Assay + S9

NA - Not active

(1R4F | EHC)

(Terpstra et al., 1998)
Ames Assay - S9

NA - Not active

(Terpstra et al., 1998)
Cytotoxicity

- 3T3 cells
- Neutral red uptake
- Test material: particulate phase and gas/vapor phase
Cytotoxicity

![Graph showing cytotoxicity](Terpstra et al., 1998)
Inhalation Study

- Rats
- 90 day exposure, 6 hr / day, 7 days / week
- Test material: diluted smoke
- Many endpoints evaluated (OECD)
- Focus is on histopathology of the respiratory tract
Inhalation

- The effects observed in animals exposed to smoke from the EHC were similar to those typically observed in animals exposed to the smoke from the 1R4F reference cigarette.
Inhalation

Rel. Biological Activity (% of 1R4F)

- body weight
- respiratory rate
- lymphocyte count
- liver enzymes
- rel. organ weights
- changes in nose
- changes in larynx
- changes in trachea and lung

(Terpstra et al., 1998)
Suitability of the Bacterial Mutagenicity Assay to Investigate Cigarette Design Parameters

Test conditions:
» cig. smoke condensate
» tester strain TA 98, + S9
» multiple linear regression analysis

(Tewes et al., 1999)
Comparison of Single Blend Cigarette Prototypes

Mouse Skin Tumorigenicity

In vitro Cytotoxicity

(Roemer et al., Tewes et al., unpublished)
Use of Exposure Data

• Experimental data provide a relative comparison of activity

• Human Data
  – Uptake
    • Biomarkers
Key Information for Assessment of Harm Reduction

• Premarket toxicology testing
  – Chemistry
  – Biology

• Exposure assessment in humans
  – Biomarkers of exposure
  – Biomarker of effect

• Aftermarket epidemiology
Summary - Testing

• Standard methods for smoke generation
• Broad spectrum of assays
• Validated for use with smoke
• Can differentiate among cigarette types
Major Diseases with Cigarette Smoke-related Mortality

<table>
<thead>
<tr>
<th>Disease</th>
<th>Cigarette Smoke-Related Mortality in US (1990)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
<td>151,322</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>179,820</td>
</tr>
<tr>
<td>Respiratory disease</td>
<td>84,475</td>
</tr>
</tbody>
</table>

(Centers for Disease Control and Prevention, 1993)
Mechanisms Related to Human Lung Cancer Development

• Initiation-promotion paradigm
  – Has not been conclusively applied to lung cancer development
  – Genotoxic events linked to initiation:
    » activation of proto-oncogenes
    » inactivation/mutation of tumor suppressor genes
  – Reversible morphological changes possibly linked to promotion:
    » basal cell hyperplasia
    » goblet cell hyperplasia
    » squamous metaplasia
    inflammation
    irritation
  – Declining lung cancer risk after cessation of smoking indicative of role of promotion
Experimental Models for Pulmonary Carcinogenesis

• Long-term inhalation studies leading to lung tumors
  – Historic attempts:
    » equivocal, mostly negative, irrespective of rodent species (Coggins, 1998)
  – Recent developments:
    » Lovelace Respiratory Research Institute studies on rats (Finch et al.)
    » UC Davis studies on A/J mice (Witschi et al.)
    » Philip Morris’ studies on rats (Haussmann, Stinn et al.)

• Mouse skin painting studies
  » initiation-promotion model (e.g., Roemer and Hackenberg, 1990)
  » restricted to particulate phase of cigarette smoke
  » suitable for specific purposes

• Short-term experimental surrogates
Long-Term Rat Inhalation Study on Cigarette Smoke and Plutonium Dioxide

(incidence of lung tumors; Inhalation Toxicology Research Institute, Annual Report 1995)

over-additive effect largely due to impairment of plutonium clearance by cigarette smoke inhalation (Finch et al., 1998)
Short-Term Experimental Surrogates

Smoke chemistry
- extended towards IARC carcinogens
  (Voncken et al., 1998)

Two-stage transformation
(Schlage et al., 1999)

In vitro cytotoxicity
(Tewes et al., 1998)

In vitro genotoxicity
- bacterial mutagenicity
- mammalian cell mutagenicity

Initiation

Promotion

In vivo genotoxicity
- micronucleus assay
  (Lee et al., 1990)

Subchronic inhalation
- irritative changes in respiratory tract
Cardiovascular Diseases

• Types
  – Coronary heart disease/ischemic heart disease
    » cardiac ischemia
    » myocardial infarction
  – Cerebrovascular disease
    » stroke
    » transient cerebral ischemia
  – Peripheral vascular disease

• Mechanisms involved
  – Atherosclerosis
  – Vascular wall changes
  – Vascular tone changes
  – Coagulopathy
Experimental Models for Atherosclerosis (Examples)

• ApoE-deficient mouse
  – Atherosclerotic process very similar to human disease in terms of
    » types and sites of lesions (Breslow, 1996)
      (i.e., fatty streaks → fibrous caps → complex lesions)
    » response to human risk factors, i.e.,
      • high cholesterol / high fat diets
      • cigarette smoke (subchronic inhalation of sidestream smoke: Gairola and Daugherty, 1999)
      » mechanistic hallmarks
        • lipoprotein oxidation

• Rabbits and birds (e.g., cockerels)
  – Pathogenesis less similar to humans
  – Reported to be responsive to cigarette sidestream smoke (Zhu et al., 1993; Penn and Snyder, 1993/1996)
Chronic Obstructive Lung Diseases

• Emphysema
  – Irreversible destruction of alveolar walls
    » disturbance of the protease - anti-protease balance,
      e.g.,
      • increased elastase activity from inflammatory cells
      • inactivated protease inhibitors

• Small Airways Disease
Experimental Emphysema Models

• Chronic inhalation of mainstream smoke in mice (March et al., 1999)
  – Morphological changes similar to those seen in man
    » increase of alveolar septa mean linear intercept
    » increase of volume density of alveolar air space
    » decrease of volume density of alveolar septa
    » accompanied by accumulation of PMNL

• *In vitro* model for emphysema
  – Inactivation of alpha-1-protease inhibitor (Pryor et al., 1990)
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- Market
  - Short-Term Human Health Effect Studies
    - Biomarkers of Effect

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Post-Market Research

- Surveillance
  - Long Term Human Health Effects Studies/Epidemiology
    - Harm Reduction Monitoring

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Time for testing, not development