International Symposium for the 30th Anniversary of the Korean Society of Tobacco Science

October 5, 2007

Contents

KOSTAS

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Characterization of Cigarette Smoke Toxicity using Rodent Inhalation Models

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Outline

- **Guiding Principle**
- **Types of Cigarette Smoke In Vivo Testing:**
  - for conventional cigarettes
    - Based on regulatory (pre-clinical) testing
  - for novel or reduced exposure/risk products
    - Disease-specific biomarker testing
      : e.g., Development of animal COPD models
- **Questions**

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Guiding Principle – Current Paradigm in U.S.

Why testing and to what extend?

"Level of concern"

: To evaluate if changes in cigarette ingredients, materials (e.g., LIP), or product designs may increase the health risks associated with smoking the new product

Guiding Principle – Current Paradigm in U.S. (cont'd)

- **Tiered approach** to consider weight of evidence
  - A battery of toxicity studies [adapted from US FDA, *Draft "Redbook II."* 1993 (updated 1997)[1]]

" Safety evaluation for a direct food additive or color additive used in food involves assigning the additive to a Concern Level (i.e., low (I), intermediate (II) or high (III)) based on information on the additive's toxicological potential predicted from its chemical structure (i.e., low (A), intermediate (B), or high (C)) and an estimation of cumulative human exposure… Frequently, exposure information has more weight than structure alert information in assigning additives to a Concern Level. If available, other information may be considered when setting the concern level for a food or color additive, and final safety decisions are made on a case-by-case basis."

  - Subject to change upon anticipated tobacco regulation by US FDA

Current Toxicological Testing Paradigm *

Toxicity Tests[1]	Concern Levels		
	Low (I)	Intermediate (II)	High (III)
Genetic toxicity tests	X	X	X
Short-term toxicity studies with rodents	X[c]	X[a,c]	X[a,c]
Subchronic toxicity studies with rodents		X[c]	X[a,c]
Subchronic toxicity studies with non-rodents		X[c]	X[a,c]
One-year toxicity studies with non-rodents			X[c]
Chronic toxicity or Combined chronic toxicity/carcinogenicity studies with rodents (available in PDF from 1993 Draft Redbook II)			X[c]
Carcinogenicity studies with rodents including *In utero* exposure phase (available in PDF from 1993 Draft Redbook II)			X
Reproduction studies		X[c]	X[c]
Developmental toxicity studies		X[b,c]	X[b,c]
Metabolism and Pharmacokinetic studies (available in PDF from 1993 Draft Redbook II)		X[b]	X[b]
Human studies (available in PDF from 1993 Draft Redbook II) including Epidemiology Studies			X[b]

[a] If needed as preliminary to further study.
[b] If indicated by available data or information
[c] Including screens for neurotoxicity and immunotoxicity (available in PDF from 1993 Draft Redbook II)

** Addative used in food; Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD , U.S.A. (June 2006)* [1]

http://www.cfsan.fda.gov/guidance.html

"...This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations..."

*For cigarette smoke testing, tests generally demonstrate **comparative toxicity testing** between Test and Reference cigarettes, in addition to comparison with the sham groups*

Guiding Principle – Current Paradigm in U.S. (Cont'd)

- **Materials Tested**
  - *Test cigarettes* with vs. *Reference (Control) cigarettes* without modification/ingredients (single & mixture)

  ⇒ Smoke (MS; SS/ETS); Smoke condensates
- **Tiered Approach**
  1. Literature
  2. Constituent chemistry analysis
  3. In vitro assay
  4. In vivo testing: inhalation; skin painting
  5. (Clinical testing & post-market surveillance)

Selectivity; Sensitivity

↑
|
↓

Relevance; Extrapolation

⇒ *Test cigarette smoke no greater and no additional new toxicity potential than reference cigarette smoke*

Representative In Vivo Toxicity Testing

90-Day Inhalation Study in Rats

90-Day Inhalation Study in Rats

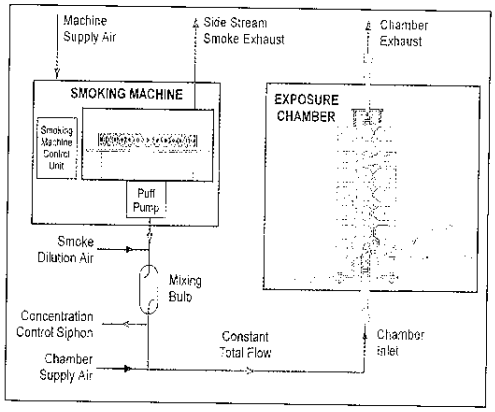
- Considered an integral part of testing for notable product modification (product stewardship for concern level II+)
- Mainstream smoke, currently under FTC/ISO
- Reference cigarettes are important to set the baseline toxicity of smoke: use control cigarettes without the modification and/or commercial reference cigarettes
- Generally performed based on internationally accepted inhalation study guidelines (i.e., OECD 413; FDA GLP) – to be scientifically dependable and reproducible

Representative 90-Day Study Design

- Adapted from OECD 413 90-day study design, with an emphasis on (upper) respiratory tract histopathology
  - 13-week nose-only inhalation exposure & 6-13 weeks of recovery
  - *1 to 6 hr/day, 5-7day/wk*
  - Up to 3 exposure concentrations (60 - 800 µg/L WTPM)
  - Test article: smoke WTPM, CO, particle size, nicotine, aldehydes; ingredient-specific?
  - Test system: M/F rats (10-20/sex/group)
  - In-life & markers of exposure (COHb, nicotine/cotinine; physiology)
  - 13-wk interim sacrifices for clinical chemistry/hematology; histopathology
  - Special endpoints: bronchoalveolar lavage (BAL) parameters

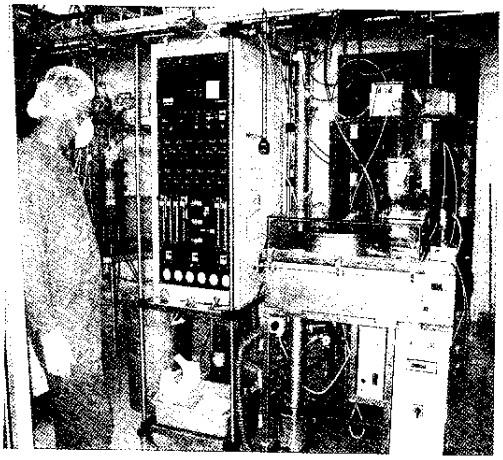
  : Provides both the acute and subchronic biological activity data

Nose-Only In Vivo Smoke Exposure System



CSES

Cigarette Smoke Exposure System



- Battelle Cigarette Exposure Suite: 2 to 4 CSES exposure units + sham unit (Ref + 3 test cigarette types)
- Compliant to OECD/GLP guidelines
- Continuous stream of mainstream (sidestream) smoke exposure up 72 animals/exposure carousel

90-Day Study: Exposure Regimens

Two representative study designs *

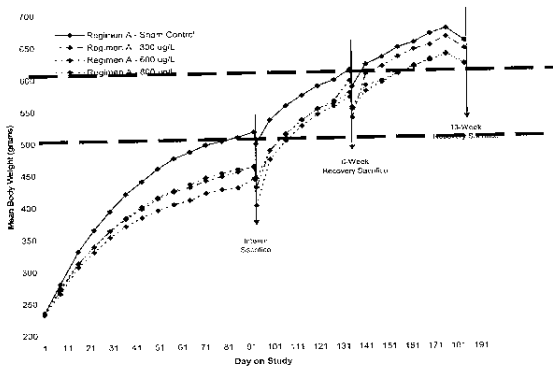
Group/Regimen		Exposure (µg WTPM/L)	Daily Exposure (hr)	Exposure Day/Week	Estimated Weekly Exposure
Sham Control	1A	0	1	7	0
	2B	0	6	7	0
Regimen A	3A	300	1	7	2100
	4A	600	1	7	4200
	(5A	*800*	*1*	*5*	*4000)*
Regimen B	6B	50	6	7	2100
	7B	100	6	7	4200

To compare the 90-day inhalation toxicity using a (2R4F) reference cigarette in male rats under two exposure regimens having ***comparable weekly doses based on [concentration x time]***

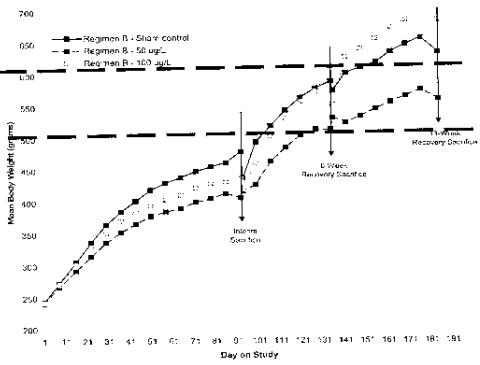
** Yoshimura et al. 2006: Comparison of Cigarette Smoke Toxicity with Various Inhalation Exposure Regimens.[2]*

Body Weight per Regimen

Regimen A (1 hr/d)



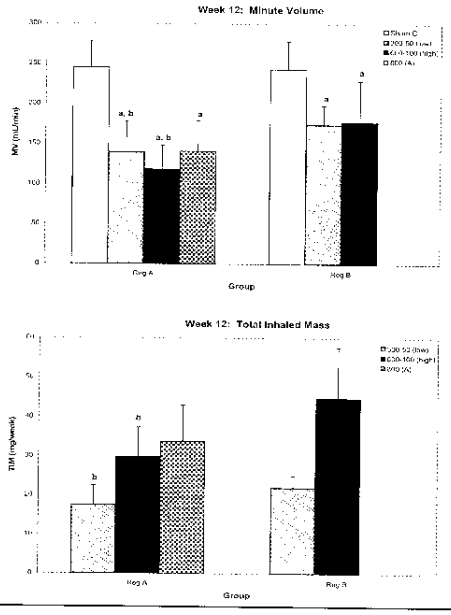
Regimen B (6 h/d)



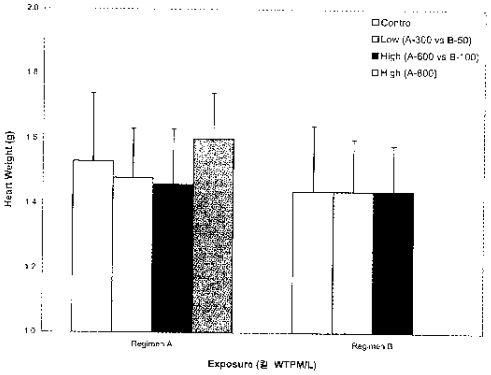
MS smoke from 2R4F cigarettes, male rats

90-Day Study: Total Inhaled Mass & Heart Weight

Total Inhaled Mass (mg)*



Terminal Heart Weight



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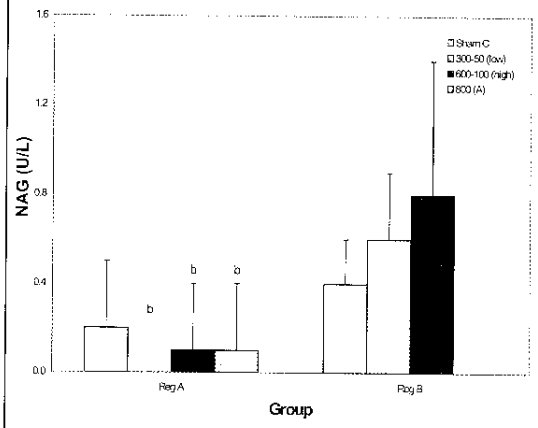
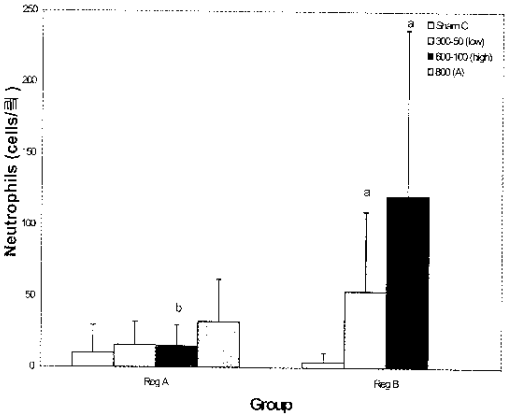
90-Day Study: Bronchoalveolar Lavage (BAL)

BAL Parameters



NAG: N-acethyl-glucuronidase



Neutrophil infiltration in the lung

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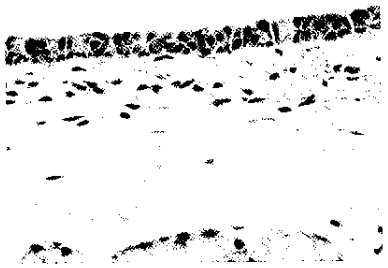
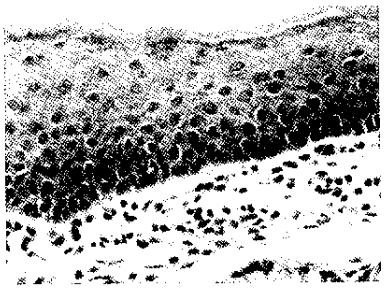
90-Day Study: Histopathology

Lesions in upper airway - Laryngeal Thickness

Sacrifice Timepoint	Target Exposure Concentration (µg/L WTPM/L)						
	Regimen A				Regimen B		
	Sham	300	600	800	Sham	50	100
Interim	0.03±0.01	0.09±0.02[a,b]	0.13±0.02[a]	0.19±0.07[a,b]	0.02±0.00	0.11±0.01[a]	0.12±0.03[a]
6-Wk Recovery	0.03±0.01	0.04±0.01[b]	0.05±0.02[a]	0.06±0.02[c]	0.04±0.01	0.05±0.01[a]	0.05±0.01[a]
13-Wk Recovery	0.04±0.01	0.04±0.01	0.04±0.01	0.05±0.01[a]	0.03±0.01	0.04±0.01	0.05±0.01[a]



Base of epiglottis, Sham control 40x.



Regimen A (800 µg/L WTPM) : 3+ squamous metaplasia, hyperplasia, hyperkeratosis

90-Day Study: Exposure Regimen Consideration

Either regimen serves the purpose with strengths and weakness: The decision may depend on the test cigarette characteristics, time and resource available

- Both exposure regimens caused mild but significant reduction in body weights
- Regimen B tended to show lower undesirable smoke effects (i.e., heart weight increase; severe respiratory reduction) but displayed greater stress response from a longer tube confinement (i.e., adrenal weight increase; lower BW increase)
- Microscopic changes of toxicological significance are comparable between regimens. Regimen B appeared to allow greater smoke delivery in the lung (higher TIM, plasma cotinine, BALF enzymes, and greater leukocyte infiltration).
- More resource-demanding for regimen B (daily exposure duration; equipments)
- Respiratory tract lesions completely or partially regressed after 6 weeks recovery for both regimens

90-Day Study: Values in testing PREP?

- **Offers "comparative" toxicity testing against conventional cigarettes**
  - *In vivo biological activity* under well-controlled exposure and responses - sufficient to support *no greater/additional* toxicity potential compared to reference cigarettes
  - Already substantial database for various cigarette types - a general consensus within the industry
- **May be limited in demonstrating specific effects in disease pathogenesis to support harm reduction potential**
  - Uncertainty in dose – esp. for PREP with notably different smoke characteristics (e.g., reduced nicotine – smoking compensation?)
  - Lack of response markers – mainly descriptive at the end of exposure with limited mechanistic information on the progressive changes

In Vivo Toxicity Testing for Novel Product Assessment

To assist early development of product candidates

Reduced harm products
Potentially Reduced Exposure Products (PREP)

PREP Assessment

Potentially Reduced Exposure Products; defined per US Institute of Medicine [3]:

... A product provides "reduced exposure" when there is scientific evidence that the product substantially reduces an average smoker's exposure to one or more tobacco toxicants, with the reduction in exposure being "sufficiently large that independent scientific experts would anticipate finding a measurable reduction in morbidity and/or mortality in subsequent clinical or epidemiological studies." A product provides "reduced risk" when there is scientific evidence that the product can reasonably be expected to reduce an average smoker's risk to one or more diseases or other adverse health effects.

⇒ *How toxicity testing assisting in demonstrating these reduced exposure/ risk/harm potentials of PREPs?*

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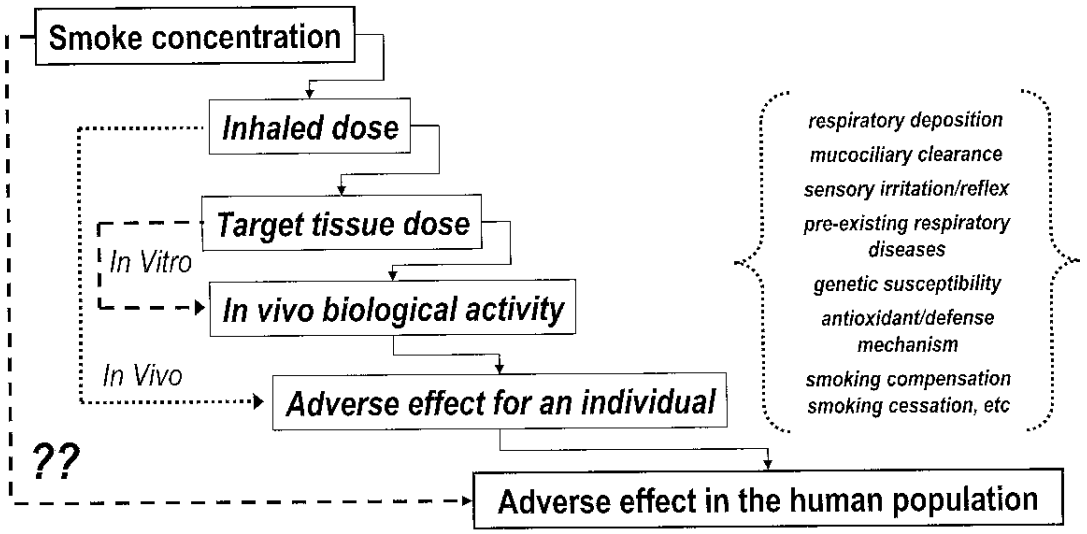
Reduced Exposure ≈ Reduced Risk or Harm?



Lung cancer; Cardiovascular disease; COPD

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Disease-Specific Toxicity Evaluation

In animal models, one needs to characterize changes in...

- Smoke constituents (total & selected constituents)
- Markers of exposure & dosimetry (tissue dose)
- The level and types of response (acute & progressive)
- Onset & progression of disease (phenotype changes over time)

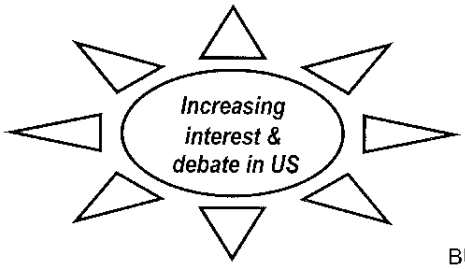
: characterize changes qualitatively and quantitatively

⇒ *Test cigarette exposures cause lower toxicity potential than reference cigarettes (but compared to the sham control?)*

⇒ *Will still have health risk regardless: an alternative to smokers to switch, or a part of smoke cessation?*

What about Smokeless Tobacco?

Potentially reduced exposure products?
Product to assist Smoke cession?



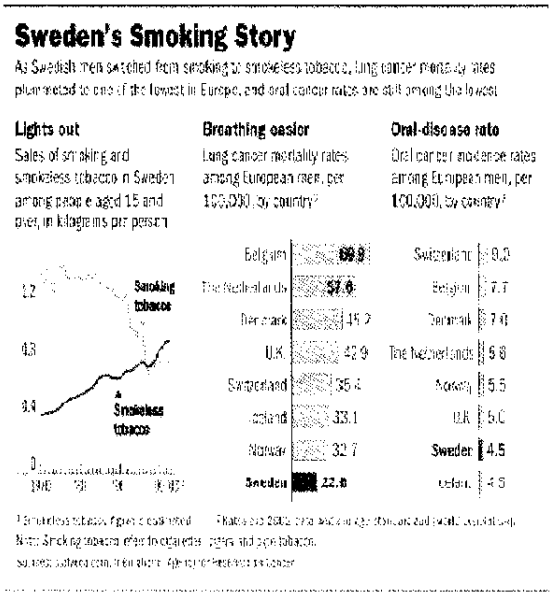
Smokeless Cigarettes

- **Increased interest and investment by tobacco industry especially under increasing limitation of public smoking**
  Example: oral (moist) snuff ; chewing tobacco; nasal snuff
  [*Skoal, Copenhagen; Grizzly; Camel Snus; Taboka; Marlboro Snus*]
- Extractant from cured tobacco: oral/nasal absorption & ingestion
- No combustion products or smoke to be inhaled
- No second hand smoke
- Lung not the target organ
- Varying levels of TSNA (NNN, NNK)
- Toxicity associated with the route of administration (oral cancer?)

⇒ ***as an alternative PREP to cigarettes for those who won't quick smoking and/or a part of smoke cessation?***

: A significant concern from the public health on promoting initiation (gateway effect) and discouraging cessation through nicotine dependence.

Smokeless Tobacco



Dr. K Fagerstrom, 2007, LSRO "*Swedish snus: nicotine, prevalence, gateway, epidemiology of harm and use for smoking cessation.*"

Disease	Smokers	Smokeless Tobacco Users
Cancer	151,000	6,000
(Mouth Cancer)	(11,500)	(6,000)
Heart and Circulatory	180,000	0
Respiratory	85,000	0
Miscellaneous	3,000	0
Total	419,000	6,000
Years of Life Lost (Average)	7.8	0.04

↑

http://www.smokersonly.org/our_harm/scientific_rationale.html; Centers for Disease Control. Morbidity and Mortality Weekly Report 42: 645-649, 1993; Rodu. B. The American Journal of the Medical Sciences 308: 32-34, 1994; Rodu, B., Cole, P. Nature 370: 184, 1994. [4]

Smokeless Cigarettes as PREP? (Cont'd)

- **Positive trends implied (e.g., Snus in Sweden) but research community, industry, and public health sides are not in agreement**

*"...**not a safe substitute** for smoking cigarettes. It can cause cancer and a number of non-cancerous oral conditions and can lead to nicotine addiction and dependence [US DHHS, 1986 [5]]"*

*" While the exclusive use of smokeless tobacco **does not increase the risk of cancer and other serious diseases as much as smoking**, the use of smokeless tobacco is a cause of significant health risks – it is not "safe" - and it is **not as safe as quitting tobacco use altogether or using clean, medicinal forms of nicotine** such as nicotine gum, patch or lozenges [Campaign for Tobacco-Free Kids, 2007 [6]]"*

- **Need to establish science-based guideline/regulation/education regarding a place for smokeless cigarettes in overall tobacco products.**

Types of In Vivo Toxicity Testing for Chronic Obstructive Pulmonary Disease

Chronic Obstructive Pulmonary Disease (COPD)

- Emphysema & chronic bronchitis: No long-term benefits from bronchodilators; corticosteroids; antibiotics
- 5th leading cause of death worldwide (example: affects ~12M Americans)
- Associated with cigarette smoking (>80% of patents are current or ex-smokers; ~15% of chronic smokers)
- Interaction of genetic and environmental risk factors: innate susceptibilty; age, exposure to air pollution, and a history of childhood respiratory infections; socioeconomic conditions
- *The reduced risk potential of PREPs is to be demonstrated against the mechanistic basis of pathogenesis* (cause & response)- Need for surrogate biomarkers for screening of PREP candidates
- ***Currently no perfect COPD animal models – needs to define the aspects of disease pathogenesis to be manipulated***

Key Mechanism & Biomarkers of COPD

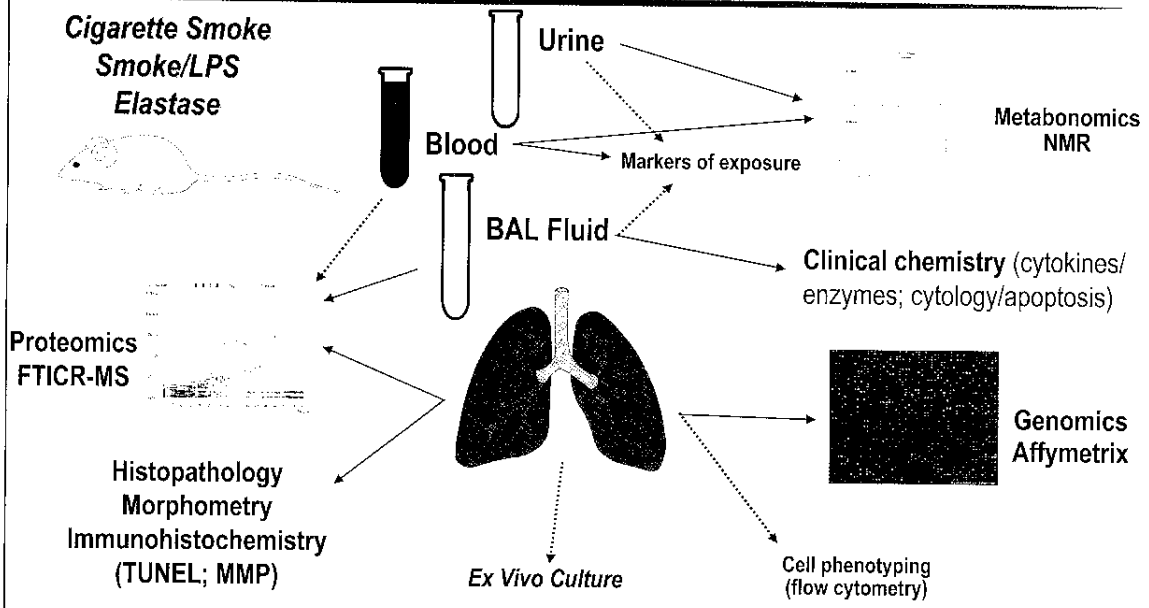
- **Inflammation** (BAL fluid & Lung)
  : BAL cytology; cytokines (IL-6; IL-8/KC, TARC; MIP-1a; TNF-a)
- **Oxidative Stress**
  : F2 isoprostane; glutathione
- **Protease & antiprotease unbalance**
  : MMP-12 & 9; TIMP-1
- **Tissue destruction & repair**
  : Desmosine, hydroxyproline
- **Immunodeficiency**
  : bacterial/vial infection during exacerbation

COPD Animal Models - Variables

A wide variety of study designs reported

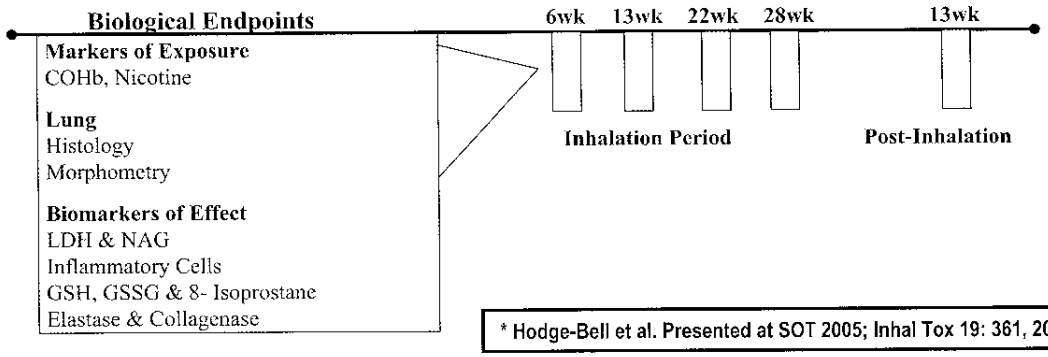
- **Test Article**: cigarette smoke characteristics
  - Types of reference cigarettes: tar content varies (2R1; 1R4F)
  - Side-stream vs. mainstream
  - Intermittent vs. continuous; aged vs. fresh smoke
- **Test System**:
  - Wild-type: AKR/J, A/J, C57BL/6, DBA2, B6C3F1 mice
  - Mutant: pallid; TNFRKO; SP-D KO; klotho mice
  - Compromised: Hypertensive rats, LPS
- **Response**: structural change (emphysema) in animal models generally mild; quantitative and consistent criteria not implemented
  - Lung morphometry (20% ↑ in mean linear intercept, Lm?)
  - Relevance to functional change (FV1)?

Systems Toxicology - Disease Biomarkers



6-Month Cigarette Smoke Mouse Model *

Animals: Male C57BL/6 & ICR mice
Exposure Mode: Nose-only inhalation for 28 Wks; 13-Wk recovery
Smoke: MS cigarette smoke, 2R4F reference cigarettes
Exposure Regimen: 0, 75, 250, or 600 µg WTPM/L; 2 h/days, 5 days/wk



* Hodge-Bell et al. Presented at SOT 2005; Inhal Tox 19: 361, 2007 [7]

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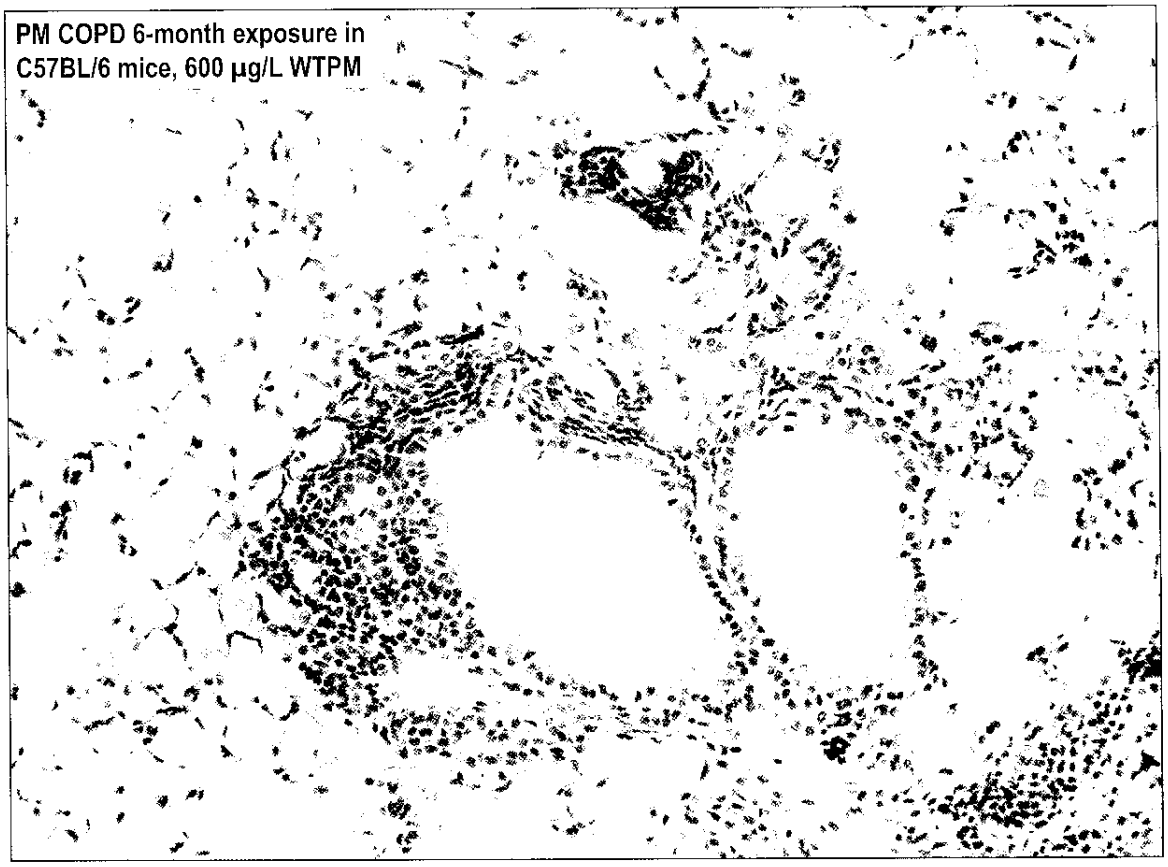
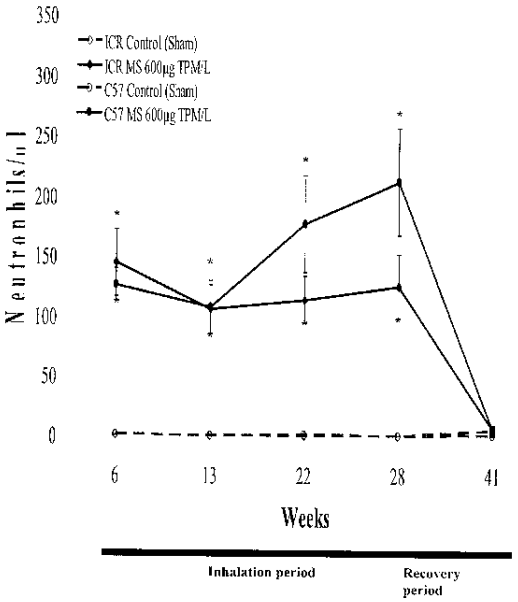
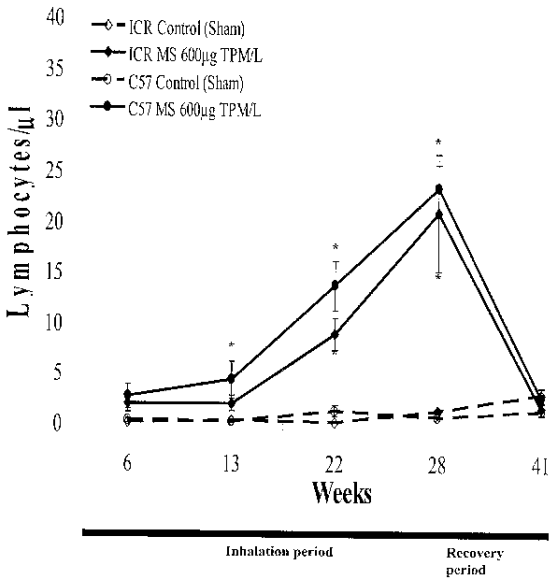


Bronchoalveolar Lavage (BAL) - Cytology





Summary: 6-Month Smoke Study

- ***Changes in inflammatory biomarkers*** primarily at 600 µg/L
- No significant changes in BALF oxidative stress (GSH, GSSG) biomarkers; elastase and collagenase activities
- ***Time-course for BAL cytology data*** *promising:* macrophages and neutrophils were consistently elevated during exposure, while lymphocytes gradually increased; consistent with reported modes of action
- ***Only mild emphysema*** in alveolar ducts at 28 wk in ICR (2/10) and C57 (1/10): minimal (13%) ↑ Lm for ICR at 28 wk compared to more stringent criteria for emphysema (i.e. Lm increase < 20% or statistically significant)
- limited in validating early biomarker candidates against the definitive morphological changes (emphysema)

Acute Compromised (Smoke + LPS) Model *

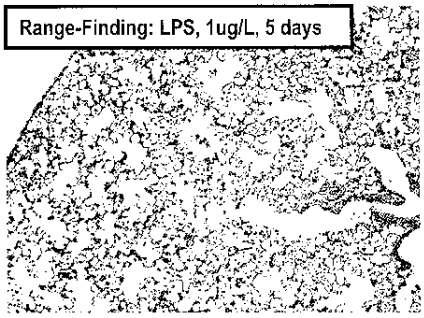
- Co-exposure of endotoxin such as lipopolysaccharide (LPS) with Cigarette Smoke (CS) augments the degree of pulmonary inflammation
- 3-Wk expsoures to develop an acute animal model that mimics the viral or bacterial insult thought to be important in COPD development and exacerbation in smokers
- Lung and BAL fluid samples collected from CS, LPS, or CS/LPS and analyzed for possible separation among treatment and for potential COPD biomarker candidates
- Study Design
  - AKR/J mice; Sham, CS, LPS, and CS/LPS groups
  - Cigarettes smoke: 250 µg WTPM/L, 5 hr/day, 5 d/wk for 3 wks
  - LPS: ~0.5 µg/mouse,1 hr/day, 2d/wk, for 3 wks

* Lee et al. 2007; Inhal Tox 19: 23, 2007 [8]

LPS Exposure (0.5 ug/L for 1 hr; twice/wk, 3 wks)

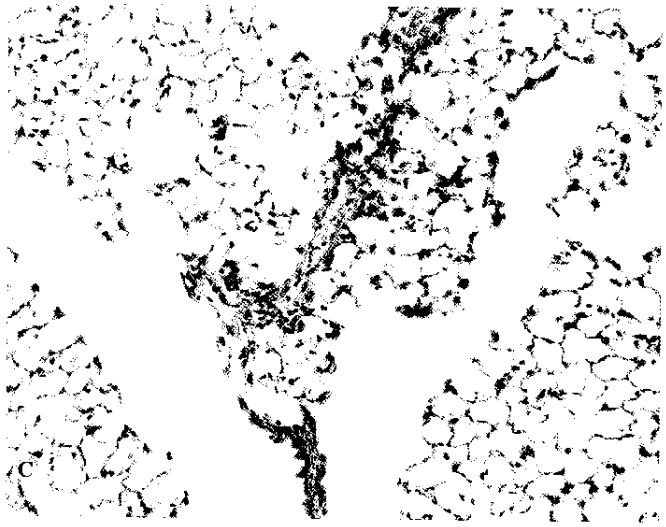
RF: Diffuse inflammation in alveoli and bronchioles, mostly neutrophils with some macrophages; Reduced response in 3-wk study

Range-Finding: LPS, 1ug/L, 5 days



Smoke Exposure (250 ug/L for 5 hr; 5day/wk, 3 wks)

Predominantly macrophage response is shown in alveolar ducts and alveoli



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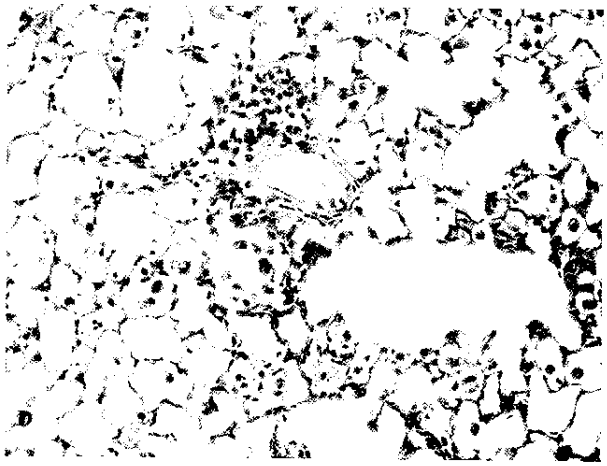
Smoke/LPS Exposure

Mixed inflammatory infiltrate composed of macrophages and neutrophils is shown in alveolar ducts and alveoli



Exposure Group	Apoptotic Cells (%)
Control	1.7 ± 0.8
LPS	5.3 ± 2.3
Smoke	5.0 ± 2.4
Smoke / LPS	17.2 ± 2.2

Period	Control[a]	LPS	Smoke	Smoke/LPS
Total Cell Count (/μL)	703 ± 229	3982 ± 1043*	1443 ± 371*	3514 ± 1173*
PAMs (% Total)	696 ± 223 (99)	1671 ± 508* (44)	1008 ± 251 (70)	2903 ± 1094* (78)
PMNs (% Total)	7.0 ± 11.3 (1)	2311 ± 823* (56)	429 ± 238* (29)	607 ± 223* (22)

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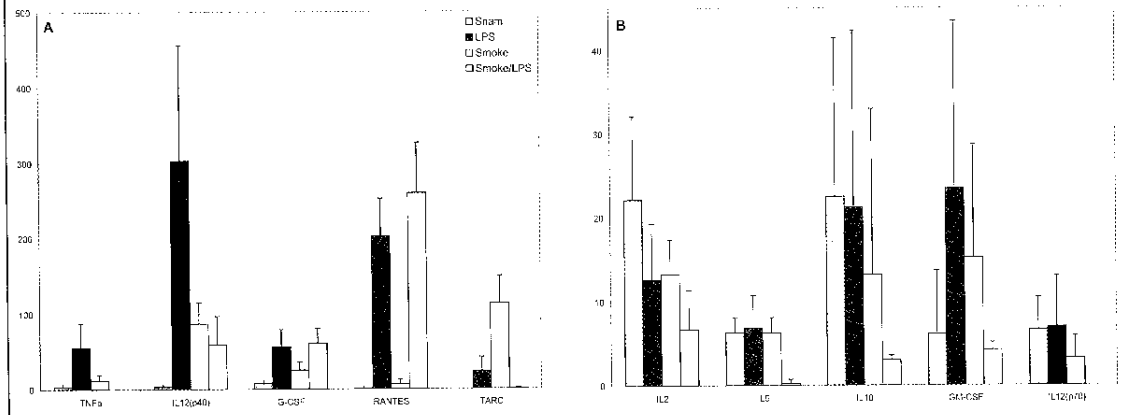
BAL Fluid Cytokines at Wk 3

A: >Sham Control

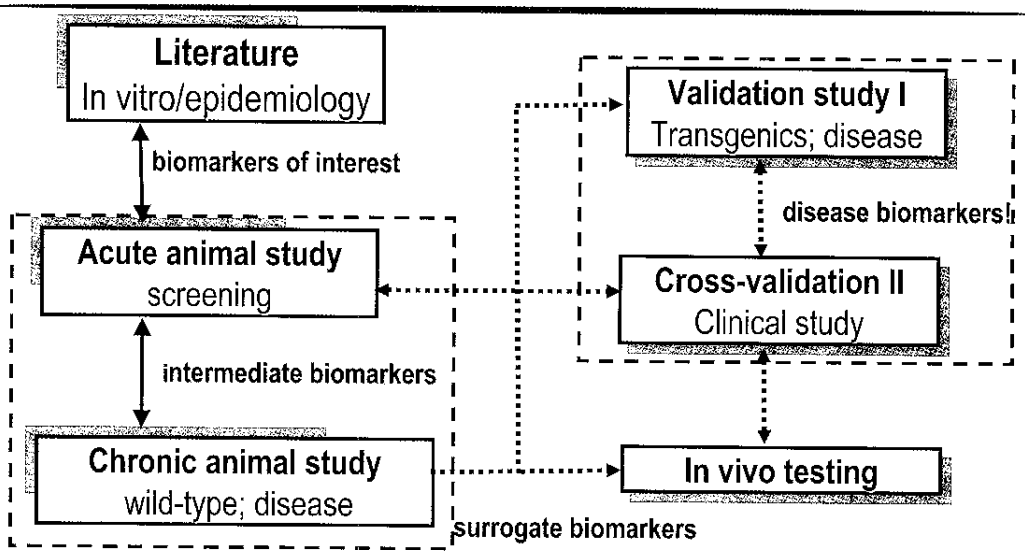
B: <Sham Control



Smoke/LPS Model: Summary

- LPS aerosol was generated, monitored, and exposed to mice via nose-only inhalation in a respirable size; Exposure regimen did not cause acute toxicity
- Histologically augmented inflammatory responses for the CS/LPS group compared to LPS or CS alone; lung apoptosis also demonstrated a similar difference
- Lung transcriptomics & proteomics identified substantial quantity of genes and proteins unique or common among different groups; The Smoke/LPS treatment displays several hallmarks of COPD, such as high levels of response to reactive oxygen species, high proteolytic activity, and dysregulation of calcium homeostasis.
- *CS appears to suppress the innate immune response to LPS treatment through decreased cytokine production by macrophages, despite increased cell numbers in the BAL fluid*

Overall Scheme on Toxicity Testing



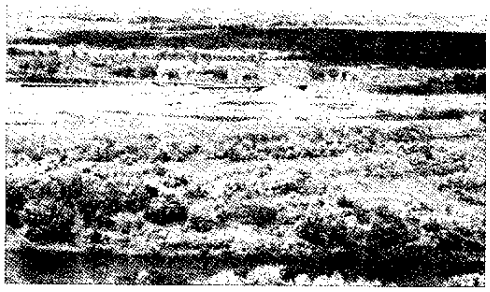
*** ITERATIVE***

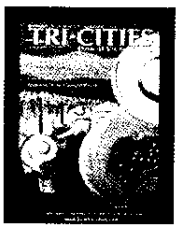
Maximize the range and the type of relevant biological responses to increase sensitivity & the discriminatory power to detect potential differences

Acknowledgements

Battelle

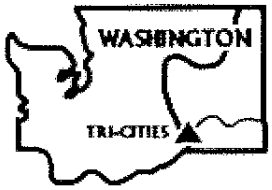
Bruce Westerberg
Ryan Meng
Roger Renne
Sam Harbo
Herb Bresler
Mark Gritz
John LaFemina





Japan Tobacco Inc.

Philip Morris U.S.A.



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KOSTAS 2007 5 OCTOBER 2007

Considerations for the Conduct of Tobacco Clinical Biomarker Studies

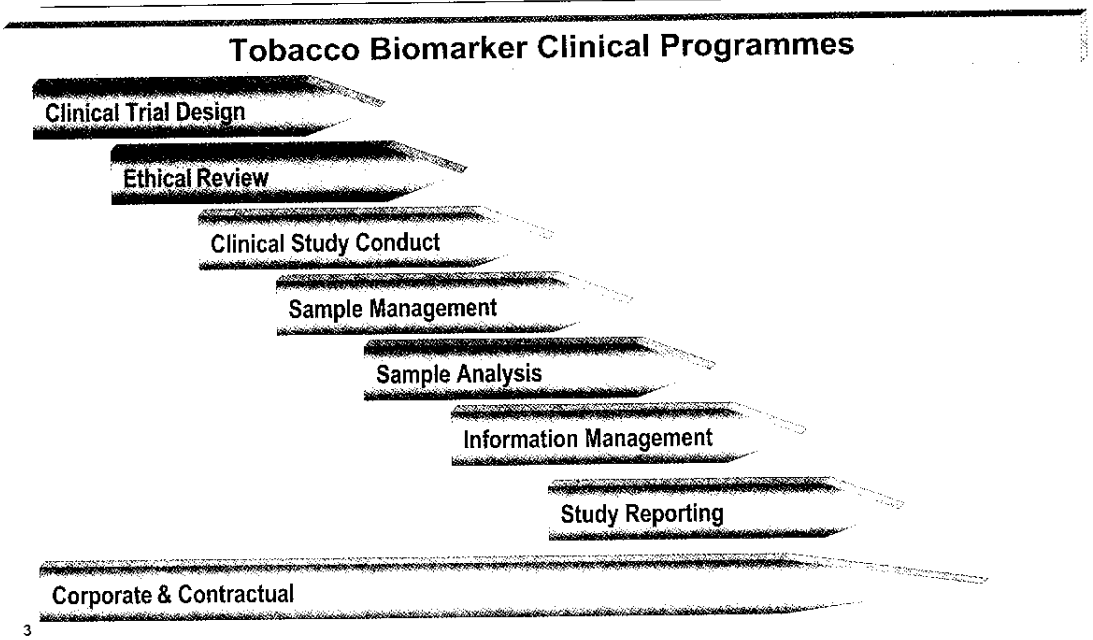
Iain W Shaw
Covance Laboratories, UK

Objectives

- Review Covance experiences to date
  - Reflect differences with pharmaceutical studies
- Highlight important factors influencing
  - Study Design
  - Set up & Initiation
  - Conduct & Performance
- Elaborate on key points for consideration by
  - Contract Research Organisations
  - Tobacco Companies

2

Overview



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Overview

	Pharmaceutical	Tobacco
Study Design	Established clinical designs	Variable study designs
Volunteers	Healthy male volunteers	Healthy male and female volunteer smokers
Entry Requirements	Preclinical Toxicology Testing	None
Regulations	National or International Approval	None
Ethical Review	Established review process	Unfamiliar review process

4

Study Design

- Considerations
  - Impacted by longevity of biomarker half-life
  - Realistic
    - smoker compliance
    - smoker availability
    - brand availability
    - study costs
  - R&D strategy of tobacco company
  - Relevant questions to determine priorities

5

Study Design

- What is the purpose of the study?
  - What data is required?
  - What population is required?
  - Is there sufficient power?
  - Are the assays sufficiently sensitive?
  - Are the assays suitably robust?
  - Will you demonstrate a difference?
- Ultimately tobacco company dependent
- Quality Standards

  Good Clinical Practice

  Good Laboratory Practice

6

Study Design

- Non-residential
  - Normal behaviour
  - Less restrictions
  - Usual smoking pattern
  - Urine collections
  - Compliance
  - Brand adherence
  - Control of test product use

  LESS CONTROL

- Residential
  - Artificial behaviour
  - More restrictions
  - Altered smoking pattern
  - Urine collections
  - Limited duration
  - Limited facilities for smokers
  - Smoking bans

  MORE CONTROL

7

Study Design

- Non-residential - Example 1:
  - Select new product
  - 7 wk study
  - Period 1 (1 wk benchmark), Period 2 (1 wk test A), Period 3 (1 wk test B), Period 4 (1 wk test C)
  - Visits @ start & end each Period
  - Washout between each Period

8

Study Design

- Non-residential - Example 2:
  - Test new product
  - 15 wk study
  - Period 1 (3 wk prelim) then Period 2 (6 wk test) then Period 3 (6 wk benchmark)
  - Visits @ wk 1, 3, 9, 15
  - No washout

9

Study Set-Up

- Research Ethics Committees (REC)
  - Does the protocol make clear the objective of the study?
    - Can the intended objective be supported with background information
  - Tailor the associated documents to address concerns
    - Quitting strategies, clarity on use & storage of samples
    - Research NOT marketing, no inducement to smoke, etc
  - Engage in dialogue wherever possible
    - Know the REC Chairman & Secretary
    - Understand their viewpoint

10

Study Set-Up

- ETHICS CASE HISTORY 1
- Exclude anyone from households with pregnant women and young children.
- Public health authorities have concluded that smoking seriously harms you and others around you, including children and unborn babies. Therefore, you should avoid smoking in the presence of children and pregnant women.
- Subjects who smoke in households containing pregnant women or children.
- I agree not to smoke in the presence of children or pregnant women.

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Study Set-Up

- ETHICS CASE HISTORY 2
- Improve accountability by collecting cigarette butts daily throughout the study
- Daily butt collection is impractical and increases the chance of non-compliance
- Proposed alternative strategy
  - Retain butt collection and analysis at intervals as detailed in protocol
  - Introduce weekly saliva analysis of cotinine to quantify cigarette consumption - a suitable method of assessment of exposure
  - Correlate data from above tests to recorded diary card consumption and cigarette returns
- Committee approved approach of weekly saliva test/cigarette supply/unused cigarette return and diary information as appropriate and practical monitoring of actual consumption

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Study Conduct

- Biomarker studies fit with Phase I clinical units
  - PROS
    - Skill set of dedicated clinical staff
    - Ability to adhere to protocol requirements
    - Volunteer database
    - Regulatory compliant study performance
  - CONS
    - Fixed geographical location (brand location)
    - Need to qualify facilities as suitable
    - Need to recognise differences
    - More costly than equivalent academic setting

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Study Conduct

- RECRUITMENT CASE HISTORY 1
- Targeting recruitment to mimic population demographics
  - Require set number of very low tar cigarette smokers
- Very low interest levels
  - Likely individuals not attracted by study

- Resolution:

  Open second clinical site & target advertising

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Study Conduct

- RECRUITMENT CASE HISTORY 2
- Very targeted study
  - Single specific brand, low market share
  - Cigarette being withdrawn from market in fixed timescale
- Impossible recruitment challenge
- Slipping deadlines

- Resolution:

15 Reduced required number of volunteers

Study Conduct

- Sample collection, handling & storage
  - Capacity of laboratory
  - Collection/ Processing/ Labeling
  - Storage/ Shipment
  - Retention
  - Stability for future investigations

  - Samples as 'Cell Bank'

16

Study Conduct

- SAMPLE CASE HISTORY 1
- Study in Continental Europe
  - Target specific smoker population
  - Laboratory struggled to handle volume of samples
  - Poor labeling and tracking
  - Confusion at analytical laboratories
  - Dissatisfaction at CRO and client

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Study Conduct

- SAMPLE CASE HISTORY 2
- Study in UK
  - Large recruitment target >1000 volunteers
  - Existing laboratory too small
  - Created additional laboratory space and dedicated staff
  - Staff focused on tobacco study
  - Other studies not impacted
  - Optimal shipments to analytical laboratories

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Study Conduct

- Data control
  - Collating data for reporting
  - Understanding management of database
  - Consistency of data capture (within & between studies)
- Data ownership
  - Client 'owns' data generated by CRO
  - Publication (or not) is client's decision

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Other Factors

- Corporate & Contractual
- Inform & involve the legal department
  - This may be new territory
  - Adds time to the process
  - Requires education
    - *Insurance and indemnification of volunteers*

20

Conclusions

- Tobacco biomarker trials are not straightforward
  - For CRO or for Tobacco Company
- Only as good as the weakest component
  - Ensure clarity of purpose & intent
  - Qualify all the parties involved
  - Balance efficiencies with thoroughness of preparation

21

Conclusions

- Key procedural differences with drug trials
  - Ensure CRO recognises differences
  - CRO committed to learning tobacco study requirements
  - Require flexibility and understanding on both sides
  - Partnership
- Designs evolve
  - As greater knowledge of biomarkers is gained
  - As different R&D strategies evolve in tobacco industry
  - Potential regulation will increase need for GCP/GLP adherence

22

Thank You For Your Attention

- Thanks to my Covance colleagues and to our various industry partners

- Questions ?

- Contact: iain.shaw@covance.com

23

WHO Framework Convention on Tobacco Control (FCTC)

Elaboration of guidelines on tobacco product regulation

Nataliya Prongue & Michael Meger

International Symposium of the Korean Society of Tobacco Science (KOSTAS), Daejeon, South Korea, October 5th 2007

1

WHO FCTC

facts – contents – process – implications

Conference of Parties (COP)

COP1 & 2 and anticipation for COP3

Guidelines on Product Regulation

COP2 discussion on FCTC Art 9 & 10

Summary

2

WHO FCTC

- is the first international treaty on public health
- has been adopted by WHO Member States in May 2003
- has been ratified by 149 Parties by end of August 2007

3

WHO FCTC

- Binding provisions, specific (time-bound measure)
  - Advertising, promotion & sponsorship – 5 years
  - Packaging, labeling (health warning) – 3 years
- Binding provisions, non-specific (but "effective measure")
  - Protection from exposure
  - Regulation on contents
  - Packaging, labeling (descriptor)
- Non-binding provisions (but "appropriate measures")
  - Price, tax policy
  - Duty free restriction, ban
  - Pictorial health warning

4

Conference of the Parties

- Parties
  - Participation with decision making right
- WHO & subdivisions (TFI, TobReg, TobLabNet)
  - Agenda
  - Technical expertise
  - Convention secretariat management
- Observers (Non-parties, NGOs)
  - Participation without decision making right

- Global frame and timeline
- Reference document
- Implementation monitoring body (COP)
- Protocol and guideline development (COP)
- "States can move further and faster"

COP 1 (6-17.02.06)	COP 2 (30.06-6.07.07)	COP 3 (10.2008)
Established working groups on Gdls to Art 8 "Protection from exposure to tobacco smoke"	Adopted Gdls to Art 8	
Gdls to Art 9 & 10 concerning tobacco product regulation	Lower priority	Will consider a progress report
Protocol to Art 15 "Illicit trade in tobacco products"	Established an *INB** to negotiate the text of the Protocol	Will consider a progress report
Protocol to Art 13.8 concerning cross-border advertising	Postponed the development of the Protocol Launched the development of Gdls to Art. 13 "Tobacco advertising, promotion and sponsorship"	Will consider draft Gdls and probably adopt
Established a Study group on alternative crops, pursuant to Art 17	Confirmed the mandate of the Study group	Will consider a progress report

*INB – Intergovernmental negotiating body

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COP 1 (6.02.-17.02.06)	COP 2 (30.06-6.07.07)	COP 3 (10.2008)
	Established working groups on Gdls to Art 11 "Packaging and labeling of tobacco products"	Will consider draft Gdls and probably adopt
	Gdls to Art 12 "Education, communication, training and public awareness"	Will consider a progress report
	Gdls to Art 14 "Demand reduction measures concerning tobacco dependence and cessation"	Will consider a progress report
	Gdls to Art 5.3 regarding protection of public health policies with respect to tobacco control from commercial and other vested interests of the tobacco industry	Will consider a progress report or draft Gdls and probably adopt

8

- COP confirmed the mandate of the WG on GdIs to Art 9 and 10, but gave lower priority to its work, due to complex technical issues, budget constraints and the need for further research
- COP extended the mandate of the WG to Art 10 (vs. initially commissioned work focusing on Art 9)
- Analytical chemistry – overall Parties agree with approach, but too early to deal with this now, more research needed
- Design features – overall Parties agree with approach, but too early at present stage to go into details

9

Options wrt standardization (WHO TFI)

- WHO as standardization body using TobLabNet
  - Independent voice
  - Embedded in FCTC COP structure
  - However, requires costs and resources
- Continuation of cooperation with ISO
  - Problem with governing structure
  - ISO TC 126 dominated by tobacco industry
  - Very inflexible to work with
- "Hybrid Option"
  - ISO TC 126 should be asked "to evolve"
  - COP should set policy and guide ISO
  - ISO TC 126 will "purely collaborate on measurement and testing"
  - ISO TC 126 should follow COP decisions

10

Countries' positions on options wrt standardization

- WHO as standardization body using TobLabNet
  Chili, India
- Continuation of cooperation with ISO
  China, Japan
- "Hybrid Option"
  Canada, European Community, Thailand

11

- Low priority to GdIs on product regulation due to cost and complex technical issues
- At this stage there are more questions than definitive answers
- ISO's further involvement is not clear at this stage
- The "hybrid option" might offer a probability

12

- Guidelines on analytical chemistry can be an important starting point for product assessment
- Guidelines and standards must be based on science and robust methodology
- ISO/COP members' national standardization bodies could provide expertise and competence in the validation process
- Public consultation would ensure transparency during guideline development
- Further research will be needed to understand biological mechanisms and to develop concepts (e.g. harm reduction)

13

Attempt to trap nitrosamines by zeolites

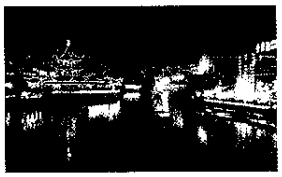
Dr. Jian Hua ZHU
Chemistry Department of Nanjing University
Nanjing, China
5 October 2007

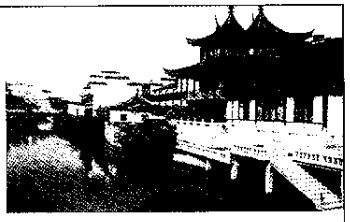
1

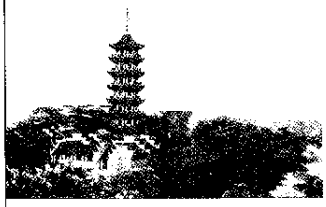
Content

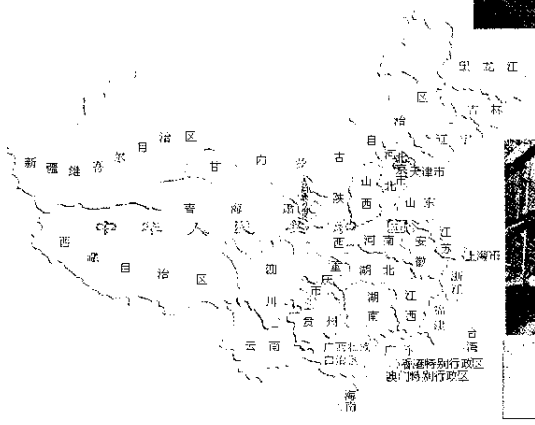
- **Briefly introduction of Nanjing and Nanjing University.**
- **Background of our research**
- **Structure of zeolites and nitrosamines**
- **Adsorption of volatile nitrosamines by zeolite**
- **Modification of zeolite to promote its capability of trapping nitrosamines**
- **Catalytic degradation of nitrosamines by zeolites**
- **Fiber-like porous materials**
- **Conclusion**

2

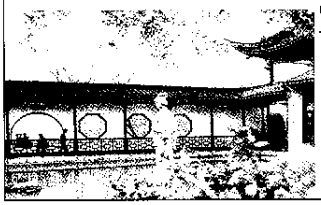


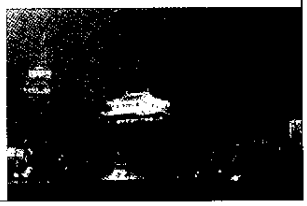






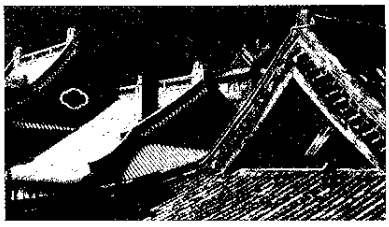
Nanjing is a beautiful city located in the eastern of China, 300 Km from Shanghai. This city has 3 million population.





Nanjing University has 17 schools with 50 departments and runs 74 undergraduate programs, 186 master's programs and 122 Ph.D. programs.

Among the nearly 2000 teaching faculty, there are 654 full professors. The university has over 41600 students, including 9964 Ph.D. and master students.



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What has motivated us to conduct this work?

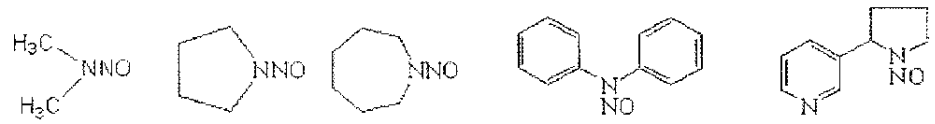
- **Smoking is popular in China. There are about 300 million people smoking in mainland, and China produced about 1.7 trillion cigarettes in 1996.**
- **However, the level of toxic compounds such as nitrosamines and CO in cigarette smoke should be reduced, in order to protect environment and public health.**
- **Our group started the research to trap nitrosamines by zeolites in 1996, with the support of Chinese Science Committee.**

5

Three volatile nitrosamines (VNA), *N*-nitrosodimethylamine (NDMA), *N*-nitrosopyrrolidine (NPYR), *N*-nitrosohexamethyleneimine (NHMI), one tobacco specific nitrosamines (TSNA), *N'*-nitrosonornicotine (NNN) and one non-volatile nitrosamine, *N*-nitrosodiphenlamine (NDPA) were utilized in our research.

Their molecular dimensions are different: NDMA~ 0.45 nm, NPYR ~ 0.56 nm and NHMI ~ 0.59 nm, NNN ~ 0.75×0.80 nm, NDPA ~ 0.61×0.95 nm.

All of the nitrosamines have the functional group of N-NO. *With this group, nitrosamines can cause cancer and many other disease .*

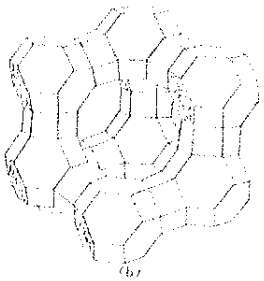


NDMA	NPYR	NMHI	NDPA	NNN
0.45nm	0.56nm	0.59 nm	0.61×0.95 nm	0.75×0.80 nm

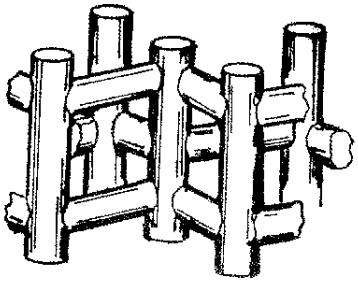
6

- ***Zeolites have uniform pore and channel so that they are able to recognize, discriminate, and organize molecules with precision that can be less than 0.1 nm. Due to their unique selective adsorption and catalysis function, zeolites play the important role in chemical and petrochemical industry as catalyst, adsorbent, and ion exchanger.***

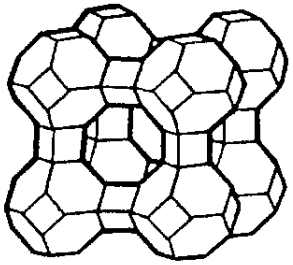
Three types of common zeolites with different pore size are used in our study to trap and catalytic decompose nitrosamines.



NaY
0.74 nm



NaZSM-5
0.54×0.56 nm



NaA
0.4nm

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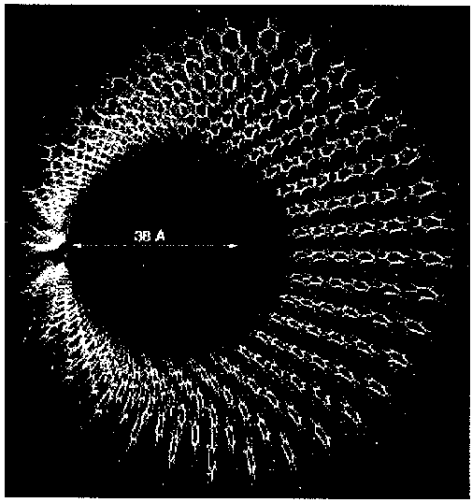
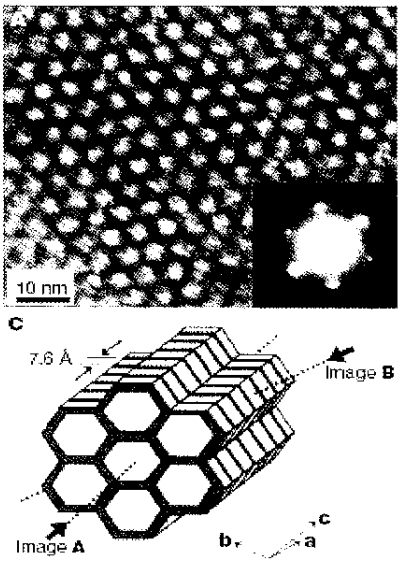
Another porous trapper employed in our research is mesoporous silica. These siliceous materials have larger pore size than zeolite, but lack of metal cations in their framework, so that they usually need to be modified.





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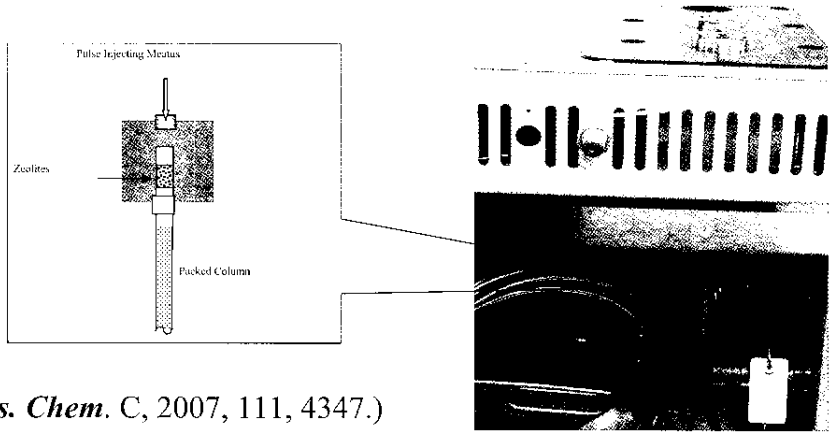
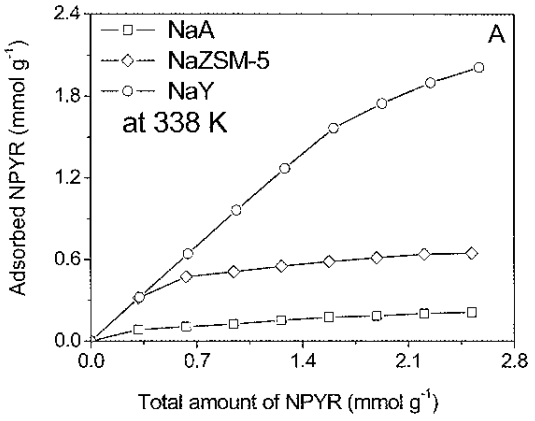
We use a new method to study the adsorption of volatile nitrosamines on zeolites. The nitrosamine solution was pulse injected and pushed by the carrier gas to pass through the zeolite bed and then to the packed column of the gas chromatography. The response at the column outlet was recorded by the GC with thermal conductivity detector, and the decrement in the ratio of solute to solvent represents the amount of nitrosamines adsorbed by zeolite.

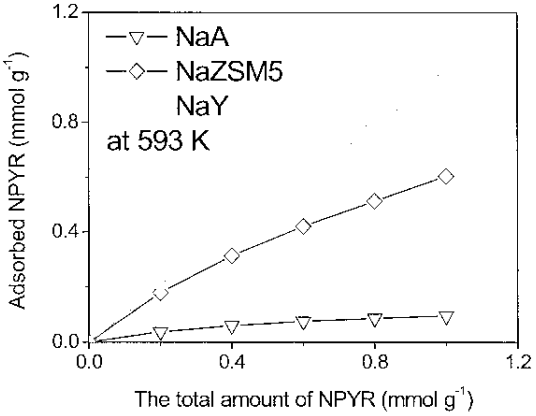


(*J. Phys. Chem.* C, 2007, 111, 4347.)

9

Among three basic zeolites, NaY shows the highest ability to trap NPYR either at 338 K or at 593 K because of its large pore size and pore volume. When 0.96 mmol/g of NPYR passed through the zeolite, all of them were trapped by NaY, whereas NaZSM-5 adsorbed about half and NaA captured one-eighth. Elevating the adsorption temperature to 593 K slightly increased the capacity of NaZSM-5, however it was still lower than that of NaY but higher than that of NaA.





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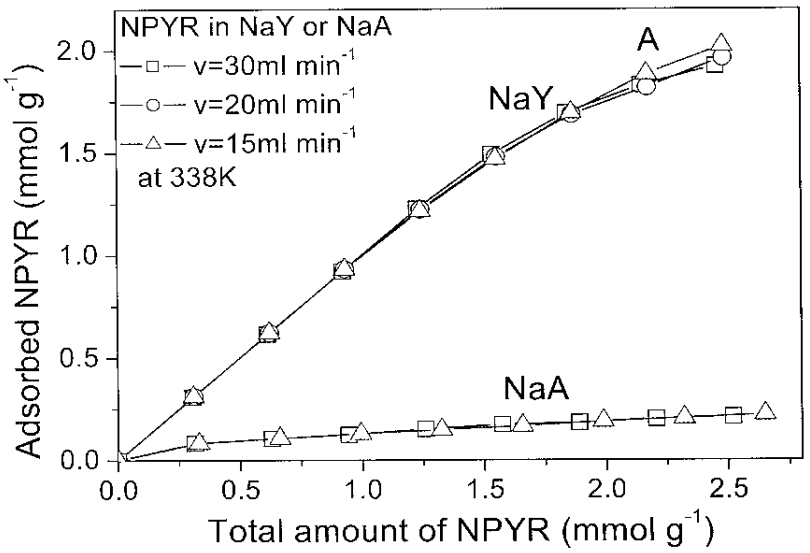
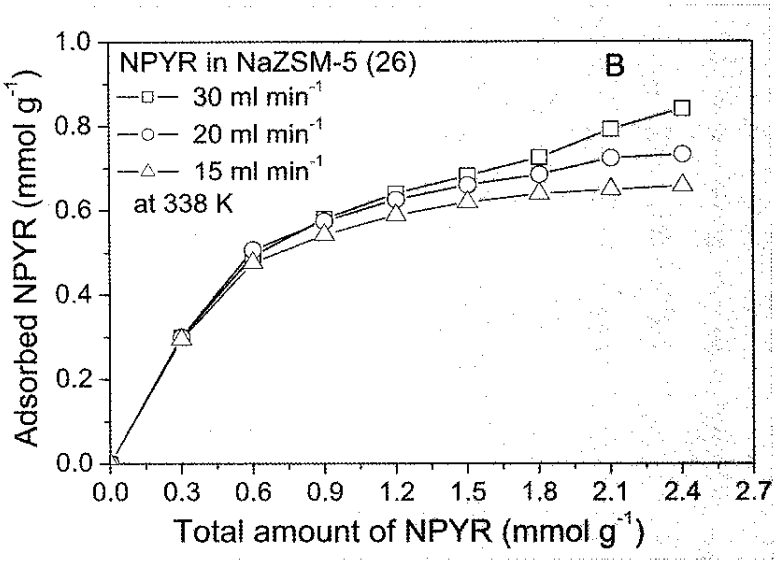


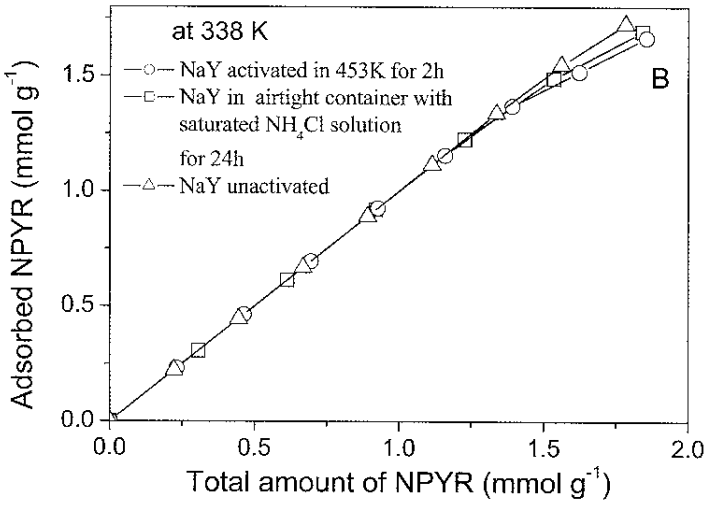
- **Contact time has a minor impact on the adsorption of NPYR in zeolite NaY and NaA at 338 K. When it changes from 0.08 sec to 0.04 sec, no significant influence is observed in the experiments.**

11



- **The higher the rate of the gas flow, the more the NPYR trapped by NaZSM-5 zeolite. The molecular size of NPYR is close to the pore diameter of NaZSM-5, so that it is difficult for NPYR to enter and diffuse in the narrow channel of NaZSM-5. Consequently, a higher kinetic energy possessed by the NPYR is beneficial for its adsorption in NaZSM-5 zeolite.**

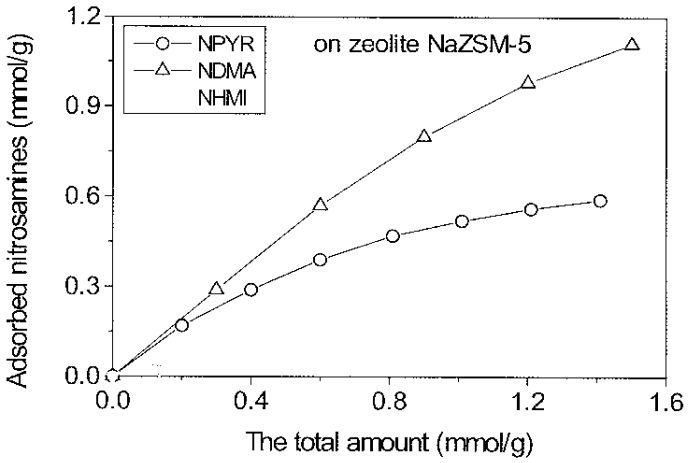
12



- **Activation is not necessary for zeolite to trap volatile nitrosamines in air-stream. NaY exhibited the similar adsorptive capacity for NPYR at 338 K no matter it was activated or not. Pre-adsorption of moisture did not hinder the adsorption of NPYR in NaY zeolite.**

13

Molecular shape-selective adsorption / catalysis arises from the presence of active sites within the intracrystalline free volume of zeolite. The geometric matching degree between the molecular conformation of nitrosamines and the pore structure of zeolite governs the adsorption of volatile nitrosamines in NaZSM-5. The larger the molecular size of nitrosamine, the slower its adsorption.

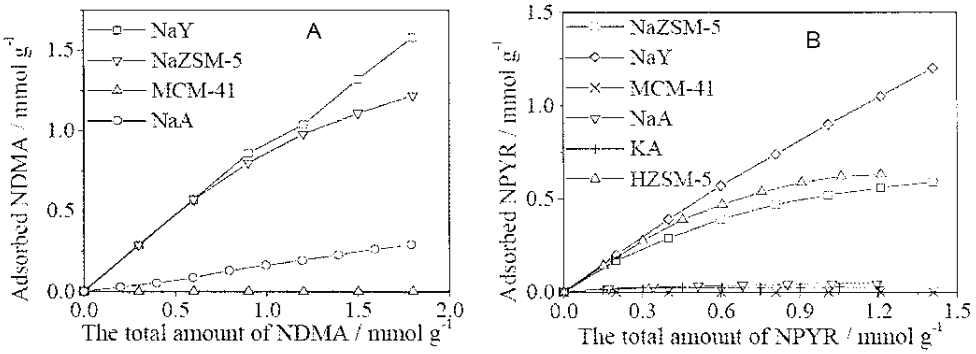


14

In general, two factors determine the adsorption of nitrosamines in zeolites. One is the geometric confinement provided by the pore structure of zeolite and another is the electrostatic field caused by the metal cations in zeolite.

Pore diameter of zeolite determines the instantaneous adsorption of volatile nitrosamines in air-stream, and the larger pore is beneficial for the enter and diffusion of nitrosamine molecules in the channel.

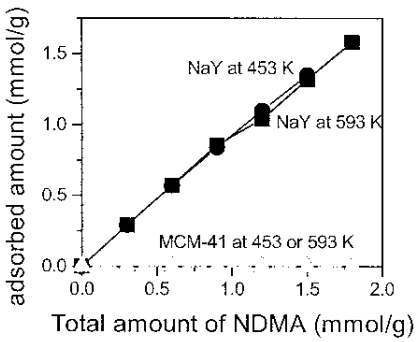
Surface acid-basicity of zeolite affects the adsorption of nitrosamines. The acidic HZSM-5 adsorbed more NPYR than the basic NaZSM-5 under the same conditions.



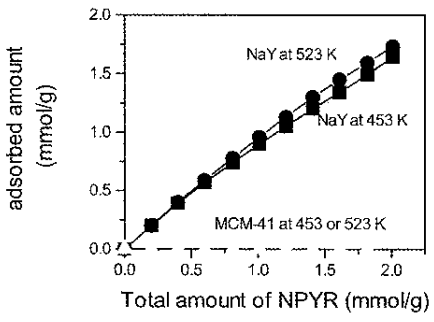
Adsorption of (left) NDMA and (right) NPYR in zeolites and MCM-41 at 453 K.

15

Mesopore cannot impose the necessary geometric constraints to volatile nitrosamines along their adsorption, so that MCM-41 fails to trap NDMA or NPYR at the relative high temperature, because the trapped nitrosamines can quickly desorb from the porous material. Once the temperature is lowered to 338 K, MCM-41 shows a capability to trap NPYR, similar to that of NaY.

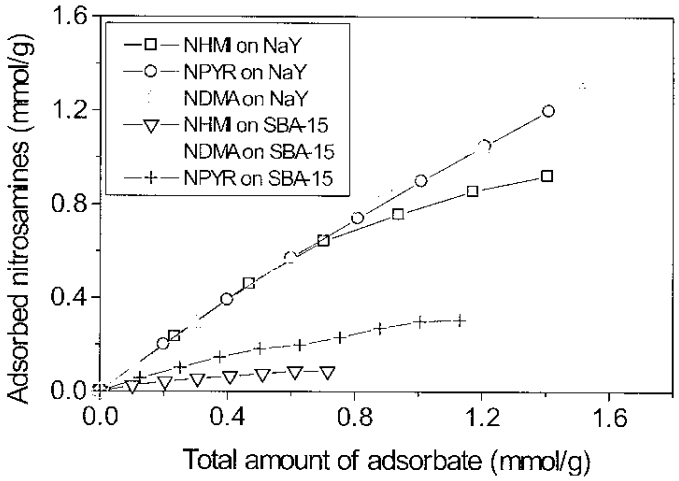


NDMA



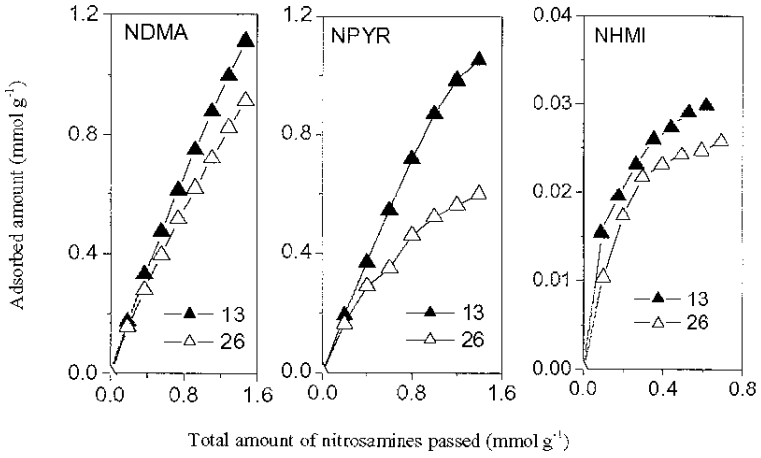
NPYR

16



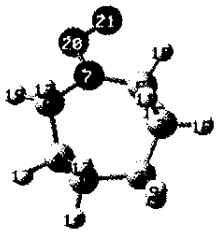
SBA-15 has a bigger pore size (6-8 nm) than MCM-41 (2-3 nm), but it can adsorb volatile nitrosamines at 453 K, because SBA-15 has some narrow micropore in its structure. Moreover, NHMI with the relative large molecular size is the most difficult one to be captures by the mesoporous silica SBA-15 among the three volatile adsorbates, which reflects the role played by the micro-pores in the adsorption.

17

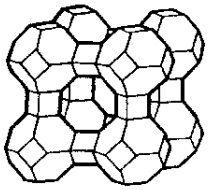


The Si/Al ratio affects the adsorption of volatile nitrosamines in zeolites. For NPYR or NDMA, the sample NaZSM-5 with lower Si/Al ratio always exhibits a higher adsorptive capacity than the one with a higher Si/Al ratio. The electrostatic force provided by the metal cation plays the important role in the adsorption, it pulls the nitrosamine molecules towards and further into the channel of zeolite.

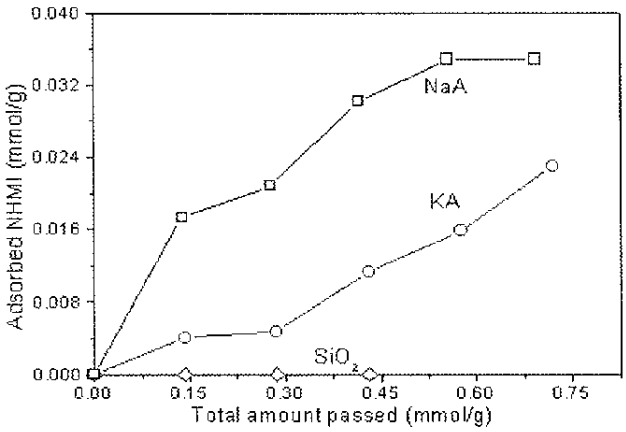
18



The structure of NHMI



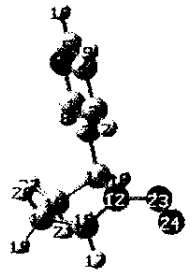
Pore structure of A zeolite



Adsorption of NHMI in gaseous phase by zeolite NaA and KA at 453 K

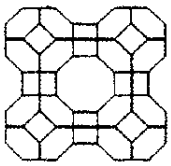
A specific adsorption behavior is observed in the case of NHMI, the nitrosamines with a big size of 0.59 nm; both NaA and KA with the pore size less than 0.4 nm can adsorb NHMI at 453 K, however the amorphous silica cannot trap it.

19

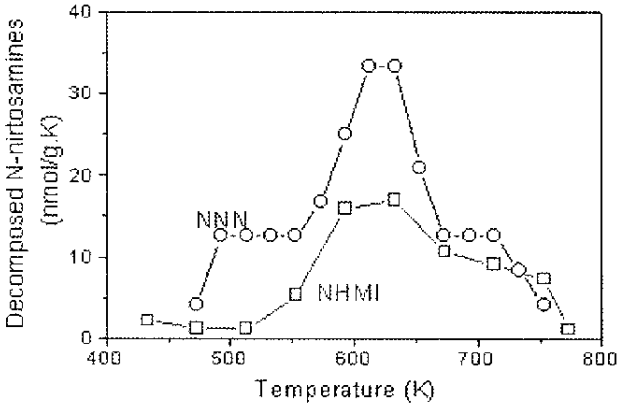


The structure of NNN

8-ring



The pore structure of KA

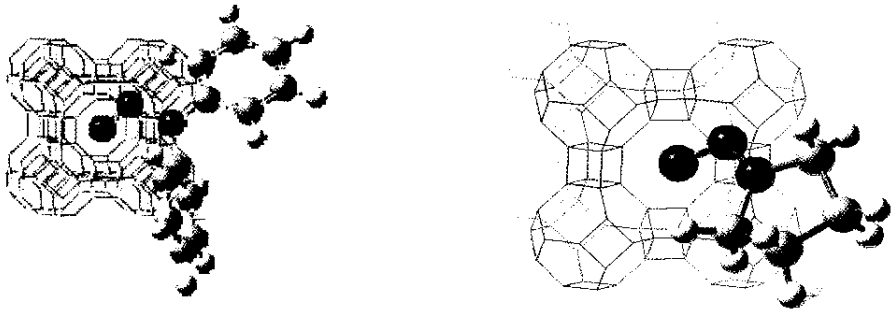


The temperature programmed surface reaction of NNN and NHMI on zeolite KA.

NNN, the TSNA with a molecular size (0.75×0.80 nm) much larger than the pore diameter of zeolite KA (0.3 nm), is still adsorbed by the zeolite and catalytic degraded as the temperature increased. Another larger nitrosamine molecule, NDPA, is also trapped and degraded by KA zeolite in the same procedure of temperature programmed surface reaction.

20

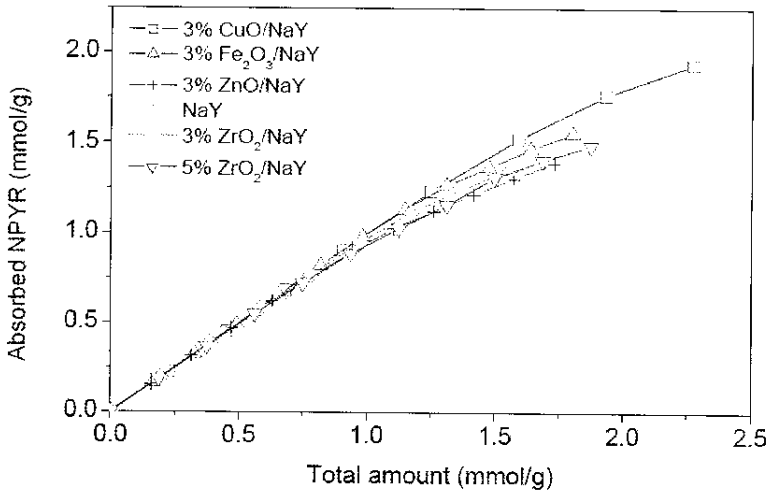
Why the bulky nitrosamines can be adsorbed by the zeolite with small pore size?



It is impossible for the whole NDPA molecule to enter the channel of KA or NaA, because the limitation of narrow pore. However, the *N*-nitroso group of nitrosamines has negative charge, so that it will be attracted by the electrostatic interaction from the cations in zeolite, consequently, the nitrosamine molecule inserts in the channel of zeolite with N-N(O) group.

So, it is feasible to increase the adsorption of zeolites by coating metal oxides inside the channel of zeolites.

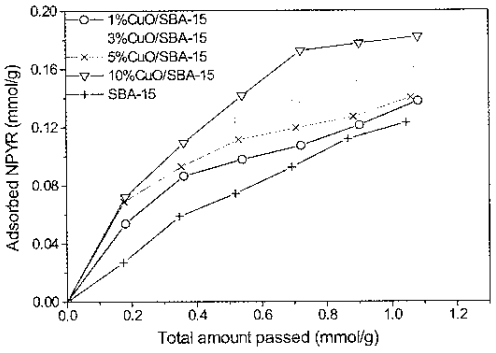
21

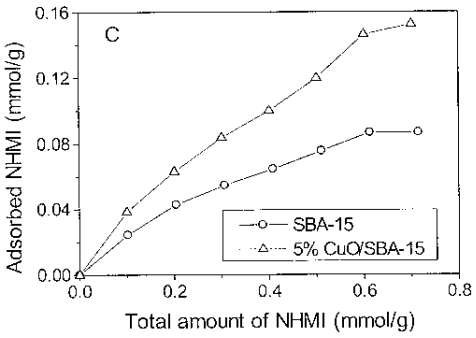


Loading metal oxide such as copper oxide, ferric oxide, zinc oxide or zirconia on zeolite NaY, can provide the strengthened interaction with the N-NO group of nitrosamines, elevating the capability of the zeolite for trapping NPYR at 453 K. Among these metal oxides, copper oxide is the best modifier for zeolite adsorbents.

22

Significantly enhancing the capability of mesoporous silica to trap volatile nitrosamines by modification of copper oxide





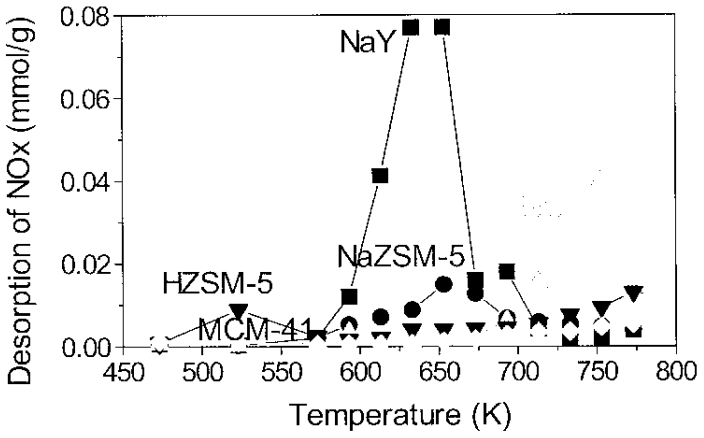
Coating copper oxide on the surface of SBA-15 can provide a lot of adsorptive sites in the mesoporous composite.

Adv. Funct. Mater. 2004, 14 (11): 1113-1123.

23

Nitrosamines can be catalytic degraded in zeolites and the first step is the broken of N-NO bond.

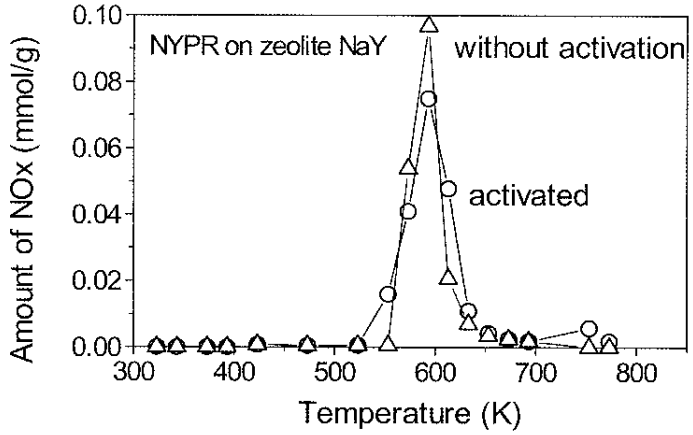
NDMA is the smallest nitrosamine with the highest volatility, but it can be catalytic decomposed by zeolites to form NOx products in the process of temperature programmed surface reaction.



Temperature programmed surface reaction (TPSR) of NDMA on various zeolites

24

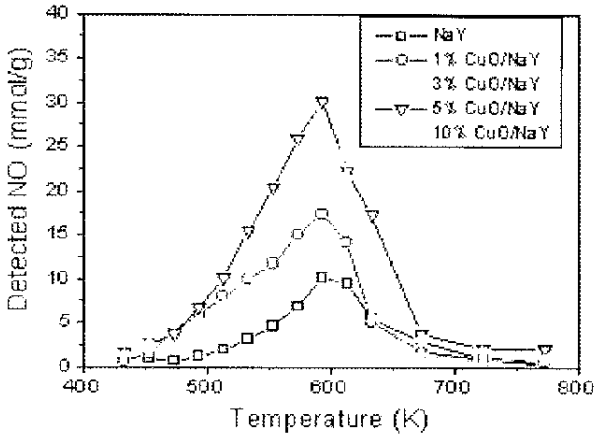
Zeolite NaY can be used to degrade NPYR without activation, so that the zeolite additive in cigarette can act as the adsorbent and the catalyst for reducing the level of nitrosamines in smoke.



Impact of activation at 773 K on the temperature programmed surface reaction (TPSR) of NPYR on zeolite NaY.

25

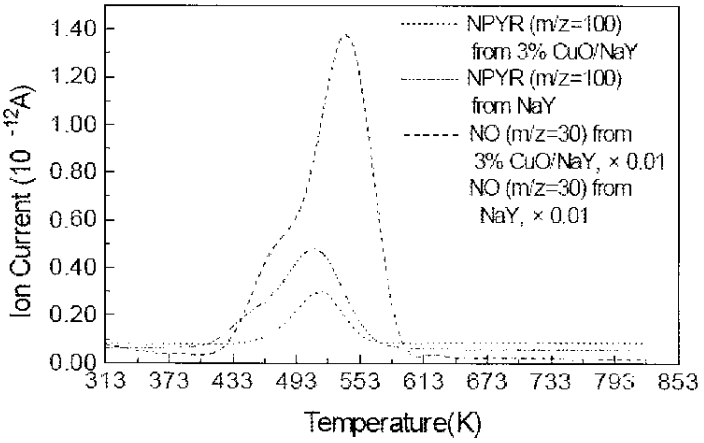
Loading copper oxide on zeolite NaY increases its catalytic activity, and more NPYR could be adsorbed and then to be decomposed to release nitrogen oxide products.



The profile of NO released during the temperature programmed surface reaction of NPYR in the zeolite NaY loaded with different amount of copper oxide.

26

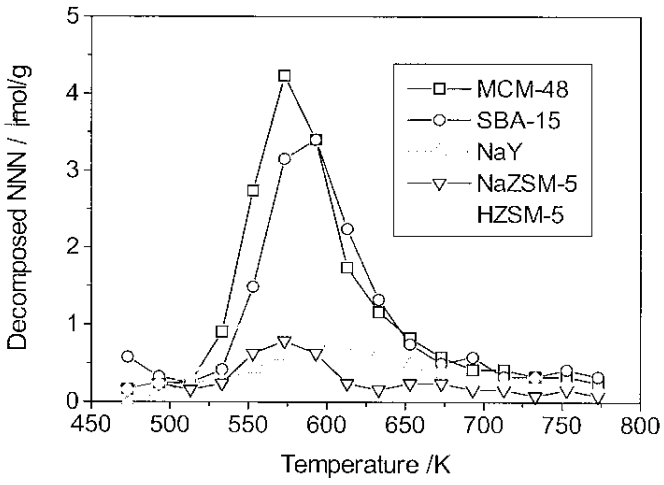
In the TPSR test of NPYR on NaY, the desorbed nitrosamines was about 1.7% in comparison with those degraded to NO. however, this proportion was significantly reduced to one third on the sample loaded with 3 wt.-% of copper oxide.



TG-MS spectrum of NPYR and NO desorbed from NaY and 3%CuO/NaY samples that adsorbed NPYR and was heated in TPSR process.

27

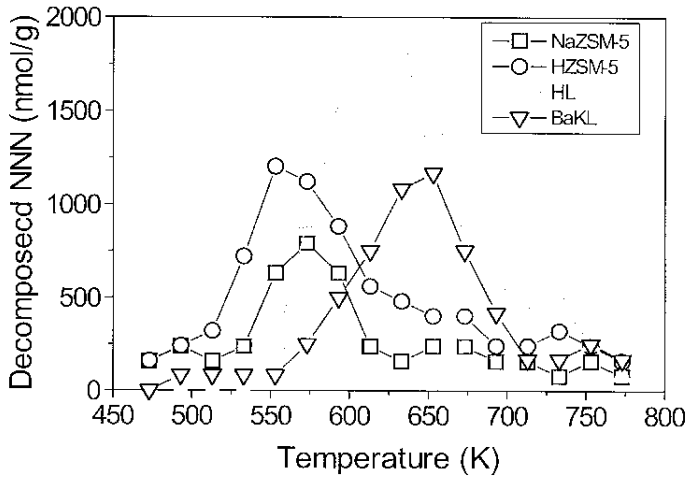
For decomposition of *N*'-nitrosonornicotine, mesoporous silica such as SBA-15 and MCM-48 exhibited much higher catalytic activity than zeolites, because of their larger pore sizes that allow the nitrosamine molecules to enter and contact with catalytic sites much easily.



Profiles of NOx liberated in the TPSR process of NNN

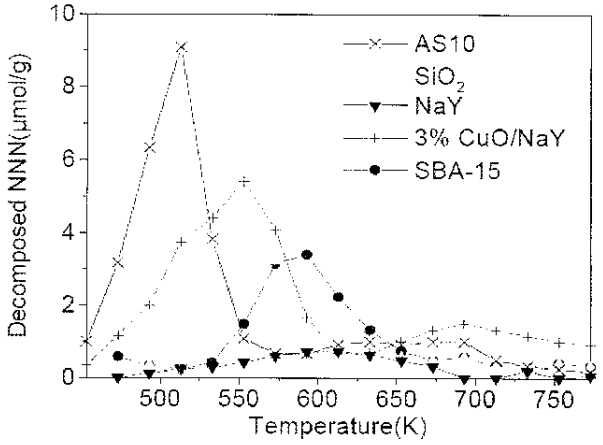
28

Surface acid-basicity of zeolite affects the degradation of NNN. Compared with the basic analogues, acidic zeolites adsorb more nitrosamines and decompose them in relative lower temperature.



Temperature programmed surface reaction (TPSR) of NNN on zeolites ZSM-5 and L.

Coating alumina on amorphous silica enables the composites to show the higher activity than mesoporous SBA-15 and the copper-modified zeolite NaY for decompose *N*'-nitrosonornicotine.



Profile of NOx released in the process of TPSR of NNN over porous samples

(***Environ. Sci. Tech.*** 2005, 39(18), 7254-7259)

we recently got a kind of porous material with fiber shape and white color.

NaZSM-5 zeolite

Crystal of GAS-1

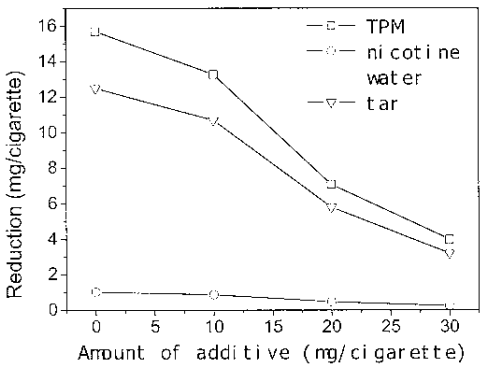
With long sticks of long plate-like crystals, the GAS-1 is not the white powder as usual zeolite or molecular sieves; rather, it looks like the short fibers, which enables it can be easily added into the filter rod. This sample has a 1-dimensional small pore system.

31

Added this material into the filter rod of Chinese Virginia cigarettes

The tar in the mainstream smoke is significantly reduced. For instance about half of tar is removed when the additive amount arrives 20 mg/cigarette, while only one third of the tar remained in the smoke as the added amount reached to 30 mg/cigarette.

This new porous material shows a higher efficiency than zeolite X or Y in reducing the tar content in cigarette smoke.



Effect of the fiber-like material in the filter of cigarette on the mainstream

32

Decrease of TSNA and B[a]P in mainstream smoke of cigarette by addition of CAS-1 in filter

	NNN	NAT+NAB	NNK	B[a]P
Control (ng/cig)	73	142	14	14.4
with 15 mg CAS-1 in filter (ng/cig)	55	110	12	7.9
Decrease (%)	24.7	22.5	14.3	54.9

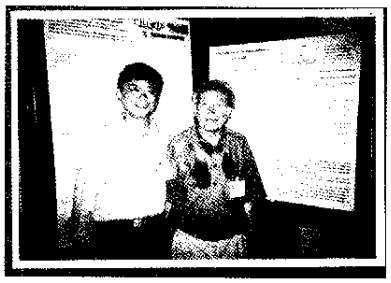
- **This fiber-like material could considerably reduce the level of TSNA and B[a]P owing to the special crystal morphology.**
- **These results implies the possibility that controlling the morphology of zeolite-like porous materials can adjust their efficiency of adsorbing tar and nicotine along with TSNA in the mainstream smoke of cigarette.**

33

Conclusion

- **Zeolite has a special interaction with the N-NO group of nitrosamines, it can capture the volatile nitrosamines in air-stream, even the contact time is less than 0.1 second.**
- **Pore structure and the metal cation of zeolite determine the efficiency of adsorbing nitrosamines. Y-type zeolite has the relative large pore size and pore volume, so that it is an excellent adsorbent to trap the nitrosamines in environment at ambient temperature.**
- **Acidic zeolites show a stronger ability to trap and to degrade nitrosamines than basic zeolites.**
- **Modification of zeolite and mesoporous silica with metal oxide such as copper oxide promotes both adsorption and decomposition of volatile nitrosamines, significantly lowering the nitrosamines level in smoke.**
- **Controlling the crystal shape of zeolite-like porous materials can adjust their efficiency of adsorbing tar and nicotine in the smoke of cigarette.**

34



Many thanks for the contribution of my group and the friends like Dr. Meier. We hope we can do more for you to reduce the risk associated with smoking.

35

KT&G

KOSTAS

October 5, 2007

SCHWEITZER-MAUDUIT

3833 /1

World Cigarette Design Study 2005

(WCDS 2005)

3831PC / 2-2505R

WCDS 2005
Contents of the presentation

- Concept and objective of WCDS 2005
- Type of analysis
- Countries analyzed in the study
- Presentation and discussion of the results

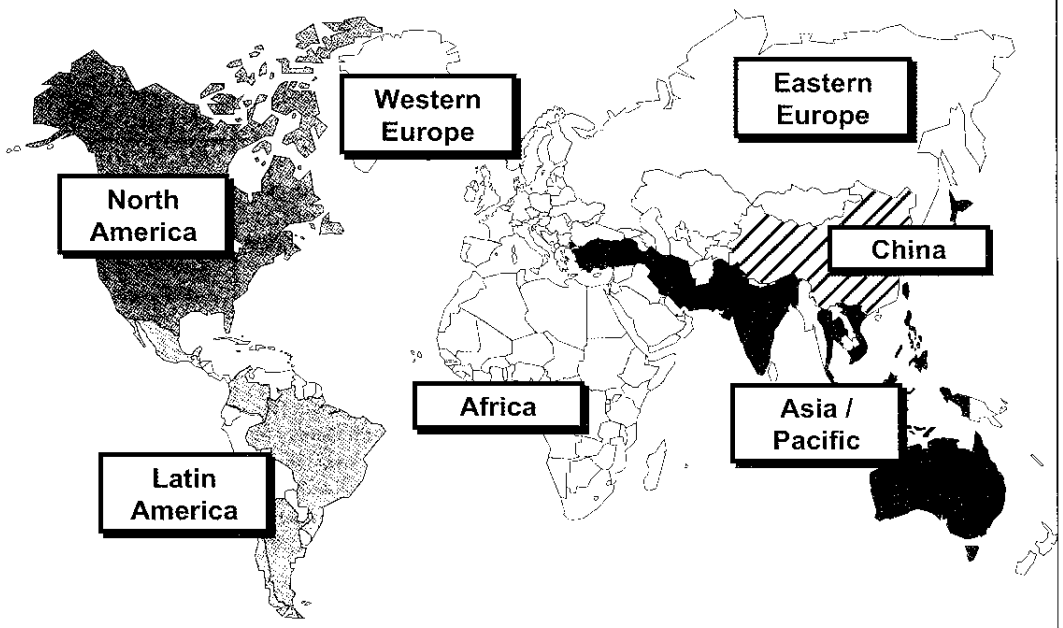
3831PC / 3-2505R

WCDS 2005
Concept and general comments

- Principle :
  - analysis of the highest sale cigarette brands per country
- All filtered cigarettes
- 868 brands
- 52 countries divided in 7 geographical areas

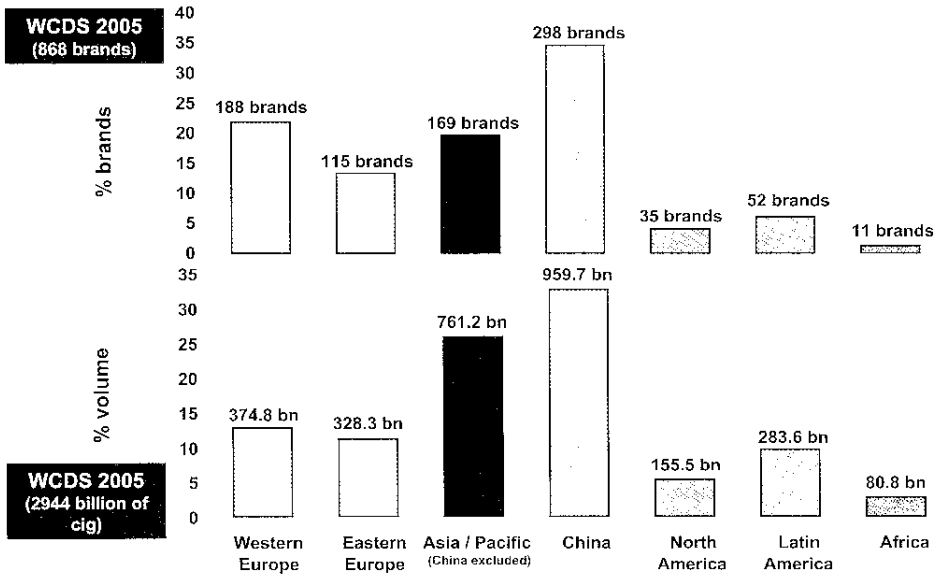
3831PC / 4-2505R

Countries selected for the study



3831PC / 5-2505R

Sample distribution in WCDS 2005
Total : 868 brands – 2944 bn



3831PC / 6-2505R

Worldwide

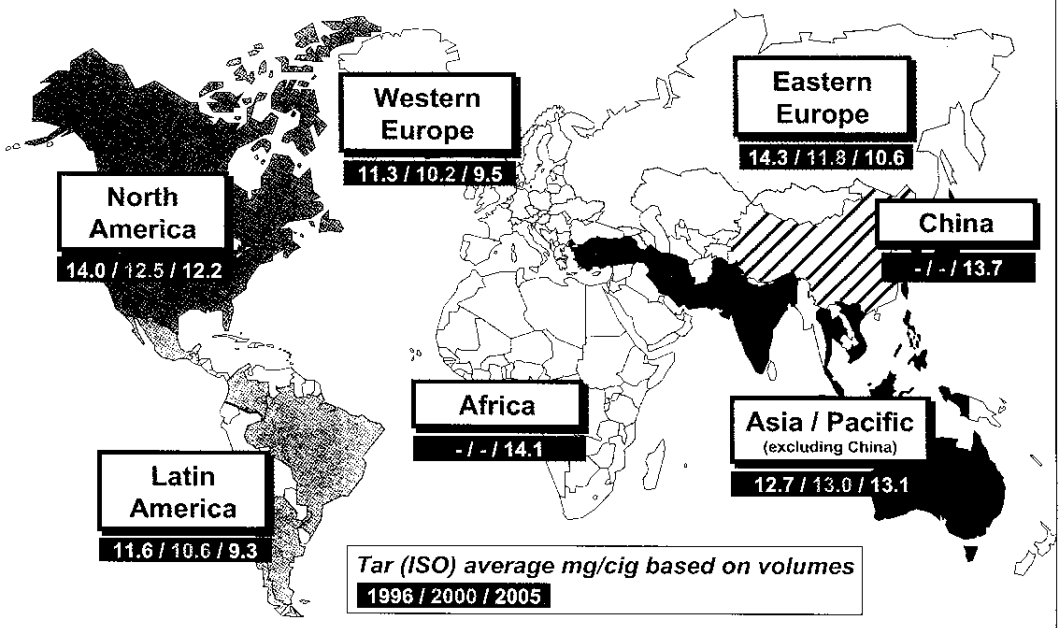
Number of filtered brands selected for WCDS 2005 : 868

	Western Europe	Eastern Europe	Asia / Pacific	Americas	Africa
Number of brands selected	188	115	467	87	11
Countries analysed (Number of brands by country)	Austria (9) Belgium (7) Denmark (5) Finland (6) France (31) Germany (36) Greece (15) Ireland (4) Italy (21) Netherlands (9) Portugal (5) Spain (6) Sweden (4) Switzerland (13) UK (17)	Belarus (8) Bulgaria (6) Croatia (4) Czech Rep. (5) Hungary (6) Poland (22) Romania (8) Russia (32) Slovakia (3) Slovenia (4) Ukraine (17)	Australia (10) Bangladesh (12) India (14) Indonesia (15) Iran (6) Japan (34) Malaysia (6) Pakistan (11) Philippines (9) Singapore (3) South Korea (14) Taiwan (4) Thailand (5) Turkey (8) Vietnam (18) China (298)	North America : Canada (23) USA (29) Latin America : Argentina (6) Brazil (6) Chile (4) Colombia (13) Mexico (6)	Egypt (3) Morocco (3) South Africa (5)

3831PC / 7-2505R

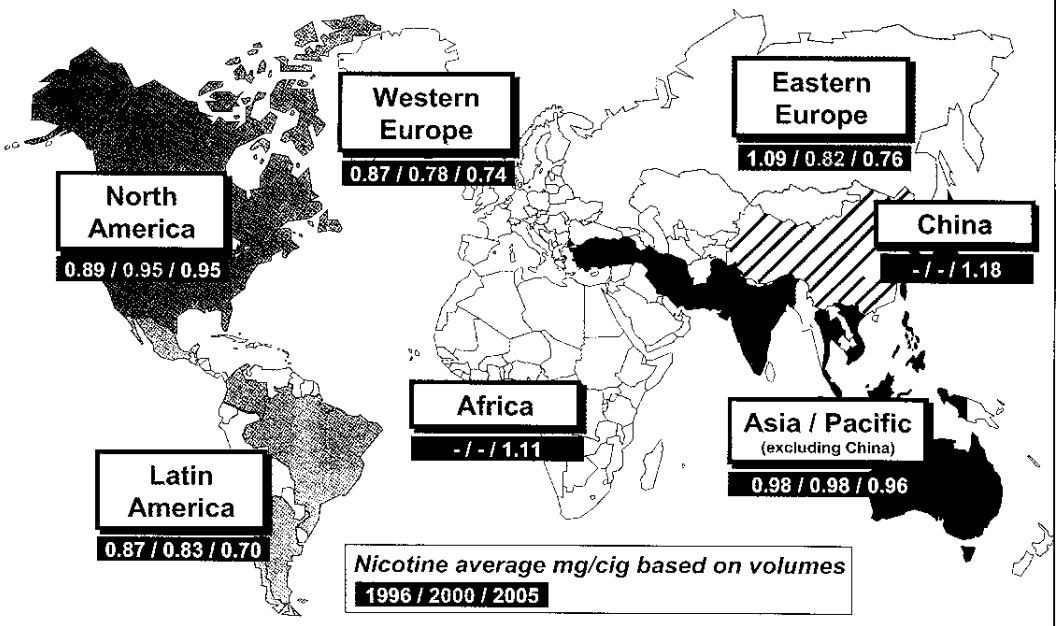
Worldwide

Tar sales weighted average across the world
Evolution VF 96 / WCDS 2000 / WCDS 2005



3831PC / 8-2505R

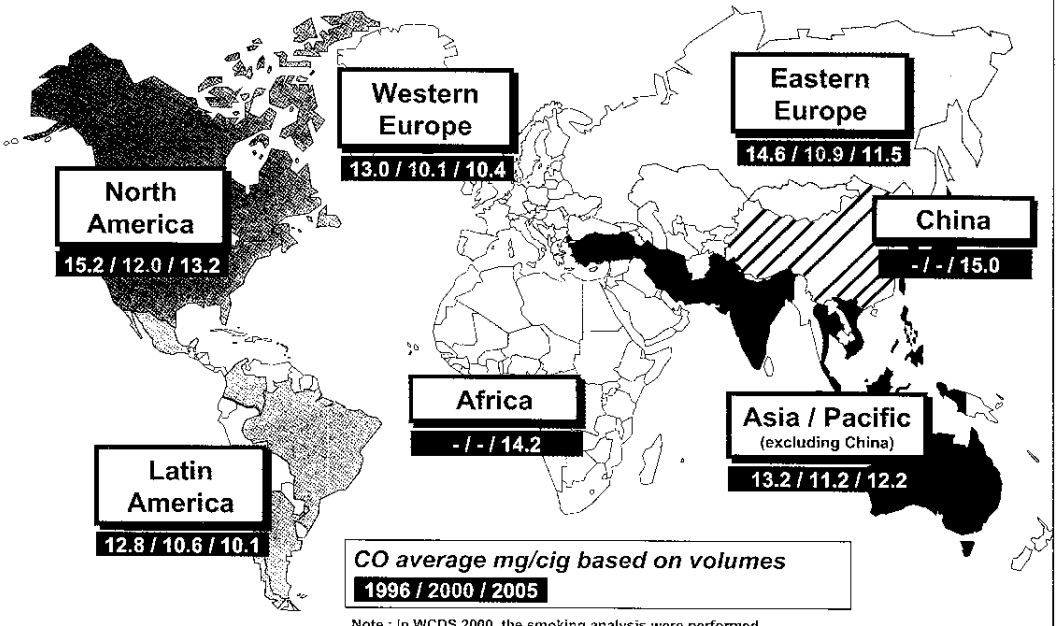
Nicotine sales weighted average across the world
Evolution VF 96 / WCDS 2000 / WCDS 2005



Nicotine average mg/cig based on volumes
1996 / 2000 / 2005

3831PC / 9-2505R

CO sales weighted average across the world
Evolution VF 96 / WCDS 2000 / WCDS 2005

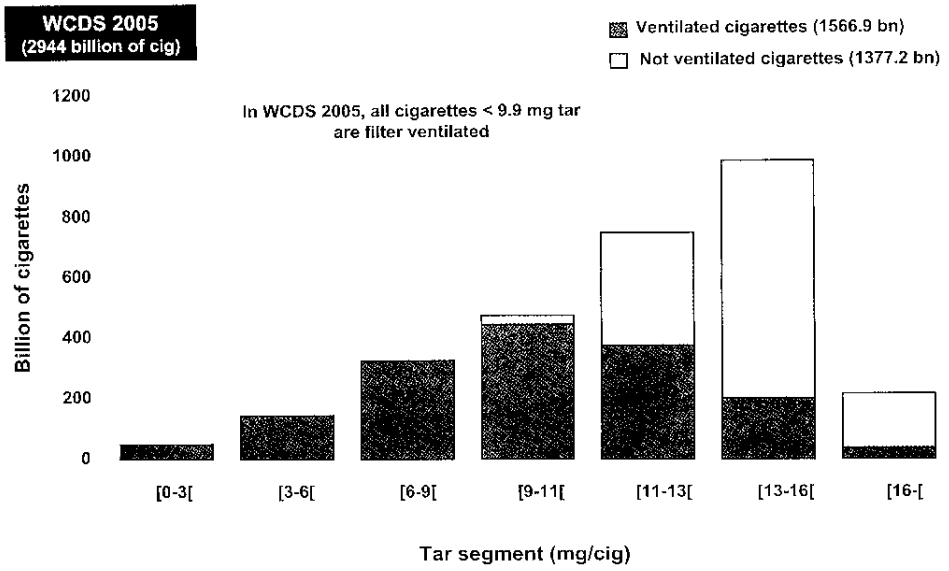


CO average mg/cig based on volumes
1996 / 2000 / 2005

Note : In WCDS 2000, the smoking analysis were performed on linear smoking machine ASM 500

3831PC / 10-2505R

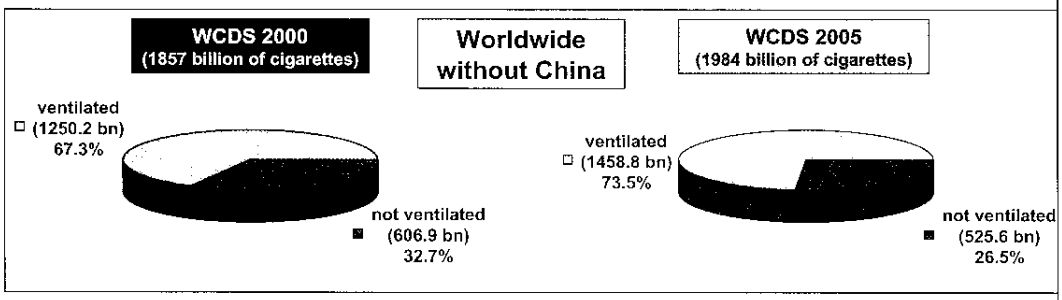
Tar deliveries distribution
WCDS 2005 (based on volume)

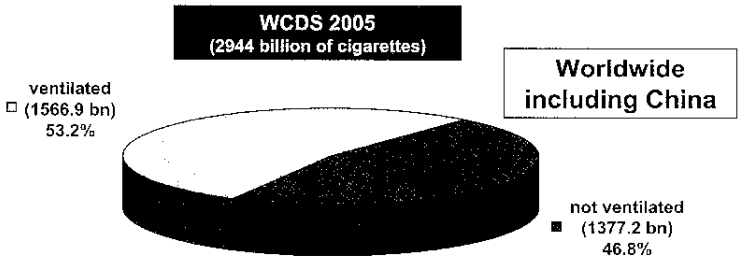


Worldwide

3831PC / 11-2505R

Filter ventilation distribution 2000 vs 2005
(based volume)

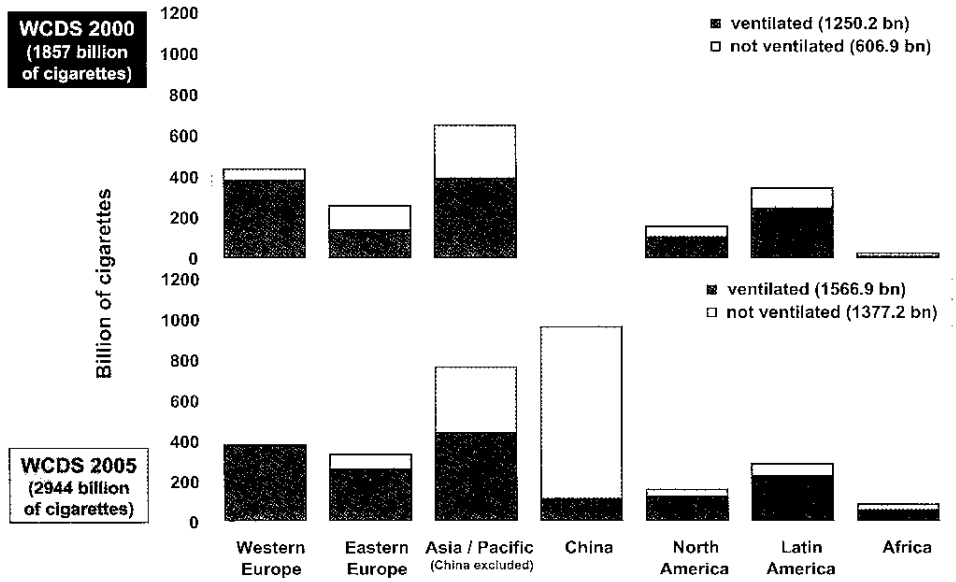




Worldwide

3831PC / 12-2506R

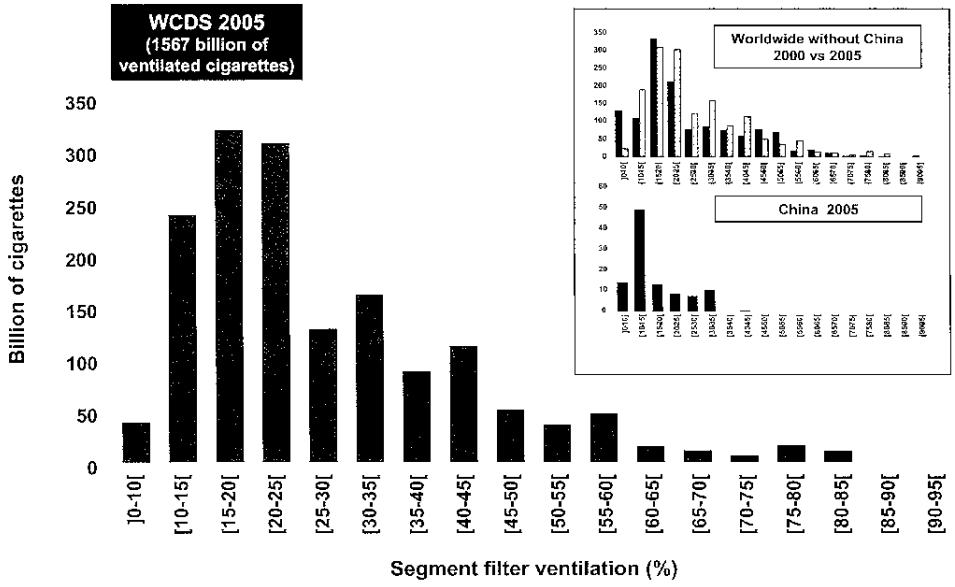
Ventilated cigarettes by area
2000 vs 2005 (based on volume)



3831PC / 13-2505R

Worldwide

Filter ventilation distribution in 2005
(based on volume) Ventilated cigarettes



3831PC / 14-2505R

Worldwide

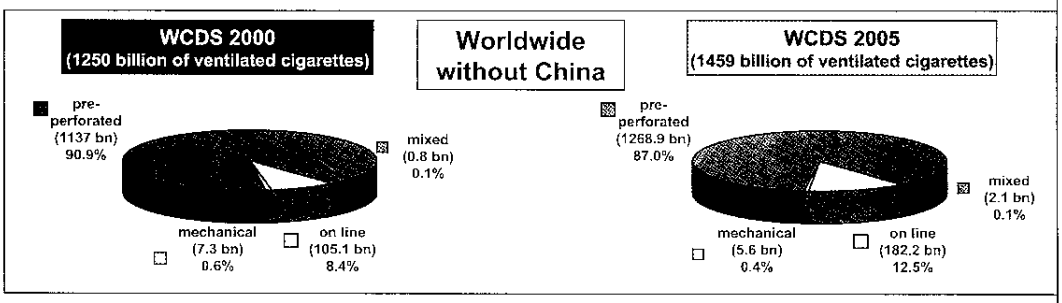
Filter ventilation weighted average
2000 vs 2005 (based on volume)

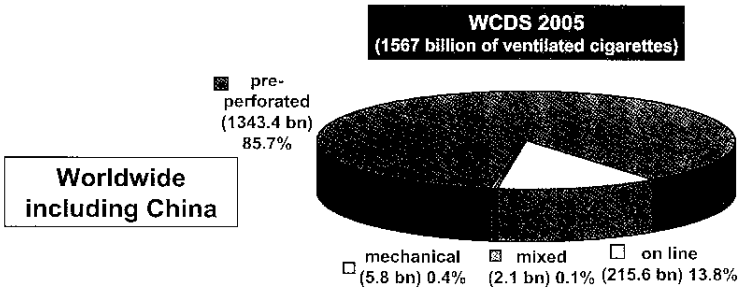
	WCDS 2000	WCDS 2005
Western Europe	27.4 %	28.6%
Eastern Europe	20.6 %	23.4%
Asia / Pacific	32.0 %	35.7%
China	-	16.8%
North America	18.3 %	20.1%
Latin America	25.5 %	29.7%
Africa	22.7 %	13.3%
Worldwide	26.2 %	27.2 %

3831PC / 15-2505R

Worldwide

Perforation type distribution 2000 vs 2005
(based on volume) Ventilated cigarettes

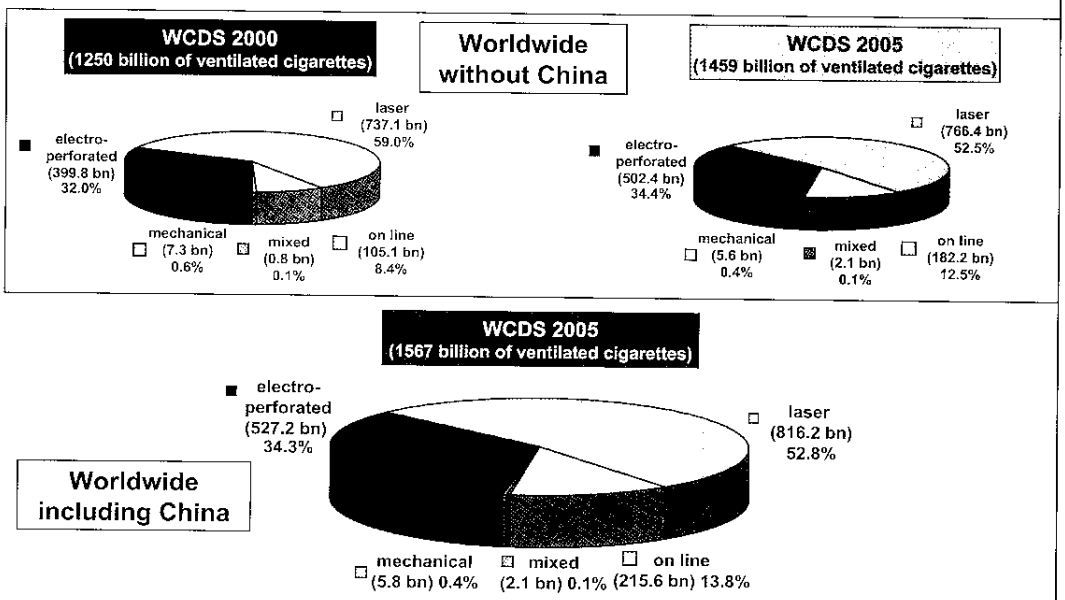




3831PC / 16-2505R

Worldwide

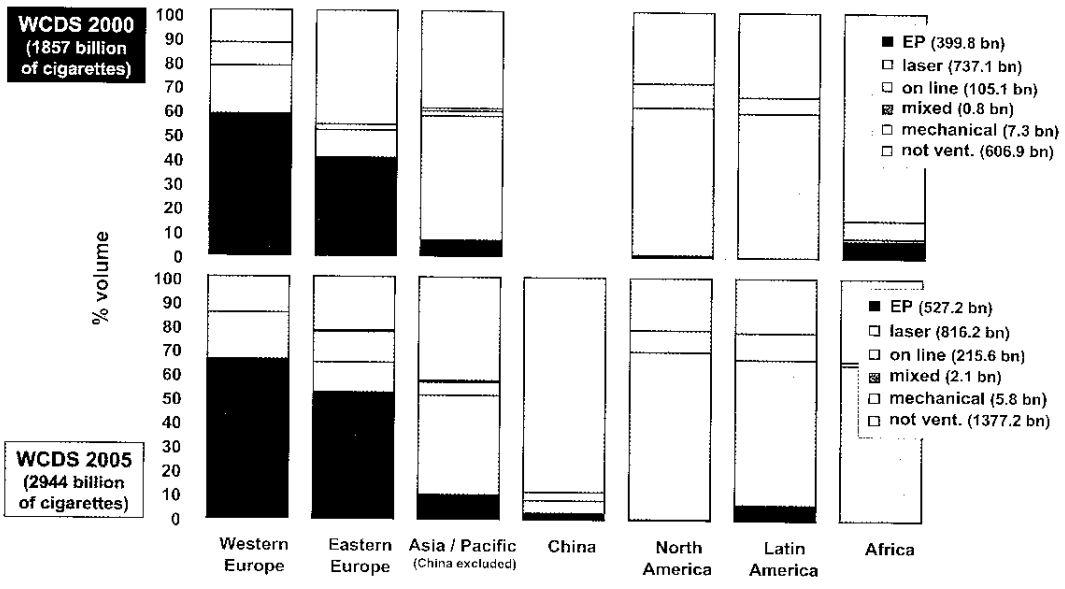
Perforation type distribution 2000 vs 2005
(based on volume) Ventilated cigarettes



Worldwide

3831PC / 17-2505R

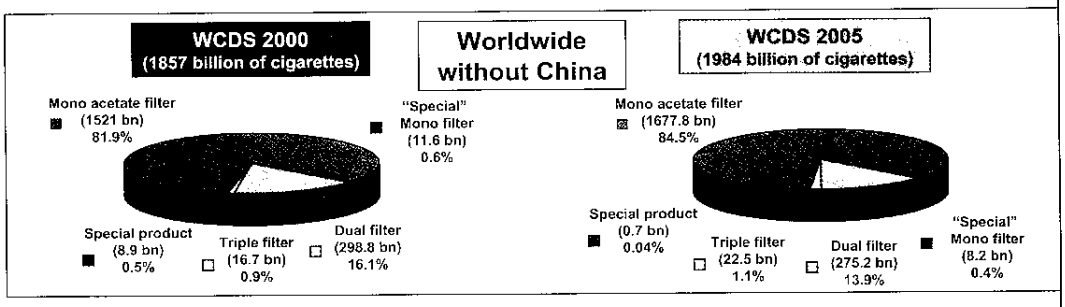
Perforation type by area
2000 vs 2005 (based on volume)

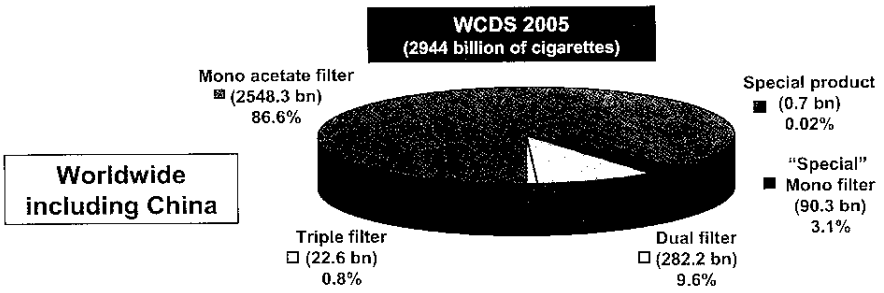


Worldwide

3831PC / 18-2505R

Filter type distribution
2000 vs 2005 (based on volume)

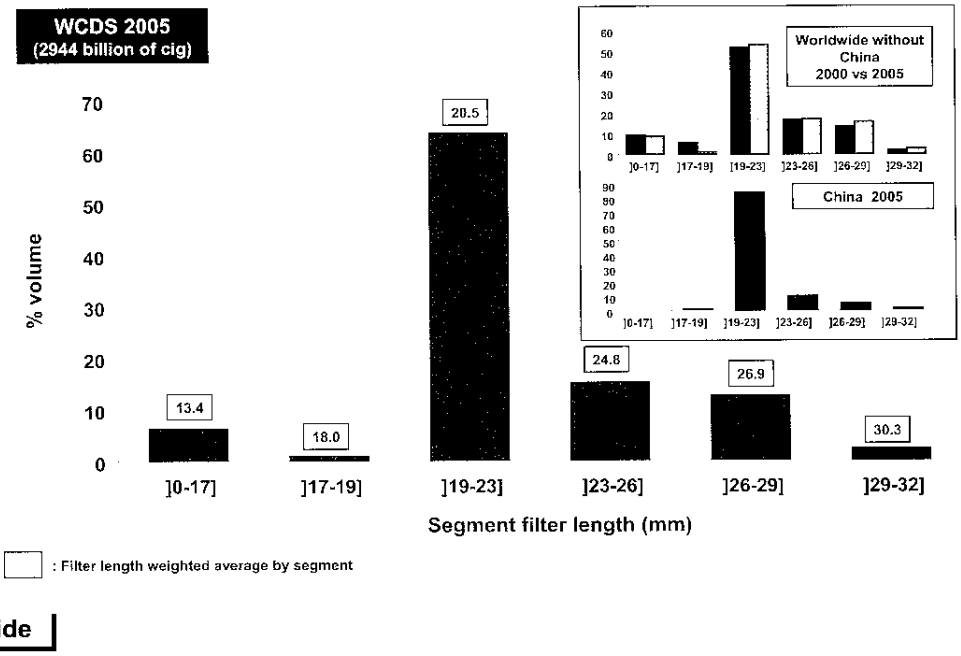




3831PC / 19-2505R

Worldwide

Filter length distribution
WCDS 2005 (based on volume)



3831PC / 20-2505R

Worldwide

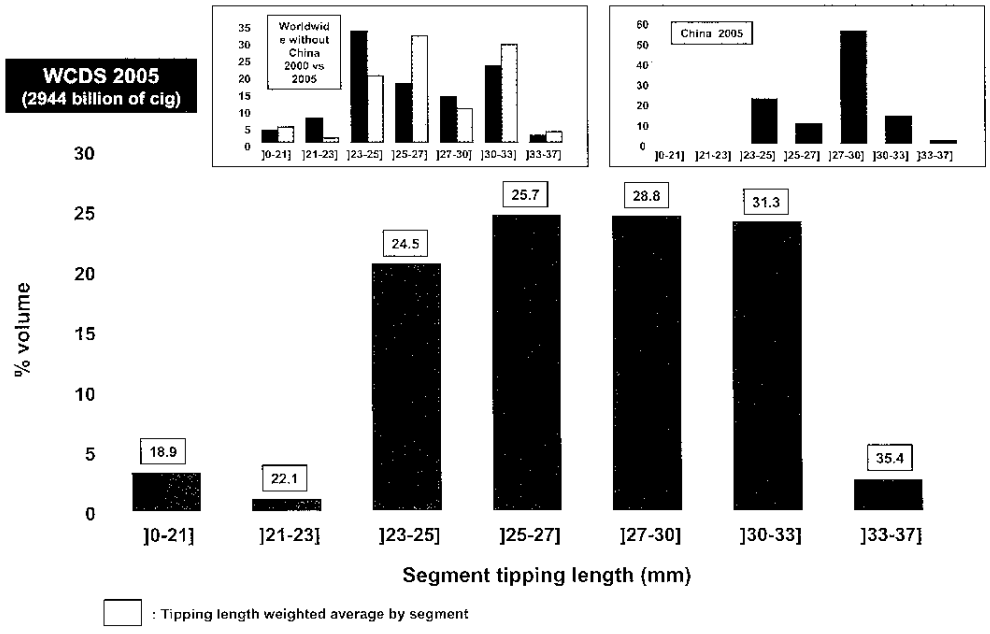
Filter length weighted average
2000 vs 2005 (based on volume)

	WCDS 2000	WCDS 2005
Western Europe	22.0 mm	22.2 mm
Eastern Europe	20.8 mm	21.9 mm
Asia / Pacific	21.9 mm	22.5 mm
China	-	20.9 mm
North America	22.0 mm	22.8 mm
Latin America	18.0 mm	19.4 mm
Africa	22.5 mm	21.3 mm
Worldwide	**21.5 mm**	**21.7 mm**

3831PC / 21-2505R

Worldwide

Tipping length distribution
WCDS 2005 (based on volume)



3831PC / 22-2505R

Worldwide

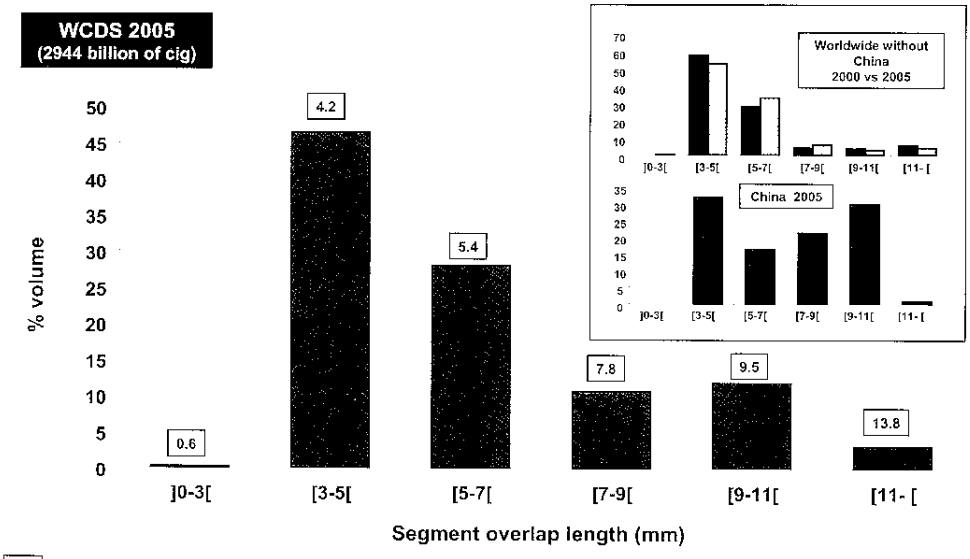
Tipping length weighted average 2000 vs 2005 (based on volume)

	WCDS 2000	WCDS 2005
Western Europe	26.4 mm	26.8 mm
Eastern Europe	26.2 mm	27.3 mm
Asia / Pacific	27.9 mm	28.3 mm
China	-	27.8 mm
North America	26.3 mm	27.4 mm
Latin America	26.3 mm	26.5 mm
Africa	26.9 mm	26.0 mm
Worldwide	**26.9 mm**	**27.6 mm**

3831PC / 23-2505R

Worldwide

Overlap tipping on filter distribution
WCDS 2005 (based on volume)



3831PC / 24-2506R

Worldwide

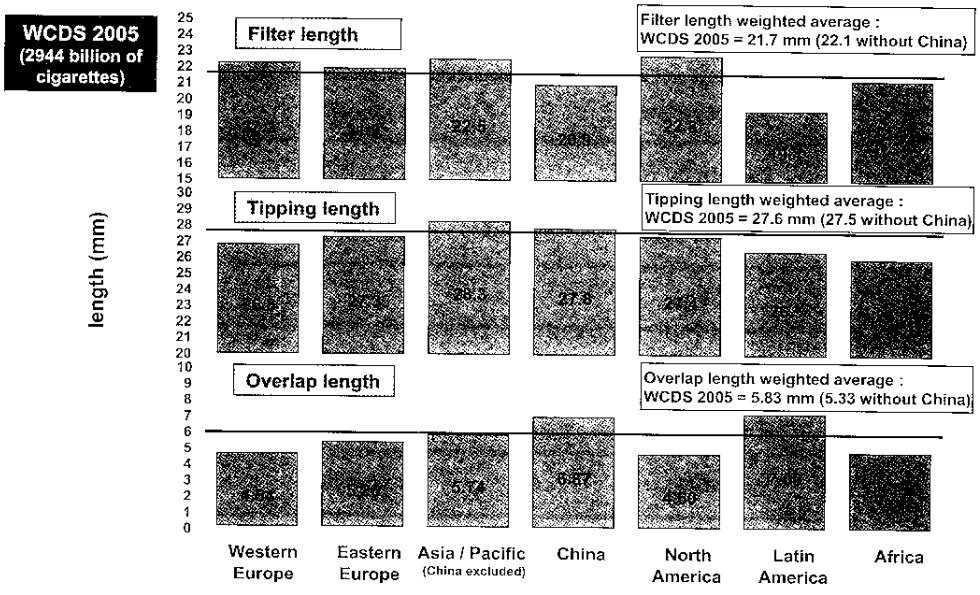
Overlap tipping length weighted average
2000 vs 2005 (based on volume)

	WCDS 2000	WCDS 2005
Western Europe	4.37 mm	4.54 mm
Eastern Europe	5.40 mm	5.26 mm
Asia / Pacific	5.96 mm	5.74 mm
China	-	6.87 mm
North America	4.34 mm	4.60 mm
Latin America	8.27 mm	7.09 mm
Africa	4.40 mm	4.74 mm
Worldwide	**5.38 mm**	**5.83 mm**

3831PC / 25-2505R

Worldwide

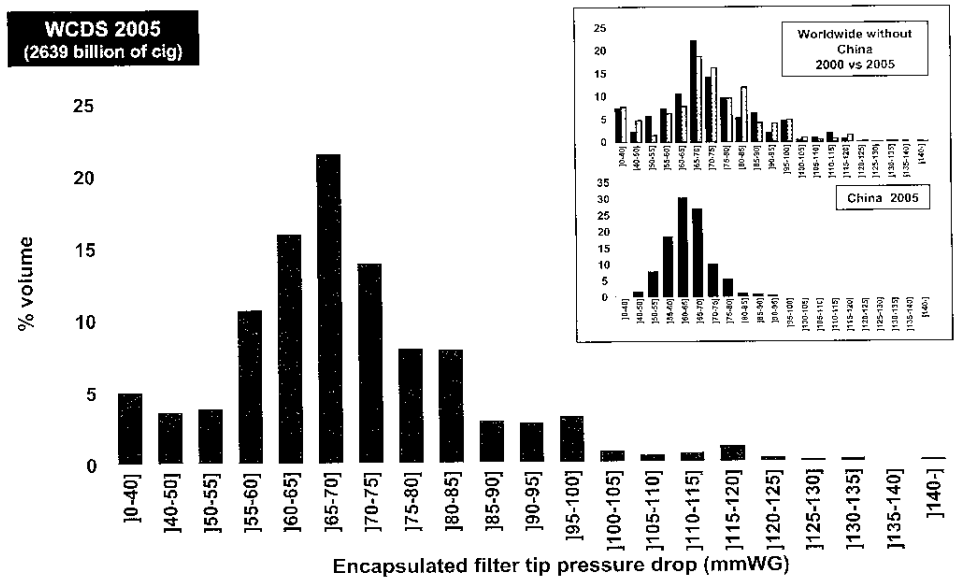
Lengths weighted average per area
WCDS 2005 (based on volume)



3831PC / 26-2505R

Worldwide

Mono filter pressure drop distribution
WCDS 2005 (based on volume) MF brands



3831PC / 27-2505R

Worldwide

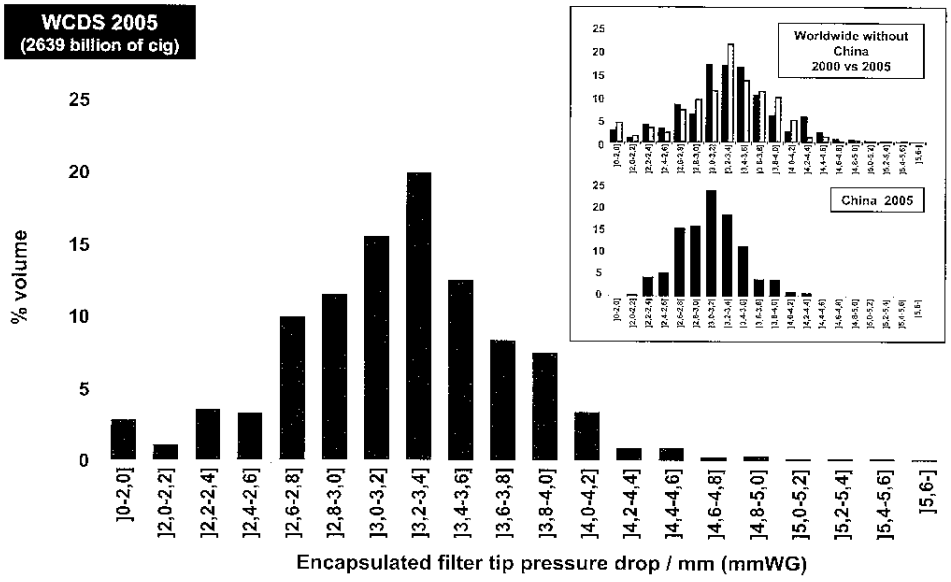
Mono filter PD weighted average
2000 vs 2005 (based on volume) MF brands

	WCDS 2000	WCDS 2005
Western Europe	79 mmWG	80 mmWG
Eastern Europe	67 mmWG	72 mmWG
Asia / Pacific	58 mmWG	63 mmWG
China	-	64 mmWG
North America	77 mmWG	78 mmWG
Latin America	69 mmWG	70 mmWG
Africa	71 mmWG	72 mmWG
Worldwide	**70 mmWG**	**69 mmWG**

3831PC / 26-2505R

Worldwide

Mono filter PD / filter mm distribution
WCDS 2005 (based on volume) MF brands



3831PC / 29-2505R

Worldwide

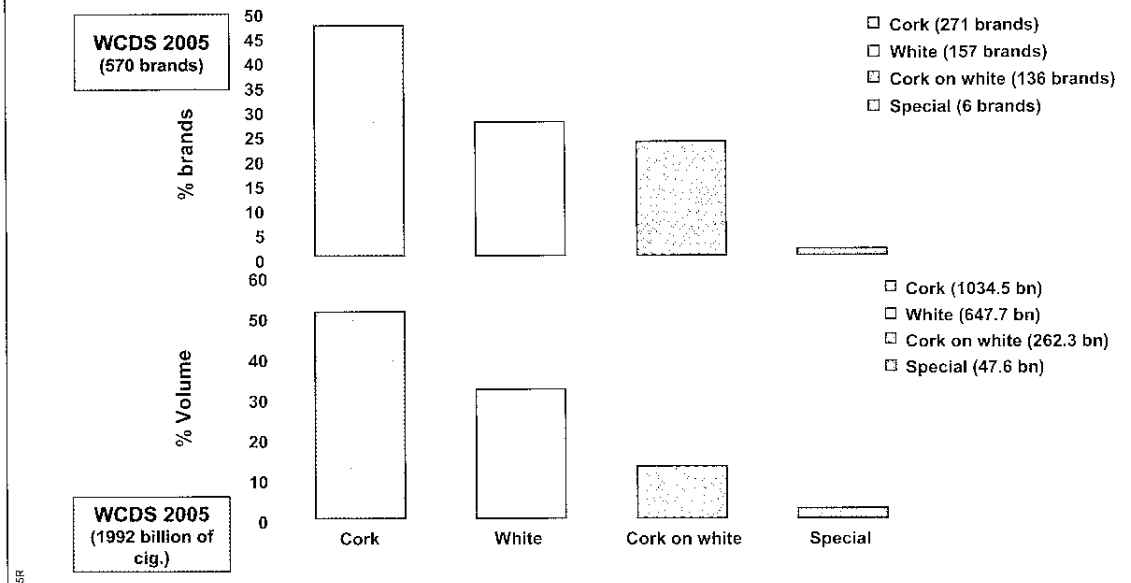
Mono filter PD / filter mm weighted average
2000 vs 2005 (based on volume) MF brands

	WCDS 2000	WCDS 2005
Western Europe	3.6 mmWG/mm	3.6 mmWG/mm
Eastern Europe	3.3 mmWG/mm	3.3 mmWG/mm
Asia / Pacific	2.8 mmWG /mm	2.9 mmWG/mm
China	-	3.1 mmWG/mm
North America	3.5 mmWG /mm	3.4 mmWG/mm
Latin America	3.8 mmWG /mm	3.6 mmWG/mm
Africa	3.2 mmWG /mm	3.4 mmWG/mm
Worldwide	**3.4 mmWG/mm**	**3.2 mmWG/mm**

3831PC / 30-2505R

Worldwide

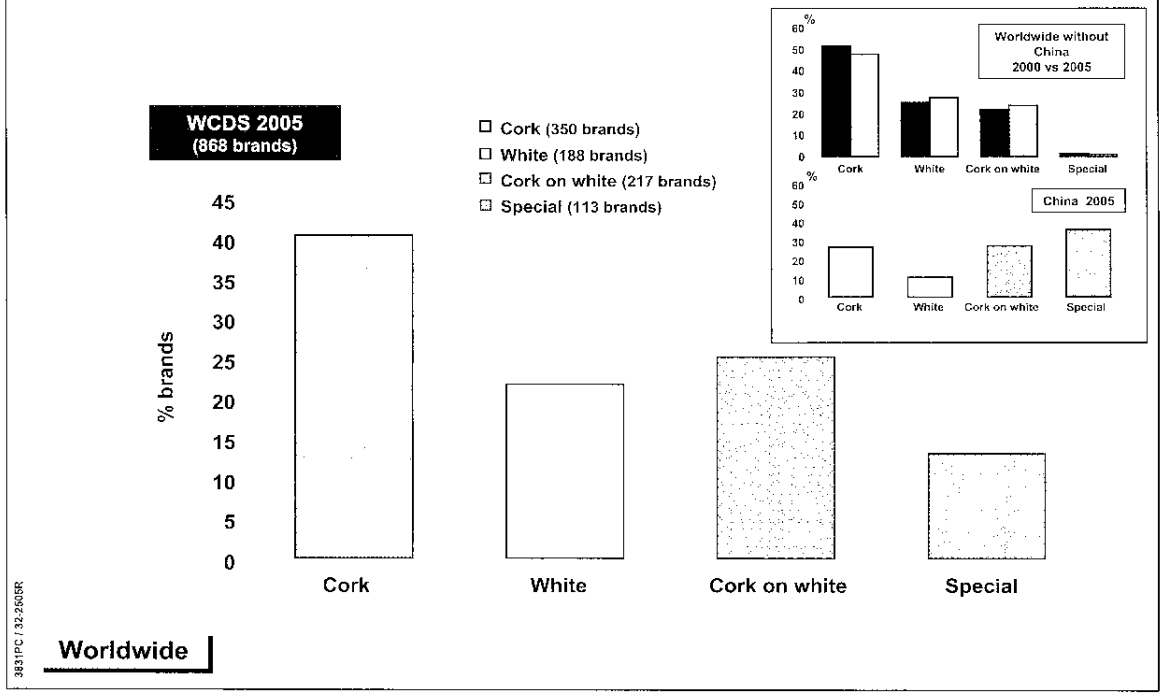
Tipping design distribution
WCDS 2005 (based on brands and volume)



3831PC / 31-2605R

Worldwide (China excluded)

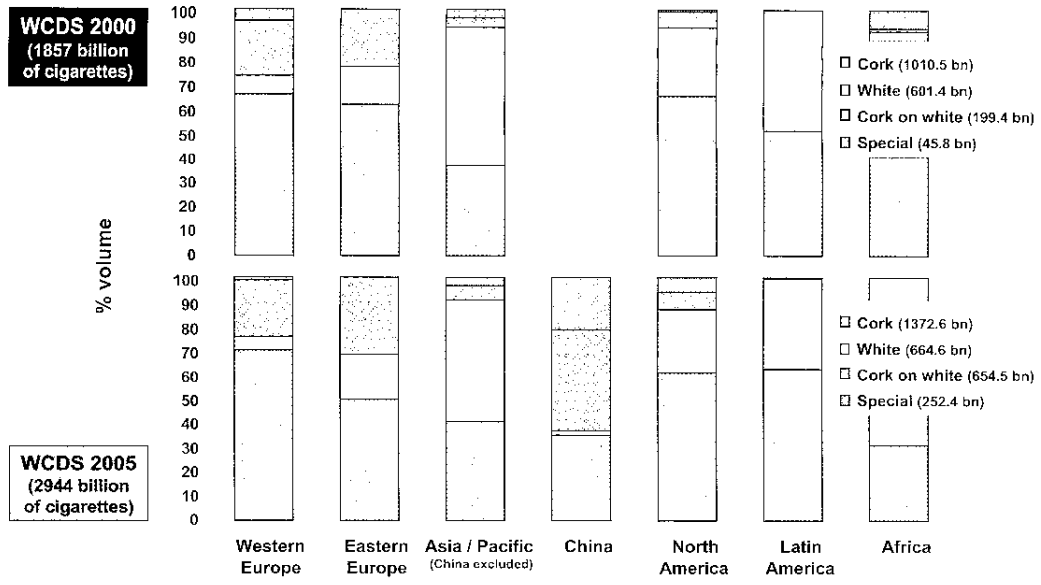
Tipping design distribution
WCDS 2005 (based on brands)



3831PC / 32-2605R

Worldwide

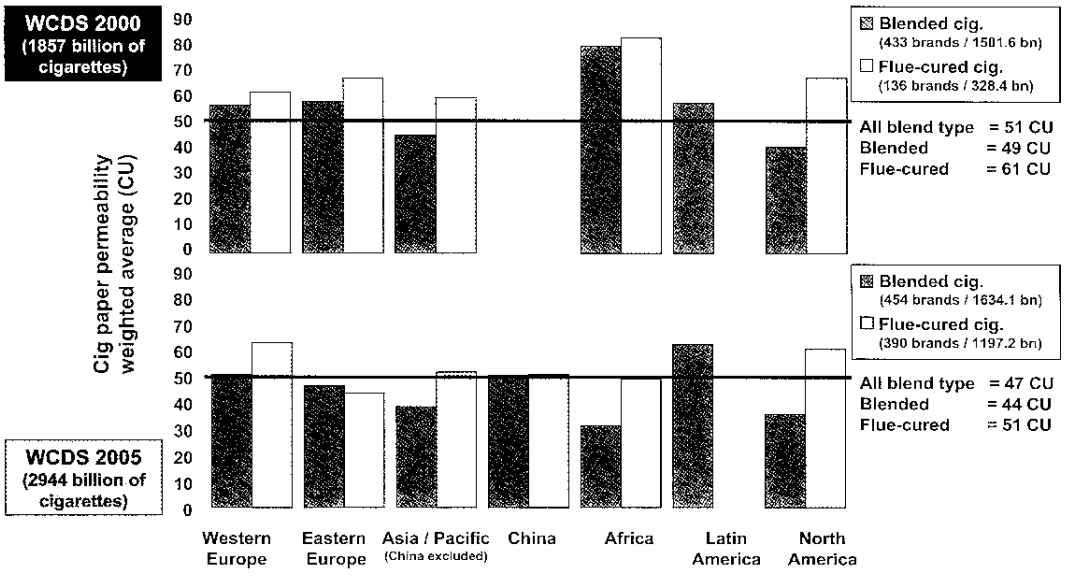
Tipping design distribution per area
2000 vs 2005 (based on volume)



3831PC / 33-2505R

Worldwide

Cig. paper permeability by area and tobacco blend - WCDS 2005 (based on volume)

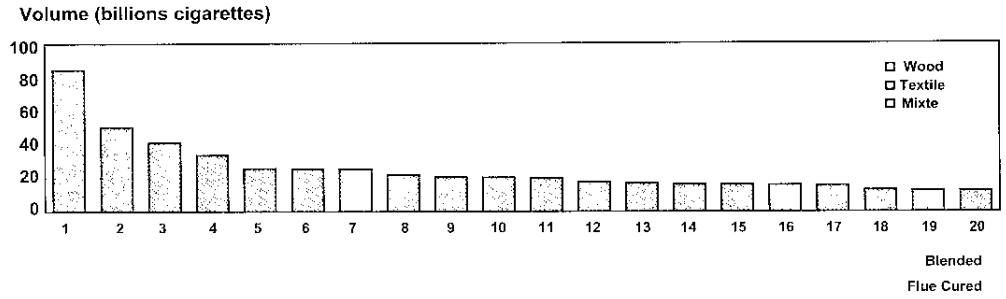


Note : Countries with number of brands by tobacco type < 3 are not reported on graph

3831PC / 34-2505R

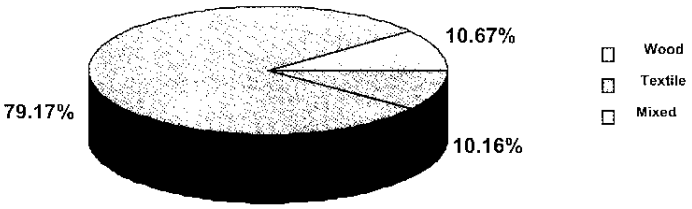
Worldwide

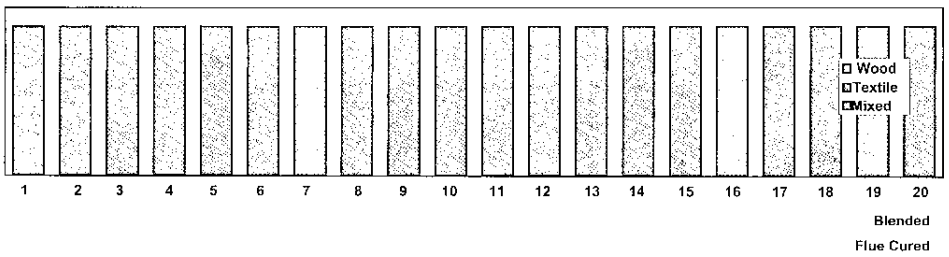
WCDS 2005 Top 20 cigarette brands worldwide
Paper composition (All blends)



3831PC / 36-2605R

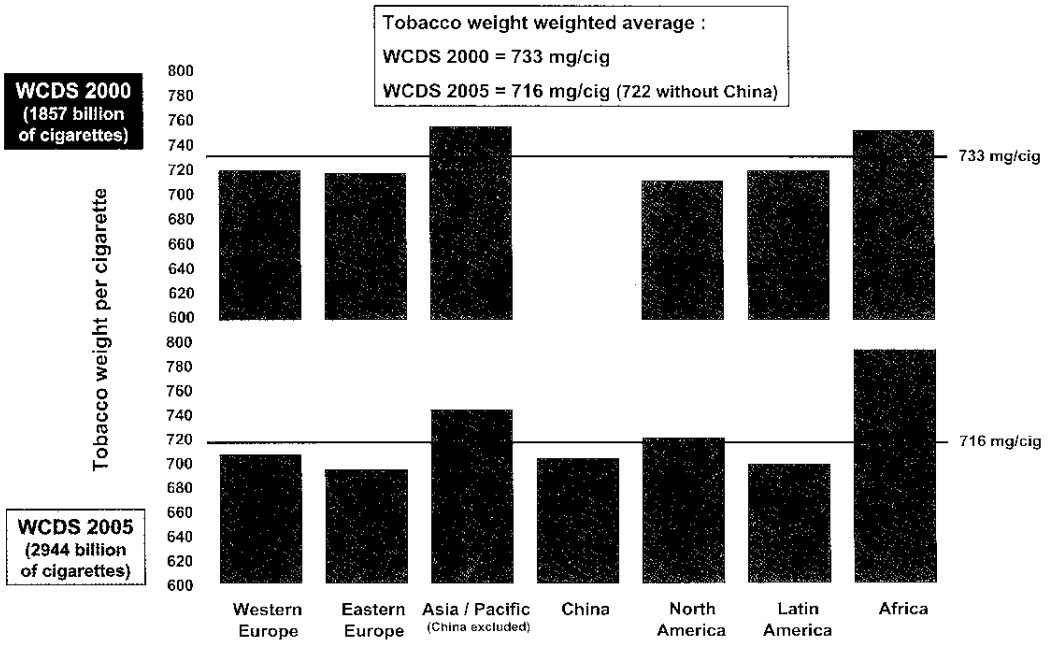
WCDS 2005 Top 20 cigarette brands worldwide
Paper composition based on volumes (All blends)





3831PC / 36-2565R

Tobacco weight weighted average per area 2000 vs 2005



3831PC / 37-2505R

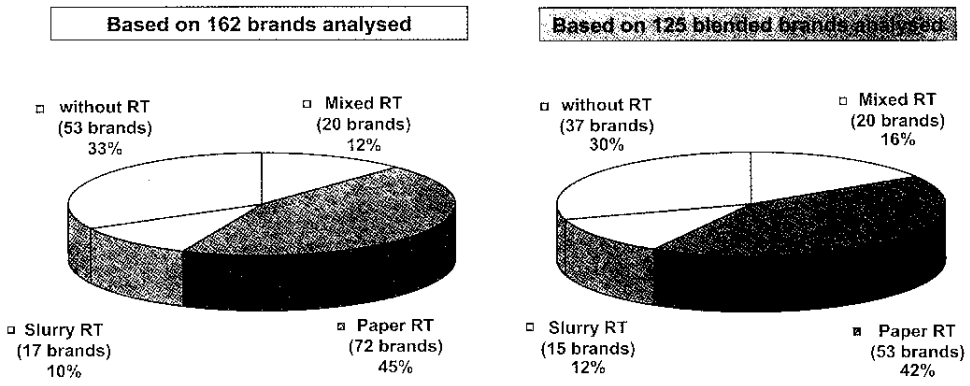
Tobacco blend sorting
Top 20 blended cigarettes

(WCDS 2005)

3831PC / 38-2505R

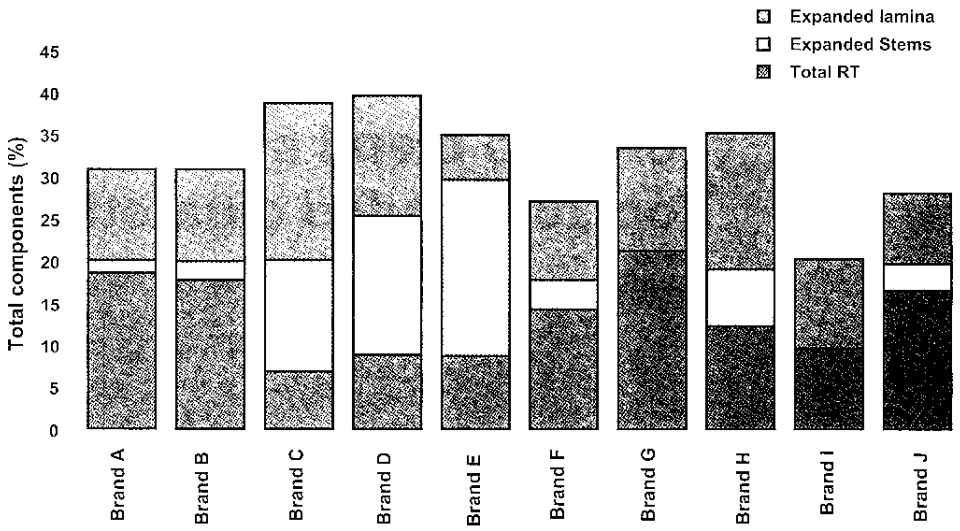
Worldwide

Tobacco composition WCDS 2005
RT type distribution (worldwide)



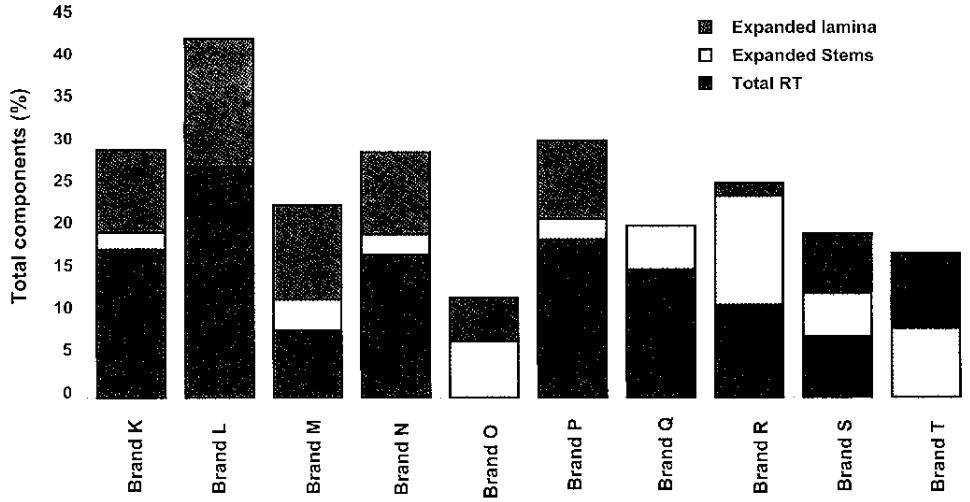
3831PC / 39-2505R

Tobacco composition WCDS 2005
TOP 10 blended brands



3831PC / 40-2505R

Tobacco composition WCDS 2005
TOP 11 - 20 blended brands

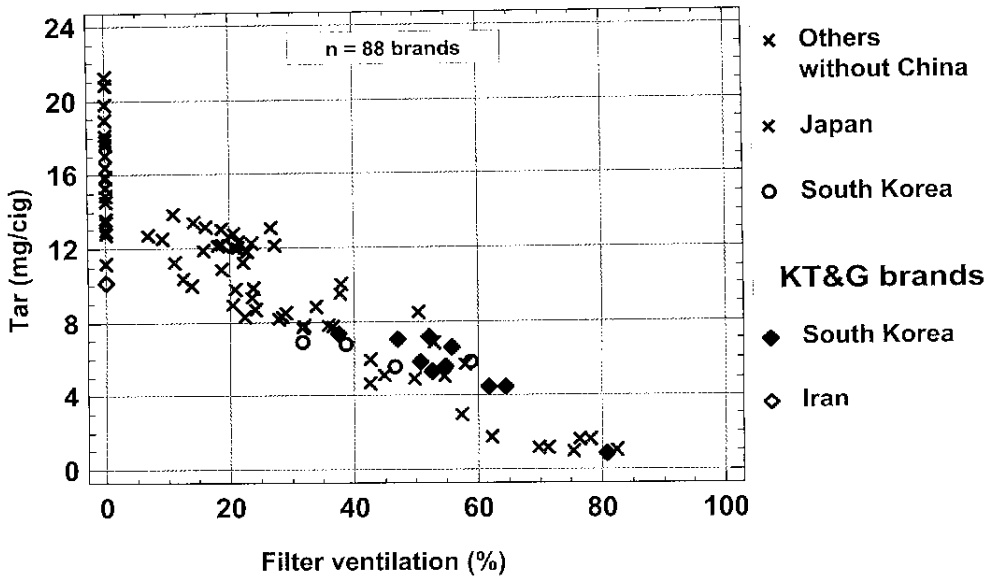


3831PC / 41-2505R

KT&G
vs Asia/Pacific area

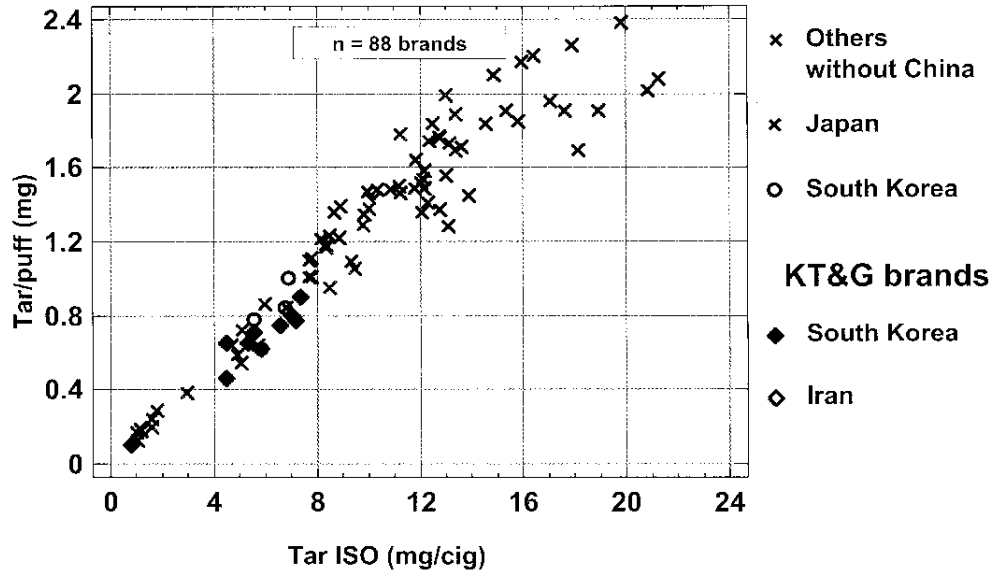
Blended cigarettes

Tar ISO vs filter ventilation



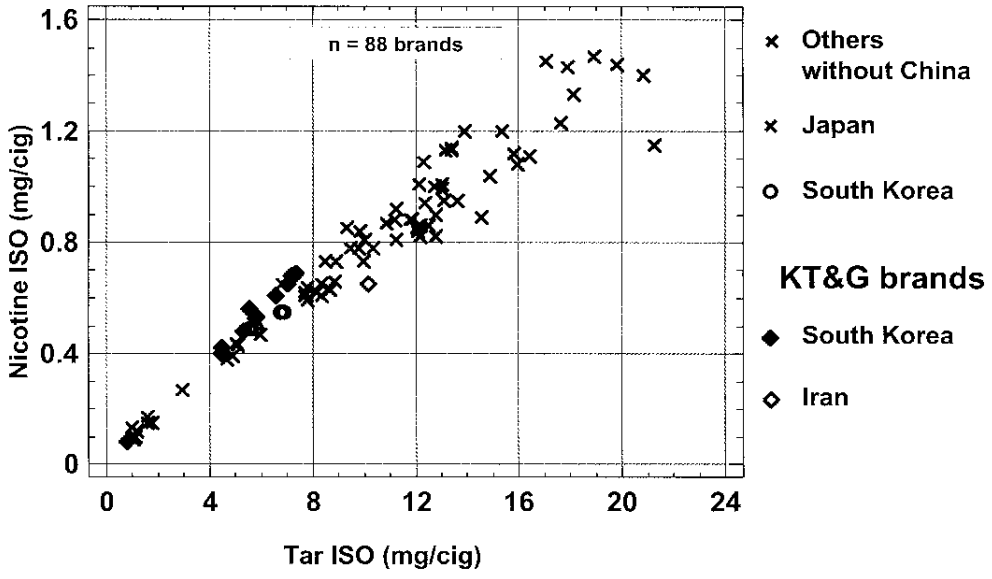
3831PC / 43-2505R

Average tar per puff vs tar delivery



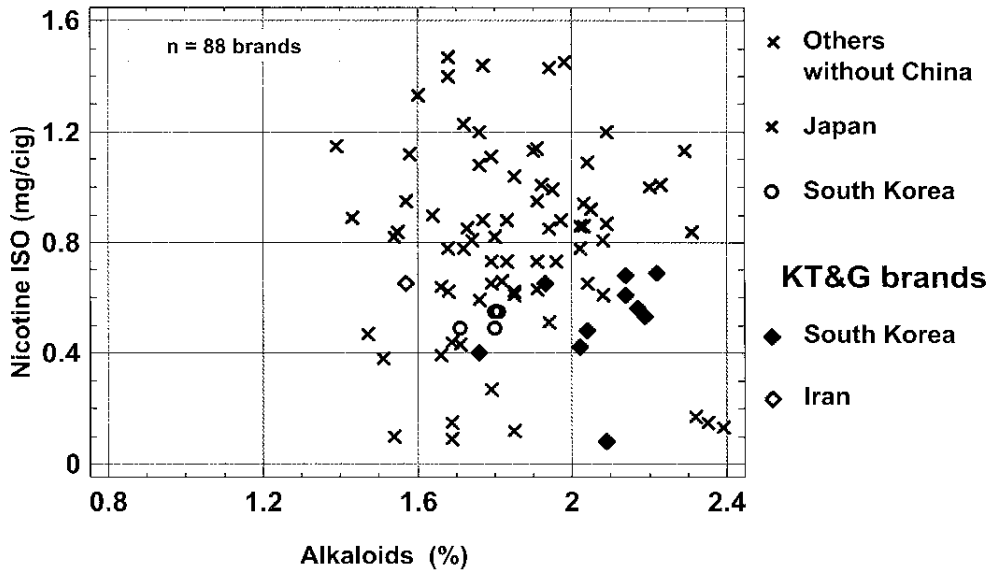
3831PC / 44-2505R

Smoke nicotine vs tar delivery



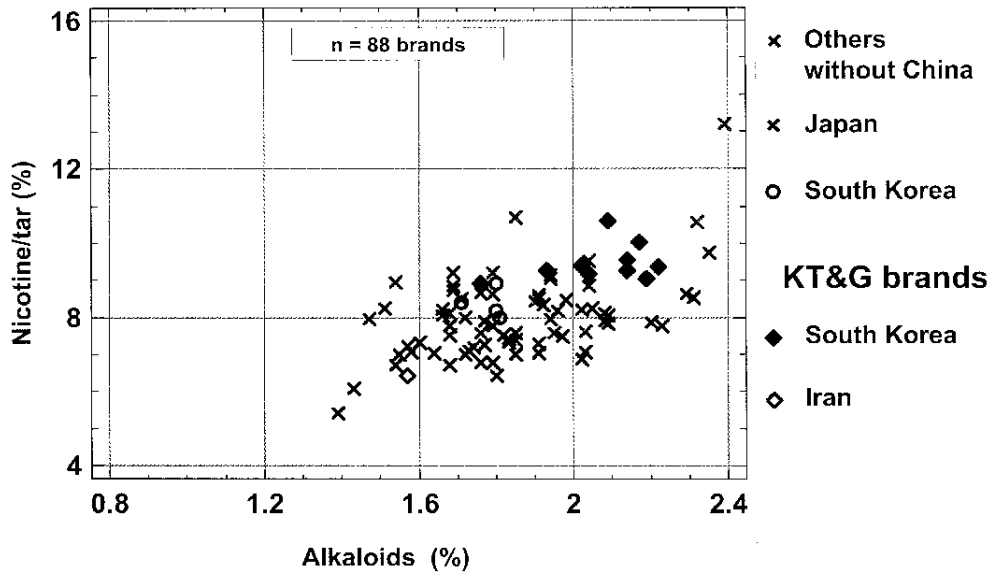
3831PC / 45-2505R

Smoke nicotine vs total alkaloids in tobacco



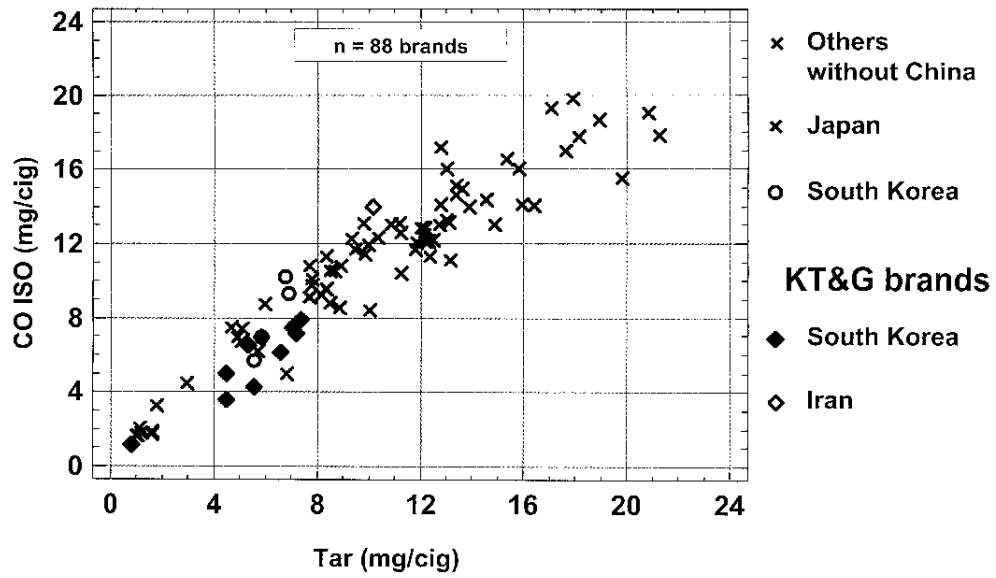
3831PC / 46-2505R

Nicotine/tar vs total alkaloids in tobacco



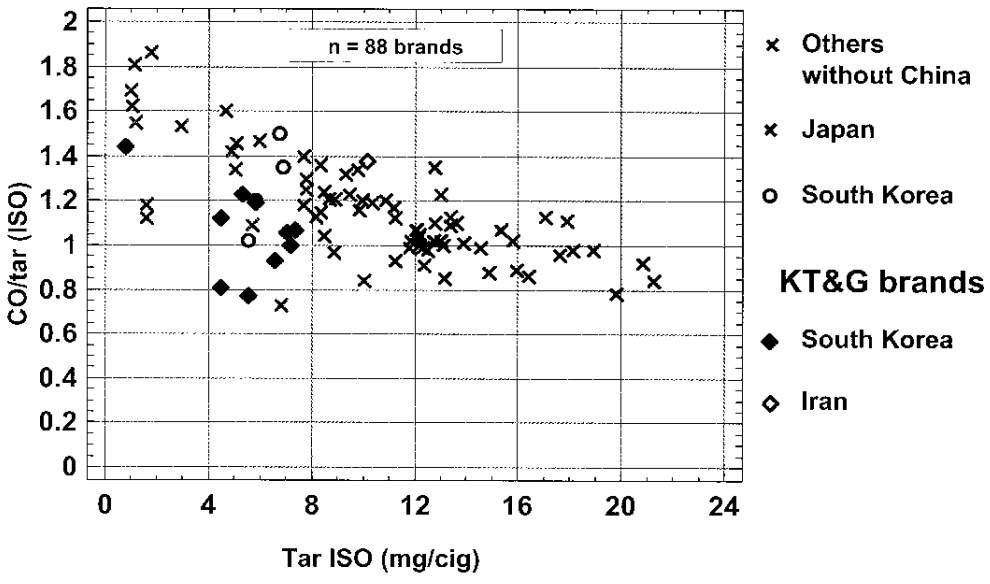
3831PC / 47-2505R

CO vs tar delivery



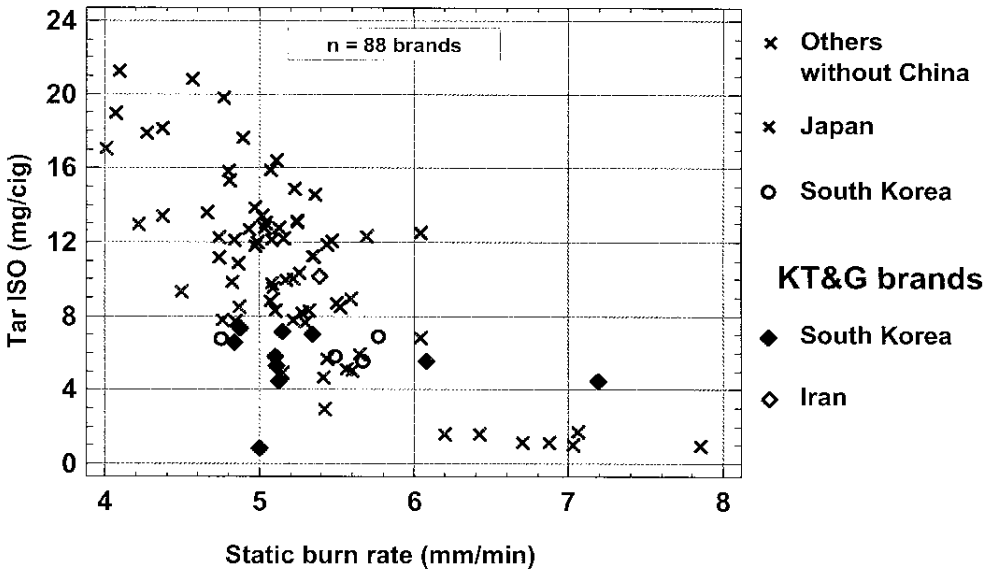
3831PC / 48-2505R

CO/tar vs tar delivery



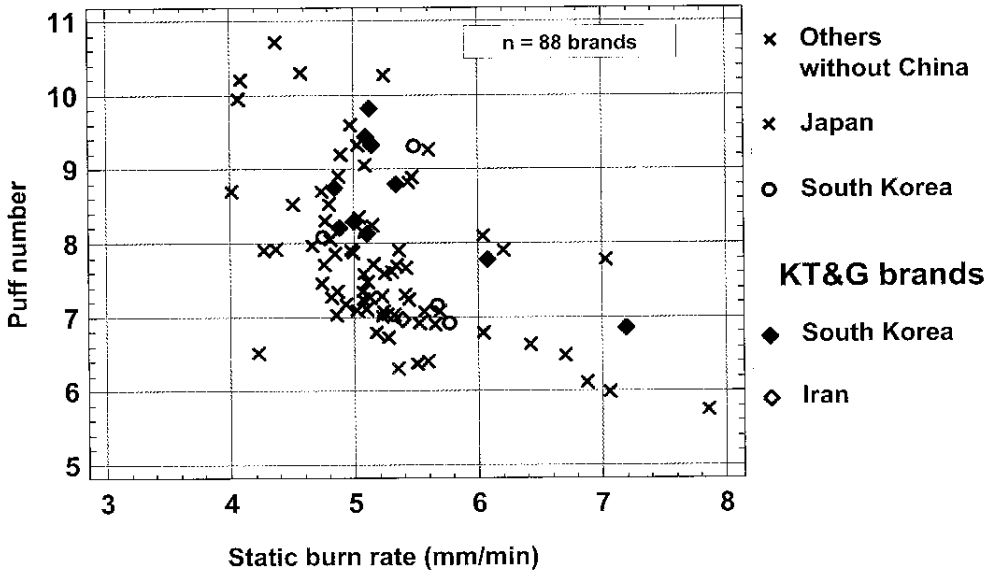
3831PC / 49-2505R

Tar ISO vs static burn rate



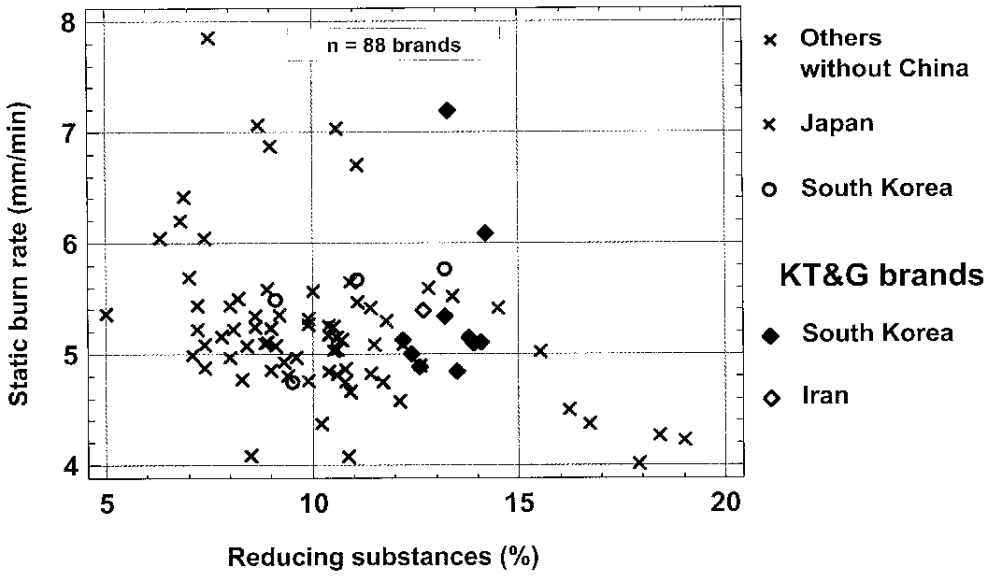
3831PC / 50-2505R

Puff number vs static burn rate



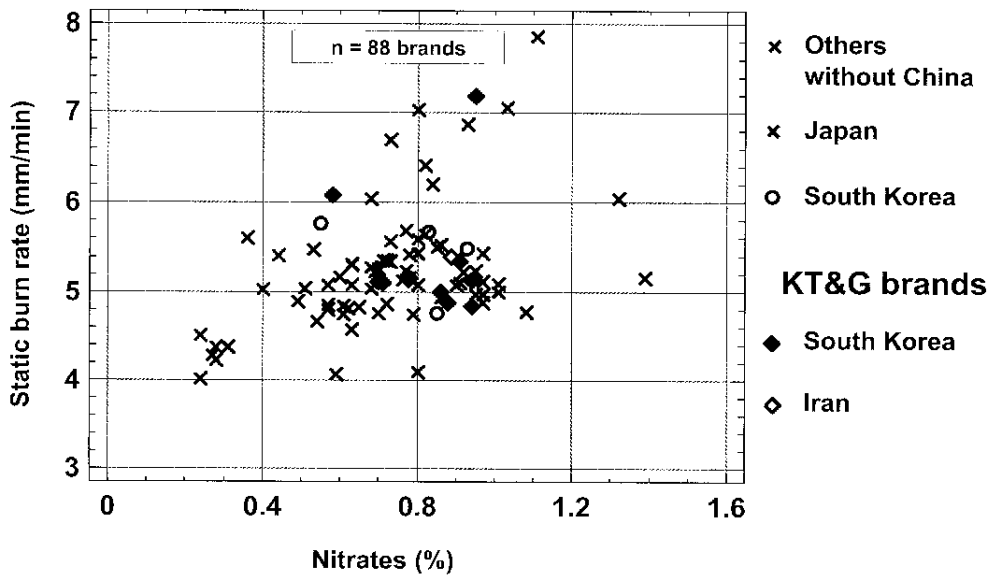
3831PC / 51-2505R

Static burn rate vs reducing substances



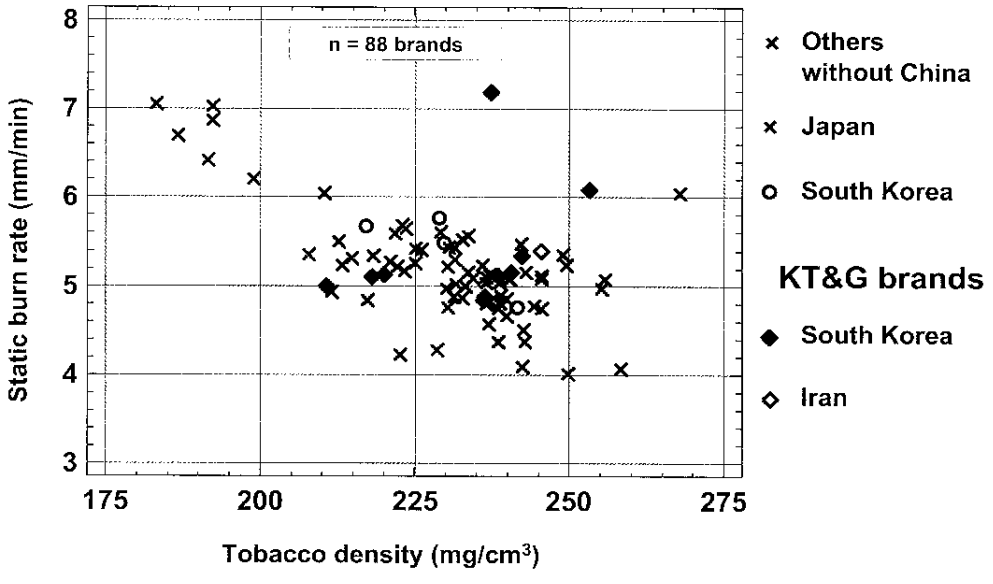
3831PC / 52-2505R

Static burn rate vs nitrates in tobacco



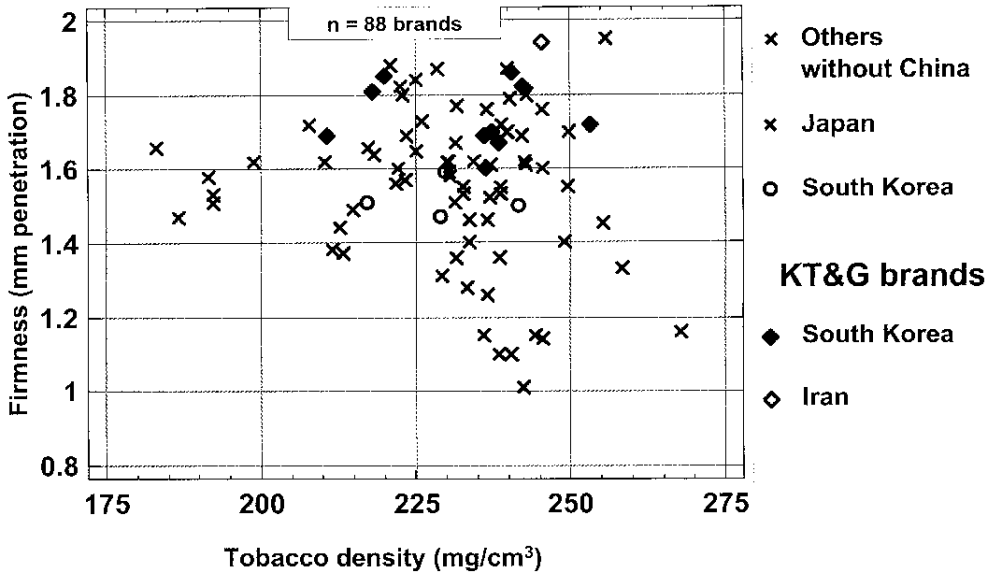
3831PC / 53-2505R

Static burn rate vs tobacco density



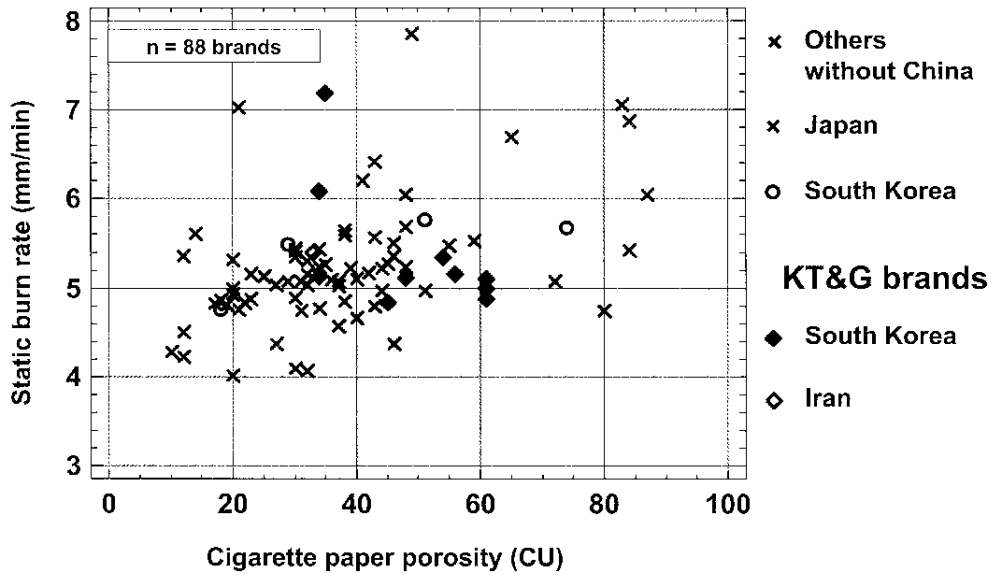
3831PC / 54-2505R

Cigarette firmness vs tobacco density



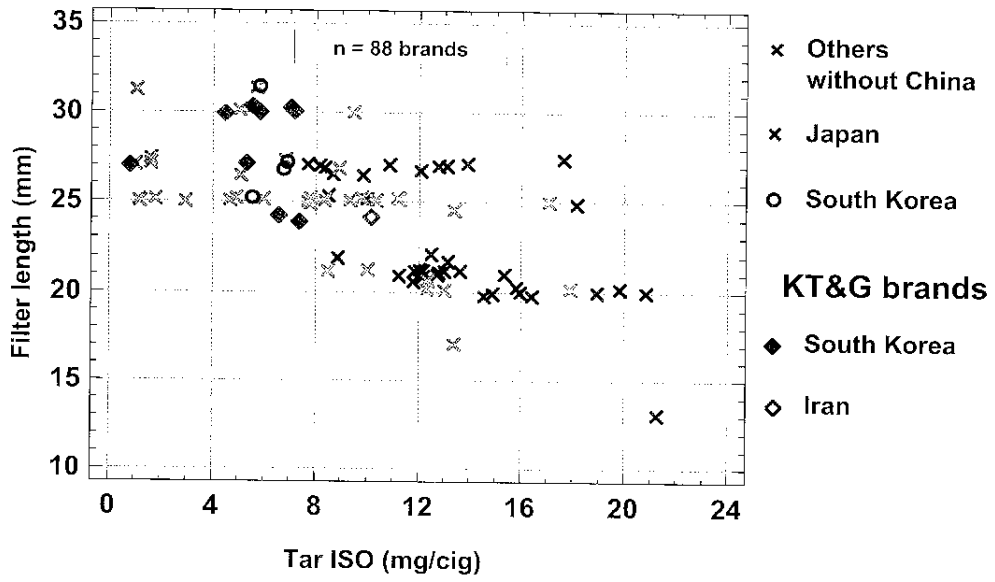
3831PG / 55-2505R

Static burn rate vs cigarette paper porosity



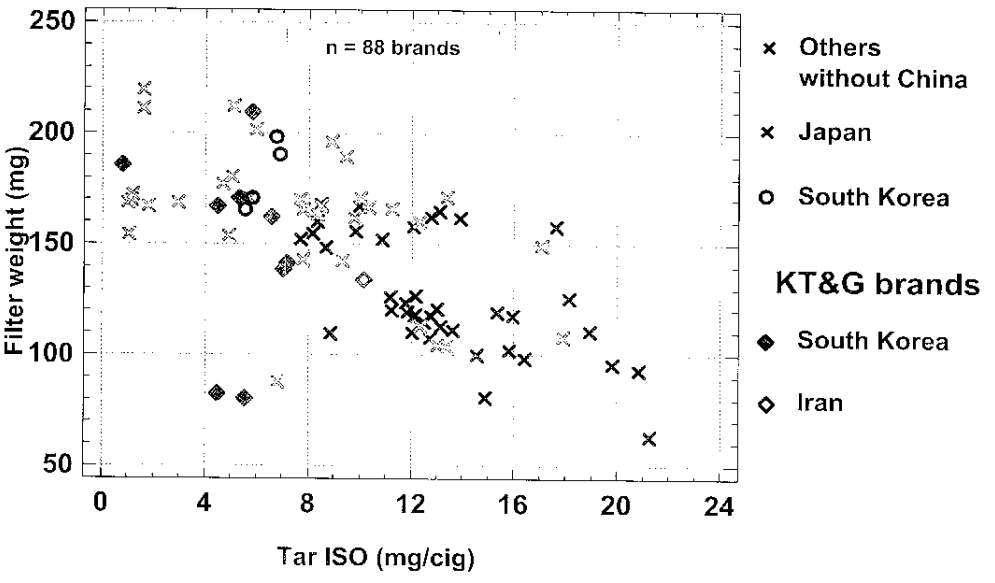
3831PG / 56-2505R

Filter length vs tar delivery



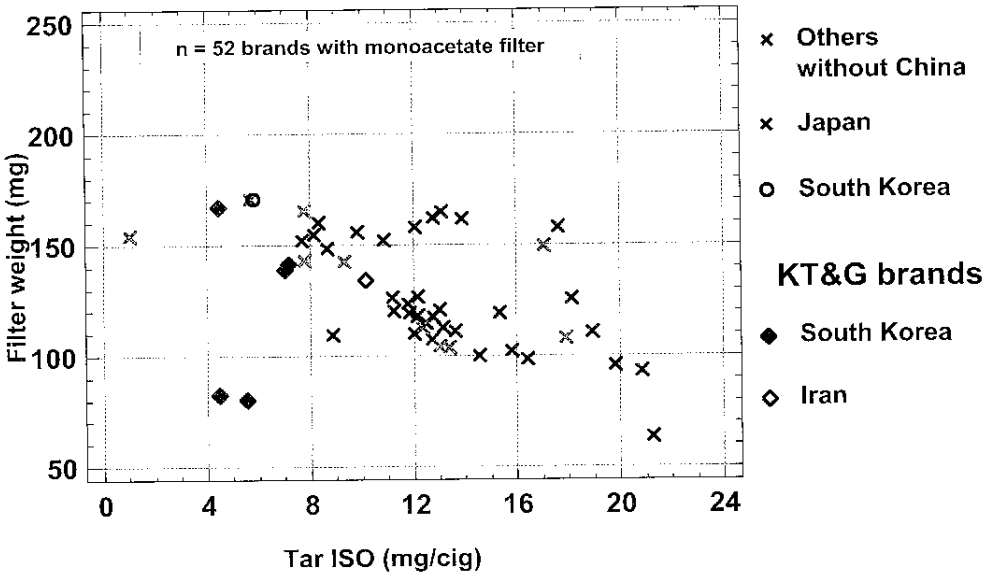
3831PC / 57-2505R

Filter weight vs tar delivery



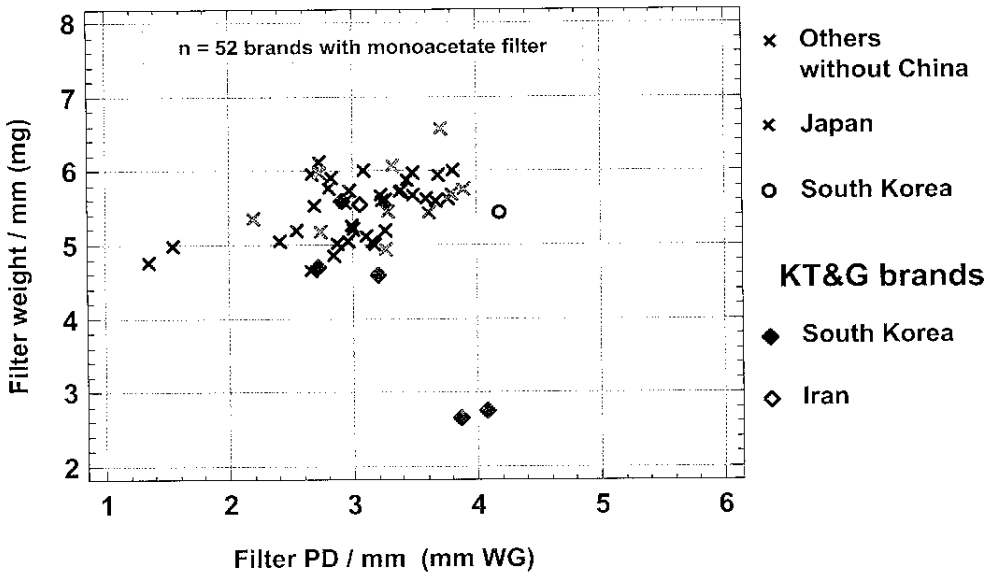
3831PC / 58-2505R

Filter weight vs tar delivery



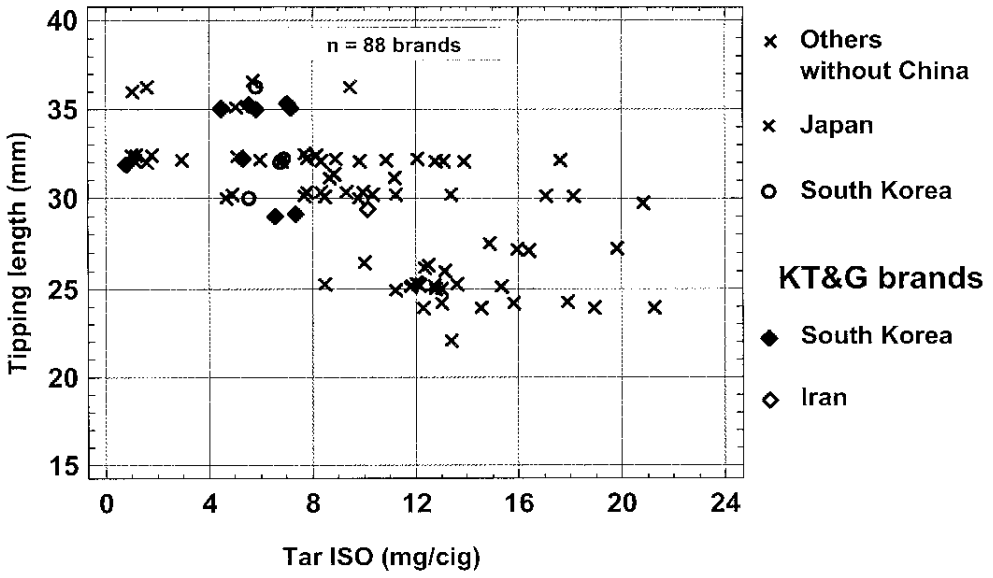
3831PC / 59-2606R

Filter weight/mm vs filter pressure drop/mm



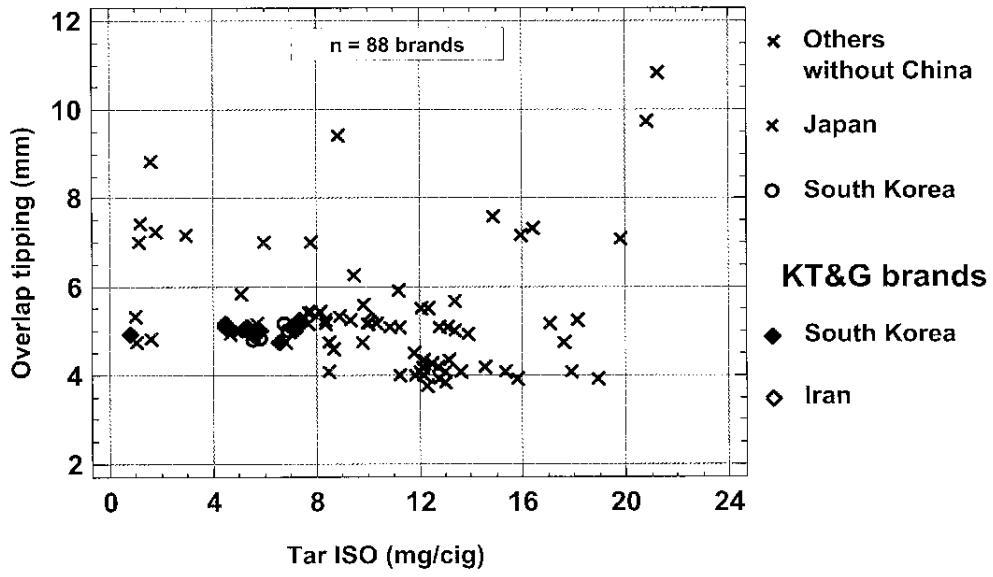
3831PC / 60-2606R

Tipping length vs tar delivery



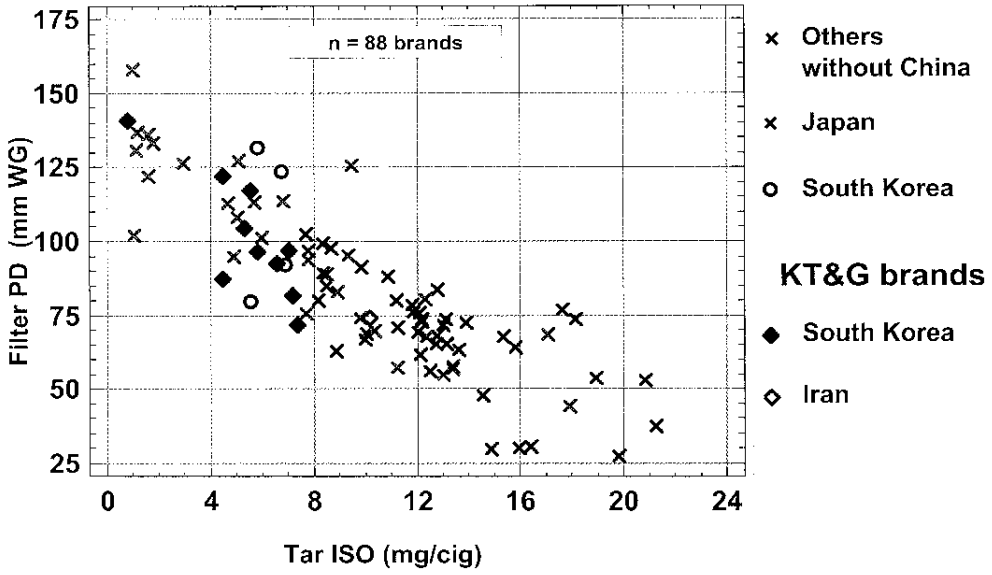
3831PC / 61-2505R

Overlap tipping vs tar delivery



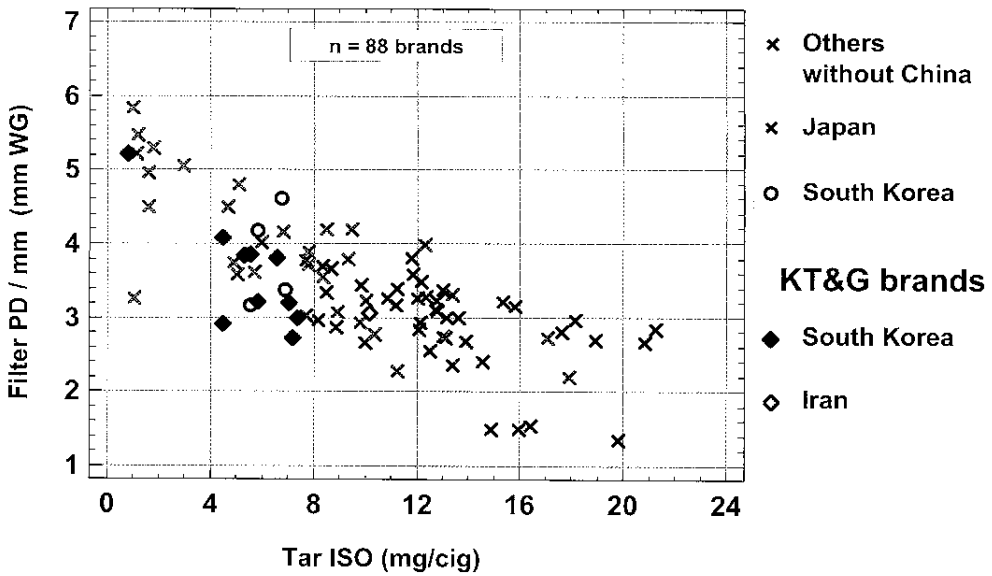
3831PC / 62-2505R

Filter PD vs tar delivery



3831PC / 63-2505R

Filter PD / mm vs tar delivery



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KT&G

Intensive Smoking Regimes

2007. 10. 05.

KT&G Central Research Institute

KT&G

2005 Joint Meeting

Contents

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II. Changes of Smoke Delivery by New Smoking Regimes

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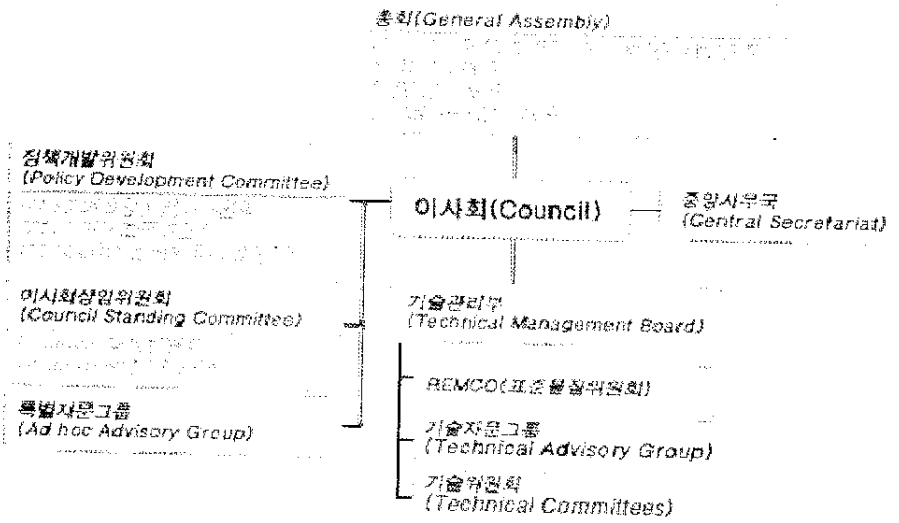
Structure of ISO
(International Organization for Standardization)



ISO Technical committee on tobacco and tobacco products (TC 126)

ISO TC 126	
Scope	Standardization of terminology and test methods for tobacco, and tobacco smoke
Activity	◆ Secretary : Kostmann, Chair : Henning Lutz ◆ Published 49 ISO standards under the direct responsibility ◆ 32 participating countries, and 29 observing countries ◆ Three working group(7, 8, 10) and two subcommittees ◆ Liaison : CORESTA, EC, ECPCI, ESTA, UN/ECE, WCO, FCA, WHO ◆ Next meeting : 15 –16th of October, Budapest, Hungary

ISO Technical committee on tobacco and tobacco product(TC 126), working group 9

ISO TC 126 WG 9	
Purpose	Development of the ISO machine smoking method
Activity	◆ Accepting WHO's recommendation, TC 126 set up WG 9 in 2004 ◆ To review worldwide human smoking behavior, and to advise a robust and practical machine smoking method ◆ Meeting was held three times in 2005 ◆ Final report submitted to TC 126 on March, 2006 (WG 9, N 047) ◆ Not reach a consensus on a single new machine smoking method (propose two alternative methods)

ISO Technical committee on tobacco and tobacco product(TC 126), working group 10

ISO TC 126 WG 10	
Purpose	Development of the ISO machine smoking method Facilitate the exchange of information between members of TC 126 and the public health sector
Activity	◆ Succeed to work of WG 9 ◆ Convener : BSI (British Standards Institute), UK ◆ Invite WHO to participate with their technical experts ◆ No draft standard is expected to be presented until the future method proposal of WHO ◆ To initiate the work in accordance with Resolution 273 and to start with the preparation of a draft Standard based on the UK proposal

Processing for the development of new ISO machine smoking method

WHO TobReg request ISO TC126 to modify the ISO machine Smoking standard for measuring cigarette emissions(2004)

↓

ISO set up Working Group 9 under TC 126

↓

Three times WG meeting was held in Geneva in 2005 and proposed 8 resolutions & 2 alternative methods

↓

26th TC 126 meeting was held in Las Vegas on May, 2006 and BSI proposed the voting for new smoking methods

↓

Many members(16/23)of TC 126 accepted Option B for new smoking methods(N836)

↓

WHO requested ISO to delay the work on developing a new smoking regime

↓

TC 126 has not yet decided on a specific smoking regime and is reviewing proposed options with the assistance of WHO

General processing for the development of new ISO standard

Process of Project	Reference		Note
	Class	Abbreviation	
Proposal stage	New propose	NP	Proposing a new work and criteria for its acceptance
Preparatory stage	Draft of WG	WD	Project represented during the preparation of the working draft
Committee state	Draft of TC	CD	Circulated a working draft to the members of TC(3 months)
Enquiry stage	Draft of Enquiry	DIS	Circulate to all national bodies for 4-months vote
Approval stage	Final draft of standard	FDIS	Circulate to all national bodies for 4-months vote
Publication stage	International Standard	ISO	Print and distribute the new standard for 2-month after passing

Purpose of TC126 WG 9 meeting

- Identify one or more sets of values for smoking machine parameters for an additional method
- Propose a smoking method more relevant to smoking behavior which could reflect maximum yields
- The exist ISO methods should be retained and evaluated as giving lower yields

Reason to make new smoking standard

- The degree of human exposure measured by standard machine smoking methods is underestimated for low-yield cigarettes because smokers tend to compensate by increasing both puff frequency and the volume of smoke inhaled.
- ISO/FTC protocol also does not take into account the important aspect of the compensatory smoking behavior of blocking the ventilation holes of ventilated or low-yield cigarettes.

Problems to set new smoking standard

- There is a large diversity in smoking behavior in smokers
- Complex relationship exists between puffing parameters, smoke constituent yields, smoke intake (i.e., mouth level exposure), and smoke constituents uptake
- A new smoking regime which is more relevant to smoking behavior should consider guidance from data on smoking topography, smoke intake, and smoke uptake

Rationale for machine smoking testing of cigarettes (Resolution No. 6)

- No machine smoking regime can represent all human smoking behaviours
- Methods are recommended which test the product under conditions of different intensities of machine smoking testing on order to collect main stream smoke
- Machine smoking testing is useful to characterise cigarette emissions for design and regulatory purpose
- Smoke emission data from machine measurements may be used as inputs for product hazard assessment, but they are not intended to be nor are they valid measures of human exposure or risks

Proposal for alternative machine smoking methods for cigarettes (WG 9, Resolution No. 7)

- A majority of 23 supported *Method B* as referred to in the report*
- Minority of 9 supported the *Canadian intense Regime*
- There were two abstentions

* *"A review of human smoking behaviour data and recommendations for a new ISO standard for the machine smoking for cigarettes"*

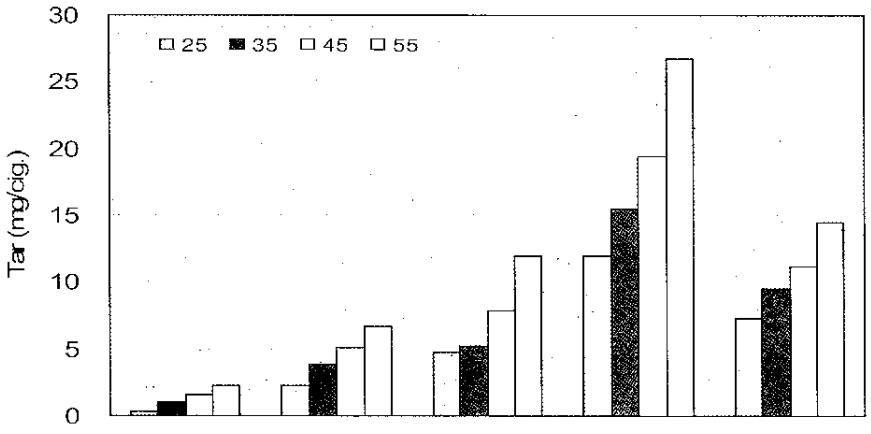
Reasons of TobReg recommended the Canadian Intense smoking regime

- The Canadian Intense provides information on the maximum amount of smoke achievable by and smoker
- TSNA yields are less variable under the Canadian Intense Regime than under the ISO smoking Regime
- There is more breakthrough of volatiles from the carbon filters under the Canadian Intense regime than with the ISO smoking Regime

Primary interest : Puffing parameters

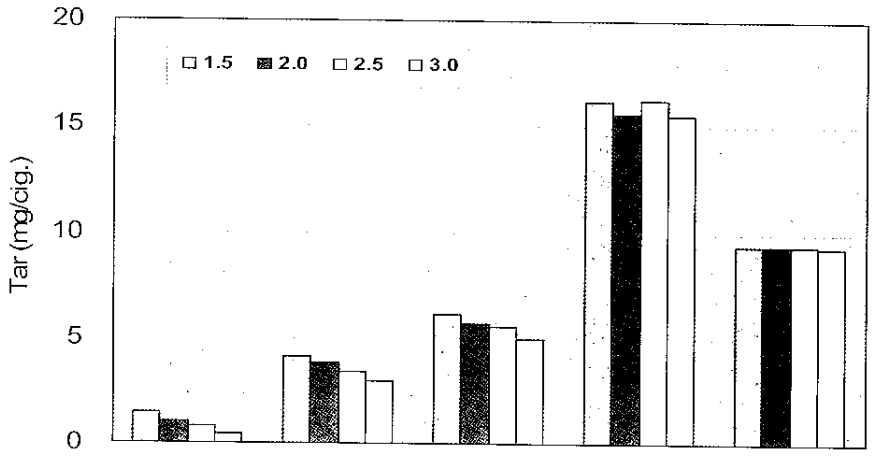
- puff volume (individual and total)
- Puff duration
- puff interval
  - Number of puffs

Changes of tar delivery yields by puff volume



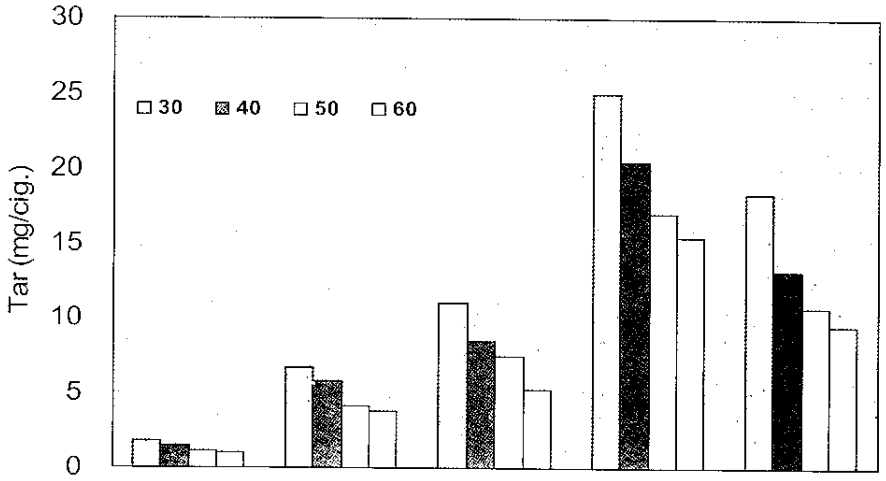
- From 2 to 10 times different yields with puff volume

Changes of tar delivery yields by puff duration



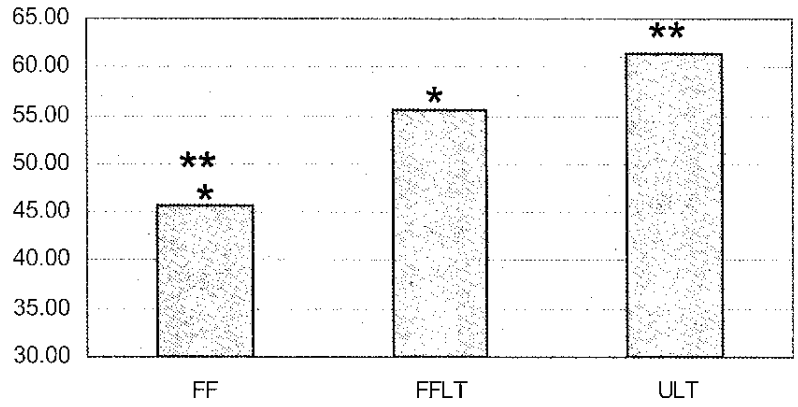
- Low tar cigarettes show a big change of tar yields with different puff duration

Changes of tar delivery yields by puff interval



- Decrease tar yields with increasing puff interval

Average Puff Volume (mL)



- Some studies find that smoking behavior varies with ISO / FTC "tar" yields.

Robinson, J.H., Ogden, M.W., Borgerding, M.F., Byrd, G,D., Heavner, D.L., Morgan, W.T., Stiles, M.F. (2004) Multiple Measures of Variability (Part 1): Experimental design and human smoking behavior endpoints. Paper presented at *CORESTA Congress*, Kyoto, Japan, (Abstract SS02).

Summary of topography by decade of publication

	Number of Data Sets	Mean Number of Puffs	Mean Puff Volume (mL)	Mean Puff Interval (s)	Mean Total Puff Volume (mL)
All data	100	13.2 ± 2.6	48.3 ± 10.8	25.7 ± 8.1	657 ± 197
1970-1980	4	10.9 ± 1.1	38.5	36.8 ± 8.7	n/a
1981-1990	38	13.7 ± 2.7	44.9 ± 12.4	25.7 ± 7.9	568 ± 158
1991-2000	38	12.5 ± 2.5	50.0 ± 9.7	26.8 ± 8.6	673 ± 208
2001-2005	16	14.6 ± 2.0	53.0 ± 7.9	21.4 ± 1.9	784 ± 166

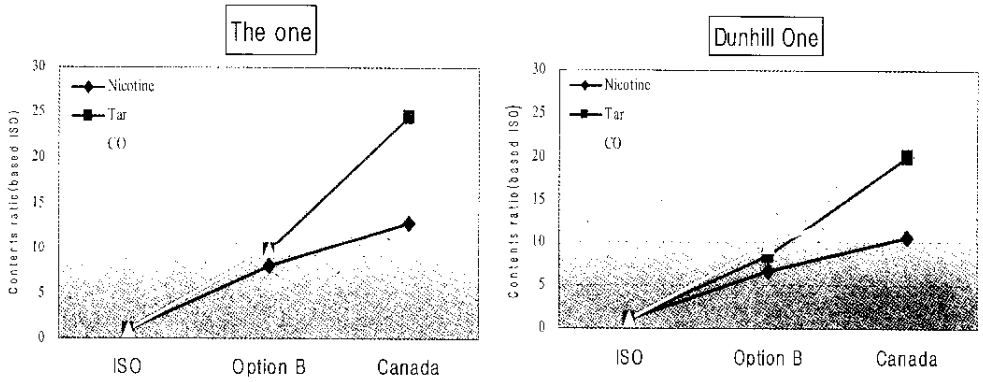
*** Matthias K. Schorp, PMI (2005)**

Changes of smoke delivery on three smoking regimes

Testing smoking condition

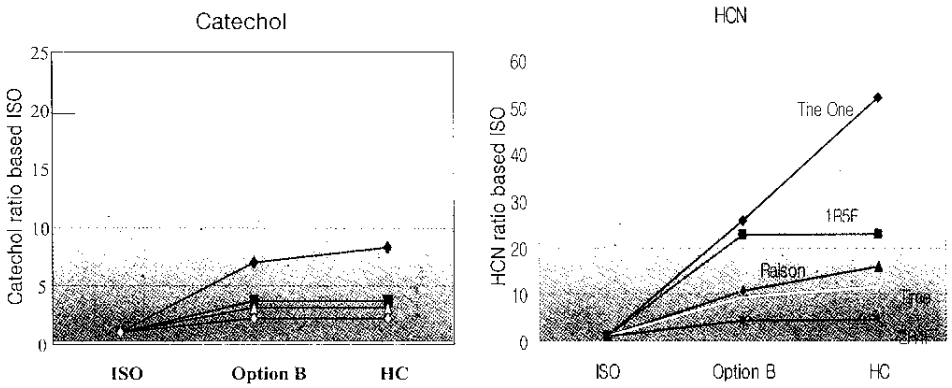
Smoking Regime	Volume (ml)	Interval (sec)	duration (sec)	Blocking (%)	비 고
ISO	35	60	2	0	ISO standard
Option B	60	30	2	50	TC 126 proposed
Health Canada	55	30	2	100	WHO recommend

Changes of Tar, nicotine, and CO on three smoking regimes (Based on 1mg of ISO)



- In case of low tar cigarette, the smoke deliver with difference smoking condition was increase.
- The maximum difference between smoking condition was 25, 18, and 13 times of tar, CO, and nicotine, respectively.

Changes of Hoffmann Analytes on three smoking regimes



- Increasing rate of HCN was higher than catechol with different smoking condition
- HCN was sharply increased on strength smoking condition.

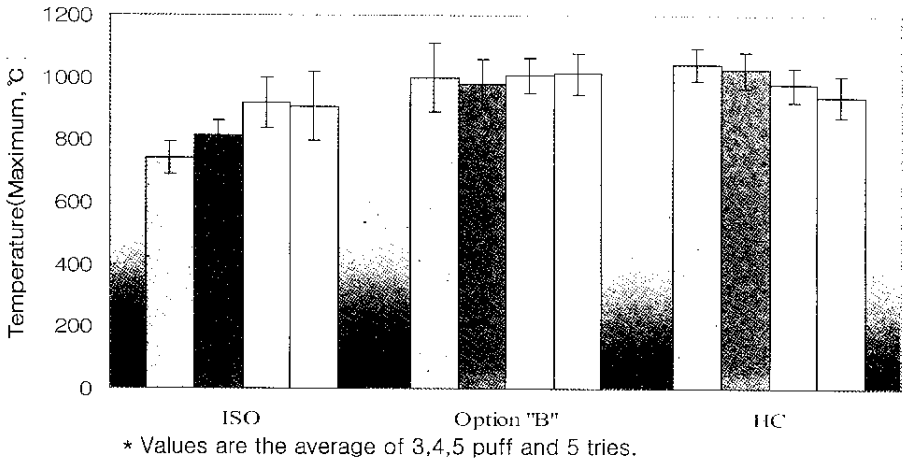
Changes of Hoffmann's Analytes based on 1 mg tar on three smoking regimes

ULT	Catechol	NNN	B[a]P	HCN	Acetaldehyde	1,3-Butadiene	Benzene
ISO	8.02	38.99	2.16	5.71	39.32	4.24	3.14
Option B	7.26	28.25	0.91	20.06	67.68	5.24	5.39
Canada	3.00	15.99	0.60	14.06	41.43	3.94	2.85

UL	Catechol	NNN	B[a]P	HCN	Acetaldehyde	1,3-Butadiene	Benzene
ISO	7.31	26.56	1.05	6.37	52.54	5.30	5.46
Option B	5.68	15.32	0.97	14.59	52.01	4.54	4.76
Canada	3.16	9.51	0.48	16.58	39.62	3.64	2.71

L	Catechol	NNN	B[a]P	HCN	Acetaldehyde	1,3-Butadiene	Benzene
ISO	6.61	26.65	1.21	6.33	51.73	4.93	4.83
Option B	4.83	14.91	0.73	14.16	43.36	3.47	3.12
Canada	3.38	10.55	0.50	10.47	35.52	3.23	2.69

Changes of cigarette cone temperature on three smoking regimes



* Values are the average of 3,4,5 puff and 5 tries.

- Burning temperature on ISO condition was a little lower than other smoking condition

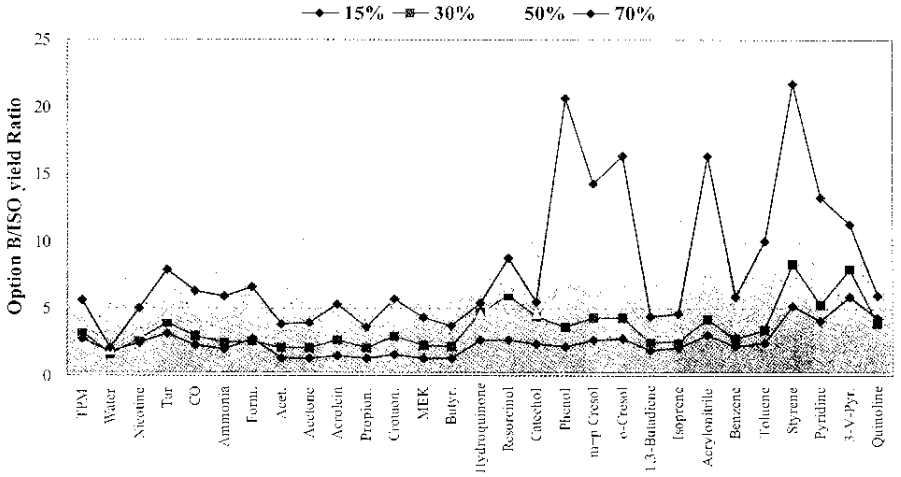
Relationship between leaf components and smoke components on three smoking regimes

ISO	Option B	Canada
Nicotine : NNK, NAT Chloride : MEK, Styrene, Nicotine CO, NNK, NAT, HCN Sucrose : Isoprene, Styrene NNK, NAT	Nicotine : Isoprene, Tolulene Styrene, Acrylonitrile Pyridine, NNN, NNK NAT, HCN Total N : Resorcinol NO_3 : Resorcinol, NAT NH_3 : Formaldehyde, Resorcinol Phenol, *m+p*-Cresol Cl : TSNA TVB : Resorcinol, Phenol Ether ext : NNN, NAB Malic acid : CO, NNN, HCN Sucrose : Isoprene, TSNA Glycerine : TSNA등	Nicotine : Acetaldehyde, Acetone, MEK Propionaldehyde, Bytyraldehyde Benzene, Tolulene, CO, NNK NAT, NAB Total Sugar : Resorcinol, Isoprene, Acrylonitrile Total N : Formaldehyde, Hydroquinone Resorcinol NO_3 : Hydroquinone, Isoprene Resorcinol, 1,3-butadiene NH_3 : Formaldehyde, Phenol, Cl : Acetone, MEK, Butyraldehyde Benzene, Pyridine, Quinoline, CO NNK, NAT TVB : Form., Resorcinol, Phenol
18 components	**62 components**	**126 components**

* Statistical analysis : 95% confidence limit $p < 0.05$

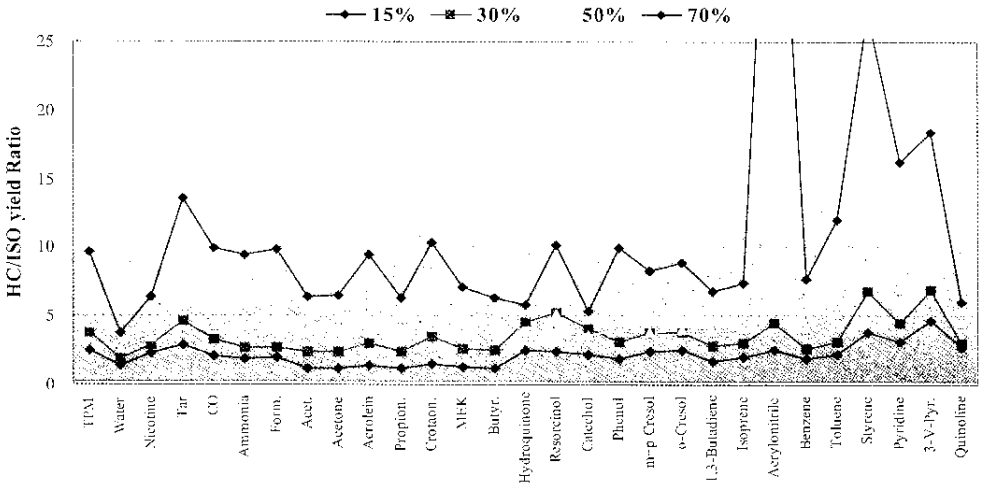
- Increase the relationship between leaf and smoke component on Canada smoking condition

Comparison of Hoffmann's analytes with a different ventilation rate on Option B and ISO smoking regimes



- As increasing ventilation rate, phenol compounds, volatile compounds were increased on Option B smoking condition

Comparison of Hoffmann's analytes with a different ventilation rate on Canadian and ISO smoking regimes

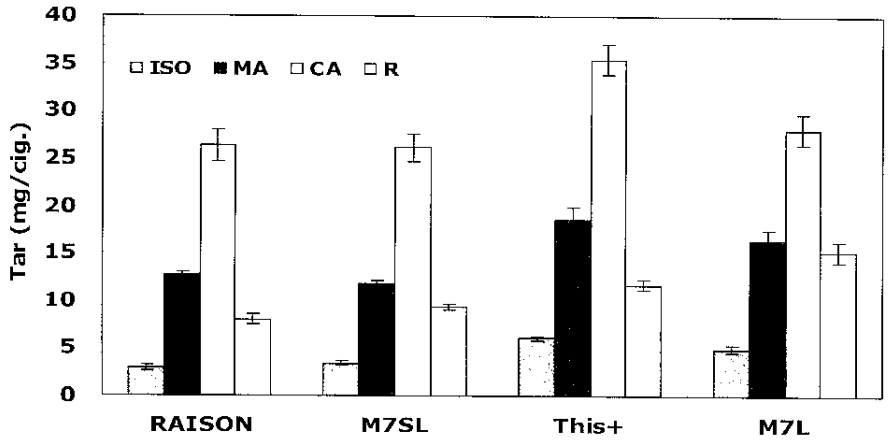


- As increasing ventilation rate, volatile compounds were increased on Health Canada smoking condition

Puffing parameters of international smoking regimes and Korean real smoking

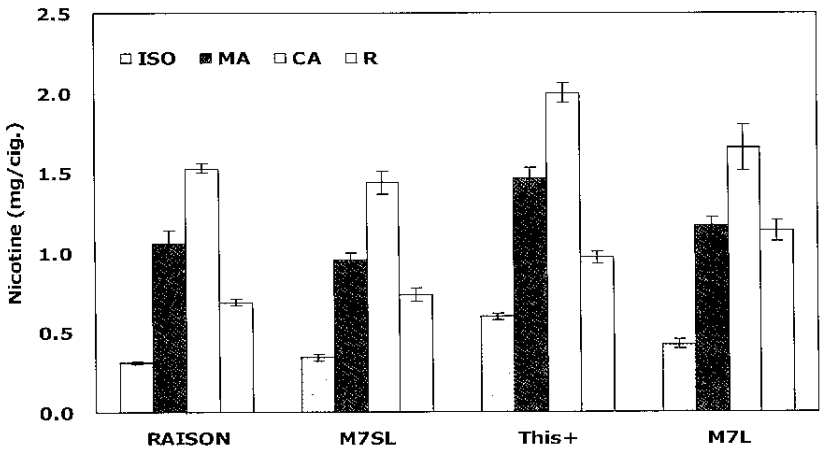
Parameters	ISO/FTC	Massachu-setts	Health Canada	Real smoking
Puff volume	35 mL	45 mL	55 mL	47 mL
Puff interval	60 sec	30 sec	30 sec	20 sec
Puff duration	2 sec	2 sec	2 sec	2.2 sec
Ventilation hole	Unblocked	50% blocked	100 % blocked	-
Butt length	Tipping + 3 mm	Filter + 3 mm	Tipping + 3 mm	46 mm

Changes of tar concentration with different smoking regimes



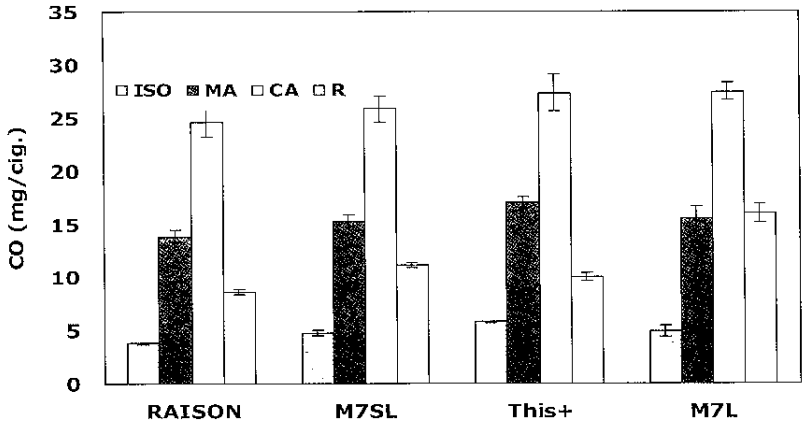
- Real smoking behavior shows similar smoke delivery with Massachusetts regimen

Changes of nicotine concentration with different smoking regimes



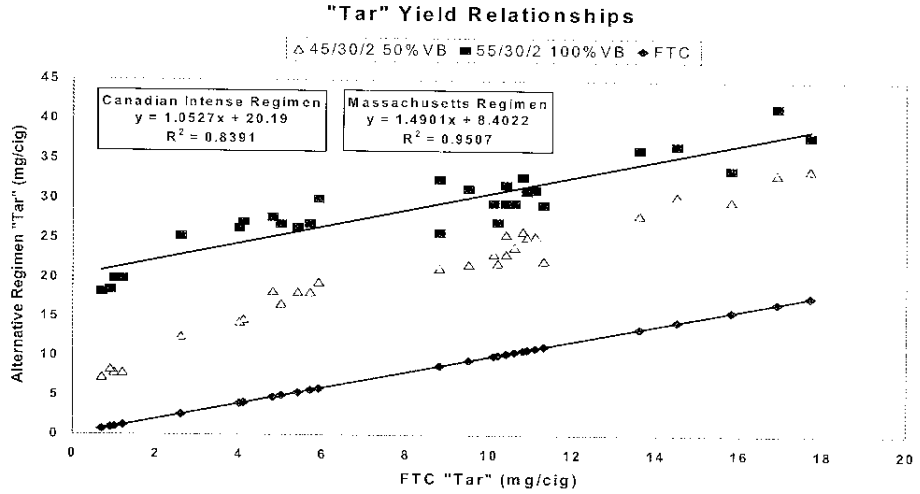
- Real smoking behavior show similar smoke delivery with Massachusetts regimen

Changes of CO concentration with different smoking regimes



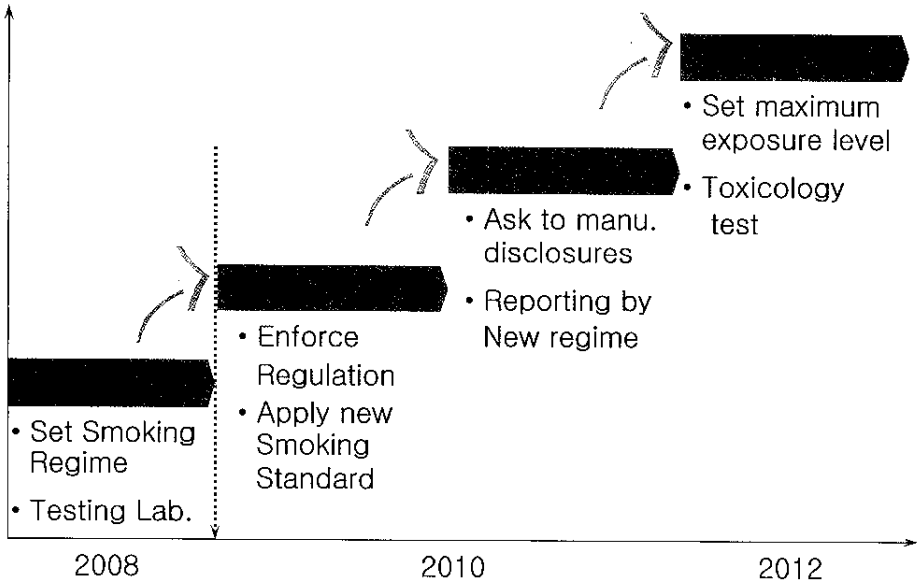
- CO show a similar smoke delivery with different smoking regimen

Changes of tar concentration with different blend of cigarettes and smoking regimes



M. F. Borgerding(R.J. Reynolds Tobacco Company 2005)

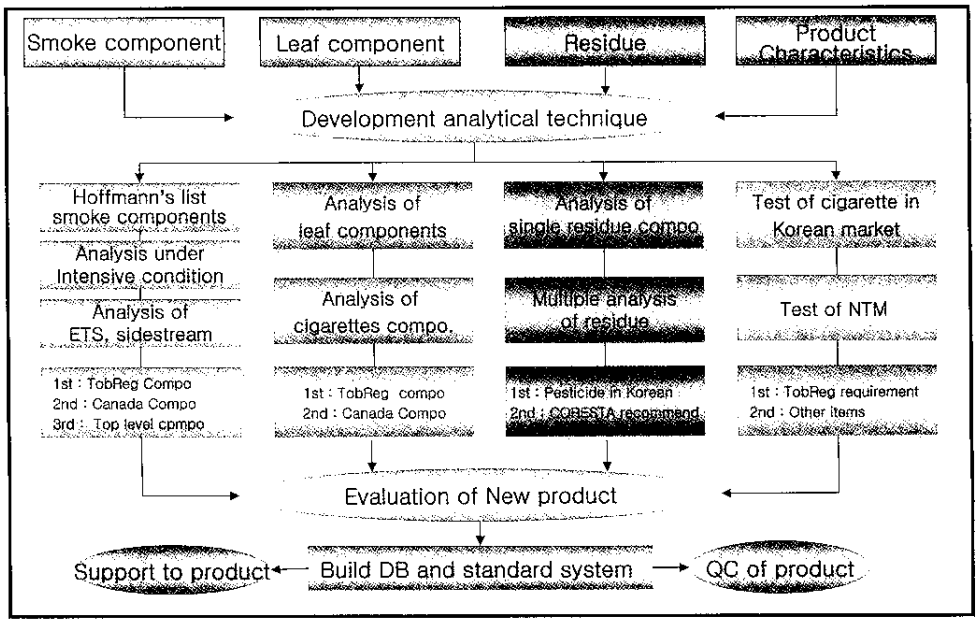
Roadmap of scientific regulation by new intensive smoking regime



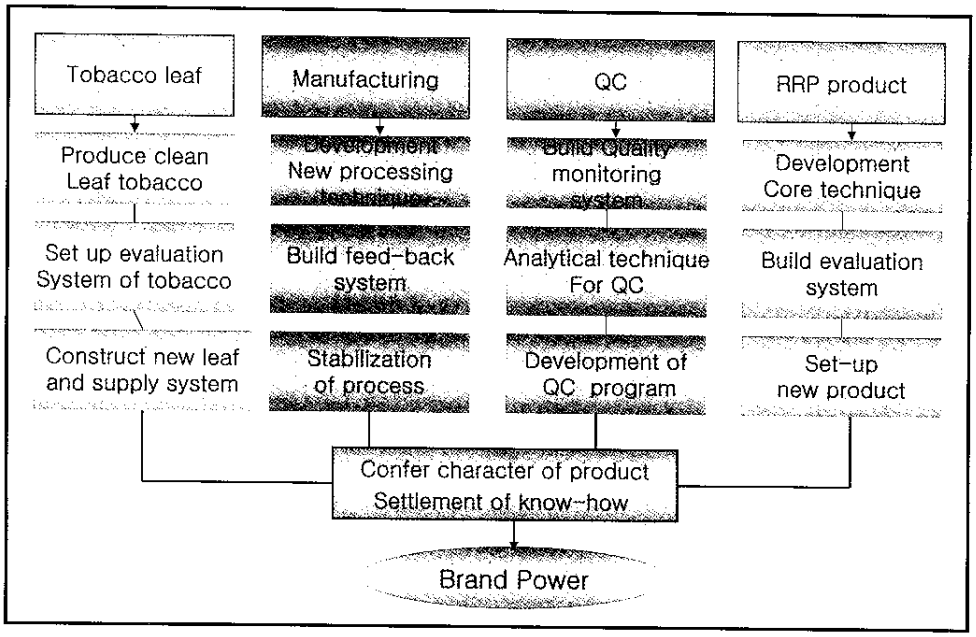
Scientific defense strategy for new smoking regime

Field	Strategy
Analysis	• Set up analysis Lab to fit intensive smoking regime • Research and development of analytical method for mainstream & sidestream • Build DB of market products • Coordination between Labs in the countries
Product	• Produce clean leaf tobacco • Set up new QC &QA system • Development RRP product • Sharing data and information between manufacturers

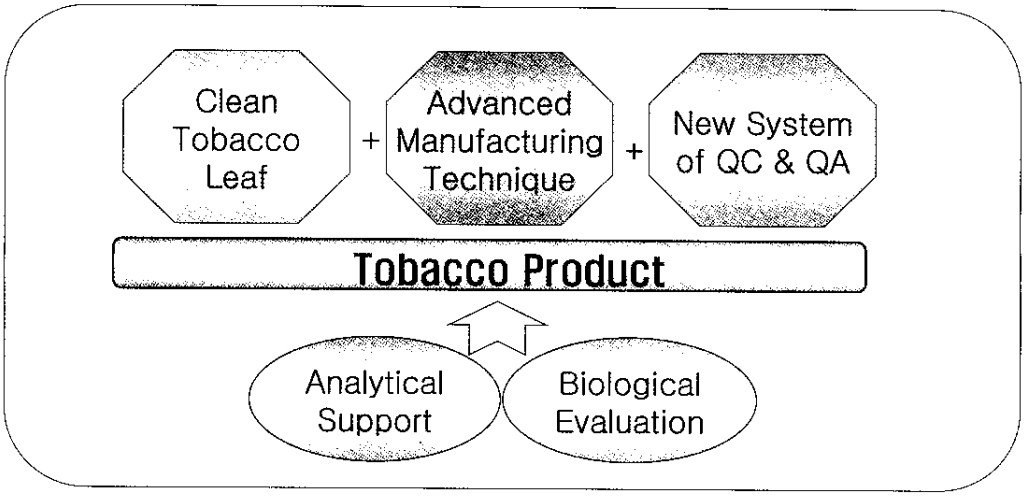
Development of analytical technique



Development of new products



Tobacco manufacturing after new regulation



Poster Presentation

1. Analysis of acrylamide in mainstream cigarette smoke and effects of reducing sugars on acrylamide content

2. Development and validation of an HPLC/UV method for simultaneous determination of 9 organic compounds

3. Evaluation of the behaviour of the anion in RECON extract by various adsorbents

4. Study of the pyrolysis pattern and the transfer rate of the organochlorine pesticide in tobacco

5. Characteristic of analytical techniques for hydrogen cyanide in mainstream smoke

6. Toxicity assessment of gas phase cigarette smoke using cell-free method

7. Adsorption behaviour of propylamine on activated carbon fibre surfaces as induced by oxygen functional complexes

8. The scavenger effects of various antioxidants in cigarette filters on the free radicals in mainstream smoke

9. Simulation of hot air fluid flow in drying precess using CFD

10. Determination of phenols in mainstream cigarette smoke by isotope dilution liquid chromatography/electrospray ionization tandem mass spectormeter

11. Development of single-tube immunocapture RT-PCR assay for early selection of resistance to *Potato virus Y* in *Nicotiana tobacum* L.

12. Breeding of low nicotine burley tobacco KB0201-10 with PVY and black shank resistance and its agronomic characteristics

POSTER 1

Analysis of acrylamide in mainstream cigarette smoke and effects of reducing sugars on acrylamide content

KIM Ick-Joong; LEE John-Tae; MIN Hye-Jeong; KIM Hyo-Keun; HWANG Keon-Joong

KT&G Central Research Institute, 302 Shinseong-Dong Yuseong-Gu, Daejeon, Rep. of Korea.

Acrylamide has been found in many foods in our everyday life. Acrylamide levels in foodstuffs were analyzed by a GC/MS after bromination of acrylamide and by a LC/MS for underivatized acylamide. In these methods, various clean up procedures are applied for the purification of the extract.

In this study, a simple and fast method for analysis of acrylamide in mainstream cigarette smoke, without the clean up step, was developed and the effects of reducing sugars on acrylamide content were observed. The analysis of acrylamide in mainstream cigarette smoke started by collecting TPM from smoking and extracting with 0.1% acetic acid solution and then detecting by liquid chromatography tandem mass spectrometry using electrospray in the positive mode. The recovery of acrylamide in 2R4F reference cigarette was 97.7% and the reproducibility was 2.5% and the limit of detection was 6.4 ng/cigarette.

Reducing sugars are considered to be the main precursors of acrylamide in foodstuffs. Cut tobacco contains substantial amounts of the reducing sugars, which may explain the occurrence of acrylamide in mainstream cigarette smoke. The effects of reducing sugars was studied in an experiment with a range of tobacco grades. The result indicated that level of reducing sugars in cut tobacco was linearly correlated to acrylamide content in mainstream cigarette smoke.

POSTER 2

Development and validation of an HPLC/UV method for simultaneous determination of 9 organic compounds

KA Mi-Hyun; KIM Mi-Ju; CHO Sung-Eul; KIM Yong-Ha; MIN Young-Keun

KT&G Central Research Institute, 302 Shinseong-Dong Yuseong-Gu, Daejeon, Rep. of Korea.

Humectants and preservatives are added to cigarettes to delay microbiological, enzymatic and chemical deterioration and to extend shelf life. This study was conducted to establish a simple and rapid method for determining preservatives that can be used in the cigarette manufacturing process. Nine preservatives were examined: dehydroacetic acid, sorbic acid, benzoic acid, methylparaben, ethylparaben, isopropylparaben, propylparaben, isobutylparaben and butylparaben. The preservatives were separated and detected simultaneously by reversed-phased high-performance liquid chromatography (HPLC)/UV-visible spectrophotometer (UV) methods. The samples were extracted with methanol and centrifuged and then filtered. The clear filtrate was analyzed by the HPLC. The analysis was carried out by isocratic elution (acetonitrile: methanol: 0.005 M CTA buffer=15: 35: 50) at pH 4.6 and wavelength of the detector was set at 254 nm. Under these conditions, nine preservatives were separated within 40 min and this method was applied to the determination of preservatives in cigarettes. The limit of detection (LOD) and limit of quantification (LOQ) were 0.12~1.20 μg/g and 0.23~5.83 μg/g, respectively. Linearity, recovery rate, repeatability and reproducibility of this method were also validated. As compared with Health Canada Method (T-313), this developed method can be used to simultaneously determine the nine preservatives and offers a simplified pre-treatment procedure, fast analysis time and excellent validation results.

POSTER 3

Evaluation of the behaviour of the anion in RECON extract by various adsorbents

HAN Young-Rim; SUNG Yong-Joo; BAEK Shin; KIM Kun-Soo; RHEE Moonsoo

KT&G Central Research Institute, 302 Shinseong-Dong Yuseong-Gu, Daejeon, Rep. of Korea.

The objective of this study is to evaluate the behaviour of anions in RECON extract by various adsorbents and several ion exchange resins using ion chromatography. The determination of ionic species in solution is a classical problem with a variety of solutions. Conventional wet-chemical methods such as titration, photometry, gravimetry, turbidimetry and colorimetry are all labour-intensive,

time-consuming, and occasionally troublesome. In contrast, ion chromatography offers the following advantages: speed, sensitivity, selectivity and simultaneous detection. The isocratic ion chromatographic method to separate and determine main ions in RECON extract was applied. The method allows the separation of anions on Dionex Ion Pac AS18 column by KOH elution and conductometric detection. The anions such as fluoride, chloride, nitrite, sulfate, bromide and nitrate were separated within 20 minutes. In this study, various adsorbents such as activated carbon, wood charcoal and ion exchanger resins were applied. The experimental parameters such as amount of adsorbent, contact time, initial anion concentration and extract concentration were tested. The composition of ions was changed depending on the properties of adsorbents. The main anions adsorbed by the activated carbon resulted in decreases of 10% in chloride ions, 10% in sulfate ions and 25% in nitrate ions. The concentration of ions was also affected by the addition amount of adsorbent and concentration of RECON extract. The optimal condition of adsorption, especially for the nitrate anion was discussed in terms of the amounts of the adsorbent addition and concentration of RECON extract.

POSTER 4

Study of the pyrolysis pattern and the transfer rate of the organochlorine pesticide in tobacco

MIN Hye-Jeong; JANG Seok-Su; KIM Ick-Joong; KIM Yong-Ha; MIN Young-Keun

KT&G Central Research Institute, 302 Shinseong-Dong Yuseong-Gu, Daejeon, Rep. of Korea.

GRLs (Guidance Residue Levels) are recommended by the CORESTA ACAC guide. In the GRLs list, organochlorine group is one of pesticide commonly used on tobacco cultivation. In this model study, the quantitative correlation between transfer rate of pesticide residue into tobacco smoke and pyrolysates was investigated by spiking cigarettes with organochlorine pesticides. The spiking concentration referred to the range of GRLs list and the organochlorine pesticides in mainstream smoke were analyzed by GC-MS and gas chromatography with selective detector (ECD). To understand the composition variation versus temperature, the behaviour of pesticides were investigated by pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). In this study, the transfer rate of pesticide residue into tobacco smoke at each spiking concentration and the composition of pyrolysates were analyzed differently. At low concentrations, pesticides in smoke were not detected by pyrolysis. At high concentrations, organochlorine pesticides were transferred into tobacco smoke in 2~10% each of component and most of pesticides were pyrolyzed during smoking. It was found that the decomposition compounds from organochlorine pesticides were composed of oxygenous and nitrogenous compounds.

This study could estimate that transfer rate of pesticides into tobacco smoke at the range of GRLs concentration recommended by the CORESTA ACAC is a very small amount.

POSTER 5

Characteristic of analytical techniques for hydrogen cyanide in mainstream smoke

LEE John-Tae; KIM Hyo-Keun; HWANG Keon-Joong; LEE Kyung-Gu; JANG Seok-Su

KT&G Central Research Institute, 302 Shinseong-Dong Yuseong-Gu, Daejeon, Rep. of Korea.

Hydrogen cyanide (HCN), formed from pyrolysis of various nitrogenous compounds such as protein, amino acids and nitrate in tobacco, is present in both the particulate phase and vapor phase of cigarette smoke. Typically the determination of HCN in cigarette smoke has been done through colorimetric and electrochemical techniques, such as fluorescence spectrometry, spectrophotometry (UV), continuous flow analyzer (CFA), capillary GC-ECD and ion chromatography (IC). The general procedure for determining HCN in cigarette smoke involves the collection of mainstream smoke through impingers filled with trapping solution and Cambridge filter pad. Most of these techniques are time-consuming and some lack specificity or sensitivity. The available results from both internal testing and reported literatures for 2R4F Kentucky reference cigarette, smoked under ISO conditions, showed a relatively wide variation ranging from 100 to 120 μg/cig of HCN. Especially, the precision and accuracy of the analytical result of HCN tended to get worse in low tar cigarettes and under intense smoking conditions.

This paper suggests an optimized analytical method including the modification of previously used methods to obtain lower detection limits and to improve accuracy and precision and therefore is applicable for a wide range of tar levels in cigarettes under ISO and intense smoking conditions. This method includes improved sample collection and quantification systems such as the number of absorption tubes, the type of extractant and reaction time for colour development. The pH of collection solution is over 11 to avoid volatilization loss of HCN and using a cooled trap to collect HCN were the recommended conditions to analyze HCN in mainstream smoke. Between seven and twelve minutes is the best effective reaction time from the addition of the colour reagent to the formation of cyanogens chloride almost reaching a maximum.

POSTER 6

Toxicity assessment of gas phase cigarette smoke using cell-free method

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In vitro toxicity tests such as cytotoxicity, mutagenicity and genotoxicity assay are useful for evaluating the relative toxicity of smoke or smoke condensates from different cigarette configurations. A major disadvantage of these tests as toxicity screening methods for tobacco product test and development is that they are relatively time-consuming and expensive. Recently, a cell-free glutathione consumption assay (GCA) as a rapid and simple screening method for the toxicity assessment of smoke has been reported by Cahours X. *et al.* (CORESTA, 2006). This study was performed to assess the capability of GCA to predict the toxicity of gas phase cigarette smoke (GVP) and to further identify individual compounds responsible for the GSH consumption. Three types of cigarettes such as 2R4F, charcoal filter cigarette (CFC), and new charcoal filter cigarette (NCFA) were evaluated by using GCA method and Neutral Red Uptake assay. The carbonyl compounds, which may contribute to toxicity, were also measured and the GSH consumption by these compounds was individually observed. The overall order of toxicity using GCA method was 2R4F > CFA > NCFA, which was consistent with the result of Neutral Red Uptake assay. The levels of carbonyl compounds of NCFA were lower than those of 2R4F and CA, indicating that GSH consumption was associated with carbonyl compound yields. A major toxicant under current study is acrolein, which contributed to more than half of the GSH consumption. Collectively, the toxicity of GVP determined by GCA method may be mainly attributed to acrolein.

POSTER 7

Adsorption behaviour of propylamine on activated carbon fibre surfaces as induced by oxygen functional complexes

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In this study, the surfaces of activated carbon fibres (ACFs) were modified by nitric acid to introduce surface oxygen complexes and to observe the influence of those complexes on the propylamine adsorption of the ACFs. It was found that the oxygen complexes including carboxylic and phenolic groups were predominantly increased, which resulted in the increase of total surface acidity. However, the specific surface areas ($\mathbf{S_{BET}}$) and the total pore volumes ($\mathbf{V_t}$) of the modified ACFs were decreased

by 5-8% due to the increased blocking (or demolition) of micropores in the presence of newly introduced complexes. Despite the decrease of textural properties, it was found that the amount of propylamine adsorbed by the modified ACFs was increased by approximately 17%. From the XPS results, it was observed that propylamine reacted with strong or weak acidic groups, such as COOH or OH, on the ACF surfaces, which resulted in the formation of pyrrolic-, pyridonic-, or pyridine-like structures.

POSTER 8

The scavenger effects of various antioxidants in cigarette filters on the free radicals in mainstream smoke

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Cigarette mainstream smoke is a complex mixture upto 4,000 chemical constituents. It is well known that the cigarette smoke can contain high concentrations of free radicals which may bind to DNA and could lead to damage of cells. This study was conducted to evaluate the effect of additives (antioxidants for free radicals reduction) in cigarette filter with various antioxidants (synthesized antioxidant : ascorbic acid, natural antioxidants : 3 species) on the delivery of free radicals of MS by ESR. Also, we analyzed Hoffmann analytes, scavenger activity according to the storage time and *in-vitro* mutagenicity and cytotoxicity. The analysis of spin number of vapor and particulate phase free radicals in MS are decreased to 14~24% and 16~40%, respectively. In result of the antioxidant potential for inactivity of vapor and particulate phase free radicals, natural antioxidants were more effective than that of ascorbic acid. Based on the result for the analysis of Hoffmann analytes for various antioxidants treated cigarette filters during the smoking, cigarette filter treated with ascorbic acid showed the lower amount of the deliveries of Hydroqiunone, isoprene and quinoline in MS than that treated with the other antioxidants. In the t-test of significant on the difference for the mutagenicity and cytotoxicity among the various antioxidant treated-cigarette filters, there are no significant differences at the 95% confidence level. Those results indicated that the antioxidants were useful for reducing free radical in MS because of the fast reaction between antioxidant and free radical in MS.

POSTER 9

Simulation of hot air fluid flow in drying precess using CFD

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CFD(Computational Fluid Dynamics) is applied to optimization of operating condition, analysis of fluid flow, diagonosis and improvement of process. Using CFD, the fluid flow of cut tobacco and hot-air in drying process was analyzed. Based on the result of the analysis, the main factors that enlarged the deviation of output moisture content in drying process was recycling area which was observed the upper part of cylinder. Recycling area was caused by unbalance of impinged hot-air. Namely, hot-air was mainly impinged to cylinder through the upper and lower part of cut outs. Also, it was simulated flow of hot air in the dryer according to input velocity(6~8m/sec). Hot-air velocity was not main factor that enlarged the deviation of output moisture content in drying process. To improve the efficiency of drying process, a mathematical model was developed to predict heat transfer phenomena in drying tobacco with impinging hot air flow.

POSTER 10

Determination of phenols in mainstream cigarette smoke by isotope dilution liquid chromatography/electrospray ionization tandem mass spectrometer

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A new technique for the analysis of dihydroxybenzenes in mainstream cigarette smoke has been developed. Isotope dilution analysis for the quantitative determination of the catechol, 3-methylcatechol and 4-methylcatechol in mainstream cigarette smoke was stable. This method used isotope dilution liquid chromatography which coupled to a tandem mass spectrometer (LC/MS/MS) with electrospray ionization(ESI) device running in nagative ionzation mode and It was significantly more sensitive than previous used methods. For the analysis of catechol, 3-methylcatechol and 4-methylcatechol in mainstream cigarette smoke, a high performance liquid chromatography (HPLC) method with automated procolumn sample preparation was developed.Mainstream smoke was collected on a cambridge filter pad and then extracted with a 50% methanol in water solution. Analysis was performed using reversed-phase HPLC under gradient mode. Eluent A was 0.5% formic acid in methanol and eluent B was 0.5% formic acid in water. The application of stable isotopomers of analytes is known to enable the correction

of compound discrimination during extraction, cleanup, chromatographic separation and mass spectrometry. This new method showed that quantitative analysis of the target compounds was possible within 25min and provided quantitative data on the amounts of dihydroxybenzenes in mainstream smoke. Compare with previous method, this new method showed high accuracy, precision, and robustness.

POSTER 11

Development of single-tube immunocapture RT-PCR assay for early selection of resistance to *Potato virus Y* in *Nicotiana tobacum* L.

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An immunocapture reverse transcription-polymerase chain reaction (IC/RT-PCR) based assay was developed for the detection of *Potato virus Y* (PVY), infecting tobacco plants. The assay could be performed in a single tube for simultaneous and sensitive detection of PVY. This detection system revealed thousand-fold increase in detection sensitivity compare to ELISA. This method could save the time and reagent cost compared with a common RT-PCR which needs several reactions and several procedures of viral RNA extractions for the same number of test plants. PVY resistant plants could be selected 7days, 10days and 15 days after inoculation with PVY construct by IC/RT-PCR, ELISA, and bioassay, respectively.

POSTER 12

Breeding of low nicotine burley tobacco KB0201-10 with PVY and black shank resistance and its agronomic characteristics

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Nicotine content is known to be a key factor of smoking taste and smokers who are concerning about their health prefer low nicotine cigarettes. Effective methods for reducing nicotine have been studied on the cigarette manufacturing process. However, the nicotine content in tobacco is affected by genetics, environmental conditions and cultural practices. As the cured leaves under 0.5% of nicotine content have poor smoking taste, it is necessary to breed a new variety which has 1-2% of nicotine content with

less unfavorable substances. The new burley tobacco, KB0201-10, was developed by crossing LAburley21 and a breeding line KB110 with PVY resistance. KRAFT's drop test method for rapid estimation of alkaloid content and isatin coloration method for identifying nicotine converters of tobacco were used in the selection procedures, respectively. The agronomic traits of KB0201-10 were very similar to those of KB108 as standard cultivar. It showed vigorous growth in the field and was almost the same as KB108 in days to flower. The yield of cured leaf was approximately 1-2% less than KB108 but it's physical characteristics were very similar to those of KB108. KB0201-10 was also resistant to PVY and black shank as well.