Chemical Composition, In Vitro Toxicity, and Theoretical Risk Estimates of Smoke from Different U.S. Blended Cigarettes

Roemer, E.[1]*, Roethig, H.-J.[2], Haussmann, H.-J.[1], [1]PHILIP MORRIS Research Laboratories GmbH, Cologne, Germany; [2]PHILIP MORRIS U.S.A., Richmond, VA, U.S.A.

* email: ewald.roemer@pmintl.com

Objective

The objective of this study was to investigate possible relationships between the total particulate matter (TPM*) yield of cigarettes obtained under different sets of smoking conditions and the chemical composition, in vitro toxicity, and theoretical risk estimates of cigarette mainstream smoke.

Concept

Eight conventional commercial U.S. cigarettes, two standard reference cigarettes, and an electrically heated cigarette (EHC) smoking system prototype were evaluated. The cigarettes were machine-smoked under two different sets of smoking conditions. Smoke was analyzed for chemical composition, in vitro cytotoxicity, and in vitro mutagenicity; and theoretical risk estimates for cancer and systemic toxicity were calculated. Regression curves were fitted to the data from the conventional commercial cigarettes to investigate potential relationships. In addition, actual human puffing profiles were determined in adult smokers for two of the conventional commercial cigarettes and the EHC smoking system prototype. Smoke was then machine-generated according to these human puffing profiles and evaluated in the same way.

Materials and Methods

Cigarettes

- Eight conventional commercial U.S. cigarettes representing a cross-section of design parameters.
- Two standard reference cigarettes from the University of Kentucky commonly used to investigate basic characteristics of cigarette smoke.
- An electrically heated cigarette smoking system prototype that produces smoke at distinctly lower burning temperatures than conventional cigarettes (www.ehcss-science.com).

Cigarette	Code	Availability	Tar Category	FTC TPM Yield ± SD (mg/cig)	Filter Type	Tobacco Amount (g/Cig.)	Ventilation (%)
Merit Ultima *	A	conventional commercial	ultra low tar	2.0 ± 0.1	ConP	0.48	67
Virginia Slims Superslims	B	*	*	6.1 ± 0.4	CA	0.46	74
Virginia Slims Ultra Lights	C	*	*	6.7 ± 0.1	CA	0.67	59
Marlboro Lights *	D	*	low tar	12.3 ± 0.2	CA	0.67	25
Benson & Hedges 100's Lights	E	*	*	13.7 ± 0.1	CA	0.83	28
Parliament Lights 100's	F	*	*	14.8 ± 0.2	ReCh	0.82	31
Marlboro	G	*	full flavor	16.4 ± 1.1	CA	0.73	11
Basic	H	*	*	26.6 ± 0.5	none	1.00	0
1R4F	4	research	low tar	10.3 ± 0.3	CA	1.07	30
1R5F	5	*	ultra low tar	2.5 ± 0.2	CA	0.85	70
Electrically Heated Cigarette *	P	prototype		2.4 ± 0.2	CA	N/A	0

Remarks: CA = cellulose acetate, ReCh =recessed charcoal, ConP = concentric paper; MERIT, VIRGINIA SLIMS, MARLBORO, BENSON & HEDGES, PARLIAMENT, and BASIC are trademarks of Philip Morris USA Inc., in the United States.
* = also used to determine human smoking conditions

Smoke Generation

- 20-Port automatic smoking machines (Borgwaldt, Germany)
- Three smoking protocols.
  - U.S. Federal Trade Commission (FTC)
    - 35 ml/puff, 2 s/puff, 1 puff/min, 0% of ventilation holes blocked
  - State of Massachusetts, Department of Public Health (MDPH)
    - 45 ml/puff , 2 s/puff, 2 puffs/min, 50% of ventilation holes blocked
  - Average human puffing profiles (see results)

*TPM = tar + nicotine + water

Human Puffing Profiles

- Determined for Marlboro Lights, Merit Ultima, and the EHC prototype
- Clinical study with 60 healthy adult male and female volunteer smokers (20/group) regularly smoking 10 to 30 cigarettes/day.
- First cigarette of the day and first cigarette after lunch measured over 8 days, number of puffs, puff volume, puff duration, inter-puff interval, and peak flow measured on Clinical Research Support System Micro (CReSSmicro) Plowshare® Technologies, Baltimore, MD.

Smoke Chemistry

- Determination of 49 smoke constituents (Rustemeier et al., 2002).
- Selection of smoke constituents based on two sources:
  - U.S. Consumer Products Safety Commission (1993), with the express purpose of evaluating the impact of cigarette design changes.
  - International Agency for Research on Cancer (1986 and 1999), compounds identified as noteworthy smoke constituents and classified as definite, probable, or possible human carcinogens.
- Validated methods with quantitation and detection limits according to the International Conference on Harmonization (1996).

In Vitro Cytotoxicity

- Neutral Red Uptake Assay according to Borenfreund and co-workers and INVITTOX Protocol 3a (1990).
- Investigation of particle phase (TPM) trapped on filter pads and water solubles of the gas/vapor phase (GVP) trapped by bubbling through phosphate-buffered saline (Roemer et al., 2002).
- Special sensitivity of the assay to chemically irritating substances.
- Assay considered relevant because of the probable link between cytotoxic activity and the promoting activity of cigarette smoke (2-stage concept of carcinogenicity).

In Vitro Mutagenicity

- Salmonella Reverse Mutation Assay according to Maron and Ames (1983) and OECD Guideline No 471 (1997).
- Investigation of TPM trapped on filter pads (Roemer et al., 2002).
- Determinations performed with tester strains TA98 and TA100 in the presence of a metabolic activation system (S9).
- Assay considered relevant because of the probable link between mutagenic activity and the initiating activity of cigarette smoke (2-stage concept of carcinogenicity).

Theoretical Risk Estimates

- Theoretical risk estimates calculated using a model based on the U.S. Environmental Protection Agency approach (U.S. EPA, 1986; Euchenhofer et al., 2003).
- Method especially recommended for comparing complex chemical mixtures.
- Weighting of smoke constituents according to Cal EPA Inhalation Risk Values and Chronic Reference Exposure Levels (Cal EPA, 2002).
- Limitations: not all smoke constituents have risk values or reference exposure levels.

Results

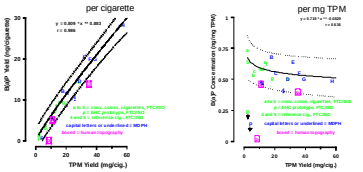
Human Puffing Profiles

- Marlboro Lights: 47 ml/puff, 1.3 s/puff, 1 puff/22 s
- Merit Ultima: 58 ml/puff, 1.5 s/puff, 1 puff/19 s
- EHC: 72 ml/puff, 2.2 s/puff, 1 puff/28 s

Smoke Chemistry

- Yields of selected smoke constituents per cigarette increased with increasing TPM yield (less than proportional).
- Concentrations of the selected smoke constituents in TPM generally decreased with increasing TPM yield (not linear).
- At a range of 5 to 20 mg TPM/cig. (which covers 90% of U.S. cigarettes marketed in 2002 when measured according to FTC conditions), mean decreases were approximately 20%.
- Constituent concentration as a function of TPM yield followed the same relationship irrespective of whether TPM yield was due to smoking conditions (FTC, MDPH, or human puffing profiles) or cigarette design.
- EHC prototype: approximately 90% lower constituent yields (human puffing profile) (N = 13) than regression analysis from conventional cigarettes would suggest.
  Exception: formaldehyde was 64% higher.

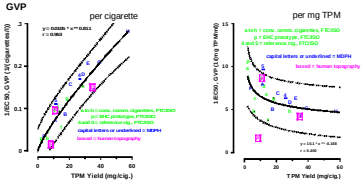
B(a)P



In Vitro Cytotoxicity

- Cytotoxic activity increased with increasing TPM yield (less than proportional).
- Cytotoxic activity per mg TPM decreased approximately 5% (TPM) and 25% (GVP) from a 5-mg to a 20-mg TPM cigarette.
- Cytotoxic activity as a function of TPM yield followed the same relationship irrespective of whether TPM yield was due to smoking conditions or cigarette design.
- EHC prototype: approximately 80% lower cytotoxicity compared to conventional cigarettes with the same TPM yield.

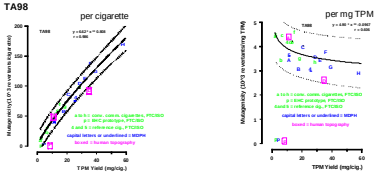
GVP



In Vitro Mutagenicity

- Mutagenic activity (TPM) per cigarette increased with increasing TPM yield (less than proportional).
- Mutagenic activity (TPM) per mg TPM decreased approximately 15% from a 5-mg to a 20-mg TPM cigarette.
- Mutagenic activity as a function of the TPM yield followed the same relationships irrespective of whether TPM yield was due to smoking conditions or cigarette design.
- EHC prototype: approximately 95% lower mutagenicity compared to conventional cigarettes with the same TPM yield.

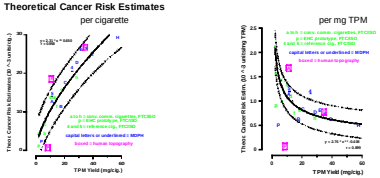
TA98



Theoretical Risk Estimates

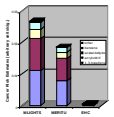
- Theoretical risk estimates increased with increasing TPM yield (less than proportional).
- Theoretical risk estimates decreased approximately 40% from a 5-mg to a 20-mg TPM cig.
- Theoretical risk estimates as a function of the TPM yield followed the same relationships irrespective of whether TPM yield was due to smoking conditions or cigarette design.
- EHC prototype: approximately 80% lower theoretical risk estimates compared to conventional cigarettes with the same TPM yield.

Theoretical Cancer Risk Estimates

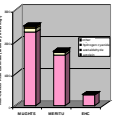


- The risk assessment-based weighting of smoke constituents provides suggestions for major contributions to theoretical cancer and non-cancer risk estimates, but is limited by the incompleteness of yield and potency data and the uncertainty of the potency data available.

Theoretical Cancer Risk Estimates



Theoretical Systemic Toxicity Risk Estimates



Discussion

- Complex mixtures such as cigarette smoke require a weight of evidence approach for toxicological evaluation because no single end point has been identified as having overriding relevance; therefore, a battery of assays was applied.
- Results for end points were consistent across the assays. This adds to the validity of each of these end points as potentially useful indicators of cigarette smoke toxicity.
- Correlations between TPM yield and the end points determined are rather tight, suggesting that relatively accurate estimates (± 20%) can be made for the same end points for other cigarettes if the TPM yields are known, provided that the blend and major design characteristics are fairly similar to the conventional U.S. cigarettes used to establish the relationships.
- Relationships between end points and TPM yield per cigarette were independent of whether TPM yield was due to smoking protocols (FTC, MDPH, or human puffing profiles) or cigarette design. This suggests that smokers of low and high yield cigarettes are exposed to similar amounts of adverse constituents or activity when they smoke cigarettes to the same TPM yield. Dosimetry studies (Jarvis et al., 2001) have shown that smokers of low yield cigarettes tend to inhale moderately lower amounts of nicotine than smokers of high yield cigarettes. Together these findings support the results of epidemiological studies (Tang et al., 1995; Kuper et al., 2002 A and B), which repeatedly found an equal or slightly reduced lung cancer risk in smokers of low vs high yield cigarettes.

Summary

- For our sample of conventional U.S. cigarettes, smoke chemistry, in vitro cytotoxicity, in vitro mutagenicity, and theoretical risk estimates for cancer and systemic toxicity can be estimated with an accuracy of approximately 20% when the TPM yield is known.
- Generally, with increasing TPM yield, the concentration of individual smoke constituents and the toxic activity per cigarette increased less than proportionally and per mg TPM decreased nonlinearly. At a range of 5 to 20 mg TPM per cigarette, the mean decreases in constituent yields were approximately 20%.
- The relationships are valid independent of whether TPM yield was due to smoking protocol or cigarette design. This suggests that smokers of low and high yield cigarettes are exposed to smoke of similar properties when they smoke their cigarettes to the same TPM yield.

Acknowledgement
The authors are grateful to the following individuals and their teams for contributing the data used in this study: J. Diekmann, C. Euchenhofer, B. Gerstenberg, T.J. Meisgen, K. Rustemeier, R. Stabbert, and D.J. Veltel. The authors are grateful to the following individuals for their contribution to the concept, design, and interpretation of the study: R.A. Carchman, C.L. Gaworski, H.-J. Haussmann, K.F. Podraza, and W. Reininghaus.

References
California EPA, Air Toxics Hot Spots Program Risk Assessment Guidelines, Part II (2002)
Euchenhofer et al., The Toxicologist 72(S-1): 392 (2003) (also view at www.ehcss-science.com)
IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 38 (1986)
IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 71 (Part 1) (1999)
ICH Harmonised Tripartide Guideline, Validation of Analytical ProceduresMethodology (1996)
INVITTOX:ERGATTIFRAME: Protocol 3a (1990)
Jarvis et al., J. Natl. Cancer Inst. 93, 134-138 (2001)
Kuper et al., J. Int. Med. 252: 206-224 (2002a)
Kuper et al., J. Int. Med. 251: 455-466 (2002b)
Maron, D.M., Ames, B.N., Mutat. Res. 113: 173-215 (1983)
OECD Guideline 471, Organisation for Economic Co-Operation and Development, Paris. (1997)
Roemer et al., Food Chem. Toxicol. 40 105-111 (2002)
Rustemeier et al., Food Chem. Toxicol. 40, 93-104 (2002)
Tang et al., Br. Med. J. 311: 1530-1533 (1995)
United States Consumer Products Safety Commission in Consultation with the USDepartment of Health and Human Services, Toxicity Testing Plan 5 (1993)
United States Environmental Protection Agency, Guidelines for carcinogenic risk assessment: Federal Register 51, 33992-34003 (1986)
United States Environmental Protection Agency, Health assessment of 1,3-butadiene, EPA/600/P-98/001F (2002)

Society for Risk Analysis: 23rd Annual Meeting, Baltimore, Maryland, December 7-10, 2003