

## **SMOKE CHEMISTRY: A USEFUL PREDICTOR OF SMOKE TOXICOLOGY?**

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### **SUMMARY**

Smoke chemistry, as a predictor of smoke toxicity, seems to have fallen into disfavor in recent times. First, industry critics claim that traditional smoking protocols seriously underestimate the doses of smoke constituents received by smokers, particularly for light and ultralight cigarettes. Second, the recent Institute of Medicine Report, "Clearing the Smoke," put emphasis on *in vitro* toxicology, human biomonitoring, and epidemiology as measures of harm reduction for Potentially Reduced Exposure Products (PREPS). Third, even if one obtains detailed smoke data under various conditions that may represent the extremes of human smoking behavior, one still has to decide what to do with the data, particularly if comparisons of potential biological activity are to be made among several products or a representative sample from a given market. However, not all is lost; there are a number of examples in the literature and company reports that show the potential relationships of product chemistry with biological endpoints. Furthermore, since smoke chemistry changes with the different smoking regimens such changes also may impact the *in vitro* biological assays, which are favored by some experts, as these rely upon essentially the same smoke collections used for chemistry studies. Finally, strategies for handling detailed smoke chemistry data will be discussed.

### **INTRODUCTION**

Smoke chemistry, as a predictor of smoke toxicity, seems to have fallen into disfavor in recent times. First, industry critics claim that traditional smoking protocols seriously underestimate the doses of smoke constituents received by smokers, particularly for light and ultralight cigarettes (1, 2, 3). Second, the recent Institute of Medicine Report, "Clearing the

Smoke," put emphasis on *in vitro* toxicology, human biomonitoring, and epidemiology as measures of harm reduction for Potentially Reduced Exposure Products (PREPS) (4). Third, detailed chemical investigations of commercial smokeless tobacco products, cigarettes, or the smoke from those cigarettes can give hundreds of analytes, many of which appear at similar levels in what appear to be very dissimilar products, thus thwarting most attempts to link chemistry with biological endpoints. Still, numerous authors have put forward hypotheses linking smoke and tobacco chemistry to diseases that have been associated with tobacco use. Many others have reviewed the literature on possible relationships among tobacco and/or smoke chemistry and the toxicology of tobacco products. Examples of recent reviews include those by Hoffmann, Hoffmann, and El-Bayoumy (5), Rodgman, Smith and Perfetti (6), and Hecht (7).

Of all the measures of tobacco and/or smoke toxicity, chemistry is probably the easiest to do. It does not require human subjects or other mammalian species. It does not require special mammalian or bacterial cell lines and those with the special training to do *in vitro* or *in vivo* assays. With the exception of needing a top-notch smoking machine, which is required for all but smokeless products and human biomonitoring studies, one can do tobacco and smoke chemistry with fairly common instrumentation, instrumentation that we would likely have on hand for general tobacco and cigarette product research, and instrumentation that many chemists know how to use. Indeed, many tobacco scientists have made their careers on being able to identify the chemistry behind subtle differences in the hedonic properties between apparently very similar smokeless or smoking products. Therefore, where have we gone wrong, if indeed, we have gone wrong, in the use of chemistry to predict the toxicology of tobacco products?

In this paper, approaches will be given to meeting the challenges of relating tobacco and/or smoke chemistry with the toxicology of tobacco products. Examples will be taken from the peer-review literature, government reports, and company reports. In this latter regard, this

paper will include citations to the tobacco company document Internet sites that were established as part of the Master Settlement Agreement. These documents can also be accessed through links from other Internet sites such as [www.tobaccoarchives.com](http://www.tobaccoarchives.com) and [www.tobaccodocuments.org](http://www.tobaccodocuments.org). These Internet sites also contain copies of public domain documents that may not be readily available from other sources.

If we go back in time to the early 1990s, we find that smoke chemistry was an important part of the program proposed by the U.S. Consumer Product Safety Commission for the assessment of the toxicology of cigarettes with reduced ignition propensity (8). At least one publication describes the comparison of the smoke chemistry and mutagenicity of two low ignition propensity cigarettes with commercial and reference cigarettes (9).

It was recognized that smoking topography could be different among different products and that smoke collection for both analytical and *in vitro* biological determinations should be done under several smoking regimens that the authors claimed would cover the range of expected smoking behaviors. Another key aspect of the proposed testing protocol was that it was designed to see if products, which incorporated technologies for reduced ignition propensity, gave a more toxic smoke than did conventional products. However, the manner in which the data from such comparison studies would be assessed was not specified. Perhaps those authors realized the conundrum an assessment body would be in if the product that had been modified for reduced ignition propensity showed, relative to the control product, more toxicity on some assays, but less toxicity on others. Obviously, if relative to the control product, the toxicity were higher on all assays (rejection of the test design) or lower on all assays (acceptance of the test design), the assessment would be easy. However, even among the *in vitro* and *in vivo* tests there can be differences. For example, all flue-cured cigarettes generally have higher biological activity than do all burley cigarettes in mouse skin painting assays for condensate tumorigenicity (10), while the converse is true for condensate mutagenicity as measured by the Ames assay (11). Furthermore, since the correlations between chemistries of

complex mixtures and the toxicology of those mixtures are often fuzzy, the toxicological significance of reductions of compounds or classes of compounds in the matrix is likely fraught with uncertainty.

It is not surprising then that much of the recent literature relating tobacco and smoke chemistry of conventional tobacco products with toxicological endpoints has been focused on showing that various design factors (i.e., tobacco additives, cigarette design features) do not increase toxicity. When considering conventional cigarette products, the data package will often contain routine tobacco and smoke chemistries, Hoffmann analytes, one or more *in vitro* assays for genetic and/or cytotoxicity, and a 13-week subchronic rodent inhalation study (12, 13, 14). Sometimes the inhalation studies are supplemented with a mouse skin painting study (15)

Outside of the major efforts associated with the assessment of non-conventional cigarette products such as Premier (16), Eclipse (17), and Accord (18), there is little in the recent literature to guide us in developing relationships among tobacco and smoke chemistries and toxicological endpoints for PREPS based largely on conventional technologies. Furthermore, if we look to the assessments that were used for Premier, Eclipse, and Accord for guidance, we find that there is little in the way of statistical correlations among various chemical parameters and toxicological endpoints.

The next part of this report will take examples from the literature to document the rightful place of chemistry in the assessment of the toxicological properties of tobacco products. It will begin with a review of the Tobacco Working Group studies of the 1970s (19). This will be followed by three examples from the literature where differences in tobacco and/or smoke chemistry have been linked to the epidemiology of tobacco-related disease. The final part of this report will cover some examples of the relationships between smoke chemistry and various *in vitro* and *in vivo* assays. In addition, examples for assessing and reporting detailed chemical data will be given along with some pitfalls in making correlations with biological endpoints.

## DISCUSSION

### *The Tobacco Working Group*

One of the great efforts in comparing chemistry data with toxicology data was the research program conducted by the Tobacco Working Group (TWG) (20, 21, 22, 23, 24, 25). The TWG was a joint program between the major cigarette companies and the Smoking and Health Program of the U.S. Department of Health, Education, and Welfare. More details about the TWG can be found in Chapter 4 of Reference 19. The TWG program began in 1968 and continued into the late 1970s. Numerous variants in blend, additives, and design were evaluated not only in terms of tobacco and smoke chemistry, but also in terms of tumorigenicity (mouse skin painting) and several measures of ciliotoxicity and cytotoxicity. In one sense, the TWG was a study of PREPS.

Table I, which was adapted from Reference 24, shows the cigarettes that were made for the program. In Experiment I, the key variables were the use of stem and various reconstituted tobaccos along with variables on cut width, cigarette paper, and nitrate addition. Variables in Experiment II included expanded tobaccos, agronomic practices, and the use of the artificial tobacco substitutes, NSM (ATS-A) and Cytrel (ATS-B). The focus of Experiment III was on paper porosity, tobacco additives, filters, and additional work with ATS-A and ATS-B. Experiment IV retested several of the concepts from the earlier experiments as well as different processing conditions and agronomic factors.

It must be pointed out that the TWG's efforts were not the first in this area. Numerous other researchers had studied the relationships among tobacco chemistry, smoke chemistry, and various indices of smoke toxicity. Some studies were summarized in the classic work by Wynder and Hoffmann, *Tobacco and Tobacco Smoke; Studies in Experimental Carcinogenesis* (26) and The Proceedings of the 3<sup>rd</sup> World Conference on Smoking & Health (27).

Table I  
Experimental Cigarettes Tested in Skin Painting Experiments

Var. No.	Cig. Code	Experiment I Cigarette Description
1	1	University of Kentucky Reference (KY1R1)
2	2	SEB I
3	3	SEB I, High porosity citrate paper (Schweitzer #505) (48 cm/min)
4	4	SEB I, Low porosity phosphate paper (Ecusta Reference A of Schweitzer Regular Verge) (11 cm/min)
5	5	SEB I, Nitrates added as KNO <sub>3</sub> , to 2X natural level of SEB I
6	6	SEB I, cut coarse (20 cuts/in)
7	7	SEB I, cut fine (60 cuts/in)
8	8	SEB I, with low porosity phosphate paper and coarse cut (combination of codes 4 & 6)
9	9	SEB I, with high porosity citrate paper, high nitrate content, and fine cut (combination of codes 3 & 5 & 7)
10	10	SEB I, Lamina only (only leaves of the formula used)
11	11	SEB I, Flue-cured Lamina only (only flue-cured leaves of formula used)
12	12	SEB I, Burley lamina only (only Burley leaves used)
13	13	SEB I, Stems only (only flue-cured and Burley stems used, rolled and cut)
14	14	SEB I, Stems only, made into RTS (reconstituted tobacco sheet) by Schweitzer paper process
15	15	SEB I, Stems and fines only, made into RTS by AMF slurry process
16	16	RTS of whole SEB I by Schweitzer paper process, no additives, medium density
17	17	RTS of whole SEB I by Schweitzer paper process, 7.5% wood pulp added, low density
18	18	RTS of whole SEB I by Schweitzer paper process, 7.5% wood pulp added, medium density
19	19	RTS of whole SEB I by Schweitzer paper process, 7.5% wood pulp added, high density
20	20	RTS of whole SEB I by AMF slurry process, no additives, medium density
21	21	RTS of whole SEB I by AMF slurry process, additives*, low density
22	22	RTS of whole SEB I by AMF slurry process, additives*, medium density
23	23	RTS of whole SEB I by AMF slurry process, additives*, high density

\*In addition to glycerine 2.81% and invert sugar 5.31%, additives were:

Methocel	7.35%
Refined unbleached sulfite pulp	4.59%
Ethylhydroxyethyl cellulose	1.84%

Table I. (Cont.)

Var. No.	Cig. Code	Experiment II Cigarette Description
1	40	University of Kentucky Reference (KYTR1)
2	41	SEB I
3	42	SEB II
4	43	SEB II
5	44	SEB II
6	45	SEB II
7	46	ATS-A, 100%
8	47	ATS-A, 50% & SEB II, 50%
9	48	RJ Reynolds puffed SEB II (Freon expansion process)
10	49	Philip Morris expanded SEB II (ammonia-CO <sub>2</sub> expansion process)
11	50	Freeze-dried SEB II
12	51	Straight Burley, normal nicotine, normal nitrogen fertilization (NN)
13	52	Straight Burley, low nicotine, normal nitrogen fertilization (LN)
14	53	Straight Burley, low nicotine, high nitrogen fertilization (LH)
15	54	Straight flue-cured, normal nicotine, normal nitrogen fertilization (NN)
16	55	Straight flue-cured, low nicotine, normal nitrogen fertilization (LN)
17	56	Straight flue-cured, low nicotine, high nitrogen fertilization (LH)
18	57	Blend 3 parts flue-cured, 1 part Burley (NN)
19	58	Blend 3 parts flue-cured, 1 part Burley (LN)
20	59	Blend 3 parts flue-cured, 1 part Burley (LH)
21	60	Hand suckered (no suckering chemicals used)
22	61	Fatty-alcohol - normal
23	62	Fatty-alcohol - 100X
24	63	ATS-B straight (100%)
25	64	ATS-B, 50% & SEB II, 50%

Note: ATS-A = Artificial Tobacco Substitute A (NSM) and ATS-B = Artificial Tobacco Substitute (Cytrel)

Table I. (Cont.)

Var. No.	Cig. Code	Experiment III Cigarette Description
1	40	University of Kentucky Reference (KY1R1)
1A	41A	SEB I
3	41B	SEB I
4	75A	SEB III
5	75B	SEB III
6	72	SEB III
7	73	SEB III
8	74	SEB III
9	76	SEB III (Schweitzer paper 489-14, Low porosity) (5 cm <sup>3</sup> /min)
10	77	SEB III (Vergé 55 paper-high porosity) (60 cm <sup>3</sup> /min)
11	78	SEB III (Schweitzer perforated paper-very high porosity) (100 cm <sup>3</sup> /min)
12	80	SEB III No sugar, with humectant
13	81	SEB III With sugar, no humectant
14	82	SEB III With 1% cocoa, no sugar, no humectant
15	83	SEB III No sugar, no humectant (no casing)
16	84	SEB III With L&M additive #1 (Magnesium Nitrate, 5.72%)
17	85	SEB III With L&M additive #2 (Zinc Oxide, 7.09%)
18	86	SEB III With L&M additive #3 (Magnesium Nitrate, 5.61%; Zinc Oxide, 6.96%)
19	87A	SEB III Burley Blend, cased with sugar, no humectant
20	87B	SEB III Burley Blend, cased with sugar, no humectant
21	88	SEB III Burley Blend, no casing (no sugar)
22	89	SEB III With dilution filter
23	90	SEB III With dilution filter and Schweitzer perforated paper (100 cm <sup>3</sup> /min)
24	91	SEB III With cellulose acetate filter
25	92	SEB III With Permanganate filter
26	93	ATS-A, 30% & SEB III, 70%, plus flavor
27	95	ATS-A, 30% & SEB III, 70%, plus flavor
28	97	ATS-B, 100% (old material, old dyes)
29	99	ATS-B, 100% (new material, no dyes)
30	0	ATS-B, 100% (old material, no dyes)
31	01	ATS-B (old material, no dyes) 50% & SEB III, 50% (casing applied to tobacco portion only)

Table I. (Cont.)

Var. No.	Cig. Code	Experiment III Cigarette Description
32	79	SEB III
33	94	ATS-A, 30% & SEB III, 70% plus flavor with Vergé 60 cm/sec paper
34	96	ATS-A, 50% & SEB III, 50% (casing applied to tobacco portion only)
35	98	ATS-B, 100% (old material, new dyes)
36	00	ATS-B, 100% (new material, new dyes)

Note: Ecusta 556 paper used on cigarette codes 72 thru 75, 79 thru 89, 91 thru 93, 96 thru 99, and on 00, 0, and 01.  
Note: ATS-A is artificial tobacco substitute A (NSM) and ATS-B is artificial tobacco substitute B (Cytrel).

Table I. (Cont.)

Var. No.	Cig. Code	Experiment IV Cigarette Description
1	30	University of Kentucky Reference (KY1R1)
1a	38	SEB III (Same as Code 75, Series III, L&M)
2	32	SEB IV
3	14	SEB IV
4	29	SEB IV
5	04	SEB IV
6	33	SEB IV, RTS, PJS paper process, no additives
7	09	SEB IV, RTS, PJS paper process, 10% additives (cellulose fiber)
8	06	SEB IV, RTS, PJS paper process, 10% additives (cellulose fiber), no return of water soluble substances
9	02	SEB IV, RTS, PJS paper process, 10% additives (cellulose fiber), nicotine reduced by proprietary process
10	28	SEB IV, RTS, PJS paper process, 10% additives (cellulose fiber), nicotine reduced and added back in form of nicotine citrate to level of Code 32, SEB IV
11	10	SEB IV, RTS, AMF slurry process, no additives except 2.8% glycerine, 5.31% invert sugar
12	17	SEB IV, RTS, AMF slurry process, 13% additives (see Note 1)
13	22	SEB IV, RTS, AMF slurry process, 13% additives plus 6% pH adjustment (see Note 2)
14	25	SEB IV, RTS, AMF slurry process, extracted with hexane-isopropanol azeotrope, 13% additives (see Note 1)
15	18	SEB IV, RTS, AMF slurry process, extracted with isopropanol-water azeotrope, 13% additives (see Note 1)
16	12	SEB IV, RTS, PJS paper process, 10% additives (cellulose fiber), waxy substances reduced
17	03	SEB IV, RTS, AMF slurry process 57%; plus 30% calcium carbonate plus Note 1
18	35	SEB IV, RTS, AMF slurry process 27%; plus 60% calcium carbonate plus Note 1
19	16	SEB IV, RTS, AMF slurry process 27%, extracted with isopropanol-water azeotrope, plus 60% calcium carbonate plus Note 1
20	27	SEB IV, RTS, PJS paper process, 10% additives (cellulose fiber) plus 25% inorganic fillers (calcium carbonate 18%, clay 7%)

Table I. (Cont.)

Var. No.	Cig. Code	Experiment IV Cigarette Description
21	19	SEB IV. RTS, PJS paper process, 10% additives (cellulose fiber) H <sub>2</sub> O <sub>2</sub> treated
22	15	SEB IV expanded stems, 100%
23	08	SEB IV expanded stems 50%, SEB IV 50%
25	24	Ecusta material 30%, SEB IV 70%
27	13	SEB IV nicotine removed
28	11	SEB IV nicotine at 0.5 mg/cig.
29	31	SEB IV nicotine at 1.0 mg/cig.
30	23	SEB IV nicotine at 1.5 mg/cig.
31	26	Burley leaf with full return of stem
32	37	Burley leaf RTS, AMF slurry process, 15% additives (see Note 3)
33	21	Burley HLC RTS, AMF slurry process, 15% additives (see Note 3)
34	20	Bright leaf with full return of stems
35	36	Bright leaf RTS, AMF slurry process, 15% additives (see Note 3)
36	05	Bright HLC RTS, AMF slurry process, 15% additives (see Note 3)
37	L8	Pesticide free tobacco
38	M6	Pesticide treated tobacco (See Note 4)
39	67	SEB IV. treated with PMO (1.5% by weight)
40	68	SEB IV. with special 100 cm/min. paper

Note 1. Additives: Refined unbleached sulfite pulp 6.05%, Galacto-Mannan Gums 5.85%, Cellulose Ether Gums 0.52%, Dialdehyde Crosslinker 0.58%, Total 13.00%

Note 2. Additives: Same as Note 1, plus Sodium Hydroxide 3.20%, Citric Acid 2.80%, Total 6.00%

Note 3. Additives: Refined Unbleached Sulfite Pulp 6.25%, Triethylene Glycol 2.25%, Galacto-Mannan Gums 4.48%, Cellulose Ether Gums 1.42%  
Dialdehyde Crosslinker 0.60%, Total 15.00%

Note 4. Additives: The soil fertility, pesticide residues, and pesticides used on Codes L8 and M6 are listed in a separate document.

RTS = Reconstituted Tobacco Sheet

PJS = Peter J. Schweitzer

AMF = AMF, Inc.

HLC = Homogenized Leaf Cured.

PMO = 3-phenyl-5-methyl-1,2,4-oxadiazole (an anti-irritant)

A short critical review of these studies was written by Slaven and can be found at the Lorillard document web site (28). For those who would like more detailed information than was published in References 20, 21, 22, 23, 24, three of the status reports can be found on the World Wide Web (29, 30, 31). Additional details on the analyses of the smoke condensates for the first two sets of experimental cigarettes can be found in a March 1975 report by Guerin and Nettesheim (32). Details of the smoke chemistry related to animal exposure systems can also be found in that report.

In the summary report of the mouse skin painting studies, factors that increased tumorigenicity relative to the Standard Experimental Blend (SEB) (in a standard nonfilter design) were identified along with those that decreased tumorigenicity or had little effect (24). Chemistry data was not given directly in that summary report, but was summarized in numerous tables in the four reports (20, 21, 22, 23). Tables II and III give some examples of the findings from the mouse skin painting studies. Each table shows the cigarette factor tested, the survival probability based upon histopathologically verified tumor data for the 25-mg/day dose groups (PFH-25), the static burn rate (SBR) in units of mm/min, the sum of benzo[a]anthracene and benzo[a]pyrene in the condensate in units of ppm, and the cigarette code for the particular factor. Table II shows some of the examples where the factors reduced tumorigenicity relative to the respective SEB reference cigarette. Data are also included for the Kentucky 1R1 reference cigarette that was analyzed in each study group. These data are in *Italics*. Data from relevant comparison products are also shown in *Italics*. Table III shows similar data for factors that increased or did not alter tumorigenicity.

Referring now to Table II, the top few rows show some of the examples from TWG I (TWG I, TWG II, etc., designations for Experiment I, Experiment II, etc.). One of the main findings from TWG I was that lamina or mixtures of lamina and reconstituted tobacco sheet (RTS) such as the SEB I cigarette gave more tumorigenic condensate than did RTS alone. In

addition, stems were found to give condensate less tumorigenic than lamina. In general, such modifications gave products with higher static burn rates than the SEB I cigarette. The use of high porosity citrate paper gave a directional improvement in PFH-25. In TWG-II, the use of freeze-dried tobacco, one type of expanded tobacco as well as low nicotine tobaccos gave condensates that were less tumorigenic than the SEB II. The largest improvement was from the artificial tobacco substitute, ATS-A. The range of responses in TWG-III for PFH-25 was more limited than in the other experiments; however, the SEB III without sugar and humectant was the only test cigarette in that experiment giving a condensate significantly less tumorigenic at the 25-mg condensate dose level than was the normal SEB III. However, when tested at the 12.5-mg condensate dose level, this effect was not seen (22). The following quote was taken from Reference 22. "This suggests that at the lower dose, sugar and humectant in combination have little effect on condensate tumorigenicity but, at the higher dose level, sugar and humectant in combination may contribute to condensate tumorigenicity." In TWG-IV, tobacco reconstitution processes gave less tumorigenic condensates in some cases as shown for the two examples given in Table II.

Table III lists some of the cigarettes whose condensates had equal or greater tumorigenicity than did the corresponding SEB. In TWG-I, none of the combinations of cut width, paper porosity, and nitrate addition gave results statistically different from those of SEB I although directional differences were seen. In TWG-II, neither of the combinations of the SEB-II blend with ATS gave condensates whose tumorigenicity was different from that of the SEB II. The cigarettes made with the SEB II blend expanded by the Freon process gave condensate of comparable tumorigenicity with that of the SEB II, while the cigarettes whose tobacco was expanded by the ammonia-CO<sub>2</sub> process gave condensate less tumorigenic than that from the SEB II. TWG-III gave some interesting findings with respect to filters and dilution devices. While the cigarettes with the dilution filter, which was essentially a hollow tube with ventilation, gave a directional reduction in tumorigenicity, the cigarettes with the cellulose acetate filter and

the permanganate filter (cellulose/potassium permanganate on aluminum oxide/cellulose) gave statistically significant increases in tumorigenicity. One of the RTS samples in TWG-IV, which would have been expected to give reduced tumorigenicity based on its formulation, was found to give condensate whose tumorigenicity was not different from that of the SEB IV cigarettes.

TABLE II

SOME CIGARETTE FACTORS GIVING CONDENSATE LESS TUMORIGENIC THAN THE SEB  
ADAPTED FROM REFERENCES 20, 21, 22, 23, 24

Factor	PFH-25	SBR	B[a]A+B[a]P	Code
SEB I, stems only, made into reconstituted tobacco sheet (RTS) by paper process	0.896*	9.09	1.95	14
SEB I, stems and fines only, made into RTS by slurry process	0.692	6.16	2.02	15
SEB I, whole blend, made into RTS by paper process, medium density, no additives	0.812*	6.41	1.90	16
SEB I, whole blend, made into RTS by slurry process, medium density, no additives	0.522	4.55	1.90	20
SEB I, citrate paper, high porosity	0.663	4.55	1.89	3
Flue-cured and burley stems from SEB-I blend	0.817*	5.62	1.94	13
KY1R1	0.454	3.84	2.06	1
SEB I	0.517	4.19	1.92	2
SEB II, ammonia-CO <sub>2</sub> expansion process	0.583*	6.30	1.10	49
SEB II, freeze-dried	0.573*	7.72	1.51	50
Straight burley, low nicotine, normal nitrogen fertilization	0.738**	9.28	1.27	52
Straight flue-cured, low nicotine, normal nitrogen fertilization	0.706**	6.10	1.41	55
ATS-A (Artificial Tobacco Substitute A) 100%	0.924**	NR	5.70	46
ATS-A, 50% & SEB II, 50%	0.411	5.20	2.51	47
KY1R1	0.343	3.89	1.79	40
SEB II	0.442	4.97	1.64	42 - 45
ATS-A, 70% & SEB III, 30%	0.557	4.17	3.63	93
SEB III with dilution filter	0.497	4.88	3.01	89
SEB III without sugar and humectant casing	0.594*	4.81	2.21	83
SEB-III with 5.72% magnesium nitrate	0.551	4.18	1.21	84
KY1R1	0.434	3.76	2.34	40
SEB III	0.449	4.15	2.26	72-75
SEB IV, whole blend, made into RTS by paper process, 10% fiber, no solubles add-back	0.814**	4.62	3.02	6
Bright leaf, made into RTS by slurry process, 15% additives (fiber, TEG, binders)	0.701**	6.05	1.68	36
Burley leaf, made into RTS by slurry process, 15% additives (fiber, TEG, binders)	0.593	6.51	1.02	37
KY1R1	0.440	3.50	2.19	30
SEB IV	0.488	4.30	1.86	4, 14, 29, 32

\*p&lt;0.05

\*\*p&lt;0.01

NR = Not reported

TABLE III

SOME CIGARETTE FACTORS GIVING CONDENSATE SIMILAR OR MORE TUMORIGENIC THAN THE SEB  
ADAPTED FROM REFERENCE 24

Factor	PFH-25	SBR	B[a]/A+B[a]P	Code
SEB I, phosphate paper, low porosity	0.565	3.55	2.29	4
SEB I, citrate paper, high porosity	0.663	4.55	1.89	3
SEB I, cut coarse (20 cuts/in)	0.449	3.93	2.31	6
SEB I, cut fine (60 cuts/in)	0.544	4.81	2.17	7
SEB I, with low porosity phosphate paper and coarse cut filler	0.532	3.27	2.12	8
SEB I, with high porosity citrate paper, fine cut filler, and 2x blend nitrate	0.654	5.84	1.75	9
KY1R1	0.454	3.84	2.06	1
SEB I	0.517	4.19	1.92	2
SEB II, Freon expansion process	0.542	6.32	1.64	48
Straight burley, normal nicotine, normal nitrogen fertilization	0.434	7.19	1.34	51
Straight flue-cured, normal nicotine, normal nitrogen fertilization	0.399	4.96	1.35	54
ATS-B, 50% & SEB II, 50%	0.455	5.62	1.86	64
ATS-A, 50% & SEB II, 50%	0.411	5.20	2.51	47
KY1R1	0.343	3.89	1.79	40
SEB II	0.442	4.97	1.64	42 - 45
SEB III with cellulose acetate filter	0.294*	4.65	3.50	91
SEB III with permanganate filler	0.298*	4.68	2.45	92
SEB III with dilution filter	0.497	4.88	3.01	89
SEB III with low porosity paper (5 cm/sec)	0.331	3.06	2.36	76
SEB III with high porosity paper (60 cm/sec)	0.407	4.49	2.43	77
SEB III with very high porosity paper (100 cm/sec)	0.413	4.62	2.21	78
KY1R1	0.434	3.76	2.34	40
SEB III	0.449	4.15	2.26	72-74, 75A
SEB IV, made into RTS by paper process, 10% fiber, 18% CaCO <sub>3</sub> , 7% clay	0.394	6.06	2.71	27
KY1R1	0.440	3.50	2.19	30
SEB IV	0.488	4.30	1.86	4, 14, 29, 32

\*p&lt;0.05

\*\*p&lt;0.01

Considerable effort was made during the TWG studies to use statistical techniques to correlate the tobacco chemistry data, cigarette property data, and smoke chemistry data with the mouse skin painting data. These correlations are detailed in each of the four main TWG reports (20, 21, 22, 23). One of the more interesting correlations was a positive correlation between the nicotine content of the condensate and biological activity. Another one was that static burn rate was negatively correlated with tumorigenicity. It must be pointed out that the tumorigenicity assays were done on a dry condensate basis. Thus, the same amount of dry condensate was applied whether or not the cigarette yield of dry condensate was 25 mg/cigarette or 10 mg/cigarette.

As pointed out by Slaven, some of the correlations did not make sense from a chemistry point of view (28). For example, various gas-phase analytes were used in some of the correlations with tumorigenicity of condensate. It was found that acetaldehyde, formaldehyde, nitrogen oxides, carbon monoxide, and acrolein were negatively correlated with tumorigenicity. Since tobacco nitrate levels were negatively correlated with tumorigenicity, and since the delivery of nitrogen oxides is very dependent on blend nitrate (33), the negative correlation of nitrate with tumorigenicity would be expected. The relationships with the other gas-phase variables and tumorigenicity appear to be less clear.

Bayne of the Oak Ridge National Laboratory took a different approach for the statistical analysis of the TWG data (34). Only data on condensate analytes determined in all four studies were used and only cigarettes containing 70% tobacco were considered. In addition, the data Bayne used from the fourth study were preliminary and differ to some extent from those finally published. Two prediction models were developed; and these are shown in Table IV, which is an adaptation of Table 9 of Reference 34. To test the simpler (e.g., Prediction Model Derived from Significant Linear Terms) of the two models, the relevant data in Bayne's report were reentered into an Excel workbook. A plot of observed PFH-values versus predicted PFH-values for the

entire data set is shown in Figure 1. As can be seen from the data in Table IV, Bayne developed second-order terms that improved the predictive power of his models. His basis to use second-order terms came from a correlation analysis that showed that there was no single variable had a correlation coefficient greater than  $r = 0.4$  with the histopathological probabilities.

When one reviews the coefficients in Table IV, one notes that except for the terms involving [nicotine]<sup>2</sup>, the coefficients of all the other terms involving nicotine are negative. This means that as the concentration of nicotine in the condensate increased, the tumorigenicity of the condensate also increased as measured by the decrease in the probability of tumor-free animals. However, nicotine is not mutagenic in the Ames assay and is it not tumorigenic (35, 36), but those factors had not been clearly established at the time of the TWG. Therefore, what was the mechanism underlying this very strong correlation between nicotine concentration in the condensate and tumorigenicity? One hypothesis was that other components in the condensate that may have arisen from the pyrolysis of nicotine were the culprits.

There was significant circumstantial evidence for that hypothesis. In 1961, Jarboe and Rosene of Brown & Williamson Tobacco reported that the pyrolysis of nicotine in an inert atmosphere at temperatures from 600°C to 900°C produced a variety of heterocyclic nitrogen compounds and aromatic hydrocarbons (37). In 1963, Kobashi, Hoshaku, and Watanabe reported on their pyrolysis studies of nicotine in air at temperatures ranging from 300°C to 800°C (38). Two fractions were collected in a cold trap. Fraction I (boiling points >200°C) consisted chiefly of myosmine, pyridylmethylketone, isoquinoline, normicotine, 2,2'-bipyridine, and nicotylene. Fraction II consisted of 3-vinylpyridine, 3-cyanopyridine, other alkyl pyridines and pyrrole. In 1979, Schmeltz, Wenger, Hoffmann, and Tso reported on the comparison of the pyrolysis of <sup>14</sup>C-nicotine in a combustion tube versus that in a cigarette (39). Those authors reported that under pyrolytic conditions in a combustion tube, nicotine underwent extensive degradation to pyridines, quinolines, aryl nitriles, and aromatic hydrocarbons. However, when

the <sup>14</sup>C-nicotine was applied to commercial KS nonfilter cigarettes, over 40% of the nicotine remained intact (considering both mainstream and sidestream smokes).

Several other factors and hypotheses were presented in that era. During the Banbury Conference on Less Hazardous Cigarettes in October 1979, F. G. Bock's paper indicated that nicotine was a cocarcinogen (40). Hoffmann, Chen, and Hecht also presented a paper on their work on nitrosamines in tobacco and tobacco smoke (41). In addition, Mizusaki and coworkers had reported in 1977 that the levels of total nitrogen, protein nitrogen, and soluble nitrogenous material in leaf were positively and significantly related to an increase in the mutagenic activity of smoke condensate (42). However, those authors also reported that nicotine and nitrate were not important factors for the mutagenicity of the condensate. Furthermore, there were reports in the literature on the mutagenicity of carbolines in cigarette smoke (43, 44).

These factors and possibly others apparently led the German Verband der Cigarettenindustrie to commission research on the fate of nicotine in the burning cigarette, and that research became known as the NPAH project (45, 46, 47, 48). One part of this project was research by Neurath to determine the amounts of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -carbolines and aminocarbolines in cigarette mainstream and sidestream smoke (49).

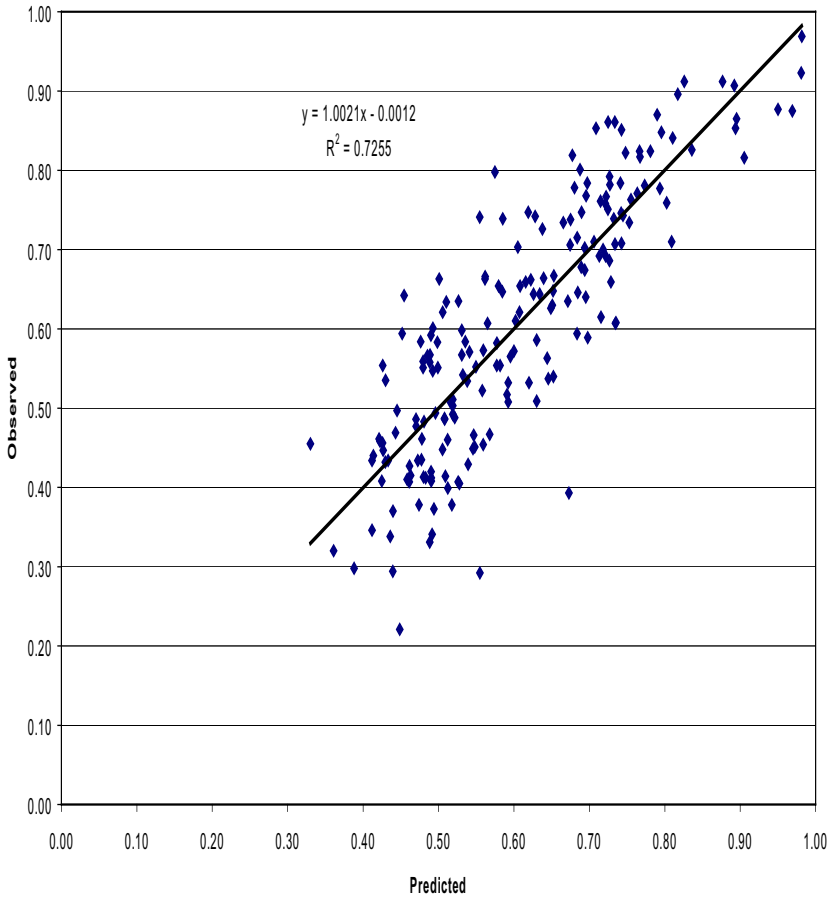
The culmination of the NPAH project appears to have been a report of research by a team of scientists that was lead by Robert Jenkins and Richard Izac of Philip Morris (50). This work used cigarettes made from tobaccos grown in a <sup>14</sup>C-CO<sub>2</sub> atmosphere. They reported between 0.13% and 0.32% of the total amount of <sup>14</sup>C-nicotine in a nonfiltered cigarette was converted to mainstream aza-arenes and between 0.14% and 0.3% of the original radiolabelled nicotine was converted to aza-arenes in the sidestream smoke. The authors did not comment on the biological activity of the aza-arene fractions that they isolated. Bleeker and coworkers have reviewed the toxicology of aza-arenes (51). Some of the compounds reported by Jenkins and Izac as pyrolysis products of the <sup>14</sup>C-nicotine are known mutagens.

TABLE IV  
COEFFICIENTS AND STANDARD DEVIATIONS OF COEFFICIENTS FOR PREDICTION MODELS OF TUMORIGENICITY  
ADAPTED FROM REFERENCE 34

Terms	Units	Prediction Model Derived from the Full Second-Order Model		Prediction Model Derived from Significant Linear Terms	
		Coefficients	SD of Coefficients	Coefficients	SD of Coefficients
1 Intercept		3.065	0.347	2.637	0.292
2 Concentration	mg/day	-0.03716	0.00266	-0.03793	0.00274
3 (Concentration) <sup>2</sup>	(mg/day) <sup>2</sup>	0.0004613	0.0000392	0.0004688	0.0000408
4 Nicotine	mg/g	-0.006746	0.000830		
5 pH		-0.4221	0.1177	-0.4434	0.0980
6 Weak acids	meq/g	0.07786	0.03471		
7 Very weak acids	meq/g				
8 B[a]P	µg/g	-0.7075	-0.244	0.1242	0.0555
9 (Nicotine) <sup>2</sup>	(mg/g) <sup>2</sup>	0.00001512	0.0000532	0.00002450	0.00000588
10 (pH) <sup>2</sup>		0.02994	0.01063	0.03663	0.00875
11 (B[a]P) <sup>2</sup>	(µg/g) <sup>2</sup>	0.4318	0.1325		
12 Nicotine x Phenol	(mg/g) <sup>2</sup>	0.0008709	0.0002073		
13 Nicotine x pH	mg/g			-0.0007078	0.0001664
14 Nicotine x B[a]P	mg/g x µg/g			-0.001770	0.000377
15 B[a]P x Phenol	µg/g x mg/g	0.1378	0.0343		
16 o-Cresol x Phenol	(mg/g) <sup>2</sup>	-0.2797	0.0554		
17 o-Cresol x m&p-Cresol	(mg/g) <sup>2</sup>	0.5581	0.1153		
18 o-Cresol x B[a]P	mg/g x µg/g	-0.9991	0.2482		
19 o-Cresol x B[a]A	mg/g x µg/g	0.8453	0.1901		
20 m&p-Cresol x B[a]A	mg/g x µg/g	-0.3238	0.0678		

FIGURE 1

Observed PFH vs. Predicted TWG All Series



Before leaving the subject of nicotine and tumorigenicity, another piece of evidence that was developed by Bock, Tso, and Fox was apparently presented at a scientific conference in 1981. The title of the presentation was, "The Effect of Nicotine on the Carcinogenic Activity of Cigarette Smoke." The slide copy can be found on the web site for the R. J. Reynolds Public Document Repository (52). The paper may have been presented at a conference in New York City in November 1981 (53).

In any case, cigarettes were made of either burley or bright tobacco containing equal mixtures of cured leaf and reconstituted sheet prepared from the same leaf. During the preparation of the reconstituted sheet, nicotine was added to the sheet providing low medium or high levels of nicotine of each tobacco type. According to the authors, the condensates from the bright tobacco exhibited twice as much carcinogenic activity as did the condensates from the burley cigarettes. However, the activity of the burley condensates depended on the nicotine content of the cigarettes while the activity of the bright condensates did not depend on the nicotine content of the cigarettes (52). Figures 2, 3, and 4 show the graphical representations of their data. The percentages shown in the Figures are the condensate concentrations.

Figure 2

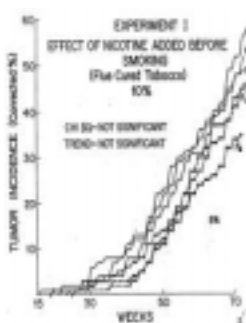


Figure 3

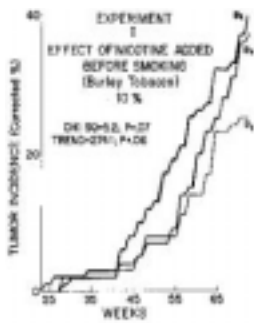
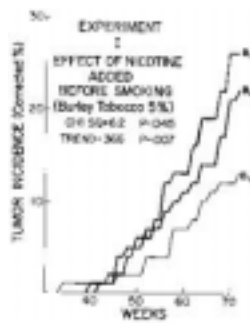


Figure 4



These data show how the correlations of nicotine with tumorigenicity developed by the TWG did not take into account the underlying chemistry of the smoke in which the deliveries of actual tumorigenic agents were likely well correlated with nicotine deliveries.

A number of other reports and publications issued as result of the main TWG program. One such list of publications, reports, and presentations resulted from the TWG can be found in Reference 31. During the TWG studies, a number of advances in the assessment of tobacco smoke were applied to some of the TWG samples. One example was the determination of condensate mutagenicity by the newly introduced Ames assay (54). Table V shows a summary of the results along with the tumorigenicity (PFH-25), static burn rate (mm/min) and the sum of benzo[a]anthracene and benzo[a]pyrene concentrations in the condensate from Reference 21.

TABLE V  
COMPARISON OF MUTAGENICITY AND TUMORIGENICITY OF SMOKE CONDENSATE  
DATA ADAPTED FROM REFERENCES 21 AND 54

Cigarette	Mutagenicity CFU/mg condensate (TA1538+S9)	Mutagenicity rank	PFH-25	Tumorigenicity rank	Static burn rate (mm/min)	Sum B[a]A + B[a]P (ug/g)
KY1R1 (TWG II-40)	58.9	2	0.343	4	3.89	1.79
SEB II (TWG II-42)	99.9	4	0.442	3	4.84	1.66
Freeze-dried SEB II (TWG II-50)	47.1	1	0.573	2	7.72	1.51
Straight burley low nicotine normal fertilizer (TWG II-52)	67.5	3	0.738	1	9.28	1.27

The data in Table V show the conundrum often faced by smoke toxicologists. Different bioassays give different rankings of products. The mutagenicity data appear to show an effect of both blend and static burn rate. Based upon percentage of burley tobacco in the blend, the rankings, in decreasing order of mutagenicity, should have been II-52 > II-50 = II-42 > II-40 (11). Differences in static burn rate appear to have changed the expected rankings of mutagenicity.

Another aspect of the TWG was the study on selective filtration and the correlations of smoke chemistry with ciliotoxicity and cytotoxicity (25). Table VI shows the basic design and smoke parameters for the twelve cigarettes in that study. All cigarettes were 85 mm in length with a 20-mm filter section. In the case of F1, the filter section was a hollow tube while in the

case of F11, the filter section was a hollow tube with ventilation holes 10 mm from the mouth end. Table VII shows the other smoke analytes determined and the results of the ciliotoxicity, macrophage inhibition, and cytotoxicity assays. All values are expressed on a per-puff basis. The last column in Table VII shows the overall ranking given the different filter designs based on the bioassays (rank 1 least, toxic; rank 12, most toxic). Unlike the mouse skin painting experiments, the ciliotoxicity and macrophage inhibition bioassays were done with whole smoke. The cytotoxicity assay was done with the water-soluble portion of cold-trapped whole smoke. The results of these assays are expressed as  $ED_{50}/\text{puff}$  as given by the following equation:  $ED_{50}/\text{puff} = (35 \text{ mL of smoke/puff})/(\text{number of mL of smoke}/ED_{50})$ . The smaller the value, the less biologically active is the smoke in the given assay.

As with the mouse skin painting experiments, various statistical tests were used to correlate the smoke chemistry results with those of the bioassays. Hydrogen cyanide and formaldehyde were strongly correlated with increased ciliotoxicity. Formaldehyde, weak acids, very weak acids (but not colorimetric phenols), hydrogen cyanide, TPM, acrolein, and particulate-phase water were significantly correlated with increased cytotoxicity. Isoprene, carbon monoxide, and carbon dioxide were correlated with decreased macrophage inhibition. Phenols (but not very weak acids) were correlated with increased macrophage inhibition. No statistical tests were apparently performed to account for possible second-order terms in the regression equations. In addition, there was apparently no attempt to correlate the results of the mouse skin painting assays with the results of the assays on ciliotoxicity, macrophage inhibition, and cytotoxicity.

TABLE VI

CIGARETTE PARAMETERS FOR TWG STUDY ON EXPERIMENTAL FILTER CIGARETTES  
DATA ADAPTED FROM REFERENCE 25

Cigarette Code	TWG III Code	Filter Type	Filter Description	NFDS Removal Efficiency (%)	TPM (mg/cig)	Water (mg/cig)	Nicotine (mg/cig)	PMW/NF (mg/cig)	CO (mg/cig)	CO <sub>2</sub> (mg/cig)	Puffs
F1		Control	Empty tube without perforations	-	38.8	8.47	1.67	28.66	15.79	47.94	8.0
F2		Mechanical	Cellulose-untreated	49.3	18.2	2.85	0.81	14.55	18.17	50.73	8.7
F3	91	Mechanical	Cellulose acetate-untreated	48.4	18.5	2.66	0.91	14.92	19.04	52.60	8.5
F4		Charcoal	Cellulose-charcoal-cellulose	44.6	20.7	3.36	1.04	16.31	16.19	46.23	9.0
F5	92	Oxidizing	Cellulose-MnO <sub>2</sub> /Al <sub>2</sub> O <sub>3</sub> -cellulose	46.5	22.5	5.70	1.25	15.52	14.47	46.66	8.2
F6		Acid affinity	Cellulose + 15% polyolimine + 10% KHCO <sub>3</sub>	51.2	17.7	1.86	1.18	14.64	15.99	47.36	8.8
F7		Acid affinity	Cellulose + 10% KHCO <sub>3</sub>	45.3	19.8	2.80	1.22	15.82	16.48	47.87	8.6
F8		Acid affinity	Cellulose + 10% polyol + 10% KHCO <sub>3</sub>	46.2	19.5	2.79	1.17	15.54	16.54	51.68	8.6
F9		Base Affinity	Cellulose + 10% glycerine + 10% citric acid	45.9	19.0	2.31	0.84	15.89	16.03	50.13	8.8
F10		Phenol	Cellulose acetate + 5% triacetin + 5% PEG	48.3	18.7	2.72	0.94	15.04	14.59	50.64	8.4
F11	89	Dilution	Empty tube with perforations	34.2	23.7	3.48	1.36	18.85	7.94	28.13	9.9
F12		Mechanical-Selective	Cellulose-magnesium silicate-cellulose	50.2	16.9	1.80	0.87	14.25	15.60	46.48	8.4

TABLE VII  
 CIGARETTE PARAMETERS FOR TWG STUDY ON EXPERIMENTAL FILTER CIGARETTES

DATA ADAPTED FROM REFERENCE 25

Code	Filter Type	Formaldehyde (ug/puff)	Acetaldehyde (ug/puff)	Acrolein (ug/puff)	Isoprene (relative)	HCN (ug/puff)	NOx (ug/puff)	Colorimetric Phenols (ug/puff)	Very Weak Acids (meq/puff)	Weak Acids (meq/puff)	Cilia-toxicity ED <sub>50</sub> /Puff	Macrophage Inhibition ED <sub>50</sub> /Puff	Cyto-toxicity ED <sub>50</sub> /Puff	Overall Rank
F1	Control	4.24	136	47.5	0.10	49.5	53.2	19.22	0.0036	0.0052	0.59	9.2	40.0	12
F2	Mechanical	3.20	129	43.6	0.10	38.0	52.5	8.31	0.0018	0.0029	0.54	6.7	27.5	10
F3	Mechanical	3.16	82	36.6	0.10	31.3	48.3	6.36	0.0021	0.0027	0.43	5.6	25.6	7
F4	Charcoal	1.87	77	13.3	0.05	11.0	64.2	8.15	0.0017	0.0022	0.13	6.2	15.9	2
F5	Oxidizing	2.29	90	24.7	0.07	19.0	20.3	7.63	0.0019	0.0020	0.20	5.8	19.1	4
F6	Acid affinity	1.72	81	29.4	0.10	5.2	50.2	6.93	0.0018	0.0021	0.01	8.3	18.2	5
F7	Acid affinity	2.65	125	38.9	0.11	19.0	51.8	8.28	0.0018	0.0023	0.15	5.8	17.8	3
F8	Acid affinity	2.36	138	34.3	0.12	7.8	57.3	4.31	0.0018	0.0025	0.09	5.6	19.3	1
F9	Base Affinity	3.90	126	41.9	0.10	39.6	41.8	8.96	0.0021	0.0031	0.33	6.2	22.9	8
F10	Phenol	3.75	134	37.3	0.10	32.9	56.6	2.81	0.0018	0.0023	0.44	6.9	30.4	11
F11	Dilution	2.35	54	13.4	0.04	19.7	22.3	12.98	0.0022	0.0028	0.10	13.5	20.9	9
F12	Mechanical-Selective		90	14.6	0.11	38.3	55.6	7.16	0.0014	0.0022	0.40	6.2	17.5	6

Some other studies that were done at around the same time as the TWG were those in support of Cytrel, a cellulose-based synthetic smoking material developed and extensively tested by Celanese (55, 56, 57, 58, 59, 60, 61). While smoke chemistry data can be found in Reference 55, more detailed information can be found in several papers published in Beiträge (62, 63, 64). Cytrel was included in the TWG studies and was given the designation of ATS-B. It was included in several sets of experimental cigarettes as the results obtained were different than expected. In each case, the condensates from Cytrel cigarettes were more tumorigenic than those from the SEB cigarettes (21, 22, 23). On the other hand, Bernfeld and Homburger conducted four studies from 1964 through 1971 and came to different conclusions. They were 1) on a weight/weight basis SC (smoke condensate) from the Cytrel products tested was no more tumorigenic and, in most cases, even less so than that of cigarette tobacco; 2) higher doses of SC from the Cytrel variants are required to cause a biologic response (benign and malignant skin lesions) equal to that of cigarette tobacco; and 3) comparison of SC from 1:1 blends of Cytrel and cigarette tobacco with that of the tobacco alone proved the blend to be less tumorigenic than tobacco alone at two lower dose levels; however, at a higher dose level they were about equal in biologic activity, but in no instance was there any evidence for synergism between the two components of the blend (61). An additional discussion of Cytrel will be found later in this paper in the section dealing with the correlation of smoke chemistry with biological activity.

### ***Epidemiology***

The recent Institute of Medicine Report, "Clearing the Smoke," put emphasis on the use of epidemiological results as possibly the best predictor of whether or not a new type of tobacco product, which has been designed to reduce health risks, truly has reduced health risks when compared with conventional products. Now, if chemistry is to be useful, chemistry results

should be able to predict the outcomes of such epidemiological studies. However, such studies may only be valid if they monitor the health outcomes over years of use. Apparently, no such studies have been done with cigarette products that are designed to be in the words of the IOM, "Potentially Reduced Exposure Products," or PREPS (4). However, there is more than enough data to see how chemistry results correlate with other biological data; and that topic will be covered later.

Epidemiology related to tobacco products does show its power to differentiate among tobacco products in three areas: 1) conventional wet snuff versus Swedish snus (a type of wet snuff); 2) cigarettes made from dark air-cured (black) tobaccos versus cigarettes made from blond (flue-cured, Virginia) tobaccos; and 3) charcoal-filtered cigarettes versus those with conventional cellulose acetate filters.

The epidemiology of Swedish snus has been studied by many researchers (65, 66, 67, 68, 69, 70, 71). One conclusion that can be taken from these epidemiological studies is that Swedish snus apparently does not have the health risks that have been ascribed to wet snuff that has been manufactured in the United States (72, 73, 74, 75, 76, 77) and other countries (78).

Hoffmann and coworkers reported on the potential toxic agents in smokeless tobacco products (74, 79, 80). Comparisons of various smokeless tobacco products are shown in Table VIII. According to Nilsson (68), the dissimilarities in the TSNA contents of oral snuff products may be the most important reason for the different outcomes of the epidemiological studies conducted in the United States and Sweden. Recently, Rodu and Cole reported that users of dry snuff had a higher incidence of oral cancer than did users of wet snuff (81). Brunneemann and coworkers reported in 1987 that the total TSNA levels in three brands of dry snuff ranged from 37 to 135  $\mu\text{g/g}$  based on wet weights while those for the five brands of moist snuff products ranged from 5 to 151  $\mu\text{g/g}$  based on wet weights (82).

TABLE VIII  
COMPARISON OF SWEDISH SNUS WITH OTHER SMOKELESS TOBACCO PRODUCTS  
SELECTED ANALYTES ON A DRY-WEIGHT BASIS

Analyte	GothiaTek® Snus (Maximum Levels) Ref. 83	Moist Snuff (Range. 5 brands) Ref. 79	Moist Snuff (Range. 5 brands) Ref. 80	Moist Snuff (Range. 5 brands) Ref. 74
Nitrite (ppm)	7	NR	NR	2 - 163
Total TSNA (ppm)	10	10 - 289	10 - 288	5 - 26
NDMA (ppb)	10	4 - 102	14 - 67	NR
B[a]P (ppb)	20	<0.1 - 63	<0.1 - 63	NR
Cadmium (ppm)	1	NR	0.45 – 1.58	NR
Lead (ppm)	2	NR	0.86 – 2.96	NR
Arsenic	0.5	NR	NR	NR
Nickel	4.5	NR	NR	NR
Chromium	1.5	NR	NR	NR

The second example where epidemiology has made a clear distinction between types of tobacco products concerns cigarettes made from dark-air-cured (black) tobaccos. Epidemiological studies have shown that smokers of dark tobacco cigarettes have higher risks for smoking-related diseases than did smokers of blond cigarettes (84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94). The results of the studies dealing with lung cancer and dark tobacco cigarettes have been recently summarized (95). That summary reported that smokers of black (dark air-cured) tobacco cigarettes have about 1.75 times the risk of lung cancer than do smokers of only blond (light) tobacco cigarettes. In addition to the epidemiological findings, condensate from black tobacco cigarettes had more activity than condensate from blond cigarettes in mouse skin

painting experiments (96). The condensate from black tobacco cigarettes was found to be more mutagenic than the condensate from blond cigarettes (97).

Why are dark air-cured cigarettes different from American-blend or Virginia style cigarettes? The first clue comes from the routine analyses of the tobaccos. The traditional black tobacco cigarettes have been reported as having low or no detectable reducing sugars (98). Very high concentrations of tobacco specific nitrosamines (TSNA) have been reported in dark tobacco cigarettes, which were high in nitrate (99). Table IX shows data on various dark air-cured, blended, and Virginia style filter cigarettes from the early 1970s (100). These data show the very low sugar content of the air-cured products relative to the other products shown in the table. Also, the air-cured products appeared to have higher blend magnesium levels. Table X shows the smoke data for the same cigarettes. The analytes that were measured at that time do not clearly distinguish among the various types of tobaccos except that the levels of smoke phenols appeared to be higher in the air-cured products as opposed to the other types of products.

Table XI shows routine cigarette and smoke data for three styles of contemporary dark air-cured products. These data were provided by Dr. J. Sarabia of Altadis. Those familiar with cigarette design will note that relatively low amounts of filter ventilation were needed to achieve the given deliveries relative to those needed for American-blend products. Another noticeable difference with American-blend products is the high carbon monoxide deliveries relative to TPM.

Table XII shows more detailed tobacco and cigarette data for two international blended products (Blended 1 and Blended 2) and a European dark air-cured product. All three products were nonventilated filter kings. These data were generated by my colleagues here at Brown & Williamson Tobacco on cigarettes that were purchased in international markets earlier this year. Relative to the two blended products, the tobacco in the dark-air-cured product showed values for humectants, sugars, and polyphenols (chlorogenic acid, rutin, and scopoletin) that were below the lower limits of quantitation for our methods. The level of alkaloids was lower than that

generally found in blended products. In addition, the tobacco from the dark-air-cured cigarette was higher in ammonia, chlorides, and nitrates. The tobacco pH was over 6.6. While the tobacco chemistry for the dark-air-cured product is atypical of blended product, the cigarette parameters are well within the values expected for blended products.

Table XIII shows smoke chemistry data for the same three products. Data were obtained under ISO smoking conditions. While not shown in this Table, the TPM delivery for Blended 1 was about 19 mg/cigarette while the TPM-levels for Blended 2 and the dark-air-cured product were about 14 mg/cigarette. There was a limited supply of these cigarettes so routine smoke chemistry data were not obtained. Also, not all analytes were determined that would have been determined if more product had been available.

The left side of the table shows the deliveries of those analytes whose values are given in terms of  $\mu\text{g}/\text{cigarette}$ . This includes the vapor-phase Hoffmann analytes and the phenolics. The right side of the table shows the deliveries of those analytes whose values are given in terms of  $\text{ng}/\text{cigarette}$ . In terms of the vapor-phase analytes and the phenolics, there were few analytes that distinguish among the three products except for the reduction in aldehydes, and the increase in phenol. The reduction in formaldehyde has been shown to be a consequence of tobacco and smoke ammonia (101). This also may be the reason for the reduction of other aldehydes relative to the other two products. The reason for the increase in phenol cannot be ascertained from the available data. There is a clear distinction between the blended product and the dark-air-cured product in terms of the PAHs and aromatic amines. In particular, the levels of 1-amino- and 2-aminonaphthalene and 3-amino- and 4-aminobiphenyl were much higher in the dark-air-cured product than they were in the blended product even though the TPM deliveries of the two products were similar. This difference in aromatic-amine deliveries has been shown to be reflective in urine mutagenicity (102). It has been postulated as a reason for the higher incidence of bladder cancer among smokers of dark-air-cured cigarettes (102, 103). The elevations in lung cancer with such cigarettes have been ascribed to NNK levels (92).

The third example of where epidemiology appears to have distinguished between tobacco products is based on the comparison of smoking and lung cancer risks in American and Japanese men (104). Djordjevic and co-workers reported that on an equi-nicotine basis, Japanese cigarettes deliver significantly less “tar” constituents including B[a]P and TSNA (105). Smoke chemistry data from their report is summarized in Table XIV. The authors stated that a statistical analysis of their data showed that there was no significant differences in the comparisons between the U.S. products and similar Japanese products for TPM, “tar”, and carbon monoxide. On the other hand, they stated that there were statistically significant differences (U.S. products > Japanese products,  $p < 0.05$ ) for NAT, NAB, NNN, NNK, and B[a]P. Unfortunately, Djordjevic and coworkers did not report on any of the vapor-phase components that would have been expected to be reduced in the charcoal filtered products available in Japan or comment on the possible correlation that the reduced vapor-phase deliveries might have had with the differences in epidemiology.

TABLE IX

BLEND CHEMISTRIES FOR VARIOUS TYPES OF CIGARETTES IN 1972  
ADAPTED FROM REFERENCE 100

Brand	Country of Origin	Blend Type	Ash (%)	PETEX (%)	Nicotine Alkaloids (%)	pH	Reducing Sugars (%)	Total Sugars (%)	Nitrogen (%)	Nitrate (%)	K (%)	Cl (%)	Mg (%)
BOULE NATIONALE	Belgium	Light air-cured	21.2	4.5	1.46	6.0	*	*	3.50	1.46	5.34	1.21	0.76
Gauloise Caporal	France	Dark air-cured	21.6	4.3	1.34	6.1	**	**	3.57	1.59	4.60	1.18	0.74
HB	Germany	German blended	17.7	4.3	1.34	5.7	10.5	2.5	2.50	1.59	3.95	0.53	0.52
Hi-Lite	Japan	Modified Virginia	16.6	3.5	1.78	5.4	13.2	18.0	2.03	0.58	4.10	1.27	0.41
Johnson	Belgium	Dark air-cured	21.4	5.0	1.62	6.2	**	**	3.85	1.42	5.63	1.19	0.83
KOOL	USA	US Blended	17.3	5.4	2.02	5.5	3.0	8.9	2.90	1.64	4.25	0.75	0.52
Marlboro	Switzerland	US Blended	16.9	5.4	1.98	5.6	8.3	11.3	2.98	1.42	3.60	0.72	0.53
Marlboro	USA	US Blended	16.9	4.9	1.92	5.7	7.0	10.1	3.20	1.73	3.90	0.78	0.53
MARY LONG	Switzerland	Medium air-cured	22.0	4.2	1.76	5.7	*	5.0	2.61	1.33	5.94	0.80	0.57
Players No. 6	UK	English Virginia	13.1	5.6	2.08	5.4	11.9	17.4	2.30	0.40	3.17	0.87	0.48
PRIMEROS	Switzerland	Medium air-cured	21.4	3.7	1.29	5.6	**	**	3.27	1.90	5.24	0.82	0.68
RIVIERA	Mexico	Sun-cured	17.3	3.3	1.12	5.3	10.8	11.5	2.45	0.84	4.08	0.91	0.56
St. Michel	Belgium	Dark air-cured	20.6	5.1	1.17	6.3	*	*	3.11	1.28	4.39	0.80	0.86
VICEROY	USA	US Blended	17.3	5.0	1.83	5.3	9.7	11.3	2.76	1.68	4.22	0.84	0.51

\* Results between 2.0 and 5.0

\*\* Results less than 2.0

TABLE X  
 SMOKE CHEMISTRIES FOR VARIOUS TYPES OF CIGARETTES IN 1972  
 ADAPTED FROM REFERENCE 100

Brand	Country of Origin	Blend Type	TPM (mg/cig)	TPM/puff	Total Nicotine Alkaloids (mg/cig)	Phenols (µg/cig)	Puffs	Smoulder Rate (mm/min)	CO (mg/cig)
BOULE NATIONALE	Belgium	Light air-cured	26	2.95	1.39	97	8.8	3.5	22.9
Gauloise Caporal	France	Dark air-cured	28	3.01	1.61	155	9.3	3.1	23.4
HB	Germany	German blended	20	2.15	0.88	53	9.3	4.9	16.8
Hi-Lite	Japan	Modified Virginia	33	3.40	1.81	90	9.7	4.1	21.7
Johnson	Belgium	Dark air-cured	24	3.28	1.54	119	7.3	4.8	20.9
KOOL	USA	US Blended	23	2.60	1.52	42	8.2	5.2	NR
Marlboro	Switzerland	US Blended	31	3.10	1.89	81	10.3	4.1	NR
Marlboro	USA	US Blended	24	2.63	1.42	58	10	4.9	15.9
MARY LONG	Switzerland	Medium air-cured	25	2.87	1.11	70	8.7	4.2	19.1
Players No. 6	UK	English Virginia	24	3.20	1.33	92	7.5	3.9	NR
PRIMEROS	Switzerland	Medium air-cured	31	3.92	1.24	59	7.9	3.9	19.1
RIVIERA	Mexico	Sun-cured	40	3.57	1.33	95	11.2	4.5	34.2
St. Michel	Belgium	Dark air-cured	34	3.36	1.60	113	10.1	3.4	18.9
VICEROY	USA	US Blended	21	2.80	1.28	47	7.5	3.9	18.4

NR = Not reported

TABLE XI  
ROUTINE CIGARETTE AND SMOKE DATA FOR CONTEMPORARY DARK-AIR-CURED CIGARETTES  
DATA FROM J. SARABIA - ALTADIS

Style	Tobacco Weight (mg)	Filter Ventilation (%)	Nicotine Alkaloids (%)	Reducing Sugars (%)	TPM (mg/cig.)	TPM/Puff	Total Nicotine Alkaloids (mg/cig.)	PMWNF (mg/cig.)	Puffs	CO (mg/cig.)
Ultraight KS	664	29	1.57	0.0	5.1	0.8	0.29	4.2	6.3	9.4
Light KS	707	12	1.49	0.0	9.7	1.4	0.53	7.6	7.0	12.6
Full Flavor KS	692	NR	1.45	0.0	13.1	2.0	0.61	10.3	6.7	14.8

TABLE XII  
TOBACCO AND CIGARETTE DATA FOR BLENDED AND DARK-AIR-CURED CIGARETTES

Parameter	Blended 1	Dark-Air-Cured	Blended 2
Ammonia (%)	0.13	0.39	0.15
Theobromine (%)	BLOQ	BLOQ	BLOQ
Glycyrrhizic acid (%)	BLOQ	BLOQ	0.02
Glycerine (%)	3.07	BLOQ	1.81
Propylene Glycol (%)	BLOQ	BLOQ	0.65
Chlorogenic Acid (%)	0.53	BLOQ	0.56
Rutin (%)	0.45	BLOQ	0.47
Scopoletin (%)	0.03	BLOQ	0.03
Alkaloids (%)	2.14	1.59	2.33
Chlorides (%)	0.88	1.68	0.70
Fructose (%)	2.75	BLOQ	3.31
Glucose (%)	1.45	BLOQ	2.34
Nitrates (%)	1.01	1.67	0.88
Phosphates (%)	0.77	0.80	0.60
Sucrose (%)	0.34	BLOQ	3.34
Reducing Sugars (%)	4.20	BLOQ	5.65
Total Sugars (%)	4.54	BLOQ	8.99
Tobacco pH	5.38	6.66	5.56

Parameter	Blended 1	Dark-Air-Cured	Blended 2
Cigarette Pressure Drop (in. water)	5.09	4.36	5.41
Tip Ventilation (%)	0.1	0.6	0.3
Circumference (mm)	24.6	25.1	24.9
Length Cigarette (mm)	83.6	83.6	83.6
Length Filter (mm)	22.3	21	20
Length Tipping (mm)	26	25	24
Length Tobacco Section. (mm)	61.3	62.6	63.6
Cigarette Weight (mg)	1022	924	925
Tobacco Weight (mg)	808	717	730
Density (mg/cc)	273	229	233
Nontobacco Weight (mg)	214	207	195
Filter Weight (mg)	124	112	109
Filter Triacetin (%)	7.99	8.27	6.94
Paper Porosity (CU)	45	45.3	42.2
Paper Citrate (%)	0.65	0.51	1.93

BLOQ = Below lower limit of quantitation

TABLE XIII  
SMOKE CHEMISTRY DATA FOR BLENDED AND DARK-AIR-CURED CIGARETTE

Analyte (µg/cig)	Blended 1	Dark-Air-Cured	Blended 2	Analyte (ng/cig)	Blended 1	Dark-Air-Cured	Blended 2
Hydrogen sulfide	38.3	25.9	24.8	Naphthalene	936	1088	632
Hydrogen cyanide	71.7	45.5	49.1	Fluorene	350	358	232
Methanol	138	23.9	115	Phenanthrene	230	258	171
Formaldehyde	40.8	21.2	39.6	Anthracene	78.4	75.2	47.2
Acetaldehyde	598	424	520	Fluoranthene	95.2	102	69.6
1,3-Butadiene	28.9	22.9	21.1	Pyrene	67.2	64.4	46.4
Acetonitrile	140	123	99.5	Benzofluorene	40.8	41.6	25.8
Acrolein	54.2	33.7	45.2	Benzanthracene	22.9	24.7	13.8
Furan	22.2	14.6	18.1	Chrysene	26.7	24.6	18.2
Propanal	35.0	22.5	28.7	Benzofluoranthene	18.9	16.8	12.3
Acetone	245	186	198	Benzofluorene	9.1	7.9	5.7
Acrylonitrile	10.5	8.6	8.2	Benzofluoranthene	11.5	9.8	7.8
Carbon disulfide	3.4	2.8	2.5	Perylene	1.6	1.1	1.0
Isoprene	187	119	160	Dibenzanthracene	0.6	0.6	0.3
Propionitrile	23.2	20.2	18.1	Benzofluorene	3.8	2.6	2.4
2-Methylpropanal	10.1	7.0	7.5	Aniline	NA	640	408
Methylethyl ketone + Butanal	66.4	42.8	54.5	o-Toluidine	NA	209	118
Crotonaldehyde	6.5	5.1	6.0	m-Toluidine	NA	294	181
Benzene	29.6	28.5	27.4	p-Toluidine	NA	271	150
Pyridine	1.2	1.0	1.0	2-Ethylaniline	NA	64.0	37.4
Toluene	18.8	20.2	17.0	2,6-Dimethylaniline	NA	6.16	2.88
Styrene	1.1	1.2	1.1	2,5-Dimethylaniline	NA	64.8	39.46
Phenol	16.4	23.0	13.5	2,4-Dimethylaniline	NA	60.4	31.8
o-Cresol	4.6	6.3	3.9	3-Ethylaniline	NA	68.4	47.2
m-Cresol	3.7	4.7	3.2	3,5-Dimethylaniline	NA	43.6	25.7
p-Cresol	8.6	13.5	6.9	2,3-Dimethylaniline	NA	9.92	5.24
Catechol	56.1	40.3	48.4	4-Ethylaniline	NA	93.2	54.8
Resorcinol	1.1	0.8	1.3	3,4-Dimethylaniline	NA	43.6	25.8
Hydroquinone	51.7	30.4	52.8	1-Aminonaphthalene	NA	75.6	38.8
				2-Aminobiphenyl	NA	10.1	5.12
				2-Aminonaphthalene	NA	141	69.6
				3-Aminobiphenyl	NA	10.6	5.52
				4-Aminobiphenyl	NA	6.56	3.88
				Benzidine	NA	0.57	0.59

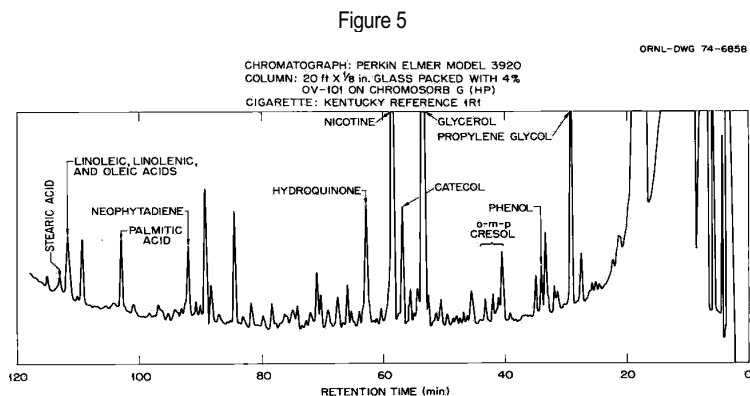
TABLE XIV  
SMOKE CHEMISTRY DATA FOR U.S. AND COMPARABLE JAPANESE PRODUCTS  
DATA ADAPTED FROM REFERENCE 105

	TPM	"Tar"	Nicotine	CO	NAT	NAB	NNN	NNK	Total TSNA	B(a)P
	(mg/cig.)	(mg/cig.)	(mg/cig.)	(mg/cig.)	(ng/cig.)	(ng/cig.)	(ng/cig.)	(ng/cig.)	(ng/cig.)	(ng/cig.)
U.S. Brands										
KS, F, SP, LT	12	10	0.8	10	183	18	165	117	483	11.1
KS, F, SP, LT	10	9	0.8	10	136	17	146	98	397	13.5
KS, F, SP, LT, MEN	11	10	0.8	12	145	12	145	92	394	9.2
KS, F, SP, LT, MEN	10	9	0.7	11	187	27	210	144	568	11.2
KS, F, SP	21	16	1.1	14	251	23	256	145	675	14.2
KS, F, SP, MEN	20	16	1.1	15	312	35	297	106	750	11.2
KS, F, SP (charcoal)	16	15	1.0	15	200	24	219	166	609	9.3
KS, F, SP	20	17	1.4	14	249	26	236	216	727	11.6
Japanese Brands										
KS, F, SP, LT	8	6	0.7	6	62	8	36	37	143	5.1
KS, F, SP, LT	8	7	0.6	6	109	13	75	49	246	5.8
KS, F, SP, LT	11	10	1.0	8	96	11	70	38	215	8.1
KS, F, SP	16	12	1.2	13	130	13	96	66	305	9.9
KS, F, HP	13	10	1.1	8	138	21	129	58	346	8.8
KS, F, SP	21	16	1.6	19	87	12	64	53	216	13.3

## Correlations among smoke chemistry and measures of smoke toxicity

It is relatively easy to generate large quantities of data on the mainstream smoke from various brands of cigarettes. Indeed, if one has the financial resources, commercial laboratories that do the job for you and present the data neatly tabulated in electronic spreadsheets or other common formats for electronic data. Since data from the laboratories that provide *in vitro* or *in vivo* also can provide data electronically, it would appear that making correlations between smoke chemistry and biological effects is a simple matter of mixing and matching data with one's favorite statistical analysis package. Obviously, this is not the case; and this has been a historical problem. Data presentation also is a problem when discussing the results of various studies with those who may be less familiar with the details of the chemistry and toxicological assays. One of the early approaches to the problem was developed by Guerin and Nettesheim (32).

Figure 5, which was taken from Reference 32, shows the resolution of the chromatograms for silylated mainstream TPM at the time of the TWG. This chromatogram was obtained with a packed column, and t much more complicated chromatograms can be



obtained with today's fused silica capillary columns. However, the numerous peaks in this chromatogram are sufficient to document the challenge of correlating chemical data with the results from *in vitro* and *in vivo* toxicological assays. Now, if one is lucky, one may get a

reasonable linear correlation with one or more of the peaks in the chromatogram and one or more toxicological endpoints. An example of this is shown in Figure 6, which was taken from Reference 32.

Figure 6

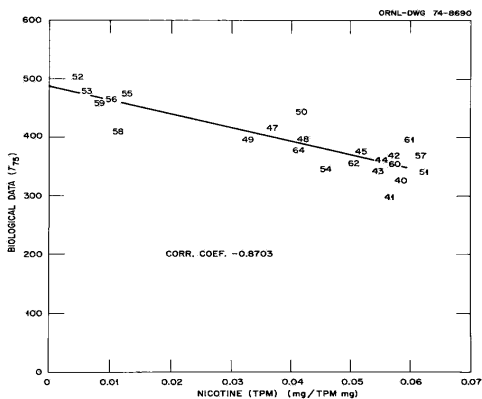
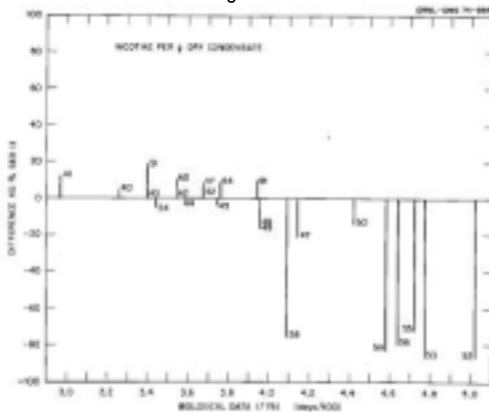


Figure 6 shows the biological response from a mouse skin painting study (T<sub>75</sub>, which is the estimated number of days from the initiation of the experiment that 75% of the mice would survive) with the concentration of nicotine in the TPM. Another way of presenting the same data is shown in Figure 7, which shows the results versus the reference product (SEB II) (32).

Figure 7



Since the mouse skin painting assays are usually done on a per unit weight of condensate basis, there is no correction for the differences in deliveries among the cigarettes in the study (i.e., nonfilter high-delivery products would be considered on same basis as ultralight products). This is a very important consideration because of the partitioning of the semivolatile components of the TPM between the vapor and particulate phases of the smoke aerosol (106, 107). If the smoke collection system used to trap the TPM allows loss of part of the semivolatiles, then the TPM becomes enriched in the nonvolatile materials. The nonvolatile components of TPM are believed to be responsible for much of the biological activity associated with the particulate phase of the smoke aerosol (5, 6, 7, 11, 108, 109, 110, 111). This may be one reason that condensate mutagenicity (TA98+S9, TA100+S9) generally increases as filter ventilation increases when condensate is trapped on the Cambridge pad (112, 113). However, increasing puff volume and puff frequency decreases condensate mutagenicity (114). Chortyk and Chamberlain reported a decrease in Ames activity with a decrease in mainstream deliveries when the smoke condensates were collected in solution (115).

Cytotoxicity studies have become more popular as more laboratories have implemented the Neutral Red Uptake assay (116). This assay has been implemented for whole smoke (117). It also has been implemented for smoke fractions such as TPM, the gas-vapor phase (GVP) passing through the Cambridge pad, and mixtures of GVP and TPM (118, 119, 120, 121). In one sense, the Neutral Red Uptake assay is a modern version of the cytotoxicity assay employed in the TWG studies, and one might expect somewhat similar findings.

In terms of the cytotoxicity of TPM, Bombick and coworkers reported in 1998 that TPM from all flue-cured cigarettes was more cytotoxic than that from all burley cigarettes and that upper stalk tobacco of both varieties gave more cytotoxic condensates than did the corresponding lower stalk tobaccos (122). The same research group has also reported that the cytotoxicities of the condensates from the Kentucky KY1R4F and KY1R5F are representative of those of the light and ultralight "tar" categories, respectively, on the domestic market (123). In

that study, the average EC<sub>50</sub> values reported for the full flavor, lights, and ultralights groups were 44.8 µg/mL, 51.7 µg/mL, and 38.5 µg/mL, respectively. The values reported for the KY1R4F and KY1R5F were 57.9 µg/mL and 42.5 µg/mL, respectively. It is important to note that the higher the EC<sub>50</sub> value the less toxic the condensate. Similar findings were reported by Tewes and coworkers although they used a different cell line in their assays (120). They found that cytotoxicities of the condensates from American blend cigarettes differed little from cytotoxicity of the condensate of the KY1R4F. Cytotoxicities of the condensates from single-grade cigarettes were higher than those of the blended products with bright tobacco giving more cytotoxic condensate than burley tobacco. There does not appear to be an explanation for the trends that have been observed nor relationships between the cytotoxicity of the condensates from the domestic brands and the smoke chemistry data provided in the same report (123). Some work has been done with pure compounds likely to be in the condensate. Bombick and Doolittle have shown that 4-vinylpyridine and 2-vinylpyridine are much more cytotoxic than pyridine itself (124). Bombick and coworkers also have shown that phenolic constituents in condensate have little impact on cytotoxicity (125).

The situation appears clearer when whole smoke or just the gas-vapor phase of smoke is considered. Acrolein and to a lesser extent formaldehyde have been found to be much more cytotoxic than the other volatile carbonyl compounds found in mainstream smoke (120, 124). Furthermore, cigarettes made with a carbon filter designed to remove such compounds from mainstream smoke had reduced cytotoxicity with respect to a conventionally filtered product (118). This finding is not a new one. In 1970, Battista and Kensler reported similar findings in an *in vivo* study using chicken trachea (126).

Changes smoke chemistry that are reflected in the *in vitro* Neutral Red cytotoxicity assay have been reported to be reflected in changes observed in rodent inhalation experiments. When apparently the same cigarette samples as described in Reference 118 were used in an acute mouse inhalation study, it was found that the cigarette with the carbon filter reduced the

irritancy of the smoke as measured by the concentration of smoke associated with a 50% decrease in respiration rate ( $RD_{50}$ ) (127).

In a rat inhalation study published in 1980, Lam found that use of a carbon-filtered cigarette versus a conventionally filtered cigarette gave some surprising results when the biological endpoint was loss of epithelial cells in the larynx (128). Lam reported that at equal particulate matter concentrations the carbon-filtered cigarette caused more pathology than did the conventionally filtered product even though the carbon-filtered product gave 73% reductions in the vapor-phase constituents such as HCN, acrolein, and formaldehyde. Lam hypothesized that the reason for the observed effect was that the carbon filter had removed part of the semivolatiles in the particulate phase thus increasing the effective dose of particulate matter. In addition, the data indicated that in the case where a significant difference in pathology was seen, the smoke concentration for the charcoal filtered cigarette was 4.00% while it was 3.50% for the conventionally filtered cigarette. There are no data in that paper on chemical measurements of the test atmospheres although the methodology for such measurements had been developed by the same laboratory (129).

In another rodent inhalation study from the same era, Coggins, Lam, and Morgan reported the results of a chronic inhalation study using cigarettes containing differing levels of Cytrel tobacco supplement (60) in a flue-cured blend typical of commercial U.K. cigarettes. Cytrel inclusion levels were 0, 25, 50, 75, and 100%. Apparently, very similar cigarettes were used as part of the evaluation of the continuous-smoking inhalation machine described in Reference 129, and the smoke deliveries of other Cytrel-containing cigarettes have been published (62, 63, 64). The relevant smoke chemistry and histopathological data are summarized in Tables XV and XVI.

TABLE XV

CYTREL INHALATION STUDY  
DATA ADAPTED FROM REFERENCES 60 AND 129

Cytrel inclusion (%)	Average puffs/cig. (per session)	Cigarette consumption (%)	Smoke dilution (%)	PMWNF (mg/m <sup>3</sup> )	PMWNF (mg/cig)	Nicotine (mg/cig)	CO (mg/m <sup>3</sup> )	CO (mg/cig)	HCN (µg/cig)	Aldehydes (mg/cig)	HCHO (µg/cig)	Phenols (µg/cig)
0	8.3	248	1.74	550	16.3	1.18	616	16.2	297	1.9	82	87
25	7.4	294	1.75	582	NR	NR	750	NR	NR	NR	NR	NR
50	6.3	325	1.94	479	8.5	0.32	807	10.9	136	1.2	62	33
100	5.3	378	5.41	619	3.1	0	1403	4.5	7	0.2	10	15

TABLE XVI

CYTREL INHALATION STUDY  
DATA ADAPTED FROM REFERENCES 60 AND 129

Cytrel inclusion (%)	Body weight male (g)	Body wt female (g)	COHb (%) male	COHb (%) female	Nasal Epithelium (0-3)	Laryngeal Epithelium (0-3)	Tracheal Epithelium (0-3)	Bronchial Epithelium (0-3)	Brown Gold Macrophages (0-3)
0	398	288	26.9	29.9	1.13	1.76	0.73	0.36	1.33
25	415	274	33.0	38.4	1.05	1.50	0.71	0.40	1.27
50	425	266	27.7	34.0	1.10	1.67	0.76	0.46	1.29
100	387	268	43.3	48.8	0.69	1.36	0.38	0.34	0.49
Room Control	652	442	NR	NR	0.27	0.27	0.10	0.03	0.01
Machine Control	506	314	NR	NR	0.16	0.19	0.08	0.04	0.00

While no statistical analyses of the data have been done, one can see that as the percentage of Cytrel in the blend increased, there was a decrease in the analytes associated with irritation and cytotoxicity. The severity of the lesions reported in the histopathological examinations for the nose, larynx, and trachea decreased as the percentage of Cytrel in the blend increased. These differences in pathology became statistically significant between the sample with 50% Cytrel inclusion and the sample that was 100% Cytrel. However, it is not known from the available data as to which, if any, of the measured analytes was responsible for the change in the biological outcomes.

It is clear from the examples in the literature that reductions in some measured analytes in some tests do correlate with reductions in biological potency in certain assays. However, generally technology that results in the reduction of a measured analyte, results in changes of other analytes, measured or not. Indeed, in the case of mainstream cigarette smoke, the unmeasured analytes outnumber the measured analytes by more than 100:1. Therefore, correlation of specific analytes with the results of biological assays is not necessarily proof that the measured analytes were the only ones responsible for biological activity. For example, Battista listed sulfur dioxide and 2,3-butanedione as having the same order of ciliotoxicity as ammonia and crotonaldehyde (130). The former two compounds are not generally measured as part of the Hoffmann analytes, but the latter two are.

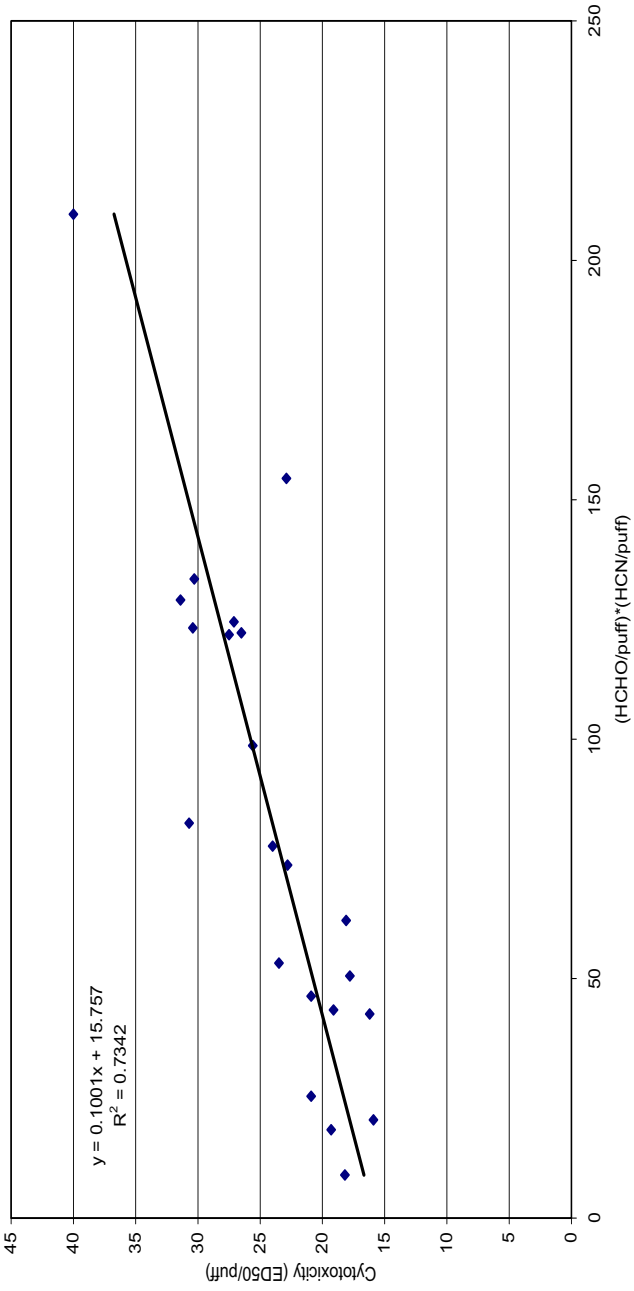
Another factor that tends to limit the usefulness of correlations between smoke chemistry and biological activity is the neglect of nonlinear relationships. First, many biological processes are nonlinear with respect to dose. For example, the dose-response curves for the Neutral Red Uptake assay of mainstream cigarette smoke are best described by a logistic function as reported by Roemer and coworkers (121). Second, there are interactions among the chemical constituents in smoke. One of these, the interaction of HCN and aldehydes, was described by James Nall of the Brown & Williamson Tobacco Corporation at the 20<sup>th</sup> TCRC in 1966 (131).

Figure 8 shows the relationship between cytotoxicity (EC50/puff) and the product of the per puff deliveries of HCN and formaldehyde for the different cigarettes designs using the straight SEB III blend and casings in TWG-III. Another known case involving the reactivity of formaldehyde that may be important in understanding biological endpoints is its reaction with ammonia (101). While there was consideration and use of second-order and cross-product terms in Bayne's analysis of the TWG mouse skin painting data (34), the significance of some of his terms (i.e.,  $[pH]^2$ ) with respect to the correlations between the smoke chemistry and the biological effect remain a matter of speculation. Therefore, correlation of smoke chemistry with biological activity should be a joint effort of chemists, toxicologists, and statisticians.

Recently, two reports detailed the results of surveys of smoke chemistry for a number of brands on the U.S. market. The first of these provided data on both chemistry and mutagenicity (113) while the latter just included smoke chemistry (132). Both reports show numerous graphs detailing the linear relationships (some better than others) of many of the Hoffmann analytes with FTC "tar", and the second report also provides an extensive compilation of smoke chemistry data for numerous cigarette brand styles. While these reports show interesting ways of processing and presenting smoke chemistry data, it is important to note that the purposes of the reports were somewhat different. The purpose of the first report was to show that the KY1R4F and KY1R5F reference cigarettes were acceptable reference cigarettes for comparative mutagenicity and smoke chemistry studies on cigarettes in the U.S. domestic market (113). The purpose of the second report was to show that the smoke constituent yields of commercially marketed cigarettes in the U.S. between 1995 and 2000 have been effectively constant (132).

Another approach to presenting smoke chemistry data has been given by Rustemeier and coworkers as part of Philip Morris's ingredient study (13, 133). They used radar charts to illustrate the differences in analytes from test and control products.

Figure 8  
Cytotoxicity (ED50/puff) vs. (HCHO/puff)\*(HCN/puff)



## SUMMARY

In this report, the complex subject of the relationships among tobacco, tobacco smoke, and a variety of biological endpoints has been covered from three perspectives: 1) a review of the studies by and associated with the Tobacco Working Group (TWG); 2) a review of the three commonly recognized relationships between tobacco or smoke chemistry and the results of epidemiological studies; and 3) a review of studies dealing with relationships between smoke chemistry and various biological endpoints and different strategies for correlating chemistry alone or with biological endpoints.

The TWG, which was essentially a study in PREPS based on conventional product technology, used many of the testing strategies that we use today in assessing the health-related aspects of tobacco products: 1) tobacco and/or smoke chemistry; 2) *in vitro* toxicology; and 3) *in vivo* toxicology. In addition, extensive use of statistics was made to identify the factors responsible for the differences in biological endpoint among the different cigarette designs. The TWG studies also pointed out the danger in using statistics as nicotine was found to be highly correlated with tumorigenicity and very significant efforts were required to show that this correlation was no doubt caused by minor components in smoke that were well correlated with nicotine.

The second part discussed the three cases of where epidemiological results have been correlated with product chemistry. The three cases: 1) Swedish snus versus conventional snuff products; 2) dark-air-cured (black) tobacco cigarettes versus blended and Virginia-style products; 3) Japanese versus U.S. domestic cigarettes show the apparent relationships between product chemistries and some diseases associated with tobacco use. These are apparent relationships and more studies will be needed to confirm the chemistries or other factors that may be responsible for the epidemiological findings.

The final section of this report covers specific examples of where smoke chemistry has been correlated with specific in vitro and/or in vivo endpoints. This section also states the need for both more in-depth chemical studies and/or the use of nonlinear statistical techniques to find better correlations between smoke chemistry and biological endpoints. In particular, results of assays of TPM in the Neutral Red Uptake assay for cytotoxicity do not appear correlated with most chemical measures employed on mainstream cigarette smoke.

In conclusion, the challenges of correlating tobacco and/or smoke chemical data with toxicological data remain after much work by numerous chemists, toxicologists, and statisticians associated within our industry. We now must use advances in analytical chemistry and toxicological sciences to continue the quest for understanding these complex relationships.

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