R.J. Reynolds Tobacco Company

Interoffice Memorandum

Environmental & Molecular Toxicology

Comments From Riley Davis

The following are comments related to the use of cotinine as a valid biomarker for ETS exposure as suggested by N.L. Benowitz:

1) RJRT has sufficient data and numerous publications in peer-reviewed journals to effectively refute the supposition that the ratio of nicotine to RSP or its fractional components, i.e., UVPM, FPM, Sol-PM, and Scop-PM. The variation of this ratio depends upon numerous factors related to air movement, room volume, etc. that have been previously discussed by us. It is critical that even if the ratio of nicotine to RSP is relatively constant, the other constituents (present in the particulate phase) are not constant with regard to nicotine. That is, nicotine cannot serve as a surrogate for particulate phase compounds generally associated with alleged health effects on humans. A. Rodgman addressed this issue from the viewpoint of dilution of ETS component compounds as compared to mainstream smoke. Benowitz states that "when a person is exposed to ETS over time, the intake of nicotine reflects exposure to other constituents of ETS"(p.189). This is invalid and must be shown to be so.
2) Benowitz's treatment of the pharmacokinetics of nicotine and cotinine appear to be reasonably sound. Note that the majority of the data are taken from active smokers with very little data for non-smokers. While he contends that smokers and non-smokers metabolize nicotine in a similar fashion, this question remains controversial especially in children and non-exposed non-smokers. I tend to agree with his premise for adults in general. We have scant data to refute this point and are not likely to obtain it without an investigation into this issue. Apparently the PBPK model cannot simulate "real-world" exposures to nicotine and the resultant cotinine body fluid concentrations at present. I differ with both Benowitz and T. Steichen regarding the ratio of serum cotinine concentration to urinary cotinine concentration in non-smokers. While Benowitz is correct in stating that this ratio is about 6 in smokers, I found that the ratio is much higher (ca. 115) in non-smokers using data from the GUM2 study (attached). Also using Benowitz's formula to estimate nicotine intake typically overestimates nicotine intake based upon G. Byrd's data from the GUM2 study (attached). Some calculations were done using Table 4 from the Pirkle paper and they are shown in an attachment.
3) T. Steichen has dealt with the Pirkle data and Benowitz' attempt to over-state ETS exposure using the London bartender data. I still am convinced that nicotine exposure can come from sources other than ETS especially at serum cotinine concentrations =/< 1.0 ng/ml. We should continue to make the arguments for dietary sources of nicotine and the off-gassing of nicotine from objects in the room where smoking occurred on some prior occasion(s).

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4) His treatment of the analytical techniques for the measurement of nicotine and cotinine is adequate. While he mentions the cross-reactivity of nicotine metabolites in the cotinine RIA, he doesn't expand on this. If cross-reactivity is operable in the cotinine RIA, then the values reported are biased high therefore presenting an exaggerated cotinine concentration. This leads to an even greater over-estimation of nicotine exposure, hence ETS exposure that serves the anti-smoking lobby well. We now know that 3-hydroxycotinine is present in serum, saliva, and is the predominant nicotine metabolite in urine. For this reason we use either ELISA or GC-NPD to measure nicotine and cotinine in urine. We and others have found that 3-hydroxycotinine is not only the major source of interference in the cotinine RIA, but that it results in an over-estimate of the cotinine concentration by 30-40%, on average use 30%. Applying this correction to R. Jenkins data (New Orleans, Dec., 1996) results in slightly lower saliva cotinine data for the four cells reported and would indicate that ETS exposure is less a problem than Benowitz et al would have the regulatory agencies believe.

Cell 1 = 1.36 ng cotinine/ml
Cell 2 = 0.62 ng cotinine/ml
Cell 3 = 0.32 ng cotinine/ml
Cell 4 = 0.11 ng cotinine/ml.

The effect of this bias on risk assessment is not known, but I suspect that it would reduce the odds ratio more closely to 1. The bias is present in a majority of the papers Benowitz cites to make his points regarding cotinine as a biomarker since the investigators used RIA or had American Health Fdtn do the analyses for them. (An aside---I would not be too surprised if Bill Rickert, Labstat, Inc. published a paper describing non-smoker exposure to ETS based upon saliva 3-hydroxycotinine concentrations.) The impact and significance of the bias present in the cotinine RIA on the ETS issue may be worth mentioning, but not as a major point.

5) The use of 3'-hydroxycotinine as a biomarker for ETS exposure suffers from the same deficiencies that cotinine does. These deficiencies have been elaborated upon by numerous authors beginning with J. Idle. Armitage, et al have recently re-examined the issue (Med. Sci. Res., 25: 3-7, 1997.) in a response to Law and Hackshaw (British Med. Bull., 52: 22-34, 1996).

I look forward to additional discussions around this topic in the near future. Should we bring forward suggestions for additional studies to bolster our point(s) of view?

Attachments.

Environmental & Molecular Toxicology

Estimates of Nicotine Intake in Non-Smokers

Note: These estimates are derived from pharmacokinetic formul
by
N. L. Benowitz as follows:

a)* Nicotine Intake (mg/24 hr) = 0.08 * $[COT]_{Bss}$, wh
[COT]Bss =
serum [Cot] at steady-state. Saliva [Cot] is convertec
taking the reciprocal of 1.3, the average plasma:saliva
ratio.

b)* Nicotine Intake (mg/24Hr) = (0.08/6) * $[Cot]_{Urine}$

c)** $[COT]_{Bss}$ = $Dose/Clearance_{Total}$ was used to estir
state;
Saliva [Cot] was estimated by multiplying Serum[Co

Analytical Data

Subject #	Sample #	Saliva [Cotinine] ng/ml	[Urine Cot.] ng/ml	Cal'd.$[Cot]_{Bss}$: $[Cot]_{Urine}$ Ratio	Estimated Nicotir Saliva mg/day	Urine mg/day
50	1	0.4	32.3	104.87	0.0246	0.41
50	2	0.3	54.9	237.66	0.0185	0.71
50	3	0.6	36.8	79.65	0.0370	0.47
50	4	0.5	73.7	191.43	0.0308	0.95
50	5	0.2	44.4	288.31	0.0123	0.57
50	6	0.3	58.5	253.25	0.0185	0.76
50	7	0.2	26.8	174.03	0.0123	0.34
50	8	0.4	72.4	235.06	0.0246	0.94
50	9	0.7			0.0431	
50	10	0.4			0.0246	
50	11	0.2			0.0123	
50	12	0.5			0.0308	
51	1	0.3	45.5	196.97	0.0185	0.59
51	2	0.2	29.2	189.61	0.0123	0.37
51	3	0.3	12.1	52.38	0.0185	0.15
51	4	0.3	18.9	81.82	0.0185	0.24
51	5	0.4	8.9	28.90	0.0246	0.11

51	8	0.3	20.9	90.48	0.0185	0.27
51	9	0.4			0.0246	
51	10	0.4			0.0246	
51	11	0.4			0.0246	
51	12	0.3			0.0185	
52	1	0.4	46.4	150.65	0.0246	0.60
52	2	0.5	43.9	114.03	0.0308	0.57
52	3	0.5	31.9	82.86	0.0308	0.41
52	4	0.5	78.4	203.64	0.0308	1.01
52	5	0.5	48.8	126.75	0.0308	0.63
52	6	1.2	85.1	92.10	0.0739	1.10
52	7	0.5	66.3	172.21	0.0308	0.86
52	8	1.3	56	55.94	0.0801	0.7
52	9	0.8			0.0493	
52	10	0.7			0.0431	
52	11	0.7			0.0431	
52	12	0.6			0.0370	
53	1	1.1	43	50.77	0.0678	0.5
53	2	0.6	47.9	103.68	0.0370	0.62
53	3	0.6	39.6	85.71	0.0370	0.51
53	4	0.4	60.2	195.45	0.0246	0.78
53	5	0.6	34.7	75.11	0.0370	0.45
53	6	1.1	46.4	54.78	0.0678	0.60
53	7	0.4	56.3	182.79	0.0246	0.73
53	8	0.2	47.3	307.14	0.0123	0.61
53	9	0.2			0.0123	
53	10	0.2			0.0123	
53	11	0.3			0.0185	
53	12	0.4			0.0246	
54	1	0.2	34.6	224.68	0.0123	0.44
54	2	1.5	93	80.52	0.0924	1.2
54	3	1.7	43.5	33.23	0.1047	0.56
54	4	1	65.5	85.06	0.0616	0.85
54	5	1.3	38.6	38.56	0.0801	0.50
54	6	1.3	61.9	61.84	0.0801	0.80
54	7	0.9	45.7	65.95	0.0554	0.59
54	8	1.4	30.5	28.29	0.0862	0.39
54	9	1.3			0.0801	
54	10	1.3			0.0801	
54	11	1.2			0.0739	
54	12	1.4			0.0862	

55	4	1.1	119.4	140.97	0.0678	1.55
55	5	1.3	52.4	52.35	0.0801	0.68
55	6	1.2	94.1	101.84	0.0739	1.22
55	7	1	92.5	120.13	0.0616	1.20
55	8	0.9	97.2	140.26	0.0554	1.26
55	9	2.4			0.1478	
55	10	1.2			0.0739	
55	11	1.4			0.0862	
55	12	3			0.1848	
56	1	1	151.9	197.27	0.0616	1.97
56	2	1.5	102.8	89.00	0.0924	1.33
56	3	0.9	79.1	114.14	0.0554	1.02
56	4	0.8	90.2	146.43	0.0493	1.17
56	5	0.9	91.5	132.03	0.0554	1.18
56	6	0.7	99.5	184.60	0.0431	1.29
56	7	0.8	59.5	96.59	0.0493	0.77
56	8	0.7	94.9	176.07	0.0431	1.23
56	9	0.8			0.0493	
56	10	1			0.0616	
56	11	0.2			0.0123	
56	12	0.9			0.0554	
57	1	0.5	52.2	135.58	0.0308	0.67
57	2	0.3	59.3	256.71	0.0185	0.77
57	3	0.3	43.6	188.74	0.0185	0.56
57	4	0.3	43.8	189.61	0.0185	0.56
57	5	0.4	36.6	118.83	0.0246	0.47
57	6	0.4	49	159.09	0.0246	0.6
57	7	0.3	49.4	213.85	0.0185	0.64
57	8	0.3	62.3	269.70	0.0185	0.80
57	9	0.3			0.0185	
57	10	0.2			0.0123	
57	11	0.3			0.0185	
57	12	0.3			0.0185	
58	1	0.2	28.7	186.36	0.0123	0.37
58	2	0.2	30.1	195.45	0.0123	0.39
58	3	0.2	39.5	256.49	0.0123	0.51
58	4	0.4	37.5	121.75	0.0246	0.48
58	5	0.3	18.2	78.79	0.0185	0.23
58	6	0.4	30.5	99.03	0.0246	0.39
58	7	0.2	9.3	60.39	0.0123	0.12
58	8	0.3	15.1	65.37	0.0185	0.19

58	12	0.3			0.0185	
59	1	0.2	35.7	231.82	0.0123	0.46
59	2	0.2	42.4	275.32	0.0123	0.55
59	3	0.5	45.5	118.18	0.0308	0.59
59	4	0.2	35.8	232.47	0.0123	0.46
59	5	0.2	31.6	205.19	0.0123	0.41
59	6	0.2	72.7	472.08	0.0123	0.94
59	7	0.2	61.5	399.35	0.0123	0.79
59	8	0.1	37.7	489.61	0.0062	0.49
59	9	0.2			0.0123	
59	10	0.3			0.0185	
59	11	0.2			0.0123	
59	12	0.3			0.0185	
60	1	0.4	32.7	106.17	0.0246	0.42
60	2	0.6	25	54.11	0.0370	0.3
60	3	0.4	22	71.43	0.0246	0.2
60	4	0.2	42.2	274.03	0.0123	0.54
60	5	0.2	26.1	169.48	0.0123	0.33
60	6	0.2	28.7	186.36	0.0123	0.37
60	7	0.3	16.3	70.56	0.0185	0.21
60	8	0.2	41	266.23	0.0123	0.5
60	9	0.2			0.0123	
60	10	0.9			0.0554	
60	11	0.4			0.0246	
60	12	0.4			0.0246	
61	1	0.3	60.6	262.34	0.0185	0.78
61	2	1.4	117.5	109.00	0.0862	1.52
61	3	0.5	55.3	143.64	0.0308	0.71
61	4	0.4	96.1	312.01	0.0246	1.24
61	5	0.6	29.8	64.50	0.0370	0.38
61	6	1.5	115.9	100.35	0.0924	1.50
61	7	1.1	65.5	77.33	0.0678	0.85
61	8	0.3	121.4	525.54	0.0185	1.57
61	9	0.1			0.0062	
61	10	0.2			0.0123	
61	11	1.1			0.0678	
61	12	0.2			0.0123	

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These data are taken from Table 4 in Pirkle, J., et al., JAMA 275: 1233-1240, 1996.

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Condition	Sample Size	Geometric Mean [Cot] ng/ml	Lower 95% C.I.	Upper 95% C.I.
No. of Smokers in home				
0	5382	0.149	0.134	0.165
1	1349	0.734	0.621	0.867
>1	600	1.24	1.07	1.43
No. of hours of Work Exposure (>17 y.o.a.)				
0	3861	0.163	0.144	0.185
1 to 3	487	0.338	0.293	0.39
>3	538	0.468	0.397	0.552
Distribution by age				
4-11 years				
No ETS Exposure	1071	0.119	0.101	0.14
Home ETS Exposure	713	1.14	0.978	1.34
12-16 years				
No ETS Exposure	379	0.113	0.097	0.154
Home ETS Exposure	268	0.808	0.622	1.04
>/= 17 years				
No ETS Exposure	3154	0.124	0.111	0.138
Home ETS Exposure	722	0.7	0.586	0.835
>/= 17 years(workers)				
No ETS Exposure	1332	0.132	0.118	0.149
Home ETS Exposure	315	0.651	0.523	0.811
Work ETS Exposure	779	0.318	0.285	0.356
Home + Work ETS Exp.	246	0.926	0.761	1.13

NOTE: For workers(>/= 17 y.o.a.) home work exposure
assumed an 8 hour work day.

These data are from the GUM2 Study and are from active smoker subjects.

Smpl	Sal.Cot. ng/mL	Tot. Nic. mg/24 h	Estim'd Nic. Intake
S01-1	345	**13.0**	21.21
S01-2	317	**10.2**	19.54
S01-3	356	**16.6**	21.92
S01-4	767	**16.2**	47.21
S01-5	617	**16.1**	37.97
S01-6	263	**15.7**	16.18
S02-1	377	**23.9**	23.22
S02-2	524	**19.9**	32.24
S02-3	445	**20.6**	27.39
S02-4	663	**22.0**	40.78
S02-5	298	**18.8**	18.35
S02-6	442	**18.1**	27.18
S03-1	335	**15.6**	20.59
S03-2	506	**23.1**	31.12
S03-3	650	**28.2**	39.99
S03-4	484	**27.1**	29.80
S03-5	460	**16.9**	28.28
S03-6	288	**17.0**	17.72
S04-1	0	**3.0**	0.00
S04-2	0	**3.9**	0.00
S04-3	0	**3.3**	0.00
S04-4	0	**1.7**	0.00
S04-5	53	**1.8**	3.23
S04-6	0	**2.5**	0.00
S05-1	771	**29.1**	47.46
S05-2	896	**32.7**	55.13
S05-3	905	**36.9**	55.67
S05-4	845	**43.4**	52.00
S05-5	543	**30.0**	33.44
S05-6	661	**28.6**	40.66
S06-1	347	**6.1**	21.38
S06-2	380	**15.1**	23.38
S06-3	395	**17.2**	24.34
S06-4	371	**14.4**	22.83
S06-5	430	**15.9**	26.44
S06-6	267	**13.8**	16.43
S07-1	825	**33.4**	50.79
S07-2	1059	**31.7**	65.15
S07-3	732	**39.8**	45.07
S07-4	1161	**30.7**	71.45
S07-5	457	**25.4**	28.13
S07-6	593	**25.3**	36.49
S08-1	876	**27.6**	53.91

S08-2	604	**24.4**	37.16
S08-3	814	**19.6**	50.07
S08-4	591	**18.3**	36.38
S08-5	375	**21.2**	23.06
S08-6	344	**24.6**	21.17
S09-1	355	**22.0**	21.87
S09-2	329	**17.9**	20.27
S09-3	203	**12.9**	12.48
S09-4	236	**11.3**	14.50
S09-5	270	**15.8**	16.61
S09-6	255	**17.0**	15.68
S10-1	134	**8.5**	8.23
S10-2	133	**7.4**	8.17
S10-3	109	**5.9**	6.71
S10-4	75	**5.1**	4.59
S10-5	128	**7.7**	7.89
S10-6	258	**7.9**	15.86
S11-1		**0.5**	0.00
S11-2	0	**0.4**	0.00
S11-3	0	**1.0**	0.00
S11-4	0	**1.0**	0.00
S11-5	0	**0.4**	0.00
S11-6		**0.4**	0.00
S12-1	343	**27.1**	21.11
S12-2	350	**27.6**	21.54
S12-3	1291	**22.3**	79.44
S12-4	1610	**35.0**	99.07
S12-5	463	**28.9**	28.52
S12-6	408	**29.8**	25.09
S13-3	540	**36.8**	33.25
S13-4	485	**41.1**	29.86
S13-5	483	**28.2**	29.74
S13-6	549	**47.7**	33.81
S13-7	447	**19.2**	27.50
S13-8	439	**55.8**	27.00
S14-3	78	**6.0**	4.82
S14-4	82	**5.8**	5.02
S14-5	90	**5.5**	5.52
S14-6	93	**4.5**	5.72
S14-7	89	**9.9**	5.50
S14-8	131	**7.3**	8.05
S15-3	445	**14.9**	27.37
S15-4	324	**12.7**	19.94
S15-5	689	**21.3**	42.42
S15-6	522	**16.0**	32.10
S15-7	379	**21.4**	23.32
S15-8	167	**12.2**	10.27
S16-3	393	**28.3**	24.17
S16-4	357	**28.8**	21.94
S16-5	479	**33.0**	29.50

S16-6	509	**35.0**	31.33
S16-7	338	**23.7**	20.81
S16-8	417	**31.2**	25.69
S17-3	314	**16.8**	19.32
S17-4	391	**13.3**	24.07
S17-5		**10.8**	0.00
S17-6	392	**9.1**	24.15
S17-7	376	**21.9**	23.17
S17-8	449	**18.2**	27.61
S18-3	707	**22.1**	43.49
S18-4	466	**19.5**	28.67
S18-5	627	**29.8**	38.57
S18-6	495	**26.7**	30.46
S18-7	1140	**19.2**	70.13
S18-8	597	**29.8**	36.75
S19-3	330	**19.8**	20.29
S19-4	376	**18.5**	23.16
S19-5	485	**24.9**	29.87
S19-6	320	**19.7**	19.68
S19-7	413	**20.1**	25.41
S19-8	366	**21.8**	22.52
S20-3	285	**12.0**	17.54
S20-4	526	**10.2**	32.38
S20-5	232	**11.5**	14.26
S20-6	496	**10.2**	30.50
S20-7	423	**9.4**	26.01
S20-8	361	**12.5**	22.23
S21-3	517	**26.9**	31.80
S21-4	526	**28.4**	32.35
S21-5	639	**43.4**	39.33
S21-6	866	**42.0**	53.27
S21-7	381	**35.2**	23.44
S21-8	823	**32.7**	50.66
S22-3	633	**9.2**	38.98
S22-4	784	**9.7**	48.26
S22-5	694	**15.7**	42.70
S22-6	560	**12.9**	34.46
S22-7	513	**15.9**	31.59
S22-8	663	**11.5**	40.79
S23-3	199	**15.1**	12.24
S23-4	345	**17.7**	21.25
S23-5	743	**23.7**	45.72
S23-6	229	**18.4**	14.12
S23-7	310	**23.3**	19.10
S23-8	331	**21.7**	20.34
S24-3	307	**22.7**	18.87
S24-4	244	**20.0**	15.00
S24-5	325	**21.1**	20.01
S24-6	235	**18.6**	14.47
S24-7	346	**24.5**	21.31

S24-8	373	**25.5**	22.93

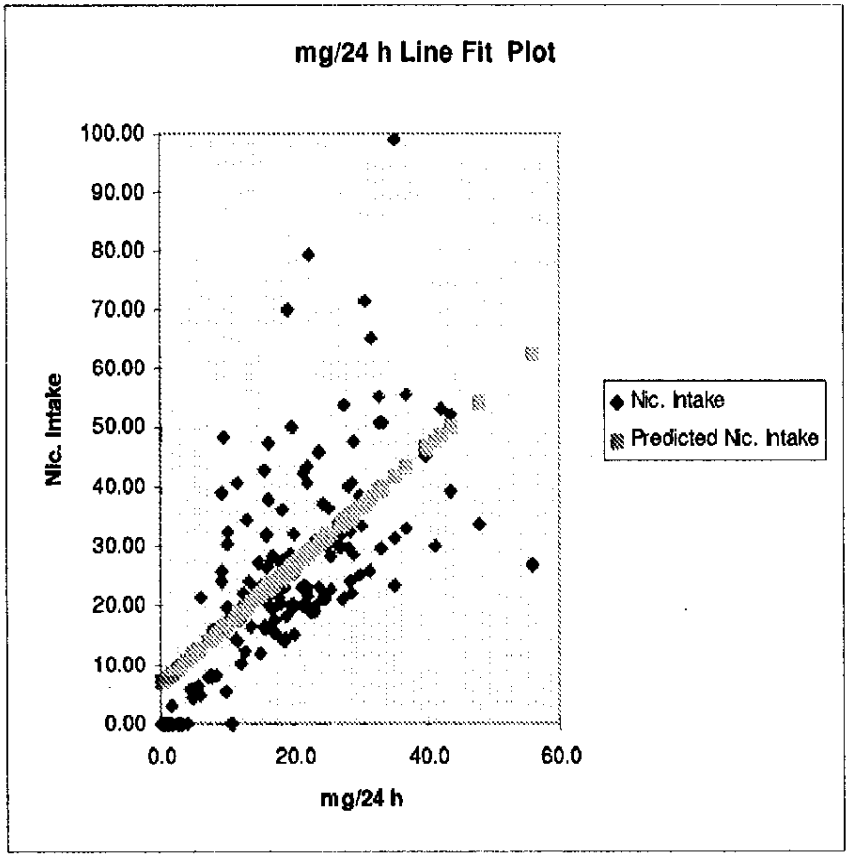
SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.632463
R Square	0.400009
Adjusted R Square	0.395784
Standard Error	13.11722
Observations	144

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	16289.13	16289.13	94.67038	1.85E-17
Residual	142	24432.73	172.0615		
Total	143	40721.86			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	6.880821	2.256997	3.048662	0.002742	2.419168	11.34247	2.419168	11.34247
mg/24 h	0.993811	0.102141	9.729871	1.85E-17	0.791899	1.195722	0.791899	1.195722



Additional Comments From Riley Davis

Charlie,

I forgot to mention the concept of steady-state serum cotinine concentrations. From a purely mathematical point of view the development of steady-state cotinine concentrations in non-smokers is possible. Tom Steichen demonstrated this to me with the ETS Levels Study data. From a pragmatic point of view the concept of steady-state cotinine levels in non-smokers exposed intermittently to various sources of nicotine or ETS appears illogical. If we accept that the half-life of cotinine is ca. 17-20 hours and the exposure concentrations are at the airborne level described by the 16 city study, then the development of steady-state cotinine levels is possible, but not probable so long as the exposures are intermittent and incidental throughout the day. Steady-state cotinine concentration would be achieved in about one to one and one-half weeks. More importantly most sampling is done as "spot samples" therefore the investigator has no idea if the individual is at steady-state. All that the investigator has is a single sample cotinine concentration such as those that were reported by Pirkle. Even Pirkle did not claim that the serum cotinine values observed in the NHANES study represented steady-state. If one can not establish steady-state conditions, then does Benowitz' pharmacokinetic treatment fall apart? I think so. His other ratio values may be challenged and have, why not examine this point and, if found to be invalid, challenge on it. Superficially it would appear that his premise that steady-state serum cotinine values are achieved and may be used to estimate nicotine intake does not

withstand scrutiny using our data. The fact that, on average, the estimates of nicotine intake are excessive implies that the cotinine values used were not steady-state cotinine values.

I would like to hear more expert opinions on this matter. Kuo-Mei and J. Don could enlighten us all.

Thanks for the opportunity to sound off.

Comments From Tom Steichen

Riley asked me to review the Benowitz paper (on cotinine as an ETS bio-marker) and then to look at the body fluid data from the ETS Levels Study to determine whether Benowitz' quantitative results seemed reasonable for our truely ETS-exposed subjects. An obvious concern in using these data is that Benowitz assumes steady-state levels -- but we did our best to force a deviation from steady-state conditions. That is, we asked our subjects to carefully avoid ETS exposures for 48 hours before a session, then we administered a relatively short-term, relatively high-level ETS exposure, and then we asked the subjects to carefully avoid ETS exposures for the 48 hours after the session. Realistically, though, many subjects did not avoid ETS, so our subjects may have been closer to steady-state conditions than we had intended.

Regardless, for the types of comparative calculations I was able to perform, these data do not provide strong evidence against Benowitz' quantitative results. Generally, I found more consistency with Benowitz than expected given our effort to break away from steady-state conditions. For example, Benowitz suggests that 80 ug would be a typical nicotine daily intake for a regularly ETS-exposed person. In comparison, the NHANES III data (as shown below) yields ~74 ug for those with both home and work exposure and the ETS Levels Study yields ~110 ug (for subjects we know received a high ETS dose). He suggests a urinary-to-serum cotinine ratio of 6. The ETS Levels data yields average 6.9 and 5.5 ratios for our two 24-hour samples (we first estimated the serum level from our salivary level using Benowitz' proposed salivary/serum ratio).

I did note, though, that Benowitz was manipulative and misleading when he characterized typical levels of cotinine from ETS and provided "corresponding" nicotine daily intake estimates and "cigarette-equivalents" (p 196). He uses data from bartenders in British pubs to suggest median nicotine intake from ETS exposure to be 630 ug/day (or, "equivalently," 0.63 cigarettes/day) and a maximal nicotine intake from ETS exposure to be 25 mg/day (2.5 cigarettes/day). Only in passing does he mention that much more appropriate cotinine levels for the US are available in NHANES III. He does not carry out the calculation of daily nicotine intake or cigarette equivalents, nor does he even note that the NHANES III are summarized by Pirkle (1996). When one carries out these calculations using Benowitz' methods, a very different picture of exposure emerges. The results are shown below:

Exposure Source	Observed Serum Cotinine (ng/ml)	Calculated Nicotine Daily Intake (ug)	Cigarette "Equivalents" cigts/day	cigts/month	cigts/year
No home or work	.13	10.4	0.0104	0.31	3.8
work only	.32	25.6	0.0256	0.77	9.3
home only	.65	52.0	0.0520	1.56	19.0

home and work 27.2	.93	74.4	0.0744	2.23

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Thus the median US work exposure level (i.e., for those people who work where smoking in allowed) suggests a daily nicotine intake equivalent to less than 1/40th of a cigarette per day (very different from Benowitz' suggestive .63-2.5 cigts/day).

Comments By Mike Ogden

My thoughts on the Benowitz paper follow, and I'll have to be very brief.

I recall 2 points that I would raise in reply; one regarding solanesol as a marker and the other regarding the import of cotinine at low levels being indicative of ETS exposure (particularly in light of possible dietary impact).

(1) Benowitz talks about extensive metabolism of solanesol, etc. as being a reason for not pursuing it as a biological marker. Of course, he is trying to torpedo solanesol as a marker (in preference to nicotine/cotinine) however in doing so he completely missed the point (or he was misled and/or misinformed by the regulatory Gestapo). We have never proposed (externally anyway) solanesol as a biomarker; we have proposed, and we have substantiated with a fair amount of research, that solanesol has many worthwhile attributes as an airborne marker of exposure. The record should be set straight.

(2) Again, whether intentional or not, the tone of the Benowitz paper suggests that we believe dietary nicotine and any cotinine derived therefrom could possibly be interfering with ETS exposure assessment at all levels of exposure. This is not our position. I do not believe that dietary nicotine has any significant impact on cotinine in people with measurable (i.e., by personal monitoring) exposure. However, I do believe that dietary nicotine cannot be ruled out as a plausible explanation for detectable cotinine in persons with no measurable levels of exposure or in person reporting they were not exposed to ETS. To broaden the issue slightly, Benowitz seems to be arguing that cotinine at all levels is indicative of ETS exposure. I do not agree. I think a reasonable statement of our position is that we do not believe that cotinine at any level necessarily indicates exposure. I would agree that cotinine above a certain level (although an exact level is unknown) is a reasonable semi-quantitative indication of exposure. However, the interpretation of cotinine levels at very low levels (particularly near method detection limits) is far from conclusive. Where all this plays out, and they want to keep it to their advantage, is in "demonstrating" the ubiquity of ETS and the large % of the population that is exposed.

Comments From Kuo-Mei Chang

Comments on N. L. Benowitz's paper titled "Cotinine as a biomarker of environmental tobacco smoke exposure" by Kuo-Mei Chang

In a recent publication, Benowitz (1996) suggested that applying pharmacokinetic basis to steady state plasma/saliva/urine cotinine data can quantitatively assess the amount of nicotine in the ETS. Based upon assumptions, Benowitz applied empirical values of pharmacokinetic parameters which derived from limited studies to evolve mathematical calculations to extrapolate ETS-nicotine exposures from a single blood measurement. The pharmacokinetic concept was either overlooked or greatly compromised in the Benowitz report for the following reasons:

The meaning for the use of cotinine as a quantitative biomarker to assess nicotine-ETS exposure is not clear and the approach is not efficient. It has been well understood that plasma cotinine can be used as an indicator for nicotine exposure. Until the meaning for the low concentration of cotinine detected in the biological samples is defined, cotinine is not useful for the quantification of nicotine exposure. The application of pharmacokinetic concepts has been applied in the field of toxicology to quantify occupational exposures to industrial chemicals to assess risks associated with exposures (Leung, 1993). Workers' exposure to an agent is more or less constant under the same environmental setting; the exposure should, therefore, be evaluated by directly monitoring the airborne concentration of that agent. Biomarkers are frequently used for purposes such as to confirm individual exposure, to establish a dose-response relationship of a chemical which causes known adverse biological effects, and to further investigate individual differences in susceptibility of that chemical caused disease in a general population. Since cotinine is not a health factor, measuring individuals' plasma cotinine to quantify amount of exposure under a general environmental setting is not an efficient approach.

A steady state blood nicotine or cotinine is seldom reached under ETS exposure. Although samples collected at steady state will ultimately reflect the rate of absorption, to maintain a constant plasma concentration; a substance must be administered at a constant rate. To reach to steady state is most reliably accomplished by infusing that compound intravenously via either drip or a pump (Rowland and Tozer, 1995). At any time during an infusion, the rate of change in the amount of that substance in the body is the difference between the rates of its infusion and elimination until the two equals. As soon as the infusion is disrupted, plasma concentration

begins to fall according to the elimination rate of that compound. While the longer half-life of cotinine (17 hours) facilitates its measurement in the blood, the slower rate of elimination does not mean that cotinine readily reaches to its steady state. Under continuous exposure of nicotine at a constant rate, it takes approximately 10 and 90 hours for plasma nicotine and cotinine, respectively, to reach their steady states (Figure 1). It will be difficult to identify someone who has continuously exposed to nicotine at a constant rate for 90 hours before a blood sample can be drawn for cotinine analysis. Thus, applying the steady state scenario of using plasma cotinine to back calculate nicotine uptake is not a good practice.

Empirically correlating a plasma concentration with an exposure concentration is very miss leading; an optimized blood sampling strategy is critical for extrapolating nicotine exposure. The pharmacokinetic principles have been extensively adapted by the pharmaceutical industry to achieve therapeutic objectives (Rowland and Tozer, 1995). In general, pharmacokinetics (PK) describes concentrations time courses of a known amount compound in the entire body after it gains its entry. The concentrations (amount) of that compound in different tissues at any specific time point are mediated by the rate of host parameters namely absorption, disposition, metabolism and excretion (ADME). Although the processes of ADME are descriptive and their meanings seem apparent, it is only within the context of experimental observation that they can be quantified. Among biologic specimens can be readily sampled in humans, blood is by far most informative. Because most of the tissue cells are richly perfused with tissue fluids or plasma, it is usually assumed that concentrations measured in the blood serve as a direct index to indicate what happens in the rest of the body. Since nicotine is rapidly and extensively metabolized to cotinine and cotinine to further metabolites, the disappearance of nicotine/cotinine from plasma and the elimination of nicotine/cotinine in biological fluids are time dependent. Concentrations measured in biological fluids are sensitive to the time of sampling. Thus spot measurements of nicotine or cotinine, in the absence of pharmacokinetic information, are useless.

Through optimized blood sampling strategies, the resultant data can be analyzed to define pharmacokinetic parameters such as bioavailability (F), the rate and extent of absorption, distribution (Vd, AUC), and elimination (renal clearance; CL_R, hepatic clearance; CL_H, and half

life $T_{1/2}$). With a set of defined PK parameter values, it is possible to relate blood time concentrations with administrated dose. A large degree of interindividual variation of nicotine and cotinine concentrations has been observed in biological samples (Byrd et. al., 1994), even when the same amount of nicotine was infused into smokers (Fig. 2) or non-smokers (Fig. 3), and when nonsmokers were exposed under the same conditions to ETS (Fig. 4). The wide spread of nicotine and cotinine observed in the biological fluids is attributed to the interindividual differences in pk parameter values for nicotine and cotinine. Under these circumstances, kinetic parameters derived from a set of plasma concentration-time data in one individual may not apply to another. Empirically correlating plasma concentrations with exposure concentrations will be very miss leading; an optimized blood sampling strategy is critical to derive AUC and clearance and that chemical uptake can be reliably estimated. As a few rules of thumb for sampling (Allerheiligen ,1994): If the absorption rate constant is to be estimated, 2 to 3 points prior to the peak are required. For Cmax, 2 blood samples before and after the peak along with 1 sample at the expected Tmax are needed. The determination of the terminal-log linear phase of the curve from at least 3 time points is critical. Since Cl and Vss are dependent upon AUC_{∞}, blood samples should be drawn over a period of at least 5 half -lives in order to obtain a good estimate of AUC_{∞}.

Cotinine in other biological fluids as biomarkers for quantitative ETS-nicotine exposure yet to be validated. Measurements of chemicals in biological fluids other than the blood, such as urine and saliva, are indirect methods to ascertain the bioavailability of a chemical. Correlations between chemical concentrations in body fluids and airborne exposure may be qualitative, but are often not quantitative. Validation for the use of these biological samples to extrapolate ETS-nicotine exposure cannot be achieved until a full description of plasma nicotine/cotinine kinetics is established. The unavailability of plasma data from non-smokers following ETS-nicotine exposure prevents the progress for the validation.

The real issues have been neglected. Two major components need to be evaluated to assess the suitability of plasma nicotine and cotinine as quantitative biomarkers for nicotine in ETS. These components are the pharmacokinetics of nicotine and cotinine at low level of nicotine exposure

and lower limits of nicotine exposure which can be adequately assessed by the measurement of markers in biological fluids. According to study results reported by Benowitz et al.(1991) and Benowitz and Jacob (1993), it was suggested that the longer clearance half-life for nicotine and cotinine is due to the slower release of nicotine bound to tissues. The existence of nonlinear pharmacokinetics at low concentrations can lead to an overestimation of ETS-nicotine exposure. Concentrations of ETS found in the field tend to be very low (Nelson et.al., 1989); the ambient ETS-nicotine level is between 0.17 to 0.73 ug/m^3 (Jenkins et al., 1994). The estimated nicotine inhaled from ETS for an 8 hour-working day would be 0.04 ug to 0.18 ug. The expected low plasma concentrations of nicotine and cotinine were further confounded by analytical limitations(Chang et al., 1994; Schepers & Walk, 1988) and residual plasma nicotine and cotinine from other sources such as diet (Davis et al., 1991). In addition, nicotine and cotinine are not ideal biomarkers for ETS particulate phase exposure (Nelson et al., 1992). The usefulness of these compounds as quantitative markers for ETS exposure is limited.

Experimental data support that plasma nicotine and cotinine at low level of nicotine exposure can not be used as quantitative biomarkers to predict the real world ETS exposure. There are many uncertainties associated with using cotinine as a quantitative biomarkers for ETS-nicotine exposure. Scientific debates, however, can not be resolved based upon individuals' opinions. Data analyzed from well designed studies can certainly support the hypothesis.

As pointed out by Benowitz, there is a body of literature reporting clinical pharmacology of nicotine from studies conducted in adult humans in experimental settings. However, most PK data analysis for parameter estimations in these studies was based on individuals. It requires an average of 8-24 sampling points from each subject (Whiting et al.,1990), and data interpretation is often limited to that very individual. Thus, these existing data sets alone could not offer utilities such as 1) extrapolation beyond the range of existing data (predictions) and 2) simulation about analogous situations which are difficult or impossible to measure directly (inferences). Nonetheless, valuable information obtained from these studies should be captured and applied to derive useful information from observational data; especially in routine clinical or nonclinical

practices where data generally consist of only a few measurements of plasma concentrations per individual which is not sufficient to estimate kinetic parameters for each individual. In order to predict and to inference outcomes from observational data to a general population, appropriate study designs and innovative data analysis methodologies must be incorporated. Advanced by the development of computational power, the science of pharmacokinetic modeling has become a very important tool for better study design, data reduction and interpolation. Since modeling is the principal conceptual tool, in an iterative process, models have to be tested by experimental data, and reformulated before it can be applied for prediction and extrapolation. With a structural nicotine model in place, the existing data can be utilized as additional information to develop and to validate the model. Thus, a PK model for nicotine will not only be useful to describe a plasma concentration-time curve but to form a scientific database that also describes human nicotine pharmacology.

A physiologically based pharmacokinetic (PBPK) nicotine model has been developed by RJR scientists (Plowchalk et al., 1992, and Yang et al., 1994) to understand the smoke chemistry of nicotine in cigarette products and to explain interindividual differences in nicotine disposition among smokers. The RJR PBPK-nicotine model was originally developed and validated in the rat (Plowchalk et al., 1992) to describe plasma and tissue kinetics of nicotine in the rat. The rat model has been scaled to human by adjusting physiologic, anatomical, and biochemical parameters according to body weight (Plowchalk and deBethizy, 1992). The human model was further refined and validated by including a forearm sampling compartment and a nested cotinine model (Yang et al., 1994) to describe nicotine and cotinine kinetics in smokers via various routes of administration.

Most recently, we have applied this model to analyze experimental data from a nicotine intravenous infusion study in nonsmokers conducted by Curvall et al. (1990) to evaluate the suitability of using cotinine as a quantitative biomarker for ETS-nicotine exposure (Chang and deBethizy, 1997). As model was tested for its capability to describe pharmacokinetics of plasma nicotine and cotinine in highly exposed nonsmokers, model

simulation of a nicotine exposure regimen in the same individual revealed the lower limit of nicotine exposure which can be assessed using nicotine and cotinine as quantitative markers. Our results provide convincing evidences that plasma nicotine and cotinine at low level of nicotine exposure can not be used as quantitative biomarkers to predict the real world ETS exposure.

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