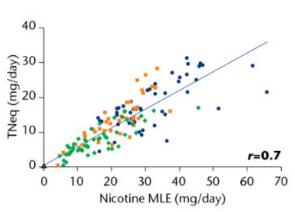




Biomarkers of Exposure (BoE) studies

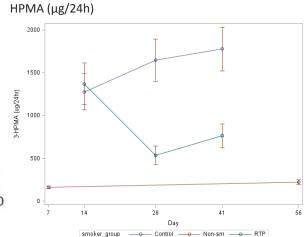
Correlation study(ref 1)

Total nicotine equivalents vs Nicotine MLE



BoE correlates to smoker mouth level Exposure (MLE)

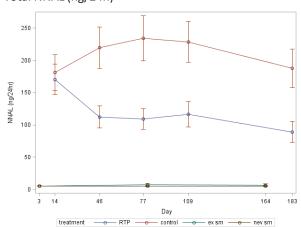
BoE 6 week study(ref 2)



Reduced
Toxicant Prototype
cigarette leads
to some reduced BoEs

BoE 6 month study^(ref 3,4)

Total NNAL (ng/24h)



Reductions in some BoEs maintained over longer study, but little change in biomarkers of biological effect



Clinical Study Approach

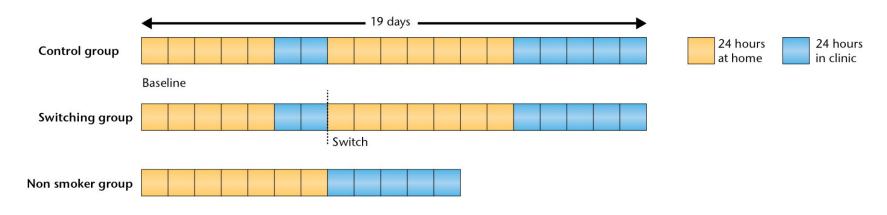
- Perform studies to Good Clinical Practice
- Clinical confinement for sample collection
- 24hr Urine, no creatinine normalisation for BoE
- Obtain Ethics Committee approval and register



Correlation Study^(ref 1)

Primary objective

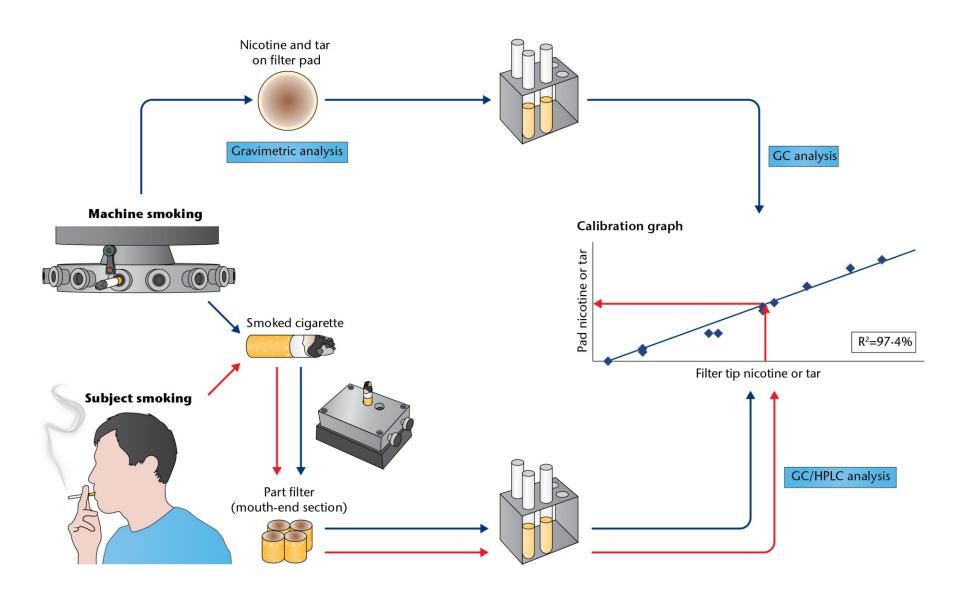
To compare the level of estimated human cigarette smoke exposure as determined by cigarette filter analysis and biomarkers of exposure in smokers and non-smokers.



- n = 50 per group
- Products 1, 4, 10 mg ISO tar (commercial products)
- 5 smoking groups
 - 3 control (continued with same product throughout)
 - 2 switching (10 to 4 mg and 4 to 1 mg ISO tar)



Filter analysis method^(ref 5)

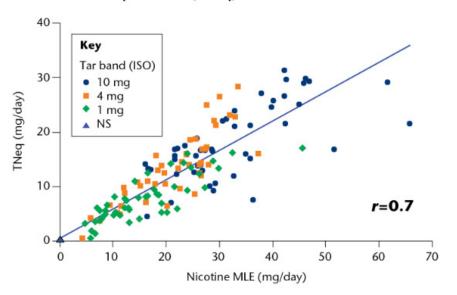




Correlation Analysis^(ref 1)

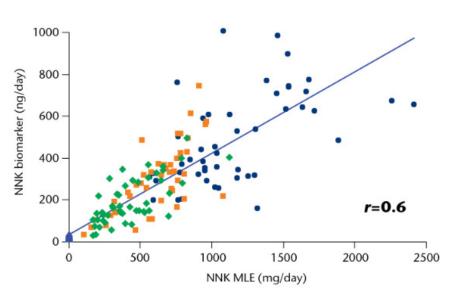
Nicotine correlation

Total nicotine equivalents (TNeq) vs Nicotine MLE



NNK correlation

NNK biomarkers vs NNK MLE

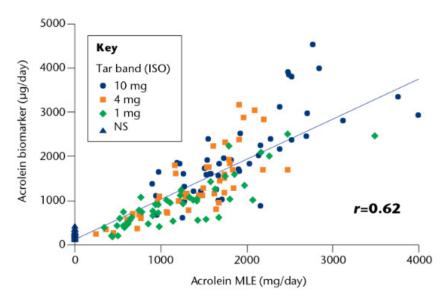




Correlation Analysis^(ref 1)

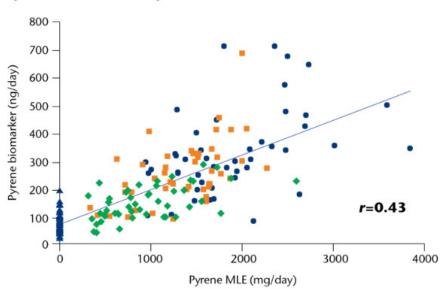
Acrolein correlation

Acrolein biomarkers vs Acrolein MLE



Pyrene correlation

Pyrene biomarkers vs Pyrene MLE





Cumulative toxicant burden Health

Product

cigarettes

Conventional cigarettes
 Reduced toxicant prototype

Canada Intense regime

7000

6000

5000

4000

3000

2000

1000

Cumulative total

BoE 6 Week Study(ref 2)

Reduced toxicant prototype with toxicant-reducing technologies

Tobacco blend

Tobacco sheet substitute (TSS) and tobacco blend treatment (BT)

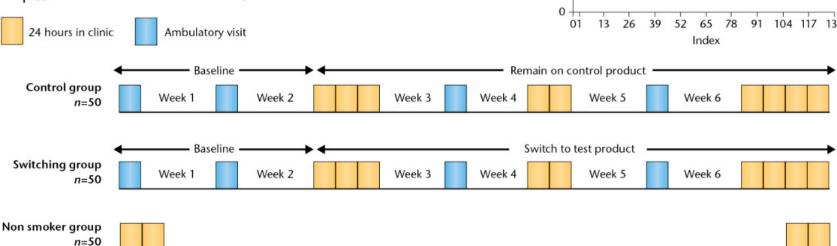
Filter

Selective filtration and synthetic carbon

1 and 6 mg ISO tar

Study registration:

http://www.controlled-trials.com/ISRCTN72157335

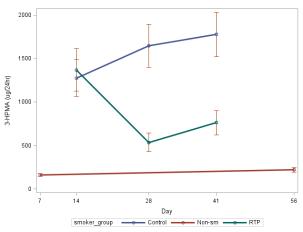




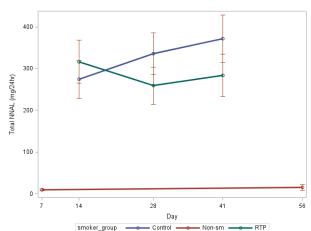
6 Week Study results – 6mg (ISO Tar) products(ref 2)

Nicotine exposure

Acrolein exposure



NNK exposure



- RTP 10.3% increase from day 14 to 41
- Control 13.3% increase from day 14 to 41
- 9% reduction in RTP yield

- RTP 45.0% reduction from day 14 to 41
- Control 34.4% increase from day 14 to 41
- 42% reduction in RTP yield

- RTP 10.5% reduction from day 14 to 41
- Control 31.4% increase from day 14 to 41
- 44% reduction in RTP yield



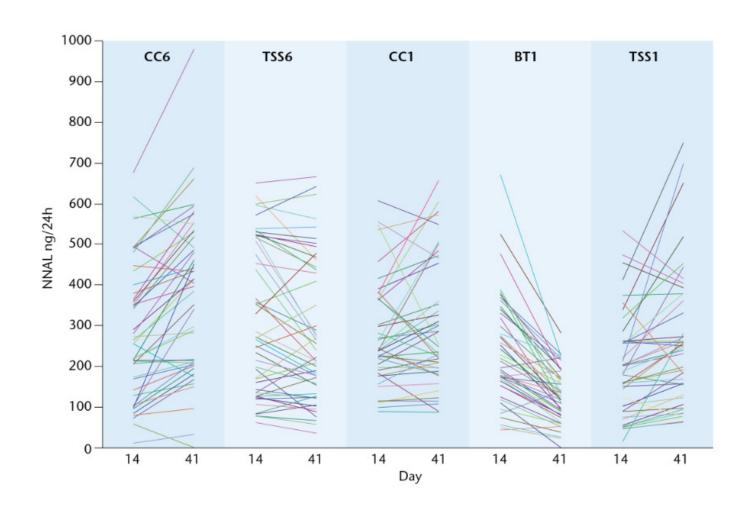
BoE 6 Week Study^(ref 2)

Smoke constituent (BoE)	Change in yield (HCI) (%)	Change in RTP group (day 14 to 41) (%)	Statistical significance of BoE change
Total nicotine (TNeq)	9% reduction	10.3% increase	Not significant
Acrolein (3-HPMA)	42% reduction	45% reduction	Significant reduction
Crotonaldehyde (HMPMA)	85% reduction	75% reduction	Significant reduction
1,3-Butadiene (MHBMA)	42% reduction	63% reduction	Significant reduction
1,3-Butadiene (DHBMA)	42% reduction	8% reduction	Significant reduction, similar in all groups
NNK (NNAL)	44% reduction	10.5% reduction	Significant reduction
NNN	50% reduction	22% reduction	Significant reduction
NAB	37% reduction	26% reduction	Significant reduction
NAT	41% reduction	24% reduction	Significant reduction
Pyrene (OH-Pyrene)	26% reduction	6% reduction	Not significant
Phenanthrene (2,3,4,1+9-OH Phenanthrene)	20% reduction	24% increase to 4% reduction	Only significant for 1+9 OH-Phenanthrene
Naphthalene (1+2-OH Naphthalene)	81% reduction	15%,11% reduction respectively	Significant reduction
Fluorene (2-OH-fluorene)	24% reduction	14% reduction	Significant reduction
2-Amino naphthalene	1% increase	11% reduction	Significant reduction
3-Aminobiphenyl	20% reduction	10% reduction	Significant reduction
4-Aminobiphenyl	13% reduction	6% reduction	Not significant
o-Toluidine	14% reduction	8% reduction	Significant reduction



Individual v Group analyses (ref 6)

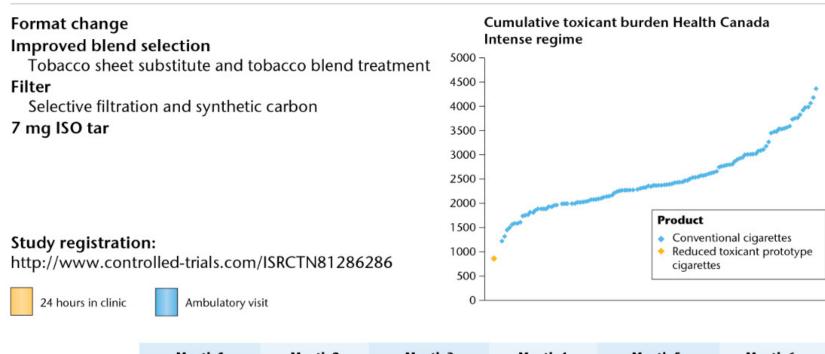
Figure demonstrates that to see significant reductions in BoE levels generally large changes in the toxicant yield from novel products are required

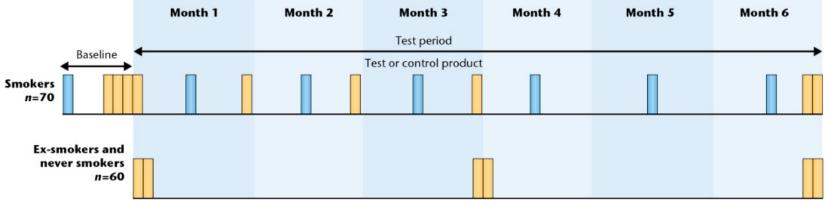




BoE 6 Month Study^(ref 3,4)

Reduced toxicant products with improved toxicant reducing technologies





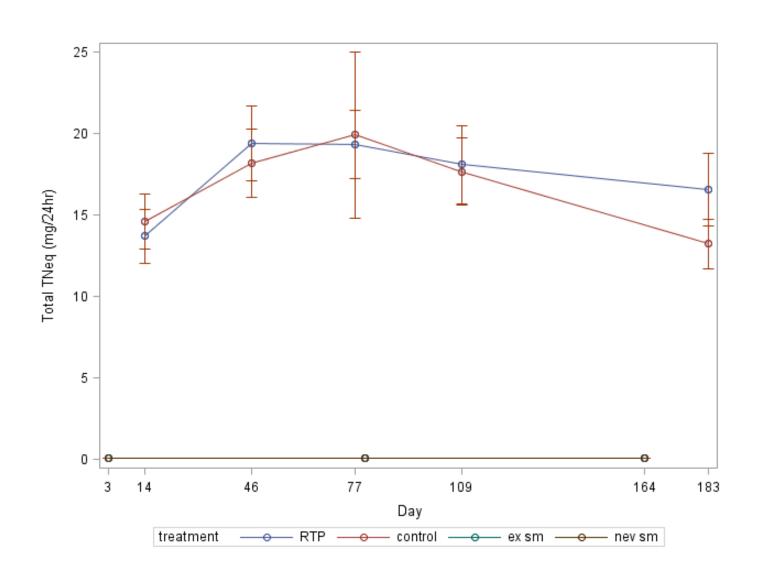


BoE 6 Month Study^(ref 3,7)

Smoke constituent (BoE)	Change in yield (HCI) (%)	Change in RTP group at EOS (%)	Statistical significance of BoE change
Total nicotine (TNeq)	7% reduction	26% increase	Significant increase
Exhaled CO	31% reduction	19% reduction	Significant reduction
Acrolein (3-HPMA)	55% reduction	34% reduction	Significant reduction
Crotonaldehyde (HMPMA)	92% reduction	75% reduction	Significant reduction
1,3-Butadiene (MHBMA)	45% reduction	31% reduction	Significant reduction
Acrylonitrile (CEMA)	80% reduction	59% reduction	Significant reduction
NNK (NNAL)	65% reduction	40% reduction	Significant reduction
NNN	85% reduction	66% reduction	Significant reduction
NAB	64% reduction	44% reduction	Significant reduction
NAT	51% reduction	28% reduction	Significant reduction
Pyrene (1-OH-Pyrene)	20% reduction	30% reduction	Significant reduction
Phenanthrene (2,3,4,1+9-OH Phenanthrene)	11% reduction	19% increase	Non-significant
Naphthalene (1+2-OH Naphthalene)	86% reduction	55% increase	Non-significant
Fluorene (2-OH-fluorene)	11% reduction	81% increase	Significant increase
2-Amino naphthalene	44% reduction	10% reduction	Significant reduction
3-Aminobiphenyl	48% reduction	32% reduction	Significant reduction
4-Aminobiphenyl	48% reduction	17% reduction	Significant reduction
o-Toluidine	42% reduction	4% reduction	Non-significant



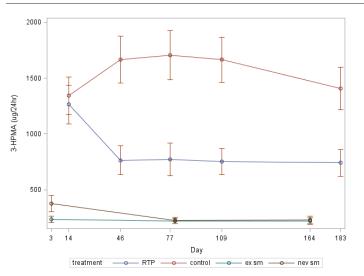
Total Nicotine Equivalents (TNeq)(ref 3)



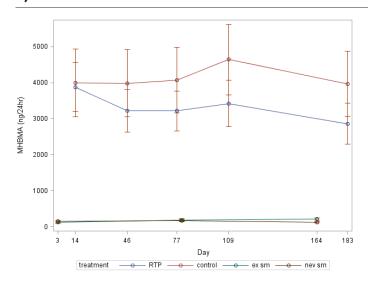


6 Month Study BoEs^(ref 3)

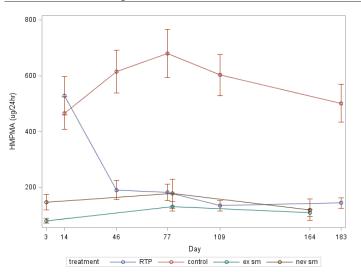
Acrolein



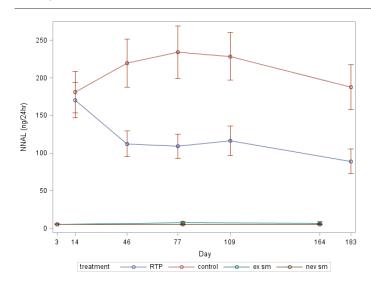
1, 3-butadiene



Crotonaldehyde

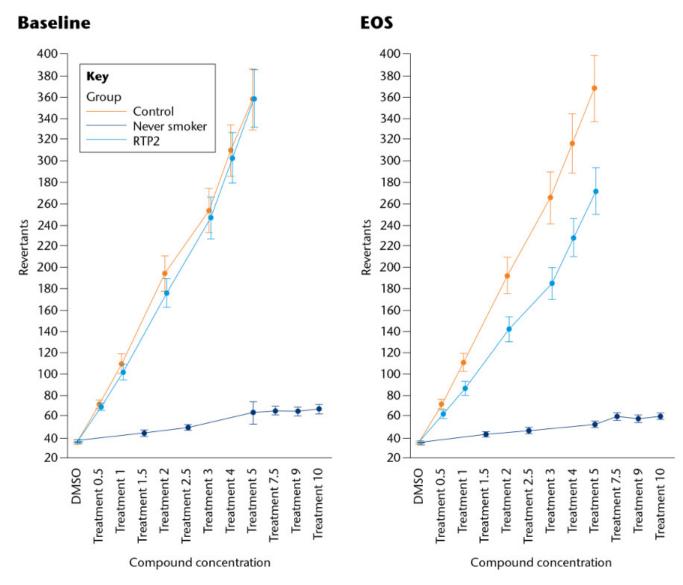


NNK





Urine Mutagenicity^(ref 3,8)



Each error bar is constructed using a 95% confidence interval of the mean



Overall changes in biomarkers in RTP and control groups^(ref 3,4,8)



RTP Smokers			
Biomarker	Change at the EOS (%)	Biomarker	Change at the EOS (%)
HMPMA ^{‡†}	-75%	4 - ABP ^{‡†}	-17%
NNN [ࠦ]	-66%	2 - AN ^{‡†}	-10%
CEMA ^{‡†}	-59%	8-iso-PGF2 type VI	-6%
NAB ^{‡†}	-44%	o-tol	-4%
NNAL ^{‡†}	-40%	WBCs	0%
3 - HPMA ^{‡†}	-34%	8-iso-PGF2 type III	3%
3 - ABP ^{‡†}	-32%	Phenanthrene ('total')	19%
MHBMA ^{‡†}	-31%	Nicotine Equivalents ^{‡†}	26%
1 - OHP ^{‡†}	-30%	Saliva Cotinine‡†	28%
NAT ^{‡†}	-28%	Naphthalene ('total')	55%
ECO ^{‡†}	-19%	sICAM-1 [ࠦ]	60%
DTBX	-19%	Fluorene ('total')	81%

^{*} Denotes % changes with Baseline vs. EOS values statistically significant as determined by evaluation of the simple effects in the statistical models that include only the smoking groups with CPD as a covariate

[†] Denotes % changes with Baseline vs. EOS values statistically significant as determined by evaluation of the simple effects in the statistical models that include only the smoking groups without CPD as a covariate.

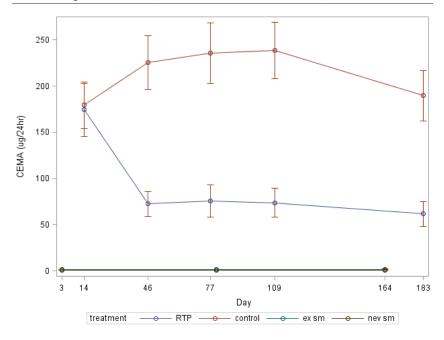
[¶] Denotes statistical significance derived from analysis excluding extreme values

^{&#}x27;total' = sum of metabolites investigated



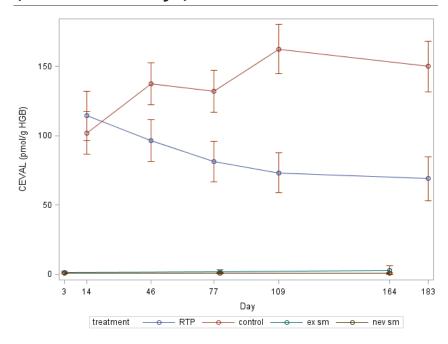
Biomarkers of Compliance^(ref 8)

Urinary CEMA (half-life 5-9 hours)



- Biomarkers of Acrylonitrile exposure
- Comparison of urinary BoE to Hb adduct
- Similar patterns for groups over time
- Indication of compliance to protocol

2-Cyanoethylvaline haemoglobin adducts (half-life ~60 days)





Conclusions

- Clinical Studies have helped identify Biomarkers of Exposure that are fit for purpose through:
 - Correlation to estimated dose at mouth level
 - Application of BoEs in both short and long-term studies including non-smoker and ex-smoker groups
- Biomarkers can also aid with quality of studies in terms of subject compliance
- Further validation of BoEs required especially inter-laboratory studies to help identify consensus values or Reference Standards to ensure comparability
- Clinical studies using BoEs can help identify levels of toxicant reduction required to see significant reductions in exposure to individual smokers
- Significant reductions in BoEs from combustible RTPs, did not lead to significant change in BoBEs



References

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- ² Shepperd at al. 2013, Reg Tox Pharma **66**:147-162 DOI:10.1016/j.yrtph.2013.02.007
- ³ Shepperd et al. 2015, Reg Tox Pharma **72**:273-291 DOI:10.1016/j.yrtph.2015.04.016
- ⁴ Haswell et al. 2014, Biomarkers **19**:356-367 DOI:10.3109/1354750X.2014.912354
- ⁵ St. Charles et al. 2009 Beitr. Tabakforsch. Int. **23**:232-243 DOI: 10.2478/cttr-2013-0863,
- ⁶ Camacho et al. 2014, ClinChem Lab Med 52:399-411 DOI:10.1515/cclm-2013-0581
- ⁷ Gregg *et al.* 2013, *Biomarkers* **18**:467-486 DOI:10.3109/1354750X.2013.821523
- ⁸ Proctor *et al.* 2014, Integrating chemical, toxicological and clinical research to assess the potential of reducing health risks associated with cigarette smoking through toxicant regulation. 68th Tobacco Science Research Conference, September 2014. www.bat-science.com



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