Integrating chemical, toxicological and clinical research to assess the potential of reducing health risks associated with cigarette smoking through toxicant regulation

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# Central question and overview of the presentation

BRITISH AMERICAN TOBACCO

Is there scientific evidence to suggest "reduced toxicant cigarettes" have a distinct place in a tobacco and nicotine risk continuum?

- The risk continuum
- Harmful and potentially harmful constituents in tobacco smoke
- Developing reduced toxicant prototype (RTP) cigarettes
- Chemistry
- Toxicology
- Clinical
- Implications for toxicant reduction regulations

sometimes
a cigarette
is just
a cigarette

Christopher Proctor

Chapter 7: Selective reduction

US Institute of Medicine

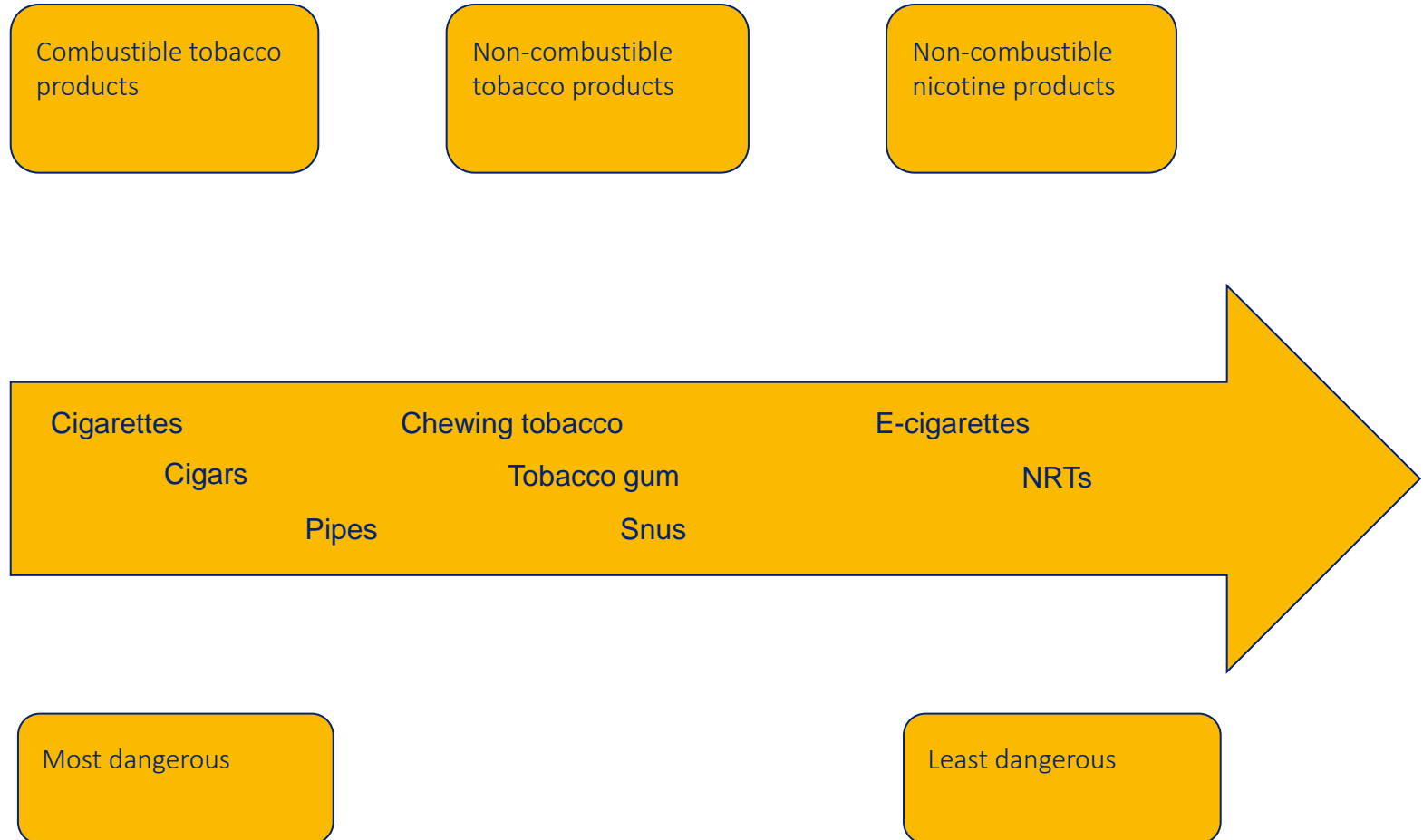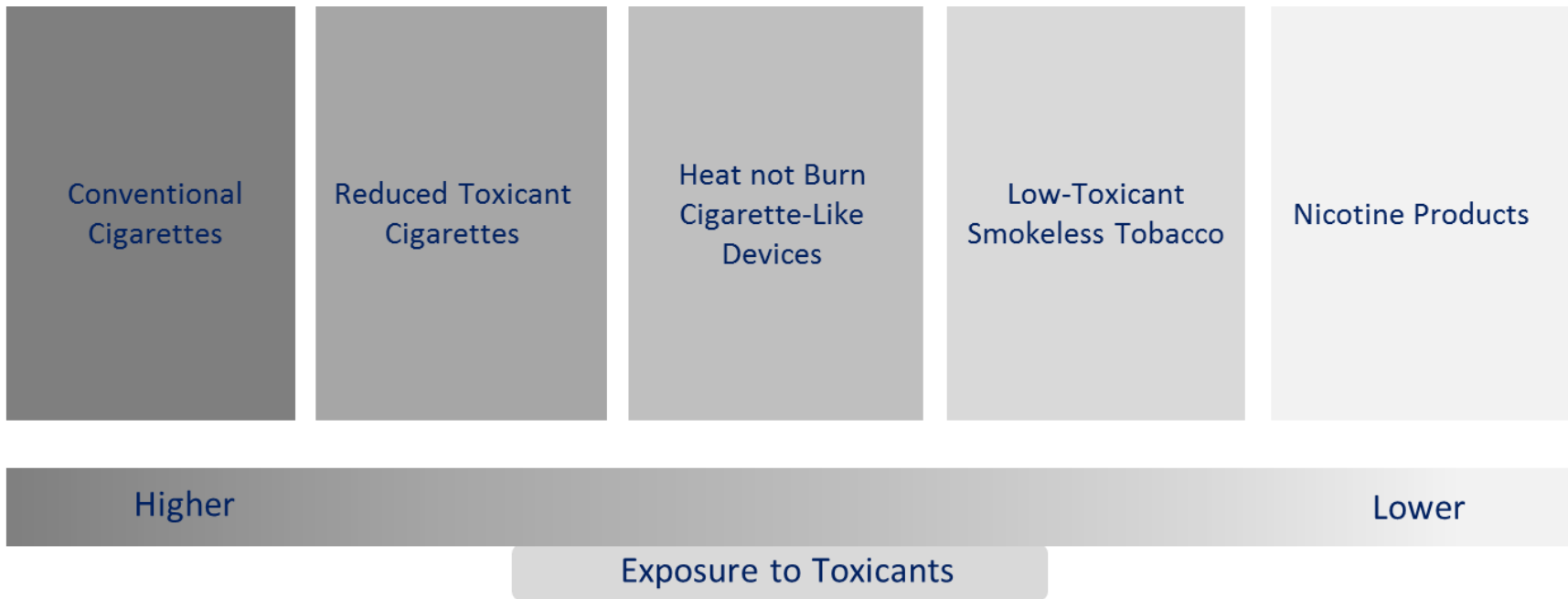**CLEARING THE SMOKE**
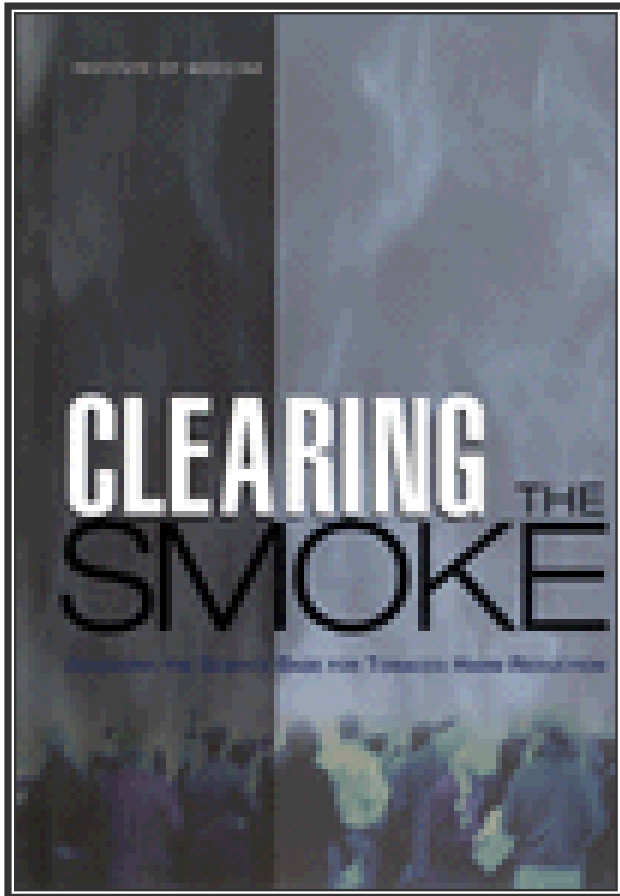
PREP: A product that (1) results in the substantial reduction in exposure to one or more tobacco toxicants and (2) can reasonably be expected to reduce the risk of one or more specific diseases or other adverse health effects.

Source: Institute of Medicine (2001) Report; *Clearing the Smoke, The Science Base for Tobacco Harm Reduction*

Reducing harm from tobacco use, Professor Ann McNeil and Professor Marcus Munafo, Journal of Psychopharmacology, October 3, 2012
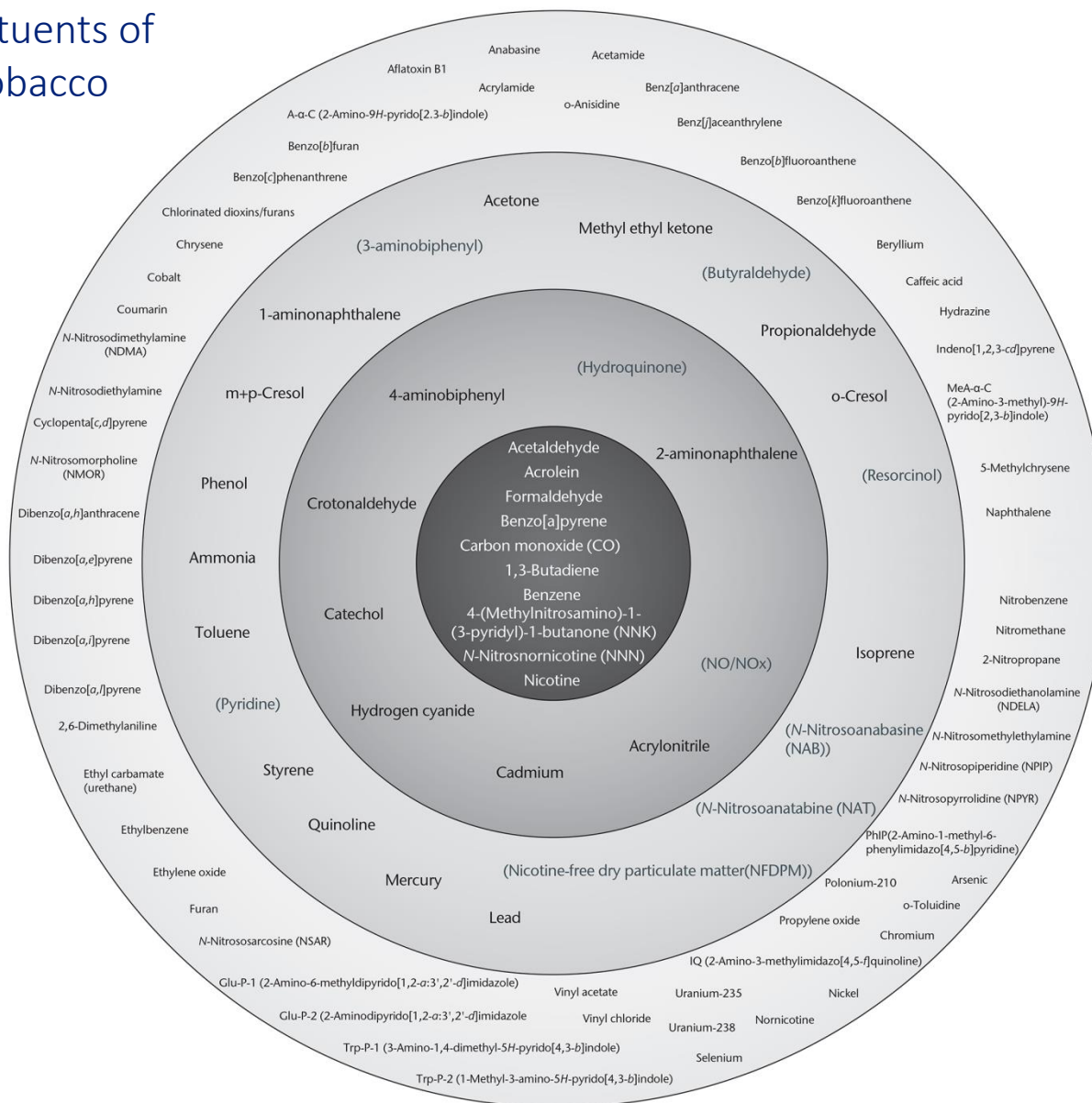
Combustible tobacco products

Non-combustible tobacco products

Non-combustible nicotine products

Cigarettes

Chewing tobacco

E-cigarettes

Cigars

Tobacco gum

NRTs

Pipes

Snus

Most dangerous

Least dangerous

British American Tobacco "continuum of toxicant exposure"

Conventional Cigarettes	Reduced Toxicant Cigarettes	Heat not Burn Cigarette-Like Devices	Low-Toxicant Smokeless Tobacco	Nicotine Products

Higher   Lower
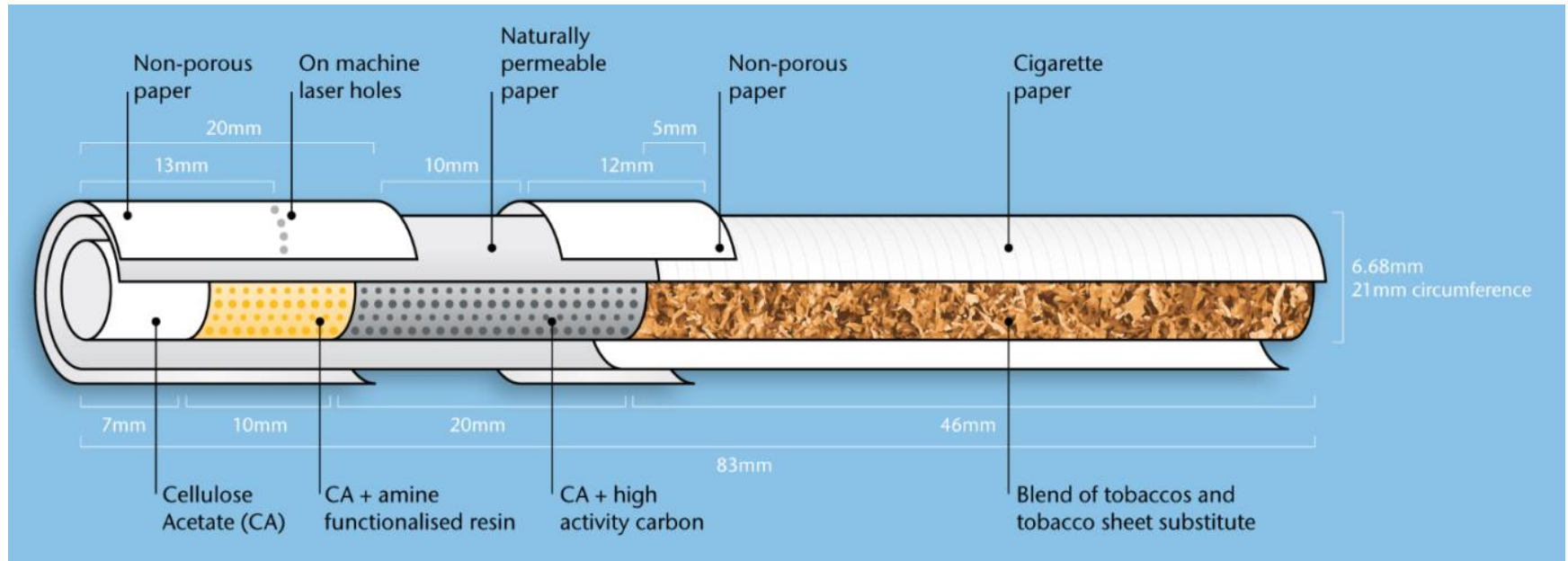
Exposure to Toxicants

The research challenge

- Develop "reduced toxicant prototype" cigarettes with substantially lower yields of as many toxicants as possible while maintaining reasonable sensory acceptability

- To do this develop new technologies capable of selectively reducing "priority toxicants"

- Evaluate whether these prototypes have the potential to reduce exposure compared to conventional cigarettes, and whether any reduce exposure is likely to be associated with reduced health risk to one or more diseases

Harmful and potentially harmful constituents of tobacco and tobacco smoke

Innermost circle:

Acetaldehyde
Acrolein
Formaldehyde
Benzo[a]pyrene
Carbon monoxide (CO)
1,3-Butadiene
Benzene
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)
N-Nitrosonornicotine (NNN)
Nicotine

Second circle:

Acetone
Methyl ethyl ketone
(3-aminobiphenyl)
(Butyraldehyde)
1-aminonaphthalene
Propionaldehyde
m+p-Cresol
4-aminobiphenyl
(Hydroquinone)
o-Cresol
Phenol
Crotonaldehyde
2-aminonaphthalene
(Resorcinol)
Ammonia
Catechol
Toluene
(NO/NOx)
Isoprene
(Pyridine)
Hydrogen cyanide
Acrylonitrile
(N-Nitrosoanabasine (NAB))
Styrene
Cadmium
(N-Nitrosoanatabine (NAT))
Quinoline
(Nicotine-free dry particulate matter(NFDPM))
Mercury
Lead

Outermost circle:

Anabasine
Acetamide
Aflatoxin B1
Acrylamide
o-Anisidine
Benz[a]anthracene
A-α-C (2-Amino-9H-pyrido[2.3-b]indole)
Benz[j]aceanthrylene
Benzo[b]furan
Benzo[b]fluoranthene
Benzo[c]phenanthrene
Benzo[k]fluoranthene
Chlorinated dioxins/furans
Beryllium
Chrysene
Caffeic acid
Cobalt
Hydrazine
Coumarin
Indeno[1,2,3-cd]pyrene
N-Nitrosodimethylamine (NDMA)
MeA-α-C (2-Amino-3-methyl)-9H-pyrido[2,3-b]indole
N-Nitrosodiethylamine
5-Methylchrysene
Cyclopenta[c,d]pyrene
Naphthalene
N-Nitrosomorpholine (NMOR)
Dibenzo[a,h]anthracene
Dibenzo[a,e]pyrene
Nitrobenzene
Dibenzo[a,h]pyrene
Nitromethane
Dibenzo[a,i]pyrene
2-Nitropropane
Dibenzo[a,l]pyrene
N-Nitrosodiethanolamine (NDELA)
2,6-Dimethylaniline
N-Nitrosomethylethylamine
N-Nitrosopiperidine (NPIP)
Ethyl carbamate (urethane)
N-Nitrosopyrrolidine (NPYR)
Ethylbenzene
PhIP(2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine)
Ethylene oxide
Polonium-210
Arsenic
Furan
Propylene oxide
o-Toluidine
N-Nitrososarcosine (NSAR)
Chromium
IQ (2-Amino-3-methylimidazo[4,5-f]quinoline)
Glu-P-1 (2-Amino-6-methyldipyrido[1,2-a:3',2'-d]imidazole)
Vinyl acetate
Uranium-235
Nickel
Glu-P-2 (2-Aminodipyrido[1,2-a:3',2'-d]imidazole)
Vinyl chloride
Uranium-238
Nornicotine
Trp-P-1 (3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole)
Selenium
Trp-P-2 (1-Methyl-3-amino-5H-pyrido[4,3-b]indole)

Constructing a reduced toxicant prototype cigarette

Non-porous paper | On machine laser holes | Naturally permeable paper | Non-porous paper | Cigarette paper

20mm
13mm | 10mm | 12mm | 5mm

6.68mm
21mm circumference

7mm | 10mm | 20mm | 46mm
83mm

Cellulose Acetate (CA) | CA + amine functionalised resin | CA + high activity carbon | Blend of tobaccos and tobacco sheet substitute

Technologies:
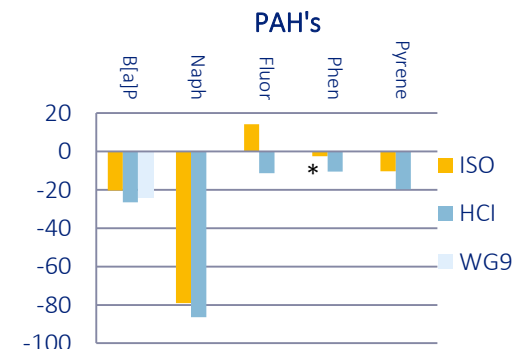High activity carbon (Branton et al, Absorp. Sci. Technol, 29, 117, 2011)
Ion exchange resin (Branton et al, Chem. Centr J., 5, 15, 2011)
Split-tipping (Dittrich et al, Springer Plus, 3, 374, 2014)
Tobacco substitute sheet (McAdam et al, Food Chem. Toxicol., 49, 1684, 2011
Blend treated tobacco (Lui et al, Food Chem. Toxicol., 49, 1904, 2011)

Chemistry of the RTP
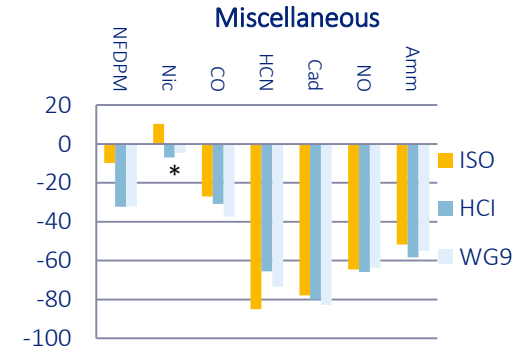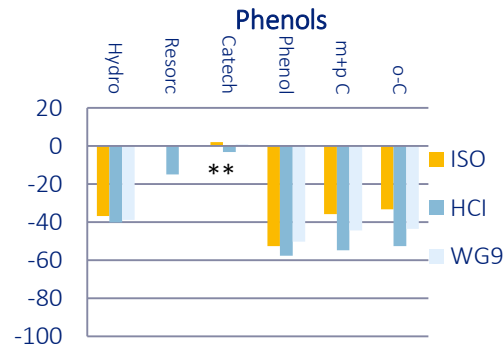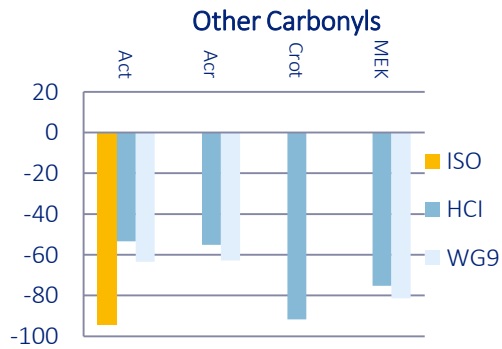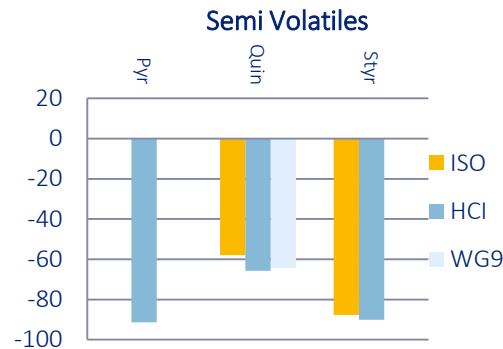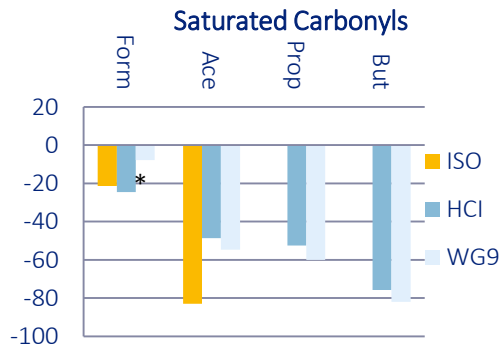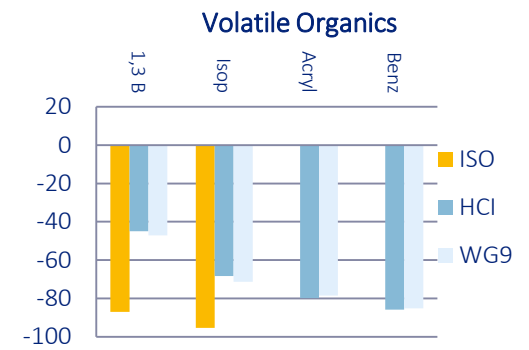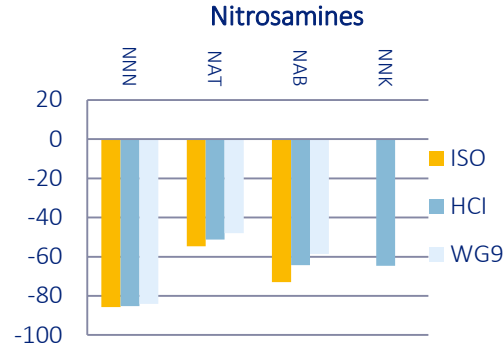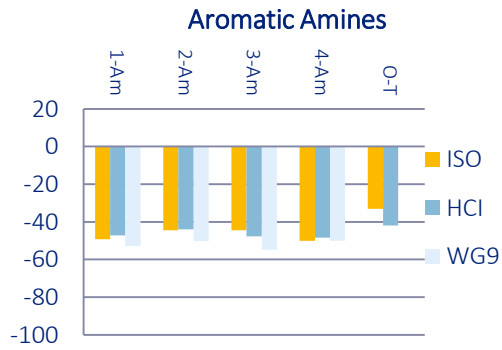
Creating a "cumulative toxicant index"

- We wanted to assess the "performance" of the RTP against a range of conventional cigarettes across a range of toxicants

- Used a database "Hoffman" analyte" yields measured at HCI of 120 commercial products from a variety of countries

- Normalised the median for each toxicant as 100, and scaled the yield for each product against this

- Summed the scaled values for all toxicants to give cumulative score

"Cumulative toxicant index" of a range of commercial cigarettes versus Reduced Toxicant Prototype cigarettes
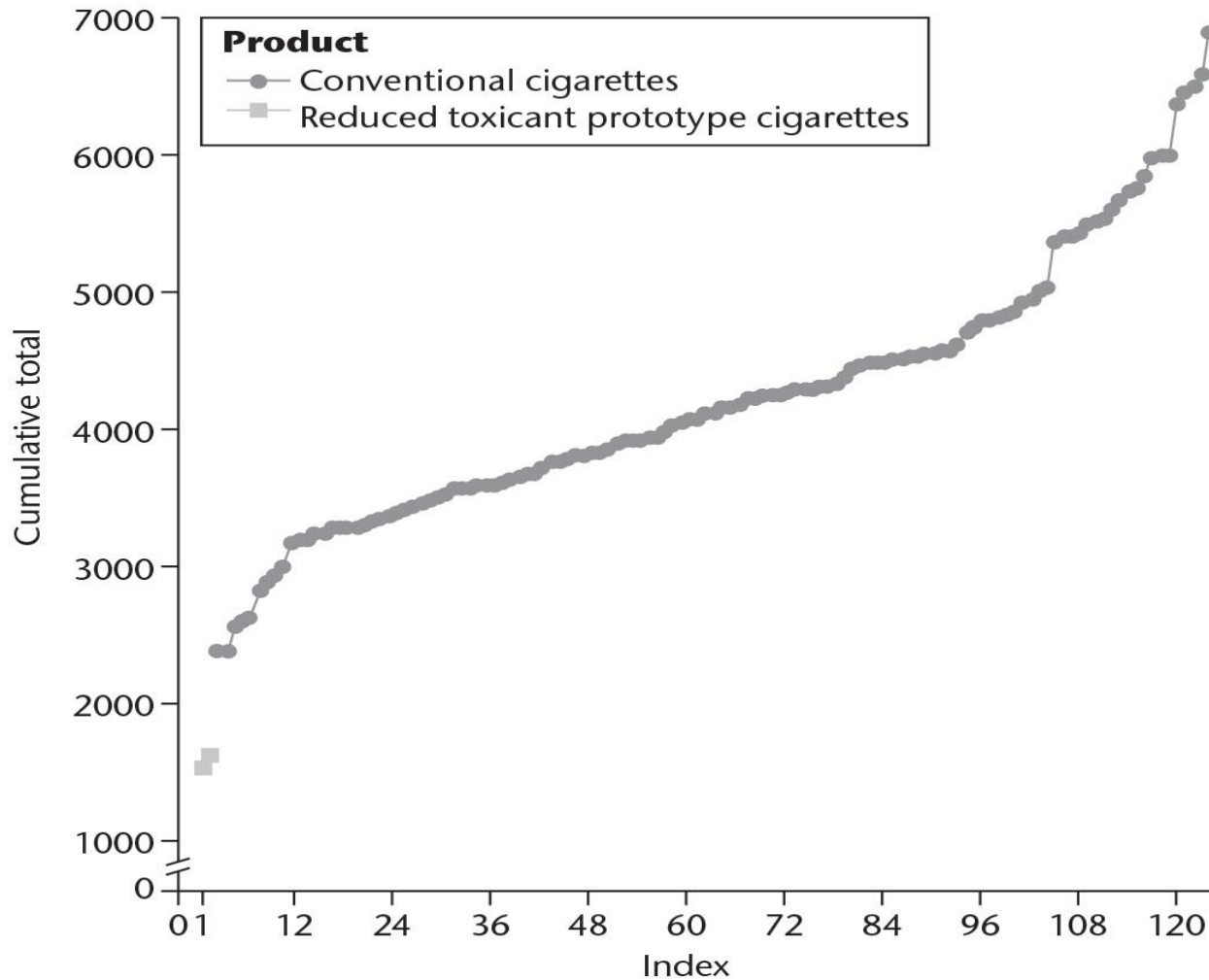
In Vitro toxicology

- We conducted a battery of *in vitro* toxicological tests

- For the particulate matter collected for RTP and control we used the Ames test, mouse lymphoma assay, in vitro micronucleus test and Neutral Red assay

- Some reductions in bacterial mutagenicity and mammalian genotoxicity were seen in the RTP compared to control, consistent with lower lower levels of some toxicants such as heterocyclic amines and the dilution of PM resulting from the tobacco substitute sheet

(Crooks et al, The combination of two tobacco blend technologies to reduce tobacco smoke
toxicant yields: Assessment in the Ames and *in vitro* micronucleus test, 10th International Conference on
Environmental Mutagens, 2014, see www.bat-science.com/Library)

In vitro toxicology

Whole smoke cytotoxicity
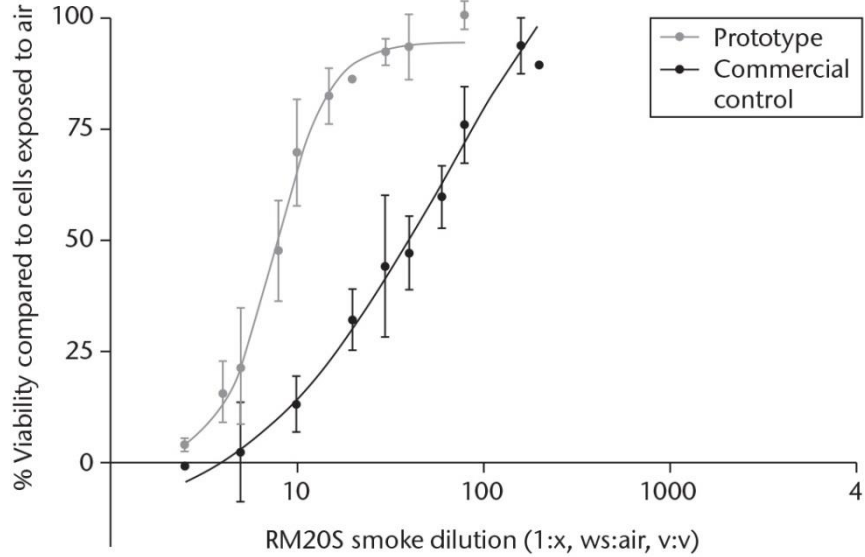
Intercellular ROS
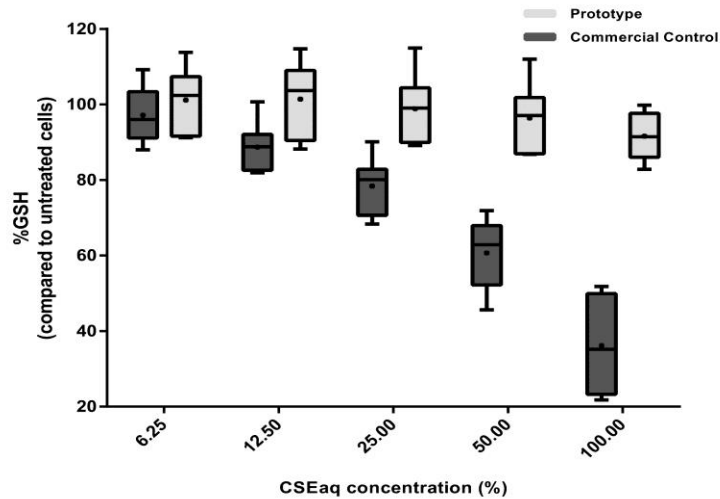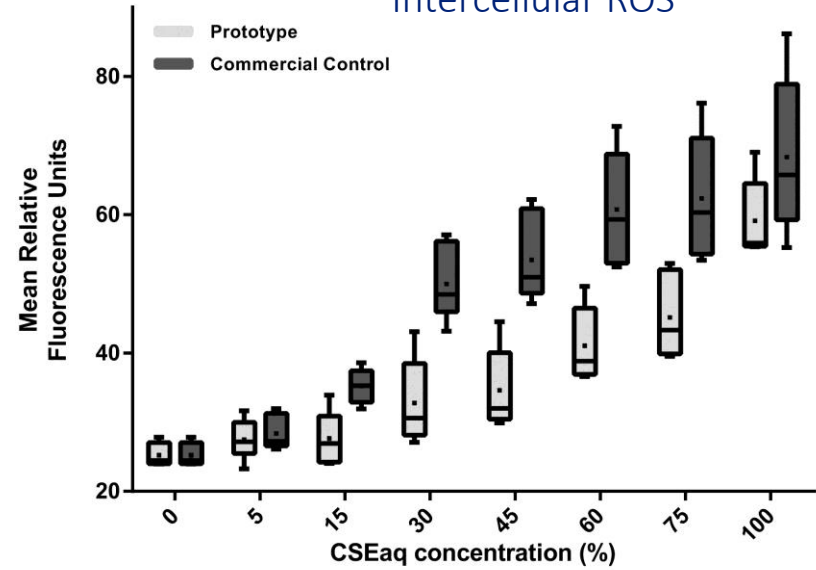
Glutathione

Computational toxicology

- We have a multi-staged approach to assessing toxicants through the use of computational toxicology

- Margins of exposure (MOE)

- Modes of Action (MOA)

- Physiologically based pharmacokinetic modelling (PBPK)

(Cunningham et. Al., A novel application of the modes of exposure approach: segregation of tobacco smoke toxicants, Food Chem Tox, 49, 11, 2921, 2011)

Margin of exposure priority assignments for a reference cigarette, commercially- based control cigarette and a reduced toxicant prototype

Smoke constituent	3R4F	Control cigarette	Reduced toxicant prototype
Acrolein	Top priority	Top Priority	Top Priority
Acrylonitrile	Top priority	Very high	Very high
Formaldehyde	Top priority	Top priority	Top Priority
Acetaldehyde	Very high	Very high	High
Isoprene	Very High	Very high	High
Styrene	Very high	Very high	High
Benzene	High	High	Medium
1,3-Butadiene	High	High	High
m- + p-Cresols	High	High	Medium
NNK	High	High	Medium
Toluene	High	High	Medium
Naphthalene	Medium	Medium	Low
NNN	Medium	Medium	Low

Estimates of toxicant yields necessary to achieve a low priority assignment in modes of exposure calculations

Compound	HCI* 3R4F yield µg/cig	MOE from HCI 3R4F (assuming 20 cigs daily)	Target µg/cig for 10,000 MOE (assuming 20 cigs per day and 100% retention)
Acrolein	155	0.3	0.0046
Formaldehyde	68.1	2	0.011
Cadmium	0.146	6	0.000086
Acetaldehyde	1534	45	6.9
Acrylamide	1.37 (ISO)	460	0.063
Benzene	104	252	2.6
1,3-Butadiene	76.5	220	1.7
Ethylene oxide	9.24 (ISO)	424	0.4
NNK	0.243	278	0.0067
NNN	0.276	2759	0.076
Benzo(a)pyrene	0.0162	16805	0.027

Overview of chemistry and toxicology

- Can substantially reduce yields of some but not all toxicants compared to commercial cigarettes

- *In vitro* toxicology gives mixed results, but generally suggest this could result in some lower toxicological activity in some assays and little to suggest increase toxicology

- Computational toxicology MOAs suggest that for some toxicants reducing the yields sufficiently to classify the levels of low priority may be impossible to achieve

Clinical studies

- Two key clinical studies, built on a series of clinical studies determining the value of biomarkers of exposure

- First was a short term switching study focused on evaluating whether reduced HCI chemistry yields translated to reduced biomarkers of exposure toxicant yields in human volunteers

(Shepperd et al, Changes in levels of biomarkers of exposure observed in a controlled study of smokers switched from conventional to reduced toxicant prototype cigarettes, Reg Pharm Tox, 66, 1, 147, 2013)

- Second was a 6 month switching study focused on BOE and BOBE

Study Design
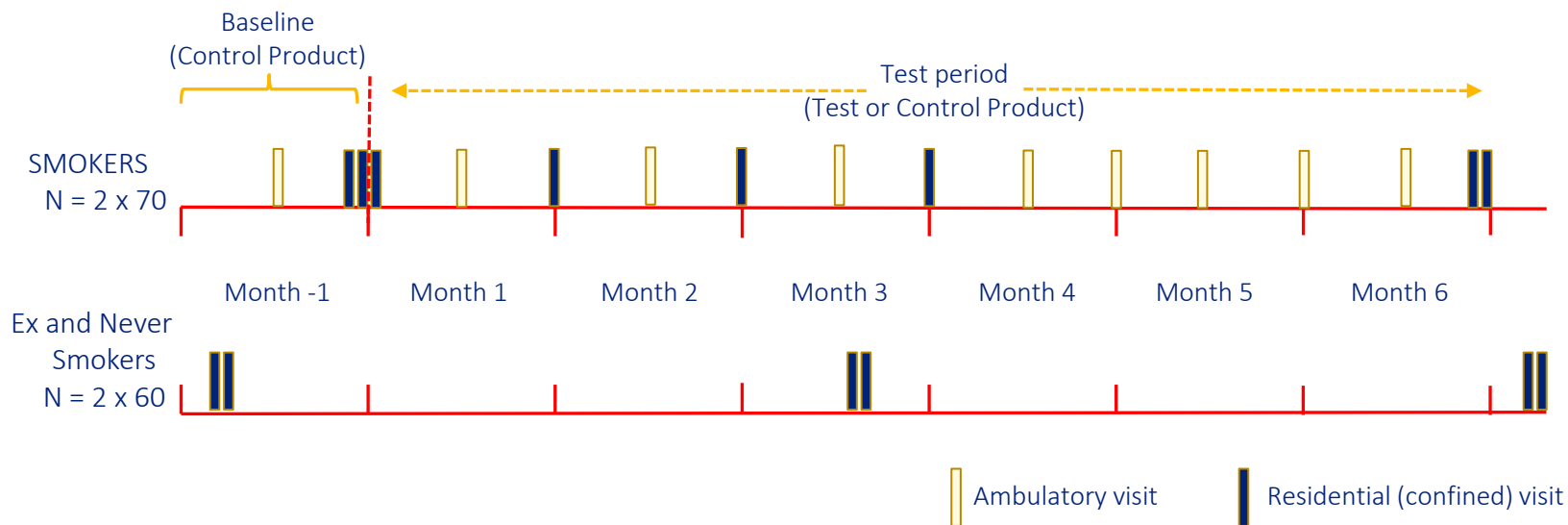
140 smokers supplied with control product for 2 weeks; baseline biomarker measures in clinic; 70 switched to RTP, 70 to visually different control (from cork to white tipping)

Clinical visits for sample collection/biomarker analysis at 1, 2, 3 and 6 months. Ambulatory visits to collect further supplies of cigarettes

Ex- and never smokers provide background levels of biomarkers of exposure and biological effect

Consumption data (baseline & on-study)
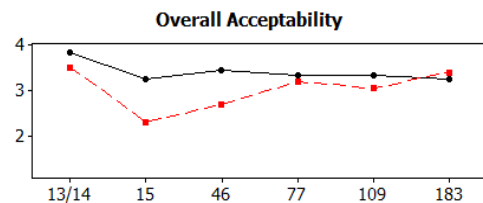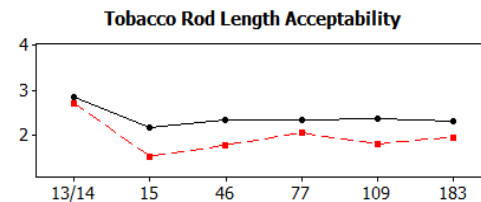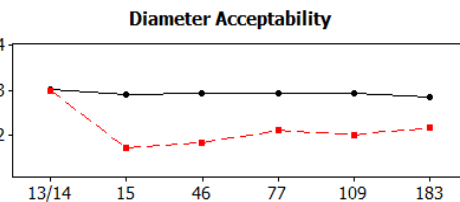
BRITISH AMERICAN

Mean CPD per smoking group
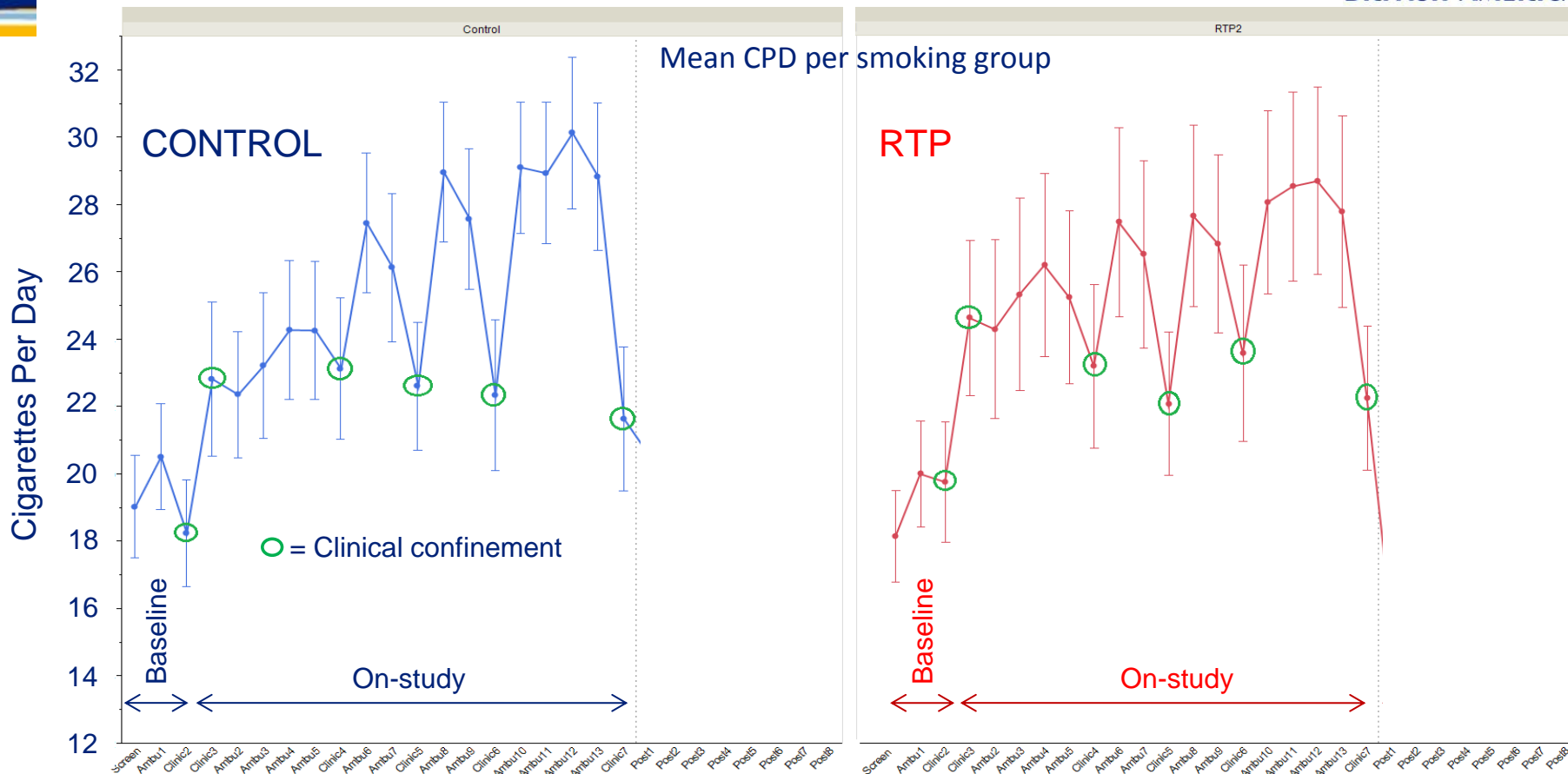
Each error bar is constructed using a 95% confidence interval of the mean.

Observed consumption change initiated:

- Increased consumption monitoring (electronic diaries) and added questionnaire
- Set-up of independent Data Safety Monitoring Board (DSMB)
- Addition of post-study monitoring of cigarette consumption

Reasons for smoking more cigarettes

Scored (4-point scale) pre-set questions

The study cigarettes don't last as long as my usual brand

I sometimes smoke one study cigarette soon after another

Study cigs. may be less harmful than my usual brand

I was in company of friends

The cigarettes are free of charge

The study cigarettes are very satisfying

The study cigarettes taste good

Being on the study

Work/job reasons such as stress, longer working hours

The study cigarettes are not very satisfying

I was on holiday

The study cigarettes taste bad

I am trying to smoke less

My lifestyle has changed

My usual brand may be less harmful than the study cigs.

Other people smoked some of these given cigarettes

The study cigarettes last longer as my usual brand

RTP (n=54)   Control (n=43)

Consumption data (baseline, on-study & POST-STUDY)

Mean CPD per smoking group

CONTROL

RTP

Cigarettes Per Day

Baseline

On-study

○ = Clinical confinement
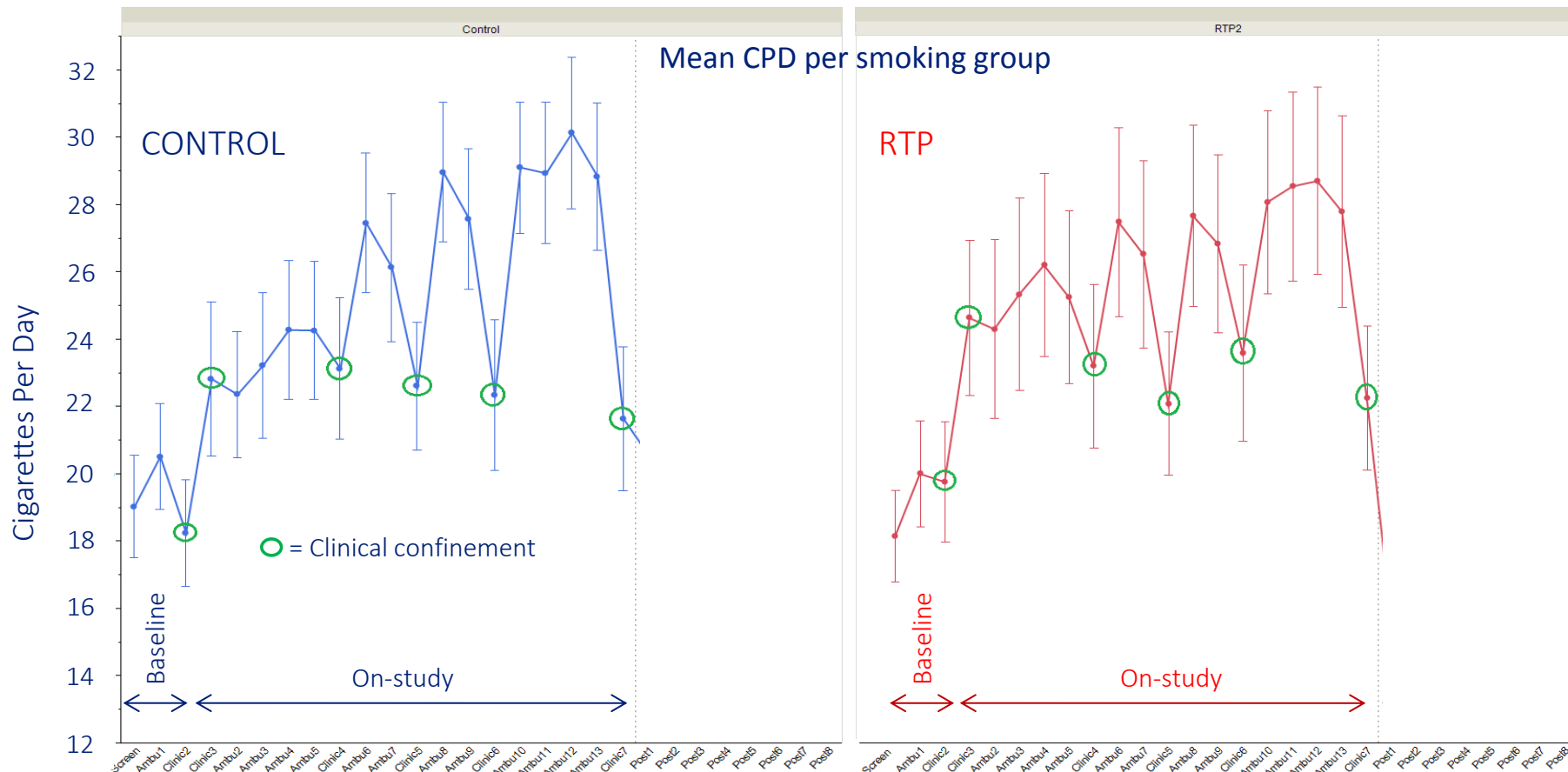
Each error bar is constructed using a 95% confidence interval of the mean.

Sensory
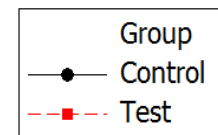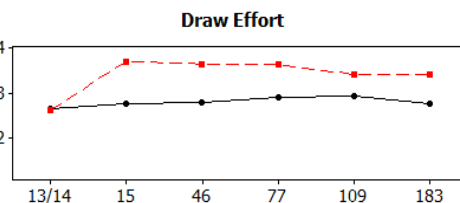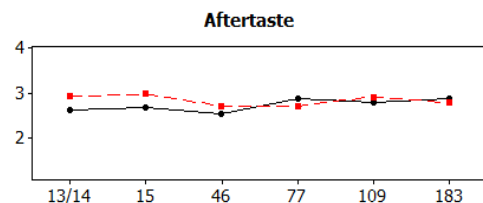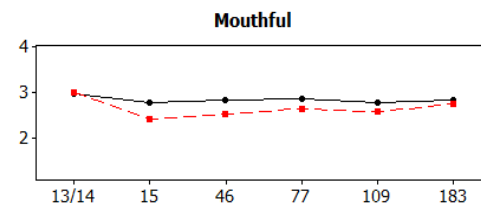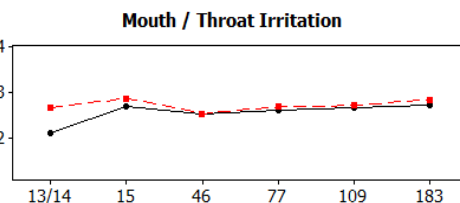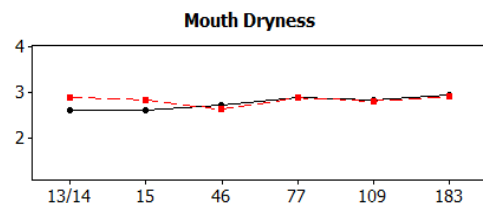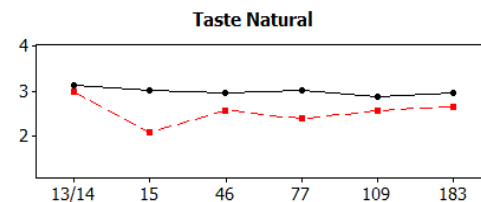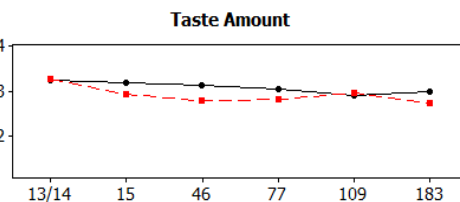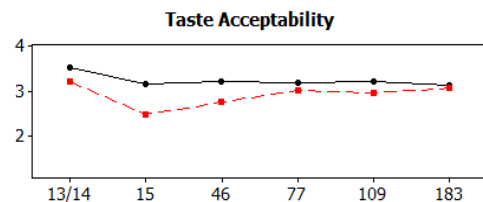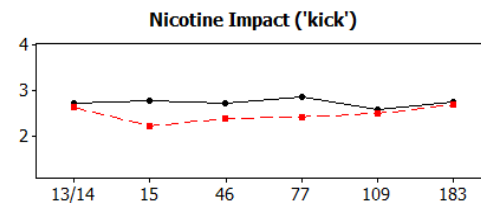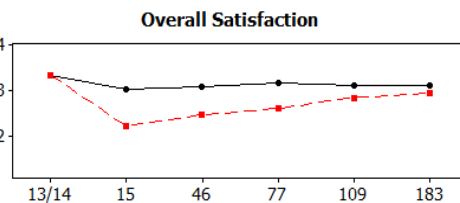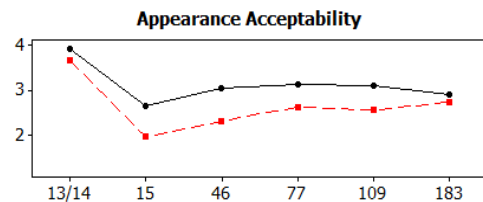
Biomarkers of effective dose

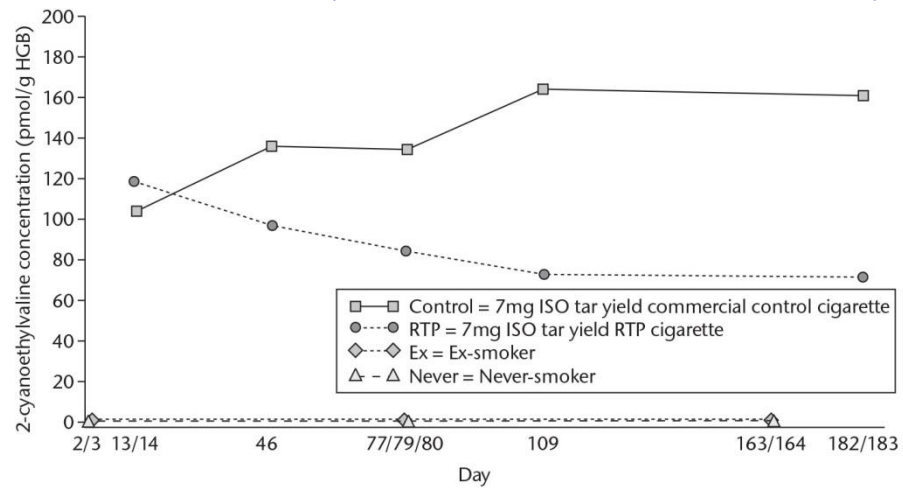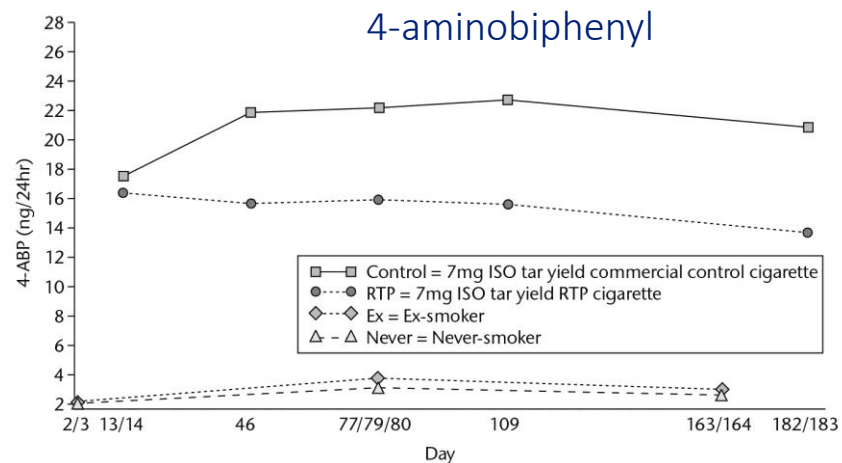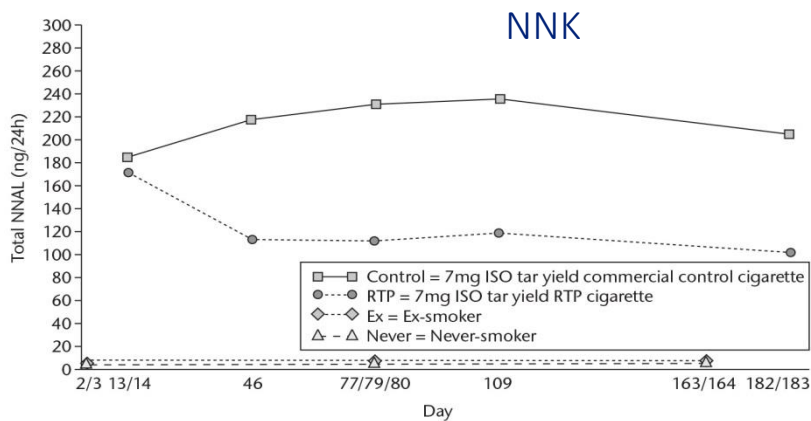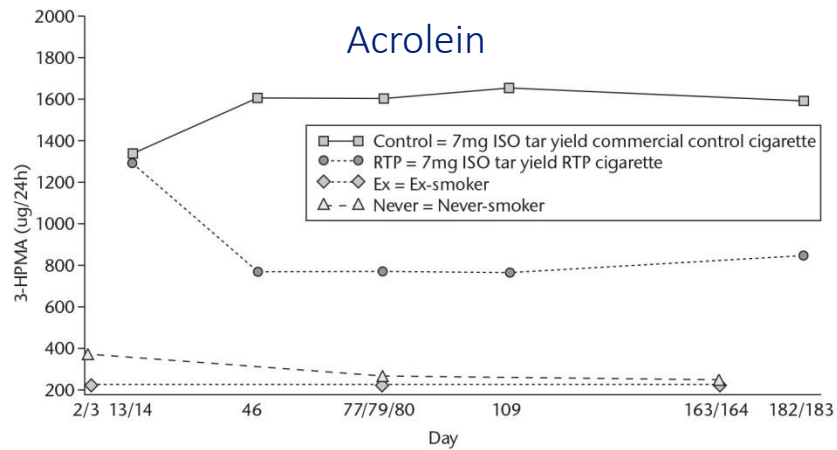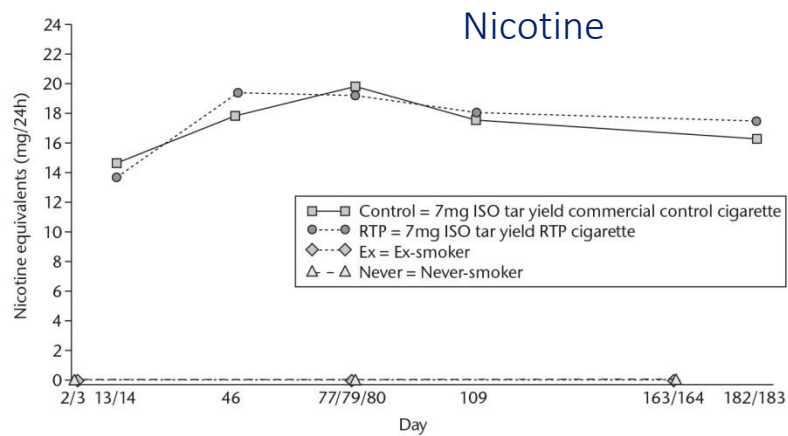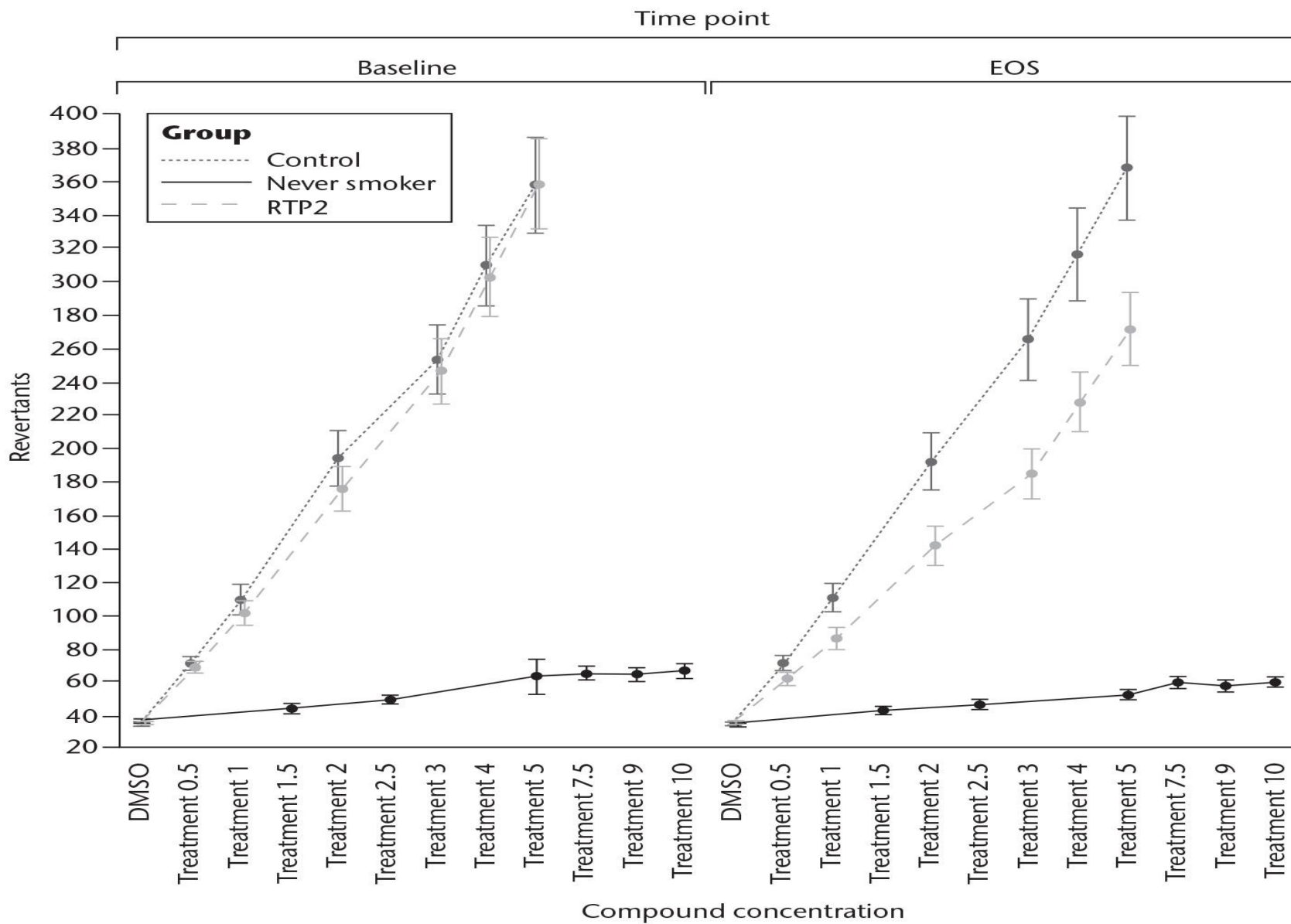Acrylonitrile BoE in urine – half life around 8 hours
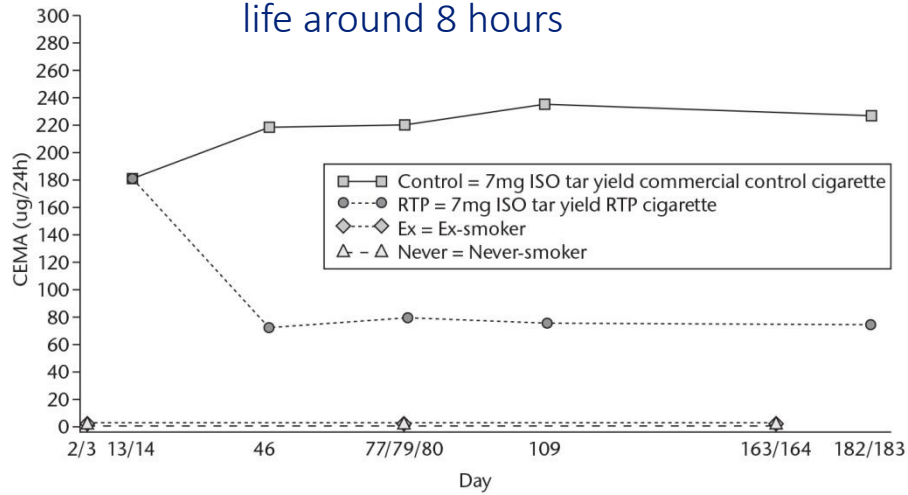
Acrylonitrile haemoglobin adduct – body residence time around 120 days
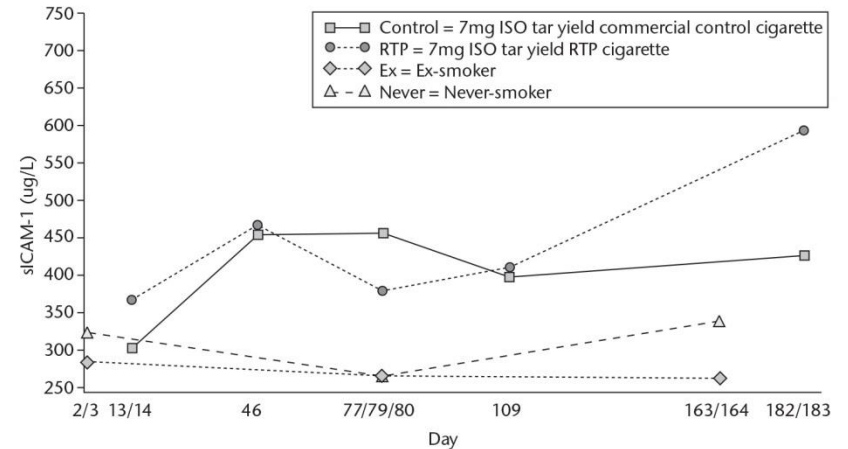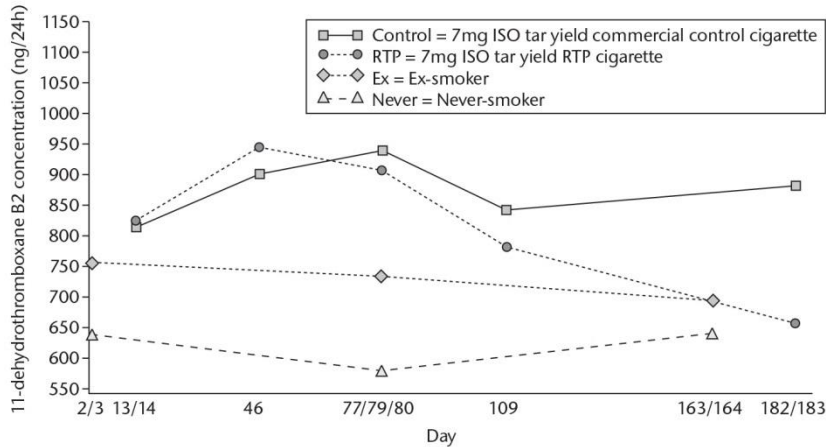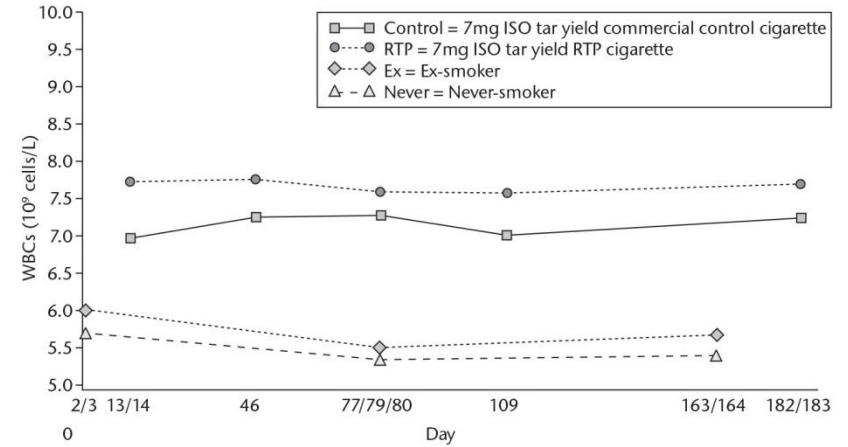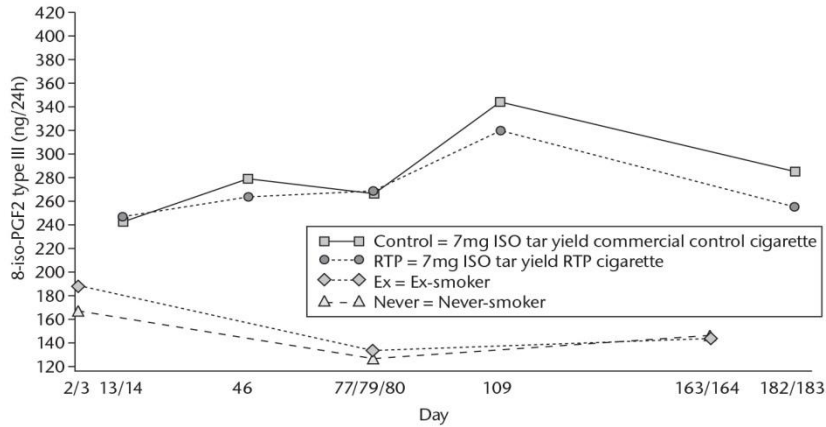
Biomarkers of Exposure

Urinary mutagenicity

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

Biomarkers of biological effect

Conclusion of clinical studies

- Switching volunteers from a conventional cigarette to a reduced toxicant prototype can result in sustained reductions in exposure to some toxicants, as measured by biomarkers of exposure

- Clinical designs of long term switching studies can cause changes in daily consumption

- Some biomarkers of biological effect clearly distinguish smokers and non-smokers

- We didn't see clear changes in BOBE in this 6 months switching study – neither increases related to increased consumption nor decreases related to lower toxicant yields

- Greater toxicant reduction may be necessary than can be achieved through modifying cigarettes

"Cumulative toxicant index" comparing a range of commercial cigarettes, reduced toxicant prototype cigarettes, tobacco heating systems and e-cigarettes
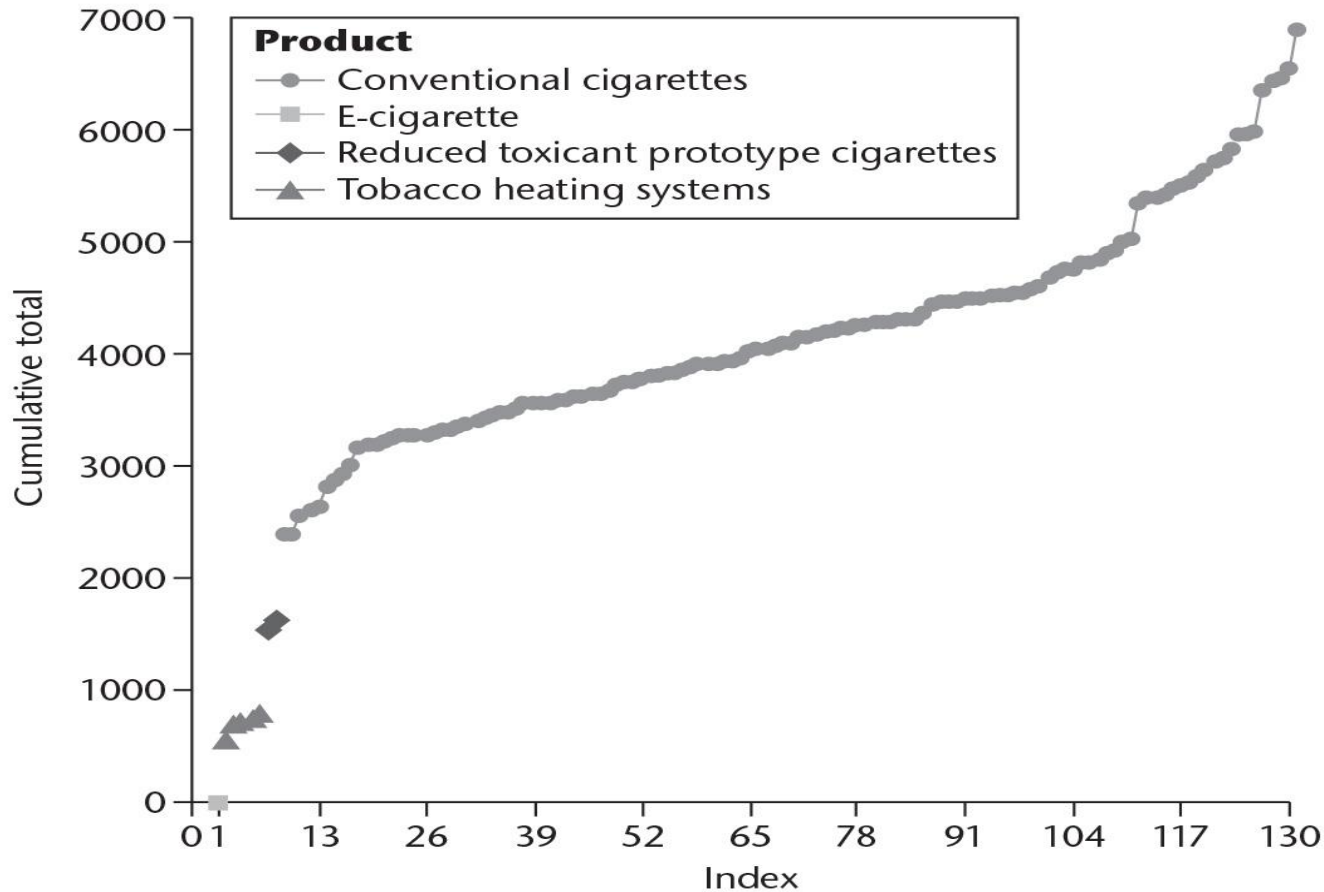
Is there scientific evidence to suggest "reduced toxicant cigarettes" have a distinct place in a tobacco and nicotine risk continuum?
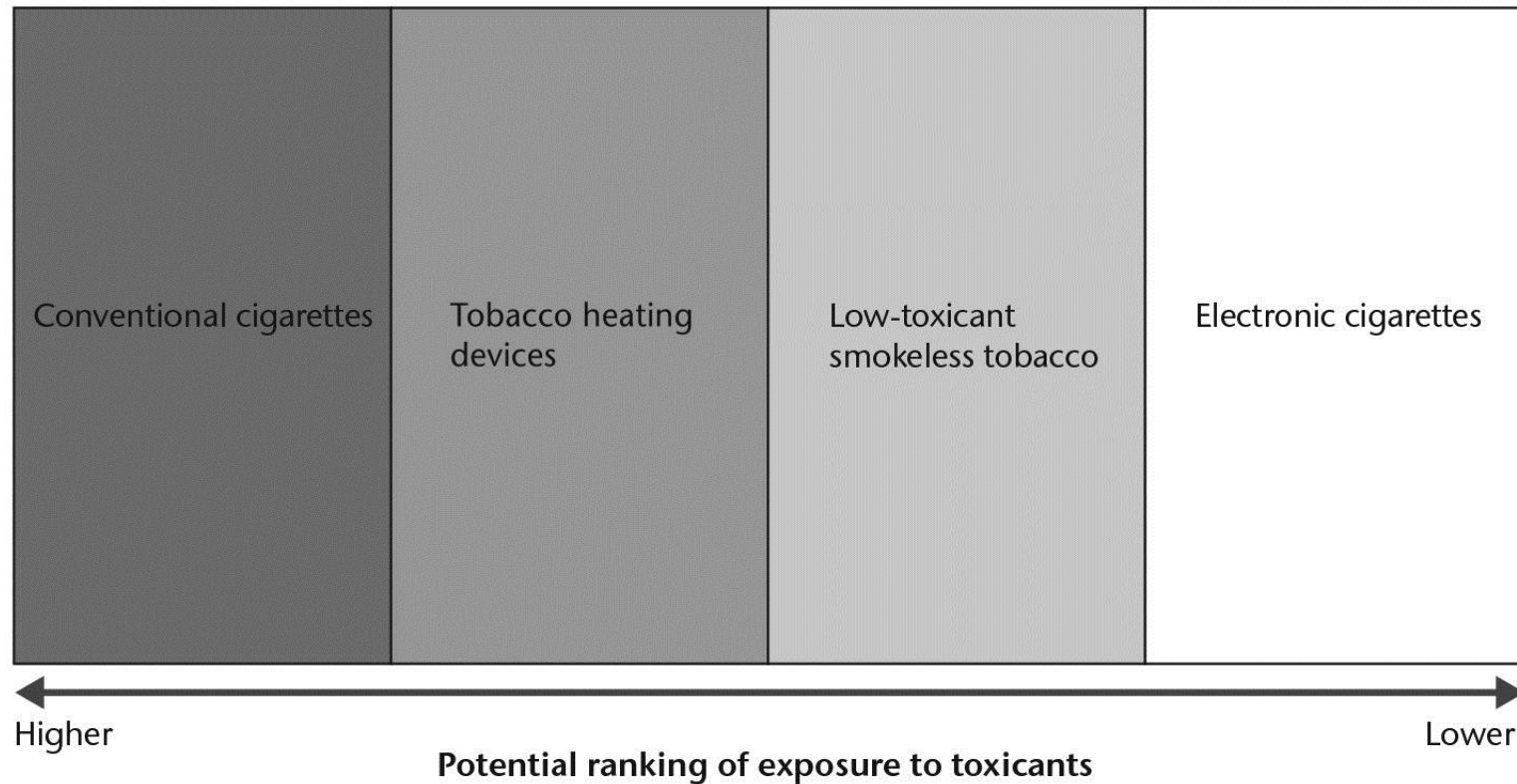
Implications for the regulation of tobacco and tobacco smoke toxicants

Potential toxicant regulations:

FDA could set product standards related to yields of toxicants

WHO's TobReg proposed a complex scheme of toxicant monitoring across a complete country/market followed by limits on nine toxicants

   NNK and NNN limited at median of the dataset
      Acetaldehyde, acrolein, benzene, benzo [a] pyrene, 1,3-butadiene,
      carbon monoxide and formaldehyde limited at 125% of the median

(or against limits identified from either an international dataset or a Canadian brands dataset)

(Burns et al, Mandated lowering of toxicants in cigarette smoke: a description of the World Health Organization TobReg proposal, Tob. Control, 17, 2, 132, 2008)

Results of our market surveys
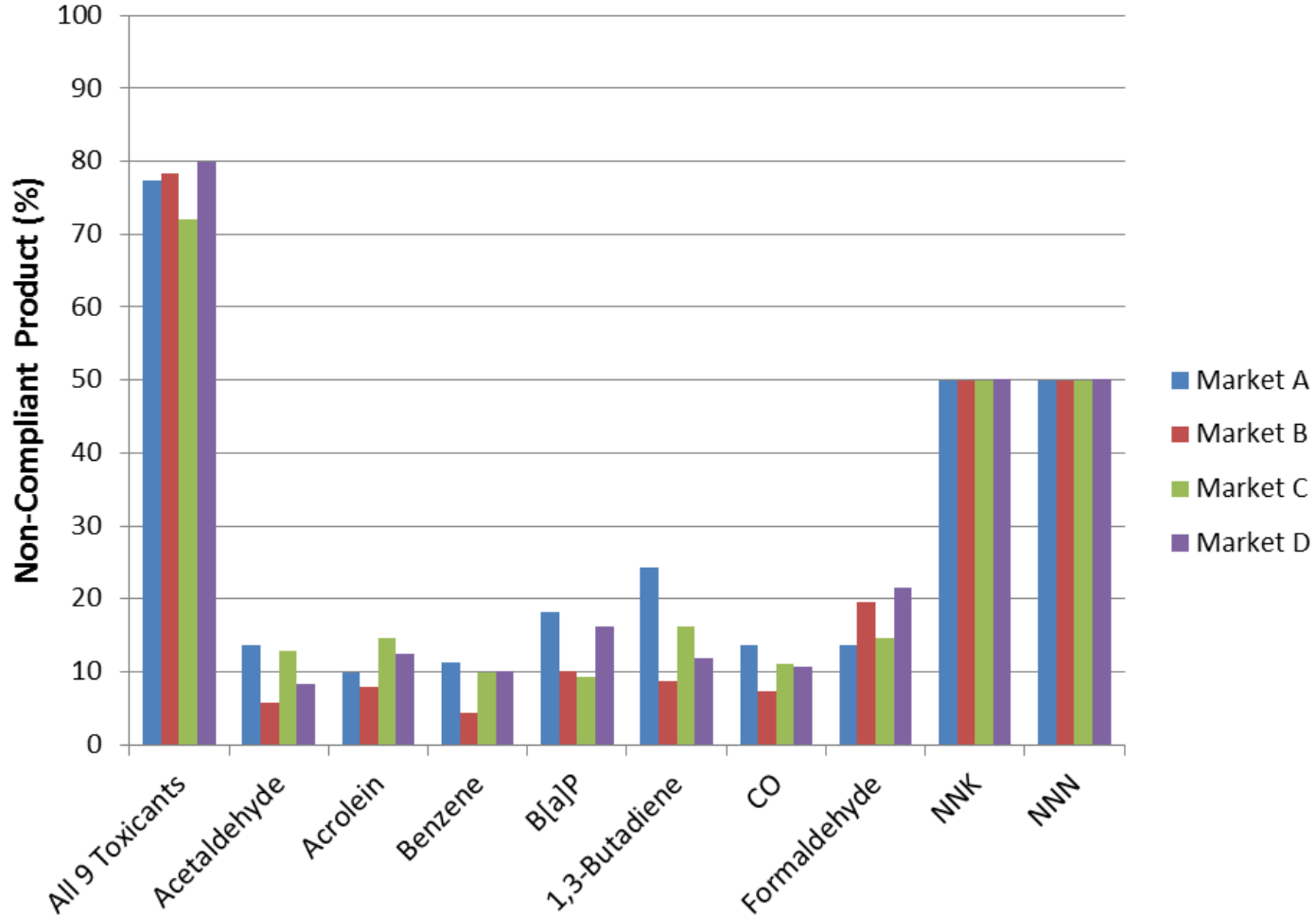
Market A – Mixed tobacco blend, low filter charcoal incidence
Market B – Mixed tobacco blend, high filter charcoal incidence
Market C – Predominantly flue-cured blend
Market D – Predominantly US blended cigarettes

Determining the potential public health impact of toxicant regulations

- Tobacco smoking causes a wide range of diseases and it is exposure to toxicants in tobacco smoke

- The epidemiology typically shows dose-response relationships, and reductions in risk following cessation

- Our studies suggest limited scope for reducing health risks substantially by modifying toxicants in cigarettes, but there are important limitations to our studies

- More research on which toxicants, or combinations of toxicants, are the drivers of smoking-related diseases, and the dynamic range of their actions, remains important

- Research evaluating the effects of tobacco heating devices and e-cigarettes may provide insights

Acknowledgements

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