National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Speciation of Metals and Metalloids in Tobacco and Tobacco Smoke

Implications for Health and Regulation

RIVM Letter report 2015-0026
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Colophon

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Publiekssamenvatting

De tabaksplant neemt metalen op uit de bodem, uit meststoffen, en uit industriële luchtvervuiling. Door roken komt een aantal van deze metalen uit tabak vrij, waarna de roker en omstanders ze inademen. Van de metalen veroorzaken arseen, cadmium, nikkel en lood de grootste gezondheidsrisico's. De mate waarin dat gebeurt, hangt af van de 'vorm' van het metaal. De vorm kan tijdens het verbrandingsproces veranderen van een weinig giftige tot een zeer giftige vorm, én andersom. Daardoor is de chemische vorm in tabak anders dan in rook. In onderzoek dat in opdracht van het RIVM is uitgevoerd, is chroom in de minst schadelijke vorm in rook aangetroffen. Arseen daarentegen is juist in de schadelijkste vorm aanwezig. Het onderzoek is uitgevoerd door de Universiteit van St Andrews in Schotland.

In het onderzoek is de chemische vorm, oftewel speciatie, beschreven van verschillende metalen in tabak en tabaksrook. Hiervoor is in eerste instantie gebruik gemaakt van een zeer krachtige deeltjesversneller, de Diamond Light Source, in Engeland. Voor deze werkwijze is gekozen omdat de speciatie van arseen en chroom moeilijk te meten is in tabak en rook. De uitkomsten van de experimenten kwamen goed overeen met voorspellingen op basis van rekenmodellen. Daarom zijn in het vervolg deze modellen gebruikt om de chemische samenstelling van andere metalen in tabaksrook te voorspellen.

Tijdens dit onderzoek heeft TobReg, het expertpanel dat de WHO wetenschappelijk advies geeft over de regelgeving van tabaksproducten, aanbevolen dat fabrikanten de niveaus van arseen, cadmium, lood en nikkel in tabak moeten testen. De resultaten van de onderliggende studie ondersteunen de keuze voor deze metalen.

Enkele voorbeelden van gezondheidseffecten van metalen zijn: kanker, lever- en nierschade.

Kernwoorden: zware metalen, tabak, rook, arseen, chroom

Abstract

Metals are acquired by the growing tobacco plant from soil, fertilisers, and industrial pollution. Smoking liberates some of these metals from tobacco into smoke to be inhaled by the smoker and bystanders. Arsenic, cadmium, nickel and lead are the main contributors to the health risks of metals in smoke. The health risk depends on the 'form' of the metal. The burning process may completely transform the metal from a low toxicity form to high toxicity, and *vice versa*. In research commissioned by the RIVM, chromium in tobacco smoke was found in its least toxic form. Arsenic, by contrast, is present in its most toxic form. The research was conducted by the University of St Andrews in Scotland.

This report describes the chemical form, or speciation, of several metals known to be present in tobacco and tobacco smoke. To this purpose, we used one of the world's most powerful synchrotrons, the Diamond Light Source, in the UK. This was necessary, as it is difficult to determine the speciation of arsenic and chromium in tobacco and tobacco smoke. The results of the experiments were in good agreement with predictions based on theoretical models. Therefore, these models were also used to predict the chemical composition of other metals present in tobacco smoke.

During our study, TobReg, the WHO expert panel set up to advise on the scientific basis of tobacco product regulation, has recommended that manufacturers test the levels of arsenic, cadmium, lead and nickel in tobacco. The results of the present study support the prioritisation of metals in their list.

Examples of harmful health effects of metals are cancer, and liver- and kidney damage.

Key words: heavy metals, tobacco, smoke, arsenic, chromium

Contents

Summary

This Report addresses the lack of information for the speciation (both valence and compound) of metals and metalloids that are transferred to the lungs in tobacco smoke. Such information is required for risk assessment. Two metals/metalloids were of particular interest, namely arsenic and chromium, because they exist in multiple valence states with markedly different human toxicities. These metals were studied using laboratory techniques (principally HPLC-ICPMS and XANES) and the findings were used to test the predictions of a thermodynamic model based on tobacco smoke pH and redox potential (Eh).

The raw materials for study were reference materials for tobacco, tobacco extracted from commercial products and tobacco plants cultivated in soil burdened with various metals in different concentrations. Burdening was successful for some metals and detailed study of As showed that the chemical speciation of moderately burdened samples is similar to unburdened and commercial tobaccos. This approach makes it possible to apply methods of characterisation that require higher concentrations to function than is normally found in commercial tobacco products. Metal burdening eventually turned out not to be necessary as the powerful third generation synchrotron used (Diamond Light Source, the UK's national synchrotron facility) was capable of detecting As at concentrations below one μg g^{-1}.

Compound speciation of As was determined using High Performance Liquid Chromatography coupled with Inductively Coupled Plasma Mass Spectrometry (HPLC-ICPMS) that discriminates between inorganic and organic arsenic species, and among the various organic arsenic species, notably methylarsonate (MA) and dimethylarsinate (DMA). Valence species for both As and Cr in tobacco, smoke condensate and ash were modelled from the positions of the edge energies of the K-alpha absorption edges of As(III) and As(V) using X-ray Absorption Near Edge Structure (XANES).

Eh-pH modelling of the species of metals in aqueous media was developed for the range of Eh and pH measured in the smoke of typical tobacco products. Agreement was good between the speciation indicated by XANES for As and Cr and the thermodynamic predictions. This encouraged application of the thermodynamic model to other metals often considered hazardous components of tobacco smoke but for which no information on speciation in smoke was available.

Principal findings of this research:

- the process of combustion and smoke generation has a major effect on speciation. Metals that partition into the smoke condensate exist in a reduced environment whereas that which partitions into ash is oxidised. This partitioning occurs regardless of the oxidation species predominating in the precursor tobacco. This has important implications for regulation based on the concentration of metals and metalloids in plants and commercial products.
- An exceedingly powerful third generation synchrotron has proved capable of successfully characterising the valence species of metals and metalloids at sub ppm levels in a range of tobacco materials including *in vivo* tobacco leaf, processed tobacco, filters and ash, and most notably smoke condensate.
- HPLC-ICPMS has also proved a powerful technique for discriminating organic and inorganic species of arsenic in a wide range of tobaccos. The same methodologies should also be applicable to other elements in which organic and inorganic species have variable carcinogenic and toxic effects.
- Simple thermodynamic modelling of metals in smoke produced results consistent with laboratory measurements, although these are currently few

in number and more complex modelling may be required as more laboratory constraints become available.

Arsenic

- In tobacco plants arsenic is present at approximately 80% inorganic species and 20% organic species (primarily DMA and MA). Valence varies between As(III) and As(V) with most plants containing a mixture of these valencies. There is no relationship between valence balance and the nature of arsenic burdening
- Arsenic in cigarette tobacco tends to be present in its oxidised form As(V)
- Combustion of tobacco causes arsenic to become reduced entirely to As(III) in smoke condensate, and oxidise to As(V) in ash. Partitioning of oxidation state is complete and no mixture of valencies has been observed in any smoked tobacco product
- Ageing of tobacco smoke for 30 minutes does not induce reduced As(III)-dominated smoke to oxidise to As(V)
- The data suggest that As may be an important smoke carcinogen

Chromium

- XANES studies show Cr to be present in tobacco as Cr(III) and Cr(0). The metallic Cr appears to be associated with fragments of steel in tobacco presumably from processing machinery.
- Cr was very difficult to detect in smoke products, but one sample of cigarette filter did indicate the presence of Cr(III). No Cr(VI) was detected in any tobacco or smoke products.
- The data suggest that not much Cr is mobilised in smoke and that little or none is in the carcinogenic hexavalent form and thus is unlikely to represent a significant hazard

Other metals and metalloids

- Modelling suggests that Cd (especially) and Ni could be important smoke carcinogens
- Modelling does not provide evidence for Be as a significant smoke carcinogen
- There is evidence that Pb may be significant toxic smoke component for non-cancer disease
- The evidence is not sufficient to include Co, Se, Mn, Cu and Hg among toxic smoke components although more research is warranted, especially on Co.

Regulatory implications:

- WHO's expert panel TobReg listed four metals recommended for regulation, namely As, Cd, Ni and Pb. This report presents evidence in support of prioritising the same four metals.
- No evidence was found for the presence of Cr(VI) in smoke and thus the inclusion of Cr in the list of elements is not recommended.
- Similarly no evidence was found to support the inclusion of other carcinogens such as Be and highly toxic metals such as Hg. At present the evidence for Co is weak but warrants more research.

1 Introduction

Smoking tobacco is the largest preventable cause of disease and the primary reason for the death of half of its users. In a co-ordinated effort to tackle this global epidemic the World Health Organisation (WHO) adopted the Framework Convention on Tobacco Control (FCTC) [1] in 2003 and 177 States have since formally signed as Parties to the Convention.

Articles 9 and 10 of the WHO's Framework Convention on Tobacco Control are concerned with the regulation of the contents of tobacco products and their disclosure, requiring the relevant authorities to "adopt and implement effective measures for public disclosure of information about the toxic constituents of the tobacco products and the emissions that they may produce" [1]. Achieving a consensus on these "toxic constituents" is a challenge. As any regulation of toxic constituents will impose burdensome requirements on the tobacco industry it is important that strong scientific evidence underpins the case for each nominated constituent, including the metals identified in this study.

Among several thousand chemical compounds documented in tobacco smoke 98 have associated risk values, and 11 of these are metals or metalloids [2]. The World Health Organisation (WHO) expert panel on tobacco regulation (TobReg) recently reviewed the published literature on metals and metalloids in tobacco and smoke, concluding that arsenic (As), cadmium (Cd), nickel (Ni) and lead (Pb) are of sufficient concern that they should be subject to regulation [3]. The panel recommended that "manufacturers … test cured tobacco purchased from each new agricultural source for levels of arsenic, cadmium, lead and nickel" and that these metals should also be analysed in tobacco blends offered for sale.

This is an important policy development. Unlike most of the 98 hazardous tobacco smoke compounds the metals already exist in (or on) the tobacco plant, albeit not necessarily in their metallic states. Smoking essentially liberates the metal from tobacco to be retained in ash or transferred to the smoke aerosol. The chemistry is complex but techniques now exist for establishing quantitative relationships between the smoker's exposure to metals and the original composition of the tobacco blend. This creates the opportunity to regulate these hazardous components on the basis of leaf composition rather than smoke composition, technically much easier to determine and within the capability of many laboratories worldwide. In some cases it may also be possible to regulate to prevent the tobacco plant acquiring high levels of metals, for example by controlling permissible fertilisers and forbidding the cultivation of tobacco crops on land severely affected by industrial metal pollution.

A large body of toxicological and epidemiological evidence indicates that the species (or speciation) of an element may strongly influence its toxicity to humans. Speciation concerns the atomic or molecular form of an analyte, its electronic or oxidation state, and complex or molecular structure. Tobacco smoke is a very complex and dynamic medium but very little research has been conducted on the speciation of metals and metalloids that are known to be transported in smoke. Arsenic is taken as an example for in-depth investigation in this report and the general concepts are applied more widely to other metals and metalloids found in tobacco smoke.

This report explores some of the new techniques and methodologies that are available for characterising metals in tobacco and smoke and modelling their behaviour. All likely metals are considered but attention is given to the four metals identified by TobReg as primary cases for regulation (As, Cd, Ni and Pb). While arsenic is studied in most detail chromium is also considered because of its multiple valence states and the role this plays in toxicity.

2 Purpose of the study

The study was undertaken for the following purposes:

- To contribute new information on the chemical form of metals in tobacco and smoke aerosol for use in assessing the risks to the health of smokers
- To make progress in establishing quantitative relationships between the species of metals in smoke and the species of metals in tobacco leaf and blends, so that regulation of metals in leaf and blends accurately reflects their contributions to the risks of smoking
- To explore where inequalities might exist in exposure to metals and metalloids in tobacco smoke on a global scale
- To identify the requirements for further scientific studies to account for the major risks attributable to metals in tobacco
- To show that generic methodologies may be applied more widely to other forms of tobacco consumption, such as oral use.

Collectively these contributed to framing policy recommendations for the control of a major but neglected class of hazardous components present in commercial tobacco products and their emissions.

2.1 Background

An important outcome of the Framework Convention has been global co-ordination of efforts to understand and mitigate the negative health effects of smoking, including scientific aspects. TobLabNet, the network of laboratories concerned with the analysis of tobacco and smoke, is a global forum for issues that can be addressed using laboratory techniques. Much of the leadership in this area comes from the EU and US, with RIVM prominent among those setting agendas and providing scientific research in support of policy. This project was an RIVM initiative to provide scientific research in an area where such research was lacking and its absence impeded informed decision-making.

The concept of metal or metalloid speciation, widely used in characterising environmental hazards, is applied here to tobacco smoke. The term speciation refers to the specific chemical form of an element defined in terms of its electronic or oxidation state and molecular structure [4]. The potential hazard of a metal or metalloid, including its mobility, reactivity and toxicity, may vary with speciation [5] and combustion can have a determining effect on speciation.

Hitherto only one major study of speciation in tobacco smoke, conducted by tobacco industry and academic researchers, has been published [6-8]. Its focus was arsenic. The present report (summarising the accompanying Scientific Report) widens the scope to other metals and metalloids, and develops the research on arsenic using new technologies not available to the earlier researchers. Experimental results are compared with thermodynamic models of speciation in smoke enabling a first order assessment of a wide range of metals and metalloids without need for new laboratory measurements.

3 Description of tobacco products

The focus of this study is tobacco as used to manufacture cigarettes designed for use by the public for the purpose of smoking.

The tobacco used for cigarettes is almost invariably the species *Nicotiana tabacum* that is cured and fermented after harvesting. Numerous different varieties of the species are cultivated, and agronomic practices include the addition of fertilisers and pesticides. Some of these factors may influence the metal content of tobacco but no attempt has been made to take these into account. As well as leaf other parts of the tobacco plant (e.g. stalk) are also utilised and the manufacturers usually include additives in the finished product. Blending of tobaccos is common and this may include reconstituted tobacco and expanded tobacco. There is no evidence that additives increase the metal content of the product and this factor is not considered in this study.

Included in this study are standard cigarettes made from individual tobacco types such as Virginia or blends such as the American blend. They are typically made from about 0.6-1.0 g of shredded tobacco wrapped in cigarette paper to make a cigarette rod about 85mm long. Mostly these are tipped with filters usually made of cellulose acetate although some designs now include activated charcoal or other constituents designed to reduce exposure to particular volatile compounds. As part of this report some tobacco was grown burdened with additional metals and metalloids, specifically to overcome analytical problems at low concentration levels. All burdened tobaccos were grown from the same KT209 seeds, a variety of burley tobacco developed at the University of Kentucky.

Not included in this study are alternative forms of tobacco use. These include smoking products such as cigars and cigarillos, hand rolling tobacco, pipe tobacco, bidis, kreteks, smokeless tobacco and mixtures used in water pipes. Also not included are oral tobaccos such as chewing tobacco and snus. Although less prevalent on a global scale some of these may be more dominant than cigarettes in certain regions or cultural groups.

4 Regional and global patterns of tobacco product use

In 2009 nearly 6 trillion cigarettes were smoked worldwide, an increase of 13% on the previous decade. This involves nearly 20% of the world's adult population [9]. Historically smoking has been more prevalent in richer countries and in the Netherlands 21% of adults smoked in 2010 (EU average 23%) down from about 70% in the middle of the 20th century. While major progress is being made in reducing smoking in developed countries there are significant global disparities, with smoking still on the increase in some developing world countries.

The risks of disease from smoking tobacco have been known for at least 50 years, nevertheless by 2030 eight million people are expected to die from the habit each year. These estimates disguise a major disparity: 80% of these deaths attributable to smoking will occur in low- and middle-income countries [9].

Various measures have proved very effective in reducing the incidence of smoking in the developed world. Most measures are based around taxation, education and/or regulation. All are important. The present report concerns regulation and the need to protect the consumer from smoking products that potentially expose the smoker (and bystanders) to particularly high levels of smoke toxins, in this case metals and metalloids.

An example of where regulation may make a difference is in the global disparity evident in the distribution of metals in cigarette tobacco. Figure 1 is based on a global database of 1380 cigarette tobaccos (unpublished, University of St Andrews). It shows the distribution of Ni, Cd, As and Pb in tobacco extracted

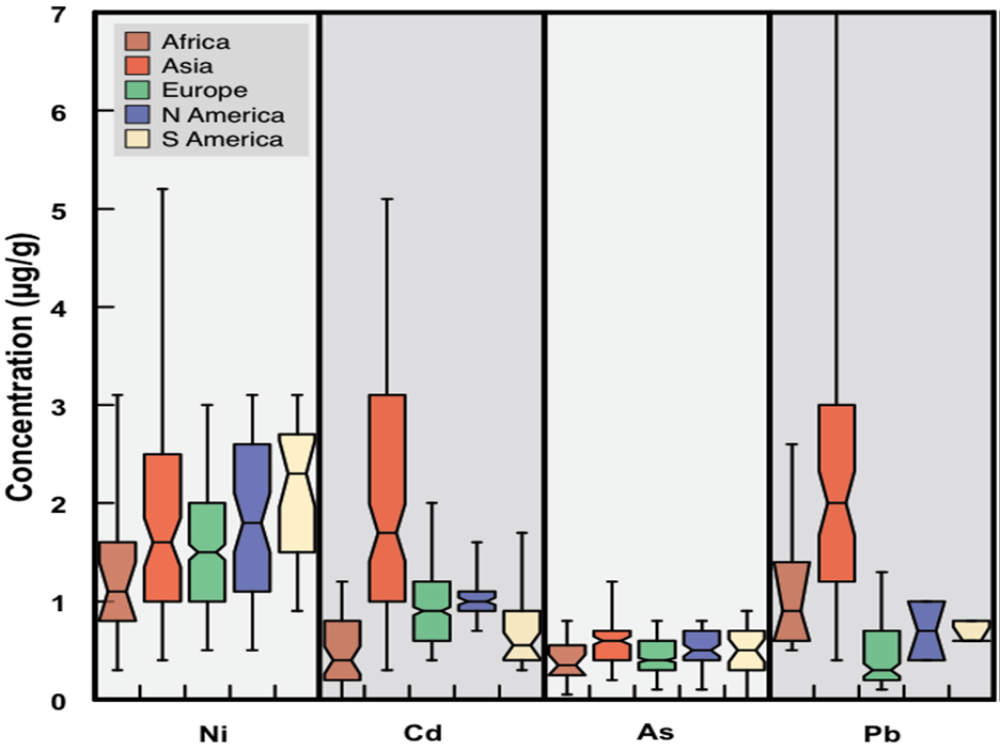

Figure 1. Box plot and 5 & 95 percentile ranges for Ni, Cd, As and Pb in global cigarette tobaccos classified on the basis of continent of purchase.

from cigarettes purchased on all the major continents showing that on average Cd and Pb are present in significantly higher concentrations in Asia than elsewhere. The large majority of metal-contaminated samples were purchased in China confirming earlier reports of metal enrichment [10].

This feature of tobacco from parts of Asia is a matter of concern as it is known that higher levels of Cd and Pb in tobacco will lead to higher exposure to these metals during smoking [11]. Cd and Pb are among four metals and metalloids recommended by TobReg for regulation because of concern for their potential harm to smokers [3]. China is home to about one third of the world's smokers, with rapidly increasing tobacco use among women and young people [12]. In this case reducing the levels of metals in tobacco could make a significant difference to the long-term health prospects of smokers in this region.

Metals and other inorganic materials incorporated in tobacco have their origins primarily in soils, agronomic activities including fertilisers, and industrial pollution. The balance between these factors contributing to high Cd and Pb in some Chinese products, for example, is not known but if primarily related to fertilisers and pollution it might be possible to reduce exposure to these metals by controlling permitted fertilisers and forbidding the cultivation of tobacco crops on land contaminated with metals due to industrial pollution. In the longer term reducing industrial pollution could also lead to declining levels of metal uptake in tobacco.

5 Impact on public health

Smoking clearly has a major effect on public health. Many components of tobacco smoke are implicated in disease but this report is concerned only with the contribution of metals and metalloids to the overall risk to health.
The report focuses on chemical species (i.e. compounds) and valence species (i.e. oxidation state) with the ultimate aim of developing a model to describe species changes in a single element along its pathway from tobacco cultivation to respirable smoke. The element arsenic was chosen for particular focus as it is highly toxic, is known to be present in mainstream tobacco smoke, and exists in multiple molecular forms and valence states.

5.1 Toxicity of metals and metalloids in tobacco smoke

Table 1 summarises the findings on metal and metalloid species along with valence in the context of health, in particular the IARC classification of carcinogens and other non-cancer adverse health effects. Also important in this context is the concentration of each metal in smoke, and the values listed are derived from various averages presented in the literature [13-15]. The combined information facilitates a first order estimation of the risk associated with these elements.

Table 1. Summary of model outcomes in context of the IARC classification of carcinogens and other forms of toxicity, and the concentration range in smoke emissions.

Element	Predicted major species	Predicted phase in ambient smoke	IARC Classification of carcinogens		Other toxicity (list not comprehensive)	ISO MS smoke (µg/g)	
			Type	Form		min	max
Arsenic	$As(OH)_3$	Solution	1	Arsenic and inorganic arsenic compounds	Cardiovascular, gastrointestinal, hepatic and renal diseases	0.004	0.100
Beryllium	BeO	Solid	1	Beryllium & beryllium compounds	Pulmonary disease	0.001	0.006
Cadmium	Cd^{2+}	Ionic solution	1	Cadmium and cadmium compounds	Stomach irritation (vomiting and diarrhoea); lung damage; kidney diseases	0.031	0.271
Chromium	Cr_2O_3	Solid	1	Cr(VI) compounds	Blood, renal and liver diseases	0.15	1.5
Cobalt	Co^{2+} (major) $HCoO^{2-}$ (minor)	Ionic solution	2B	Cobalt and cobalt compounds	Contact dermatitis mutagenic effects.	0	0.4
Copper	Cu	Solid	Not known to be carcinogenic		Blood, kidney, gastrointestinal disease	0.013	0.013
Lead	Pb^{2+} (major) $Pb_6(OH)_8^{4+}$ (minor)	Ionic solution	2A	Lead compounds (inorganic). Metallic lead (2B)	Neurological damage; renal disease; cardiovascular and reproductive effects	0.032	0.41
Manganese	Mn^{2+}	Ionic solution	Not known to be carcinogenic		Neurological; liver function	0.002	0.003
Mercury	Hg	Solid	3	Mercury & inorganic mercury compounds	Nervous system, kidney damage	0.006	0.292
Nickel	Ni^{2+}	Ionic solution	1	Nickel compounds	Skin disease, allergies	0.001	0.887
Selenium	Se	Solid	3	Selenium & selenium compounds	Gastrointestinal disease, neurol. damage	0.002	0.319

The scientific report applies simple thermodynamic models to determine the metal and metalloid species most likely to be present in smoke under measured conditions of pH and oxidation potential at 25°C. The results are summarised in Figure 2 as bars that reflect species concentrations in smoke emissions and coloured according to the principal host phase.

The diagram indicates that chromium can be present in quite high concentrations in smoke and is transferred primarily as Cr_2O_3, the trivalent form of chromium which is associated with low toxicity. Arsenic is transferred as $As(OH)_3$, a soluble trivalent species considered to be highly toxic. Cadmium, nickel and lead may be present as aqueous ions, all likely to be bioavailable and potentially toxic.

Those metals that are bioavailable, toxic and present in significant concentrations require further investigation to establish the risks that they pose. Other metals such as beryllium, though toxic, may pose little risk in that they may exist in insoluble forms and/or are transferred by smoke in very low concentrations.

This interpretation highlights five metals and metalloids, namely As, Cd, Co, Ni and Pb for consideration. On the other hand chromium may not be readily bioavailable and is modelled in smoke as Cr(III) rather than the highly toxic Cr(VI) species.

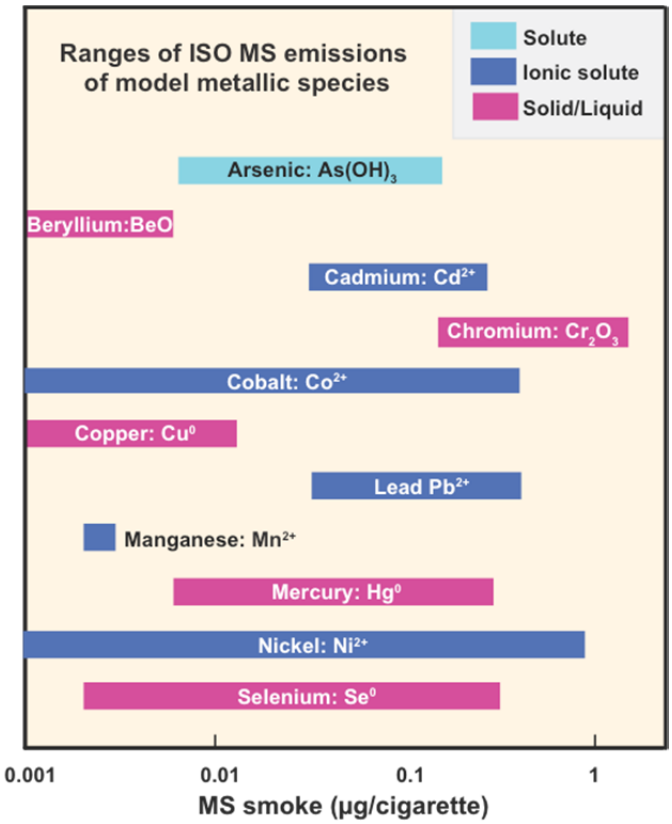

Figure 2. Ranges of mainstream smoke emissions (ISO) quoted in the literature for the metals analysed in this study. The range bars are annotated with the model species of each metal. Note that the ranges are plotted on a logarithmic axis.

5.2 Health risks of metals and metalloids in tobacco smoke

To provide context to the findings they are compared with published studies of the risk of cancer and other diseases due to individual smoke components [13, 16]. Although such assessments are necessarily simplistic they are informative when compared over several orders of magnitude.

Using published cancer potency factors derived for air quality risk assessment Fowles & Dybing (2003) estimated a "cancer risk index" of individual components based on their concentrations in mainstream tobacco smoke [13]. More recently Behera *et al.* presented new data for metals in two US and two UK brands purchased in Singapore, and used the data to calculate the "incremental cancer risk" of individual metals [16]. A comparison of the two risk indices is presented in Figure 3, with the cancer risks spanning three orders of magnitude.

Figure 3 indicates that high risks are associated with Cr, Cd, As and Ni while lower risks associated with Be and Pb. This outcome highlights the problem of using concentrations of components without allowing for speciation. Although Cr indicates the highest cancer risk, as discussed above the risk data are specifically associated with exposure to Cr(VI), i.e. hexavalent chromium, trivalent chromium having little or no toxicity in humans. Using a powerful synchrotron the Cr found to be present in smoke condensate is primarily in Cr(III) state with minor particles of Cr(0) from steel fragments of machinery. No Cr(VI) was detected in the synchrotron studies. Modelling in this report also predicts chromium to be present as the Cr(III) species in smoke. Thus chromium should have a much lower ranking in these comparisons of cancer risk.

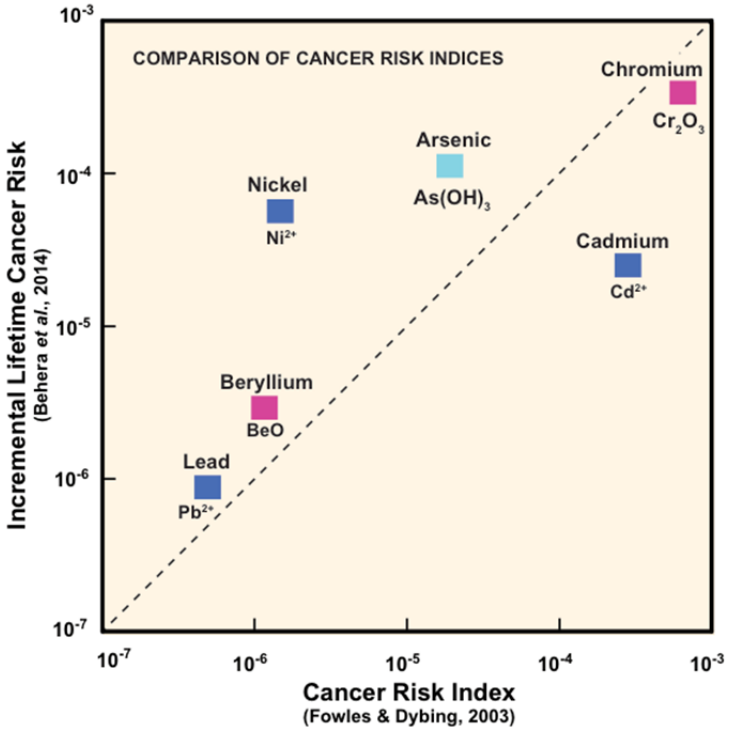

Figure 3. Comparison of values using two different cancer risk indices for various metals found in tobacco smoke. Dashed line indicates 1:1 agreement. Incremental Lifetime Cancer Risk based on the mean analysis of four brands in the paper by Behera et al. (2014). Colour coding as in Fig.2. See text for more details.

In contrast arsenic, an element that also exists in multiple valence states in nature, has been shown by the synchrotron studies to be present in smoke condensate exclusively as As(III) (Figure 4).

The cancer risk comes entirely from As(III) in inorganic compounds, and arsenic in tobacco is approximately 80% inorganic and 20% organic. Thus arsenic should be firmly established as one of the most important metallic carcinogens. These cancer risk assessments can be re-evaluated using the speciation data generated in this study. Among tobacco smoke components the study suggests that As, Cd and Ni should be regarded as the most important metal and metalloid carcinogens in tobacco smoke.

Behera *et al.* (2014) also developed an analogous risk index for non-cancer diseases and applied their model to four popular international brands purchased in Singapore [16]. Their data suggest that Cd and Pb contribute most to the risks of non-cancer disease. An analogous re-evaluation of their findings taking metal speciation into account supports these inclusion of these metals and does not identify any other metal or metalloid as high risk components.

These conclusions are provisional as there are insufficient data to evaluate all the metals and metalloids. It is noteworthy that cadmium features in the lists of metals and metalloids for both cancer and non-cancer disease risk.

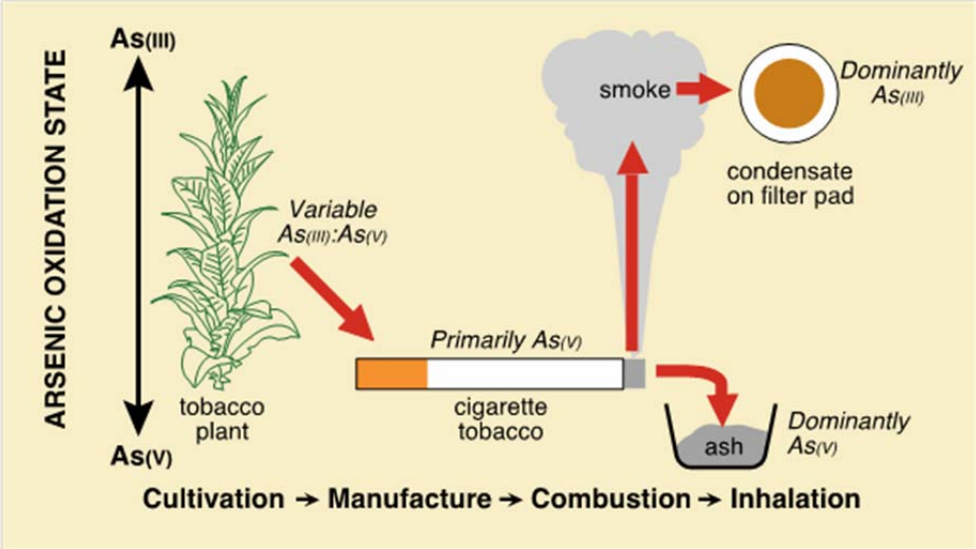

Figure 4. Model pathway of the variation in arsenic valence state (As(III) and As (V) from cultivation to exposure. Combustion converts the arsenic involved to the As(III) state prior to transfer in smoke.

6 Science base and conclusions

A more substantial Scientific Report of this study is available which includes summaries of previous research on metals in tobacco and smoke, descriptions of the methodologies used, and a presentation of the results of laboratory analysis and thermodynamic modelling. The information is analysed in the context of risk analysis of smoking involving a wider range of metals and metalloids.

Also detailed in the Scientific Report is a detailed investigation of arsenic in tobacco, charting its changes in speciation from the harvested plant to the commercial product, finally to the components of smoked cigarettes (smoke condensate and ash). The methodology included cultivating tobacco plants burdened with additional arsenic. The value of developing a detailed model for arsenic lies in the demonstration that the process of combustion and smoke generation has a major effect on speciation. Metals that partition into the smoke condensate exist in a reduced environment whereas the fraction that partitions into ash is oxidised. This partitioning occurs regardless of the oxidation species predominating in the precursor tobacco. This has important implications for regulation based on the concentration of metals and metalloids in plants and commercial products.

The methodology developed to model the behaviour of arsenic is applicable to other elements, sometimes with adaptations, and should be applied to those elements in tobacco smoke that may be present in toxic concentrations in order to gain a fuller understanding of the potential hazard.

The Science Report also compares laboratory and literature results on metal/metalloid speciation with predictions based on thermodynamic models using known ranges of oxidation potential and pH in cigarette smoke. The agreement is very good for arsenic and chromium, the only elements for which synchrotron results for valence speciation are available. This encourages application of thermodynamic modelling to other metals and metalloids for which no laboratory data are yet available.

7 Research needs

In its review of the literature on metals TobReg identified a number of research requirements, many of which relate to the toxicological response to metals (and metalloids) in smoke [3]. This list is not repeated here. A few general areas where research could improve understanding the processes and consequently the accuracy of risk assessment relevant to the present study are highlighted below:

7.1 Speciation analysis

Laboratory characterisation of compound and valence speciation Cd, Ni and Pb in tobacco and smoke is a priority given their inclusion in TobReg's list of elements recommended for regulation. Similar studies of Co and Mn are also warranted.

Thermodynamic modelling of speciation based on Eh (oxidation potential) and pH was based on old estimates acquired before cigarette design parameters such as filter ventilation were introduced. These may have a profound effect on smoke conditions, as might certain additives. Also required for modelling are better estimates of the conditions in aged smoke, second hand smoke and environmental tobacco smoke. More comprehensive models would be based on measurements of potentially-modifying anions such as Cl and S. Further refinement of the modelling would be possible with measurements of Eh-pH conditions in smoke-lung fluid interactions.

7.2 Inhalation toxicology

Better mechanistic understanding of the role of smoke metals and metalloids in pathways to disease is required. Much current understanding is based on the effects of elements in their metallic state and the role of speciation is poorly understood. In vivo and in vitro model studies are required to elucidate the role of metal speciation during inhalation exposure to metals within the smoke aerosol.

Risk assessment is largely based on parameters derived from occupational exposure and may inadequately represent the risks associated with long term, low dose exposure to metals transferred in the complex smoke aerosol. Much could be gained through collaboration with expertise in other forms of particulate exposure.

7.3 Linking concentrations of metals in tobacco with those in smoke

TobReg's recommendation for regulating metals in tobacco is based on the assumption that high concentrations of metals in tobacco equate to high concentrations in smoke. There is evidence to support this [11, 17] but predictive models for all regulated metals and metalloids covering the range of global compositions are required. These models also need to account for the effects of cigarette design (tobacco mass, filter ventilation etc.) on the concentration in smoke [18]. No quantitative models linking tobacco to smoke yet exist in the open literature.

A related issue concerns the global variations in metals such as Cd and Pb. It is important to determine whether populations smoking products known to contain higher average concentrations of these metals (e.g. China [10]) are actually exposed to higher levels in smoke, and whether chronic exposure to these products can be correlated with any disease patterns.

7.4 Non- smoking exposure to tobacco metals

The laboratory and modelling methodologies developed for this study may be adapted for non-smoking tobacco consumption, including oral (chewing or snus) and water pipe methods. Some of these methods may also have applicability to e-cigarette products in cases where metals or metalloids appear to be transferred in the aerosol [19].

8 Regulatory Recommendations

Articles 9 and 10 of the WHO's Framework Convention on Tobacco Control require the relevant authorities to "adopt and implement effective measures for public disclosure of information about the toxic constituents of the tobacco products and the emissions that they may produce" [1]. Guidelines for the implementation of these Articles aim for "strengthening … tobacco-control policies through regulation of the contents and emissions of tobacco products" [20]. The European Union explicitly adopted these Guidelines in its recently revised Tobacco Product Directive [21].

This report provides some of the scientific basis for identifying "toxic constituents" among the metals and metalloids known to be present in significant concentrations in tobacco smoke.

TobReg, the WHO expert panel set up to advise on the scientific basis of tobacco product regulation, has recommended that regulatory authorities require the testing by manufacturers of the levels of arsenic, cadmium, lead and nickel in crops and products, and that these are reported and verified as appropriate [3]. Regulatory authorities are recommended to take action when the levels of these metals and metalloid change significantly. The present report has reviewed the TobReg list of recommended elements and others using some criteria not applied by the panel and finds additional scientific underpinning to support the list.

Consideration should be given to the method of implementing the recommendation. There is an underlying assumption that a smoker's exposure to metal is directly related to the concentration of that metal in the tobacco leaf and blends. Analysis of the former is assumed to be a reliable predictor of the latter. There is some evidence to support this but it is not a simple relationship, being made more complex by cigarette design features such as filter ventilation. It is suggested that prior to implementation accurate predictors should developed, possibly by means of multivariate relationships of measurable parameters in common smoking materials. Alternatively manufacturers could be required to supply verifiable analyses of metal concentrations in emissions from their products according to an agreed protocol. Presently very few laboratories worldwide have the capability of producing accurate analyses of metals in smoke thus implementation of a regulation requiring such analyses may come up against a considerable short term capacity problem.

In summary the regulation of arsenic, cadmium, nickel and lead in smoking products could contribute significant improvements in public health as anticipated by the FCTC, however there are presently some problems in TobReg's proposed method of implementation, i.e. analysis and reporting of the metal content of tobacco. The quantitative relationships between metals in tobacco and smoke need first to be established. The alternative of direct analysis of metals in smoke is considered presently to be impractical in the short term if required on any large scale.

9 Declarations and Acknowledgements

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11 Annex 1: Scientific report

11.1 Background and Context

11.1.1 *Metals in tobacco and smoke*

Among several thousand chemical compounds documented in tobacco smoke 98 have associated risk values, and 11 of these are metals or metalloids [1]. The World Health Organisation (WHO) expert panel on tobacco regulation (TobReg) recently reviewed the published literature on metals and metalloids in tobacco and smoke, concluding that arsenic (As), cadmium (Cd), nickel (Ni) and lead (Pb) are of sufficient concern that they should be subject to regulation [2]. The US Food and Drug Administration has a longer list of "Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke" including beryllium (Be), chromium (Cr), cobalt (Co), mercury (Hg) and selenium (Se) [3]. The WHO panel recommended that "manufacturers … test cured tobacco purchased from each new agricultural source for levels of arsenic, cadmium, lead and nickel" and that these metals should also be analysed in tobacco blends offered for sale.

11.1.2 *Importance of speciation to health*

The concept of metal speciation (the term metal is used loosely to include metalloids) has been widely developed in the environmental sciences because of its importance in identifying hazards, their impacts on health and on remediation of contaminated ground.

The International Union of Pure and Applied Chemistry (IUPAC) defines speciation as the process yielding evidence of atomic or molecular form of an analyte [4]. This includes the specific form of an element defined in terms of its electronic or oxidation state, complex or molecular structure and isotopic composition [5].

The potential hazard of a metal (mobility, reactivity, toxicity) varies with speciation and may change with time and environmental conditions [6]. This is important in smoking as combustion can have a large effect on speciation, but so also may other factors such as time. While a large body of data exists on metal concentrations in tobacco and smoke (as summarised in the literature review, section 11.2) only three detailed papers have been published on speciation of potentially toxic metals in smoke. These three papers involve the same authors and concern the form of arsenic and to a lesser extent cadmium in smoke [7-9]. In this report we focus on chemical species (i.e. compounds) and valence species (i.e. oxidation state) with the aim of developing a model to describe species changes in a single element along its pathway from tobacco cultivation to respirable smoke. The element arsenic was chosen for particular focus as it is highly toxic, is known to be present in mainstream tobacco smoke, and exists in multiple molecular forms and valence states. The methodology developed to model the behaviour of arsenic is applicable to other elements, sometimes with adaptations, and should be applied to those elements in tobacco smoke that may be present in toxic concentrations in order to gain a fuller understanding of the potential hazard.

11.1.3 *Speciation and regulation*

Proposals to introduce new regulations are routinely challenged by the tobacco industry and it is important that regulators are informed by scientific research on the risks posed to smokers by heavy metals in smoke. Presently the research literature (reviewed in section 11.2) comprises numerous reports on the concentrations of heavy metals in tobacco and a few studies of heavy metals in cigarette smoke but very little attention has been paid to speciation. Such shortcomings are potentially exploitable by those opposing regulation because

speciation influences bioavailability, reactivity with cellular materials and detoxification mechanisms, thus strongly influencing toxicity. Reactions within the growing plant and subsequent curing may modify metal species while the extreme redox conditions of combustion can lead to major changes in speciation. Such factors may markedly alter the risk of toxicity associated with a given heavy metal, depending on the way that tobacco is consumed. Risk assessment also requires quantitative models of toxicological response to these heavy metal species and these are rarely available for the relatively low concentrations to which tobacco users are typically exposed.

The study of arsenic as the exemplar presented in this Report underlines that even at low concentrations some metals may be present in smoke in their most toxic possible forms. The results strongly support the inclusion of arsenic among the four metals recommended for regulation by TobReg [2]. Speciation studies of the other metals identified for regulation (cadmium, nickel and lead) could similarly strengthen each case for inclusion (or possibly weaken it), and the case for other metals such as chromium, not currently included on TobReg's list, should also be investigated.

11.1.4 *Stucture of the Report*

The literature of metals and tobacco is reviewed in Section 11.2. After presenting the background much of the literature is condensed into tables that highlight the important features and enable comparison between metals.

The presence of metals in tobacco is addressed in Section 11.3 and chemical speciation is the subject of Section 11.4. The focus of the research was to quantify the inorganic and major organic species in 14 tobacco samples. On the basis of these analyses a predictive model was developed for the relative quantities of toxic and less toxic species in tobacco.

Section 11.5 addresses valence speciation of arsenic in both tobacco leaf and smoked products using synchrotron techniques. The results demonstrate the importance of combustion in determining the relationship between trivalent and pentavalent arsenic in smoke condensate, and underlines possible differences in arsenic toxicity between oral and smoking products.

Section 11.6 concerns Eh-pH (Pourbaix) modelling of equilibrium species for model solutions under oxidation potential and pH conditions measured in smoke at ambient temperature. Predictions are compared with the speciation results obtained in sections 11.3 and 11.4 and the results are consistent. Building on the successful modelling of arsenic, cadmium and chromium models for other metals are developed.

Section 11.7 summarises the findings and considers them in terms of health and proposals to regulate the concentrations of particular metals in tobacco crops and in commercial tobacco products. Areas where further research is required are identified.

11.2 Literature Review

11.2.1 *Introduction*

The literature on metal speciation in tobacco is sparse compared with the total literature on tobacco and tobacco smoke constituents. Here the available literature relevant to the concentration of metals in tobacco and their potential transference to tobacco smoke is reviewed. The health implications of metals inhaled in tobacco smoke are also considered.

The anthropological significance of tobacco is explored leading via the definition of toxic heavy metals with relevant examples to consideration of how metals in

the environment may be sequestered by plants which, in various ways, may be consumed by humans and result in the transference of metals. The concept of speciation is then defined and explored with reference to key elements of known human toxicity. This is followed by a section detailing the translocation and transference of some of these elements through the various stages from harvesting to smoking, with some details on the effects in the receptor.

This review primarily concerns tobacco in cigarette form as this is the form of consumption of greatest relevance to health, but some research may have relevance for alternatives such as chewing tobacco, snus and other potential reduced exposure products (PREPs) that use tobacco as their source of nicotine, although they are not considered in the same detail.

11.2.2 *Tobacco (Nicotiana tabacum L.)*

11.2.2.1 Botanical characteristics of the tobacco plant

Domain: Eukarya
Kingdom: Plantae
Phylum: Magnoliophyta
Class: Magnoliopsida
Order: Solanales
Family: Solanaceae
Genus: Nicotiana
Species: Nicotiana tabacum

Tobacco *(Nicotiana tabacum)* was first described by Carl Linnaeus in c. 1560 [10]. The genus Nicotiana is considered to have evolved 75-100 million years ago (mya), and the species, *N. tabacum*, 6 mya [11]. Humans are thought to have been aware of the plant, and a closely related species *N. rustica*, 18 thousand years ago (kya), and were cultivating both species between 5-3 kya [12]. From details in historical records, inferences derived from observations of physical characteristics (such as day-neutral photoperiodism and the transient photo-dormancy of freshly harvest seed), and genetics, tobacco is presumed to have originated from forest margins at mid to low altitude in the Peruvian/Ecuadorian Andes of Central and South America [12-14].

The species travels well, with a seed viability of 25 years under air-tight conditions, and dimensions so miniscule that tobacco seeds can number 10-13k g^{-1} [14]; attractive attributes for agriculture. In fact, with a mature, individual plant typically producing 12-15 g of seed, and typically five times the number required sown to produce enough viable seedling transplants, an area of 0.25 km^2 of mature plants could be grown from the seeds of a single plant [14].

The classification of tobacco flower is 'perfect' (containing both androecium and gynoecium), and pollen is not anemophilous, so usually a self-pollination to cross-pollination ratio of 20:1 occurs, though inbreeding depression is uncommon and heterosis is limited even where hybrids are produced from inbred lines [13]. General descriptive botanical characteristics of the species will not be explored further in this review.

Within the species *N. tabacum*, many varieties (as well as 'lines' and crossbred lines) are grown for specific qualities. These can include morphological properties, alkaloid content, disease resistance and so forth, which can be the basis on which the final tobacco blends are prepared. Tobacco is usually blended to achieve certain flavour, packing density and pyrolysis attributes in finished products such as cigarettes (Figure 1).

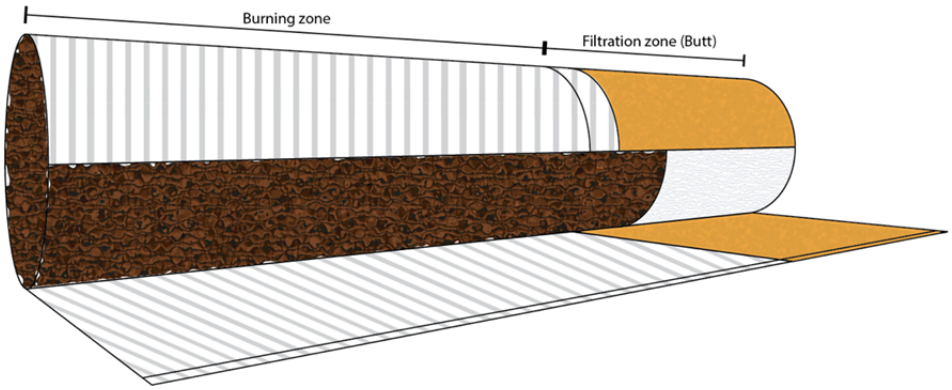

Figure 1. A tear-away diagram of a king-size cigarette with indication of the burning and filtration zones.

11.2.2.2 The tobacco industry as part of the global economy

Tobacco (Nicotiana tabacum L.) is an unusual crop, in that a consumer combusts the leaves instead of ingesting the fruiting bodies or seed; nevertheless, it has become of immense importance to the international economy as a key source of financial income to farmers in many countries [15]. Over 50 species of tobacco are known to be cultivated globally although a few commercial varieties dominate [14, 16, 17].

The plant is ideal for horticulture and agronomy at latitudes between 55°N and 40°S as the progeny from seeds (weighing ~0.1 mg) from a single plant could populate roughly 6 ha, growing to maturity after transplanting; requiring 120-140 days of frost-free field growth, adequate irrigation, 100 days of temperatures above 13 °C and preferably between 27-32 °C during the day in order to flourish [14].

In recent times, breeding or genetic engineering to enhance or achieve advantageous characteristics, such as ameliorating agronomic performance to meet trends in the global demand shifts, has resulted in the tobacco industry being more diversified and segmented as an industry [15].

11.2.2.3 Germination and seedlings

With adequate moisture, and temperatures between 18 and 23°C, a fragile embryonic tobacco radicle will germinate from the tiny seed in 7 to 12 days, in order to access a source of moisture. At this sensitive point it may succumb within 4 hours to an excess of temperature or evaporation, or an excess of moisture whereby the respiration during metabolism of seed reserves is prohibited [13]. Deviation from the optimal temperature range can reduce the rate number of successful germinations, taking up to an extra 2 weeks [13].

Most commercially available seeds for research purposes have a coating which can be dissolved in water, and the seed sterilised with sodium hypochlorite (NaOCl) if desired [18]. The majority of seedlings will gestate in two weeks in an incubator or growth chamber under standardised conditions of 16 hours of daylight at 23°C, to 8 hours of night at 20°C, light intensity of 250 mol photons m-2s-2, and a relative humidity of 75-80% [18].

11.2.2.4 Plant requirements

Providing plants with the potential for maximum possible leaf expansion is fundamental in tobacco agriculture. To achieve this, the plant generally requires aerated, loose soil with a relatively open structure which promotes free moisture movement, abundant water, and an adequate nutrient supply [14]. Light sand-loam soils are generally ideal, however burley varieties grow successfully on heavy silt loam soils [14].

Tobacco is a higher plant, and therefore requires at least 20 elements to be present in the soil within certain concentration ranges, including the elements boron (B), carbon (C), calcium (Ca), chlorine (Cl), copper (Cu), iron (Fe), hydrogen (H), potassium (K), magnesium (Mg), molybdenum (Mo), nitrogen (N), oxygen (O), phosphorus (P), sulphur (S), and zinc (Zn) [17]. Poor development is expressed in nutrient deficient soils, or toxicity in excess. Examples of this phenomenon include Zn and Cu, for which deficiencies are rare in agriculture, but are toxic above 20 ppm, with both scenarios reported in acidic soils [19].

11.2.3 *Elements, nutrients and toxic heavy metals*

11.2.3.1 Description of metals groups and terms

This research is principally concerned with 'heavy metals' and more specifically 'toxic heavy metals', although 'macro nutrient' and 'micro nutrient' elements also play a key role. A report by IUPAC [20] has described in detail the technical misnomer of the term 'heavy metals', however the term is deeply entrenched in the literature and for the purposes of this research they are taken to mean the metal and metalloid elements capable of biological, ecological and environmental harm.

Harm with regard to contamination of land is well defined in The Environment Act 1995 of UK law, however the definition may be vary between countries. The application of 'toxic' to an element usually refers to it not being biologically essential; thus either the dosage (or concentration), exposure pathway, compound or combinations thereof can result in phyto- or zootoxicity [21]. It is important to note that the term does not refer to all compounds (or 'species'; see section 11.3) of a heavy metal and all of its compounds, as there will be different biological and physicochemical properties depending on the chemical state, and therefore different toxicological characteristics [20]. Again common usage is lax in this respect. The best defining property for the term is the atomic density of an element, with recommendations for >6g cm^{-3} (with exceptions) or >5g cm^{-3} [21, 22].

Heavy metals can also be biologically essential elements, otherwise known as 'trace' elements in nutritional circles, and 'macro nutrients' and 'micro nutrients' in scientific nomenclature [21].

Human toxicity has been investigated for many diseases. The International Association for Research on Cancer (IARC) has categorized the carcinogenicity status of many agents [23] (Table 1) and this classification is used in a summary of the various metals and metalloids found in tobacco that may be implicated in disease pathways, arbitrarily listed in alphabetical order in Table 2.

Table 1. World Health Organisation (WHO) International Agency for Research on Cancer (IARC) Carcinogenicity Classification.

GROUP	DESCRIPTION
1	Carcinogenic to humans
2A	Probably carcinogenic to humans
2B	Possibly carcinogenic to humans
3	Not classifiable as to its carcinogenicity in humans
4	Probably not carcinogenic to humans

Tobacco plants acquire elements from their growing environments, some of which are non-essential or toxic [24]. Ambient environmental conditions, the growing region, and even the soil characteristics are reflected in the composition of trace elements in tobacco [16]. The most important transition metals in tobacco as cobalt (Co), Cu, Fe, manganese (Mn), nickel (Ni), and Zn [25].

The following subsections of the chapter are principally concerned with total elemental concentrations. Reference to the toxicity of individual compounds, oxidation states, species, or valence states where relevant is made elsewhere.

11.2.3.2 Sources of metallic and metalloid elements in tobacco

The atmosphere, hydrosphere and lithosphere contain natural and anthropogenic metal and metalloid contaminants [4], some of which are important for living organisms in the ecosystem, others may be toxic to the plant, and others play no part in the plant's metabolism.

Plant tissues are not resistant to the permeation of many elements, and this is particularly the case for species that are selected and grown for leaf material. Indeed leaves, roots and stems accumulate more metals than edible seeds and fruit [26]. The relative barrier strength of plants to some elements (that is, the ability of the plant to resist the uptake of that element) can be ranked, and the observed trend is chromium (Cr), lead (Pb), mercury (Hg) > Cu > cadmium (Cd), Ni, Zn > Mo, thallium (Tl) [26].

Tobacco naturally contains both trace macro- and micro-nutrient elements, such as Co, Cu, Fe, Mn, Zn, and heavy and toxic heavy metals such as Cd, magnesium (Mg) and platinum (Pt) [27, 28]. Table 3 lists the observed ranges for selected elements in unburned tobacco, tobacco smoke and ash.

The most significant routes for human exposure to toxic heavy metals are primarily ingestion of food and beverages, and inhalation of contaminated air [29]. Tobacco leaves provide an entry route for elements into the body system that may possess toxic properties, therefore composition characterisation is important [30] and smoking tobacco has been modelled as a exposure route for some heavy metals [31]. Heavy metals inhaled during smoking have a long half-life in the body depending on bioavailability, detoxification and excretion mechanisms [32]. These issues are discussed elsewhere in this study.

Table 2. Summary of elements of inorganic origin found in tobacco, their nutritional importance and some known toxic effects.

ELEMENT	TYPE	NUTRITION	TOXICITY	IARC GROUP	REMARKS	REFS
Aluminium (Al)	post transition metal	no known function	occasionally phytotoxic	1	There is an unproven link between Al and Alzheimer's disease; interesting due to the relatively high concentrations in tobacco. Microcytic anaemia, osteomalacia, inflammatory and oxidative events are triggered on involvement of Al.	[32, 33]
Antimony (Sb)	crystalline metalloid	no known function	known toxic species	2B or 3	Poorly described in the tobacco literature.	[34]
Arsenic (As)	metalloid	possible function at ultra-trace	toxic to most phyla	1	Cancer (e.g. lung and skin); cardiovascular, gastrointestinal, hepatic and renal diseases. The nature of this component of tobacco smoke is defined elsewhere in this study.	[22, 34-38]
Beryllium (Be)	alkali earth metal	no known function	toxic	1	Possible role in tobacco is not well characterised.	[39]
Bromine (Br)	halogen	suspected function	variably toxic in some concentrations	3	There are limitations on selected crops and products of 250 mg Br kg^{-1}	[34, 40]
Cadmium (Cd)	transition metal	no known function	toxic	1	Stomach irritation (vomiting and diarrhoea); lung damage; kidney diseases; cancer (probably). An archetypal highly toxic heavy transition metal. Boiling point significant for pyrolysis temperatures of a cigarette coal (800+ °C). Carcinogenicity status in upheld by the United States National Toxicology Program but contested elsewhere. Interactions occurring between Cd and the essential element Zn in plants, due to the similar physical characteristics of the two dicationic metals, have been defined as prevalently negative. Suspected to accumulate in a variety of plants. Actively transported via metallothionein (MT) proteins from the soil into plants and crops in potentially large concentrations with little sign of phytotoxicity, which could be grown for human consumption. Similarly, there is a relationship between contamination of cigarettes with Cd and subsequent concentration in the blood of smokers.	[19, 22, 33-35, 38, 41-53]

					Existing evidence does not demonstrate causality of cancer by Cd in cigarettes. Smoking contributes about half of smokers' daily intake of cadmium.	
Calcium (Ca)	alkali earth metal	essential (macronutrient)	rare	none	Similar physical dicationic properties to Cd and Zn, including ionic radius (0.99 and 0.97Å respectively), therefore elemental substitution in organisms could be a mechanism for toxicity. Other sources comment that high Ca concentration may reduce the phytoremdiation potential of tobacco plants for some heavy toxic metals due to the detoxification mechanism whereby trichomes on the leaf surface excrete minerals that contain Ca and Zn which can accumulate in phytotoxic levels, and lower relative concentrations of Cd, which can accumulate in very high concentrations without extensive signs of phytotoxicity.	[[18, 53, 54]
Chromium (Cr)	transition metal	micronutrient (essential)	known toxic species	1 (Cr(VI)) & 3 (Cr(III))	Genotoxic carcinogens (Cr(VI)); lung cancer. Cr(III) required in carbohydrate metabolism in animals and humans, and ameliorates mild glucose intolerance.	[38, 44, 50, 55]
Cobalt (Co)	transition metal	micronutrient (essential)	known toxic species	2A & 2B	Contact dermatitis mutagenic and carcinogenic effects. It is also is essential for enzyme catalysed reactions as a co-factor.	[19, 34, 38, 40, 44, 56]
Copper (Cu)	transition metal	micronutrient (essential)	rare	none	Blood serum levels of Cu are reported to increase from smoking. Inhibition of dihydrophil hydratase (in haemopoiesis); accumulation in liver and kidney.	[19, 32, 38, 57-59]
Iron (Fe)	transition metal	essential (macronutrient)	known toxic species	1, 2B & 3	Human carcinogen in occupational Fe smelting work environment, associated with asbestos or silica dusts Fe is well known to be an essential element for human health as a component of heme proteins or ferritin. Females are known to have lower stores of Fe relative to males and an increased absorption as a compensation mechanism. Excess Fe absorption from diet, exposure or supplementation can produce pro-oxidant interactions in the body which can be as harmful as anaemic deficiency. Potential harmful nature of compounds and dust in conjunction with smoking tobacco.	[40, 59-61]
Lead (Pb)	post transition metal	no known function	toxic	2A, 2B & 3	Irreversible neurological damage; renal disease; cardiovascular effects; reproductive toxicity. Pb(0) (2b), organic compounds (3). Evidence for carcinogenicity is weak, compounds and Pb(0)can be	[22, 32, 35, 38, 50, 53,

					highly toxic to animals, humans and the environment. Increases of Pb in the blood of smokers have been identified relative to non-smokers; this is significant due to its prevalence as a pollutant and involvement in mechanisms deleterious to health.	58, 62, 63]]
Manganese (Mn)	transition metal	micronutrient (essential)	not known	none	Essential human nutrient as well as toxic to the nervous system. Neurological symptoms; affects liver function. As yet no relationship has been reported between smoking and the Mn content in blood.	[32]
Mercury (Hg)	transition metal (liquid state)	no known function	known toxic species	3	Organic compounds exhibit toxicity in humans, though Hg(0) and many Hg compounds have been designated Group 3. Tests conducted on Hg in humans smokers failed to highlight any directly correlating health implications, apart increased sister chromatid exchange (SCE) frequency (possible proxy for mutagenicity). Due to the relatively lower boiling point, almost unique in the vapour phase of smoke as other metals are largely represented in the particulate phase (Se also behaves in a similar way).	[32, 33, 35, 49, 50, 64-66]
Molybdenum (Mo)	transition metal	essential (macronutrient)	not known	none	Essential element in both plants and animals.	[67]
Nickel (Ni)	transition metal	no known function	known toxic species	1 & 2B	Possible human carcinogen in metallic and alloys. Known Group 1 compounds induce SCE. Some Ni compounds are reported to be toxic to human health. Ni increases have been associated with inhaling cigarette smoke. Accumulation in lungs.	[32, 33, 38, 44, 68, 69]
Polonium (Po)	radioactive metalloid	no known function	known toxic species	2B	Alpha and beta radiation. Higher relative concentrations found in bodily tissues of smokers than non-smokers.	[32]
Radon (Rn)	radioactive noble gas	no known function	toxic	1	A decay product of uranium (U) or thorium (Th) producing alpha particles. Environmental hazard reported to contribute to carcinogenicity of tobacco and tobacco smoke.	[34]
Selenium (Se)	non-metal	suspected function	rare	3	Gray, Alpha and Vitreous forms. Se is reported to be an anti-carcinogen. Reported reduction of blood and serum Se concentration of smokers compared to non-smokers.	[32, 33, 59, 70]
Thallium (Tl)	post trans. metal	no known function	toxic	none	Known to be toxic and non-essential in humans	[14, 26]
Tin (Sn)	post trans. metal	no known function	known toxic species	none	Has organic compounds that are relatively more toxic than inorganic compounds	[26]
Titanium (Ti)	transition metal	no known function	known toxic species	2B or none	A single compound designated Group 2B. Reported to be toxic to humans. Included in analyses as proxy for soil contamination of	[44, 55]

					plant material for correction purposes.	
Vanadium (V)	transition metal	no known function	known toxic species	2B or none	A single compound designated Group 2B. Not known to be essential in humans, but has soluble forms that are well absorbed in the lungs with properties reported to mimic human hormones.	[33]
Zinc (Zn)	transition metal	micronutrient (essential)	toxic	none	Inhibition of copper absorption; nausea, vomiting, loss of appetite, abdominal cramps, diarrhoea, headaches. An important nutrient for plants and humans. Phytotoxicity (from excess), or cytotoxicity (in deficiency), exhibited out with a limited range of concentrations. Biological ligands compete for Zn and Cd, which makes it important to this study due to understanding phytoaccumulation mechanisms. Not accumulated in many human tissues or fluids, however higher relative concentrations proven to occur in kidney cortex of smokers over non-smokers.	

Table 3. Concentration and source information for the more common inorganic elements found in tobacco and smoke.

ELEMENT	TOBACCO		Refs	SMOKE		Refs	ASH		Refs	SOURCE OF METAL	Refs
	Min	Max		Min	Max		Min	Max			
	µg g^{-1}	µg g^{-1}		µg g^{-1}	µg g^{-1}		µg g^{-1}	µg g^{-1}			
Aluminium (Al)	16	1200	[22, 71, 72]	-	-		110	260	[71]		
Antimony (Sb)	0.06	33.62	[16, 24, 40, 73]	0	1.018	[16, 24, 74]	0.356	57.4	[40, 75]		
Arsenic (As)	0	3.4	[16, 24, 32, 73, 76-79]	0.004	0.1	[24, 80-82]	-	-		Occurs naturally in air, rock, soil and water. Natural sources estimated at 8000 tonnes per annum with anthropogenic sources almost triple that including agrochemicals, computer chips, fossil fuel burning, herbicides, glass manufacture, insecticides, mineral debris, mining waste, non-ferrous metal smelting, rodenticides, and wood preservatives.	[22, 36, 37, 50]
Barium (Ba)	1.15	188	[16, 32, 34, 72, 83]	2.2	4.3	[16]	10	635	[7, 17, 63]		
Beryllium (Be)	-	-		0.001	0.006	[82]	-	-		Naturally found in aluminosilicate minerals. Anthropogenic sources include Be salts used in dental prostheses, golf clubs, nuclear weapons and space shuttles.	[39]
Boron (B)	10	25	[72]	-	-		7.6	26	[72]		
Bromine (Br)	12	577	[16, 24, 34, 40, 72, 73, 76, 83]	0.34	75	[16, 24, 73]	1.37	647	[16, 34, 40, 72, 73, 76]	Methyl bromide was widely applied in controlling nematodes and other pests in tobacco crops but is gradually being phased out following the Montreal Protocol as an ozone layer-depleting compound.	

Cadmium (Cd)	0	11.87	[24, 29, 47, 49, 62, 72-74, 77, 79, 84-91]	0.031	0.271	[25, 51, 74, 80, 81]	0.085	14.3	[30, 74, 76, 85]	Cd has a mean annual global emission rate of 5400 tonnes of which two thirds is anthropogenic. Contamination of soils can result from application of manures, phosphatic fertilizers sewage sludge, and soil amenders in agriculture, aerial deposition, contamination of livestock feeds by supplement addition, liming and consequent excretion mining, ore-dressing, electroplating, fuel combustion, galvanizing, metallurgy of Cu, Pb, Zn and other metals, use in pigments, rechargeable batteries, and stabilization of PVC products.	[19, 22, 51, 52, 61, 66, 85, 86, 92-96]
Cesium (Cs)	0.053	0.64	[16, 83]	0.003	0.082	[16]	0.024	0.419	[16]		
Calcium (Ca)	5000	78000	[16, 32, 34, 72, 73, 76, 83]	-	-		9900	365000	[16, 34, 72, 76]		
Cerium (Ce)	0.47	5.2	[16, 24, 72, 83]	0.09	0.19	[16]	0.57	21.7	[16, 72]		
Chlorine (Cl)	5000	50000	[76]	-	-		-	-			
Chromium (Cr)	0	10.3	[16, 24, 28, 32, 40, 73, 79, 83]	0.15	1.5	[16, 73]	1.82	107	[16, 28, 40, 73, 76]	Liberated from parent material and cycled through organisms into the soil. Anthropogenic sources include fossil fuels (hexavalent Cr), and alloy production.	[96, 97]
Cobalt (Co)	0.138	6.66	[16, 24, 29, 32, 40, 73, 83, 91]	0	0.4	[16, 24, 73]	0.069	36.8	[16, 29, 40, 73, 88]	Anthropogenic sources include by-products of refining copper, iron, lead, nickel and silver ores.	
Copper (Cu)	0.24	510	[16, 24, 34, 72-74, 76, 77, 88]	0.013	0.013	[24]	1.8	750	[16, 34, 72, 76,		

									88]		
Europium (Eu)	0.003	0.27	[16, 73, 83]	0.004	0.022	[16]	0.085	0.86	[16]		
Gallium (Ga)	1	3.8	[72, 76]	-	-		0.88	5.7	[72, 76]		
Gold (Au)	0.08	0.23	[40]	-	-		0.22	0.33	[40]		
Hafnium (Hf)	0.004	2.25	[16, 40, 83]	0.02	0.08	[16]	0.003	8.62	[16, 40]		
Iron (Fe)	80	7859	[16, 24, 29, 32, 34, 40, 72, 73, 76, 79, 83, 84]	0.014	127	[16, 24, 73, 84]	103	24349	[16, 29, 34, 40, 72, 73, 76, 84]	Fe has been included in this descriptor of elements due to its ubiquitous nature in the environment, animals and plants.	[98]
Lanthanum La	0.39	10	[16, 72, 73, 83]	-	-		0.54	6.07	[16, 72]		
Lead (Pb)	0	43.66	[24, 29, 46, 49, 62, 72-74, 76, 77, 79, 84, 88, 89]	0.032	0.41	[24, 49, 80, 81, 84]	0.58	80.2	[29, 72, 76, 84, 88]	Anthropogenic sources include building material, ceramic glazing pigments, plumbing, emission of vehicle combusting tetraethyl leaded fuel (declining trend in developed countries). General population exposed to Pb from air and food with a spatial heterogeneity borne from the widespread distribution of roads to agricultural areas and population centres, though apparently there may not be a linear relationship between concentrations in soil and tobacco plants due to	[22, 49]
Lithium (Li)	1.5	20	[72]	-	-		1.3	17	[72]		
Magnesium (Mg)	1300	5400	[32, 72]	-	-		1600	4200	[72]		
Manganese (Mn)	55	540	[24, 32, 72-74, 76, 79]	0.002	0.003	[24]	66	12909	[72, 76]		

Mercury (Hg)	0.007	0.334	[16, 49, 73]	0.006	0.292	[16, 49]	0.085	0.4	[16]	Historically a component of fungicides utilised in tobacco production. Hg enters water from the geological process of crustal degassing. Anthropogenic sources associated with coal consumption, dental amalgams, gold mining in non-developed countries, and industrial pollution.	[22, 33, 35, 49]
Molybdenum (Mo)	0.84	1.08	[67]	-	-		-	-			
Nickel (Ni)	0.26	400	[29, 32, 72, 76, 88, 91, 99]	0.001	0.9	[24, 82]	0.47	40.4	[29, 72, 76, 88]	Liberated to the environment from weathering of rocks and soil, and volcanic emissions of Ni containing dusts.	[69]
Niobium (Nb)	1	1.2	[76]	-	-		1.76	2.85	[66]		
Phosphorus (P)	1840	3180	[16]	30	40	[16]	7830	13200	[16]		
Potassium (K)	1300	118000	[16, 24, 34, 72, 73, 76, 83]	39	4420	[16, 24]	6300	617000	[16, 34, 72, 76]		
Rubidium (Rb)	4.5	100	[72, 73, 76, 83]	2.1	3.38	[16]	4.6	170	[16, 72, 76]		
Samarium (Sm)	0.01	0.88	[83]	-	-						
Scandium (Sc)	0.085	9.1	[16, 24, 40, 83]	0.01	0.05	[16, 24]	0.35	6.61	[16, 40, 74]		
Selenium (Se)	0.18	1.8	[16, 24, 32, 76]	0.002	0.319	[16, 24]	0.072	0.296	[16]		
Silicon (Si)	140	180	[72]	-	-		230	460	[72]		

Sodium (Na)	39	1910	[16, 24, 72, 73, 100]	1.2	590	[16, 24]	94	4930	[16, 72]		
Strontium (Sr)	27	300	[16, 32, 34, 72, 74, 76, 83]	-	-		25	1593	[34, 72, 76]		
Thallium (Tl)			-	0.011	0.011	[24]					
Thorium (Th)	0.011	0.834	[[16, 72, 83]	0.052	0.087	[16]	0.013	4.295	[16, 83]		
Tin (Sn)	0	2.1	[79]	-	-						
Titanium (Ti)	0.76	378	[34, 72, 74, 76]	-	-		3.4	1515	[34, 72, 76]		
Uranium (U)	0.005	0.01	[72]	-	-		0.009	0.041	[72]		
Vanadium (V)	0.49	5.33	[99]	-	-					Primarily anthropogenic sources associated rural and urban air pollution from industrial processes.	[99]
Zinc (Zn)	8.1	169	[16, 24, 32, 40, 74, 76, 77, 83, 84, 91]	0.07	17	[16, 24, 74, 84]]	14.7	1189	[16, 40, 74, 76, 84]		
Zirconium	8	13.9	[83]	-	-	-	40.3	55.5	[83]		

11.3 Metals and plant production

11.3.1 *Bioavailability and toxicity*

Toxic substances may migrate through food chains to humans as receptors at their terminus [46]. From the soil, entry of metals into the food chain depends on the amount and source of the contaminant, soil properties, the magnitude and rate of plant uptake and/or extent of absorption by animals [21]. This relationship becomes further complicated when one considers that tobacco is combusted and inhaled, or simply chewed for consumption by humans through the respiratory system and/or saliva through the digestive system, instead of harvested for consumption exclusively in the digestive system as with similar agricultural crops that are cultivated for their leaves.

Bioavailability to a plant and the solubility of pollutants from the soil are ultimately governed by biological and chemical properties of the soil, and climatic factors [26], as well as organic matter content and pH [101], which also affect the chemical and physical properties of tobacco [16].

Tobacco is thought to have evolved about six million years ago [11], and in addition to the conditions above, the plant has survived successfully in environments and growth media which are rich in potentially toxic contaminants. Tobacco plants can accumulate toxic elements from polluted air and soil, the concentration of which is dependent on the extent of the pollution and the element-specific uptake rate [102].

Cell metabolism perturbations, due to the inhibition of proteins and enzymes, can be expressed in plants exposed to high concentrations of metals, resulting in reduced or inhibited root and shoot development and elongation, leaf chlorosis and tissue death [31]. Some metallic elements absorbed by higher plants can be 'bound' to a certain extent by a variety of mechanisms, the majority of which are based on phytochelatins [103], which partially detoxify potential toxins. Phytochelatins are oligomers of glutathione, a tripeptide anti-oxidant which prevents reactive oxygen species (ROS) from causing damage to important cellular components.

pH of soil is the dominant property affecting Cd availability to plants [42], and the effects could be doubled under acidic conditions [19]. The concentration of Cd and some other elements including Cu, Ni, Se, Mn and P, redox conditions and sorptive capacity of the soil also affect plant uptake rate [51].

Climate and biological activity also play a major role. Climatic effects include the positive correlation between uptake of heavy metals Pb and Cd by plants from soil with increasing temperature, and biological effects include the potential immobilisation of some metals through microbial activity [101].

In certain locations Cd concentrations can be well above the historical background, such as at the Rothamsted Experimental Station, UK where levels in the plough layer depth soils increased by 20-55% over a period of 130 years [48]. An increase in Cd concentrations in tobacco shoots following a fallow year was observed following an experiment [48]. When considering that tobacco is a natural accumulator of Cd, one can hypothesise that the crop might also act as a remediator when harvested; removing a portion of the Cd deposited from the environment to the soil each year.

11.3.1.1 Effect of soil properties on heavy metals

Soil characteristics, including the total elemental concentrations of some transition metals, are influenced by aeration, anthropogenic inputs, climatic conditions, geochemical and biogeochemical processes, local irrigation and hydrology, local parent-material geochemistry, partitioning between solid and

aqueous (or otherwise soluble) phases at the soil particulate level, and soil pH [16, 58, 101].

Soil has the capacity to buffer the effects that contaminants may have, by binding these agents to soil constituents, or chemically converting them to inactive or soluble forms: these factors alone make for complex dose-response relationships in crops [48]. Cr and Pb for instance, have low solubility in soil and limited uptake through plant roots [26].

Interactions between soil and heavy metals include absorption to organic and mineral soil colloids such as clay particles and transition metal sesquioxides, immobilisation to inorganic and/ or hydroxide forms, and complex formation with organic matter [104]. At the microscopic level, the cation exchange capacity (CEC) of soils becomes fundamental to the bioavailability or holding of nutrient (or contaminants) cations exchanged from the soluble phase. CEC is determined by the prevalence and composition of clay and humic colloids, and pH. Plant roots hairs cells have a corresponding CEC which will be explored in the following section. Physically, metals can be redistributed throughout the soil profile by processes including weathering [101], making certain elements differently accessible to plant roots in the wild, and making ploughing a complicating factor in agriculture.

The pedogenic (geogenic) processes of semi-natural soils generally result in low levels of pollutant loading, as naturally occurring heavy metals occur in forms that are chiefly unavailable for plants to uptake [21, 101]. Soils which are either heavily managed, or contaminated with point source/localised high concentrations of pollutants, are regarded as typically having higher bioavailability to plants due to surface site saturation; sources can include disposal of domestic and industrial waste, industrial and manufacturing processes [21, 101, 102]. The accretion of undesirable components to soils through addition of animal manures, herbicides, pesticides and phosphatic fertilizers, and atmospheric deposition [48], are regarded as diffuse source pollution in agricultural areas that represents a major source of concern for heavy metal introduction to the food chain or potential release to water bodies [21, 105].

11.3.1.2 Uptake to roots

It has been ascertained that undesirable contaminants exist in tobacco, which may be ingested by humans as a receptor in the food chain. The contaminants exist in soils, accumulated from natural and anthropogenic sources, which can become or remain bioavailable to plant uptake, as found in this study. It is known that tobacco plants accumulate metals in the leaves by absorbing them from the soil through the roots [99]. For example, when bioavailable, Cd and Pb can be easily absorbed from soil and concentrate in tobacco leaves [45]. In this case the source is explained and the receptor defined, however the pathway for this phenomenon needs to be understood.

The fields of plant and plant cell physiology, uptake and translocation mechanisms of substances, and their associated biochemistry are explored briefly in terms of the foundations as well as some specifics for individual ions, compounds or groups of substances pertinent to the present study in the later sections. This following section is concerned with the interface between the environment and plant root.

Before a substance (an amino acid, compound, ion or molecule) can reach the cytoplasm, it must negotiate, or be introduced by channels or mechanisms within the plant cell wall and membrane physiology. Principal uptake mechanisms of substances in normal metabolism (excluding for the moment gases, water and contaminants) include active transport and passive mediated

transport. In active transport, energy is required to transport a substance across the root cell membrane against a concentration gradient. In passive-mediated transport, protein channels and transport proteins, mediate diffusion of soluble substances that would otherwise be prohibited by the hydrophilic nature of cell walls and membranes. Once inside the plant root epidermis cell walls, substances are translocated through apoplastic and symplastic pathways within tissues or organs. Longer distance transport occurs through the xylem, initially at the symplasm level through the epidermis tissue, then at tissue level through the cortex to the endodermis, to the pericycle, then into the xylem.

Nutrient cations are exchanged at the interface of soil colloids and plant root hair cells carboxyl and hydroxyl groups, and to a lesser extent phenolic and amine groups determine the CEC of roots [18]. The decrease of cation exchange affinity on root cell walls has been reported as H>Cu>Ca>Zn and H>Cu>Zn>Ca [18]. Plant root cell walls are involved in the acquisition or exclusion of mineral elements including heavy metals as a tolerance mechanism [18]. This property limits the translocation of a significant proportion of an accumulated contaminant to the fruit, leaves or seed [26]. However, the substance uptake and translocation rate characteristics could be unique for the interactions between different soil types, plant groups or substance compositions at any level of observation.

Cadmium uptake
The non-essential nutrient Cd is accumulated in crop species predominantly from soil, at a rate largely regulated by the concentration and pH in the soil, but also influenced by CEC, microbial activity, mycorrhizal interactions, presence of competing elements such as Zn, redox potential and type of soil. Reduction in Cd translocation to above-ground plant tissues could be achieved by sequestration to the roots in the form of Cd-metallothionein, though elements with similar atomic characteristic (such as Cu) may interfere with this aspect of nutrient metabolism [19].

In tobacco it has been found that leaf Cd concentration correlates negatively with plant yield, and that uptake may be reduced by liming the soil [51]. However, leaf hairs of tobacco exposed to specific Ca and Cd isotopes produce grains containing the same isotopes as an excretion mechanism, adding a further dimension to the interaction of tobacco and environmental Cd, and heavy metal tolerance in the plant [31].

Zinc uptake
Zn can occur at very high concentrations in soil before precipitation occurs [101]; however it is a micronutrient at normal concentrations in soil, deficiencies being rare. Levels higher than 20ppm can become phytotoxic [19, 58], when plant growth above and below ground become affected and chlorophyll content reduction has been observed [31]. Sources can include fertilizers and fungicides applied to agricultural land, at various intensities and frequency of input.

In toxic conditions there is a systematic increase in tobacco leaf vein and trichome Zn concentrations; subsequently grains are created and excreted from the trichomes [31].

11.3.1.3 Environment to plant

Currently, the most significant source of inorganic, organic and radioactive environmental pollutants concentrated into tobacco from the environment is from anthropogenic energy conversion, and subsequent waste by-products liberated to the atmosphere by burning fossil fuels [14]. So, in addition to the uptake of these pollutants from their accretion in soils through roots into shoots, aerosol particulates can accumulate on shoots and leaves from atmospheric

deposition [31]. In this way, metals may dissolve and be transported throughout the plant through entering the stomata [48].

Effects of fertilising

Soil pH and fertilization imbalance are known to affect the availability of certain nutrients and cause disease in plants, therefore tobacco has been studied extensively in nutrition, and especially nutrient deficiency studies. For instance excess N has been found to protract the vegetative phase which is important information when dictating the balance between yield and quality, and excess K2O reduces the tar and nicotine delivery without affecting yield or grade [14].

In an ideal world, fertilisers would be pure: primary nutrient fertilizers containing just N, P and K, secondary S, Ca, Mg and/or Na, and micronutrient B, Co, Cu, Fe, Mn, Mo and/or Zn [105]. Also, concentrations in plant leaves would increase proportionally with application rates; however, in tobacco, leaf Mn concentration increases with increasing Mn application to soil, but decreases with increasing soil pH (decreasing acidity) [32].

In the real world, manure and sewage sludge are cheap, effective and widely exploited sources of nitrate (NO3)-, and phosphate (PO4)3- fertilizers are chiefly derived from rock. Both fertilisers have been implicated in heavy metal accumulation in soil [30]. Interactions are complex, so too the biological and chemical immobilisation of metals can be achieved through application of 'exceptional quality biosolid' organic compounds, lime and (PO4)3- reducing bioavailability of heavy metals [21, 48]. Field grown tobacco crops require high N fertilisation, a process which transiently acidifies soil through microbial nitrification of ammonium (NH4)+, increasing the uptake and therefore concentration of Cd in tobacco leaves [19]. Cigarette manufacturers also discourage the use of fertilizers containing high levels of Cl [14].

Phosphatic fertilizers and heavy metals

Positive and negative effects have been observed with phosphatic fertilizers applied in tobacco cultivation. The same plots were used repeatedly and excess phosphatic fertilizer was applied [106]. In these circumstances, cultivated land can be increasingly contaminated by traces of Cd, Cu, Mn, Ni, Pb and Zn and other naturally occurring metals that exist in (PO4)3- rocks from which the fertilizers are derived, through increasing partitioning to the soluble phase as soil acidification can occur simultaneously [47, 107]. Excess (PO4)3- and sulphate (SO4)- ions have also been found to reduce soil solution Pb and Cu concentrations [101].

Sludge application and bioavailability

There are views on the application of fertilizers to agricultural soils in the form of sewage sludge or biosolids, in relation to contaminant bioavailability. The positive perception is that application could limit the transfer of potentially toxic elements to plant roots, because sludge solids and soils have a greater affinity to bind them [26], and that plant growth is promoted significantly with this treatment compared to commercial fertilizer application [58]. Notwithstanding the positive aspects, the negative perspectives are becoming increasingly numerous.

Industrial wastes, municipal solid wastes and sewage sludge are applied due to the 'beneficial use' philosophy; the understanding whereby these materials have beneficial characteristics for agriculture in the short-term, whilst being known to have undesirable properties and/or contaminants (perhaps in significant concentrations) that could cause contamination at local and regional scales in the long term [26, 48]. Some of the potential hazards identified from sludge application are listed below (Table 4).

Table 4. Effects of sludge application to agricultural soils for some toxic elements.

ELEMENT	MEDIA	EFFECTS OF APPLICATION	REF
Arsenic (As)	Sludge	Becomes largely unavailable due to rapid fixation in soils	[110]
	Industrial waste	Repeated application or disposal can lead to an accumulation in soil, and through the trophic chain	[21, 58, 104]
Cadmium (Cd)	Sludge	High mobility in soils for plant uptake under the right circumstances	
	Sludge	Generally, crops produced from soils amended with sewage sludge contain higher concentrations	[68]
	Sludge	In one example, a soil with a 1 mg Cd kg^{-1} background, had a final concentration of 44 mg Cd kg^{-1} after amendment with sewage sludge	[42]
	Sludge	An experiment indicated that guideline limits in clayey, loamy and sandy soils were not exceeded	[58]
	Sludge	There is a correlation between increasing Cd concentration with decreasing pH	[22]
	Sludge	Quality of the sludge and suitability of the soils must be scrutinised as the resultant tobacco concentration will be consequential	[90, 110]
	Industrial waste	Repeated application or disposal can lead to an accumulation in soil, and through the trophic chain	[21, 58, 104]
	Sludge	Consumption of plants with high Cd concentrations as a result of being grown on sludge treated soils can increase Cd concentrations in human kidney and liver tissues directly or indirectly through the trophic chain	[96]
Chromium (Cr)	Sludge &	Becomes largely unavailable due to rapid fixation in soils	[110]
	Industrial waste	Repeated application or disposal can lead to an accumulation in soil, and through the trophic chain	[21, 58, 104]
	Sludge	Usually low concentration of from diffuse sources can be increased to very high levels in sludge amended soils with long term degrading of organic matter; at high pH Cr may be oxidised to chromate (Table 5).	[26]
Copper (Cu)	Waste (especially Industrial)	Repeated application or disposal can lead to an accumulation in soil, and through the trophic chain	[21, 58, 104]
Mercury (Hg)	Sludge	Becomes largely unavailable due to rapid fixation in soils	[110]
	Waste (especially Industrial)	Repeated application or disposal can lead to an accumulation in soil, and through the trophic chain	[21, 58, 104]
Nickel (Ni)	Waste (especially Industrial)	Repeated application or disposal can lead to an accumulation in soil, through the trophic chain; tobacco effectively absorbs soil Ni, accumulated it in the leaves	[21, 32, 58, 104]
Lead (Pb)	Sludge	Becomes largely unavailable due to rapid fixation in soils	[110]
	Waste (especially Industrial)	Repeated application or disposal can lead to an accumulation in soil, and through the trophic chain	[21, 58, 104]
Tin (Sn)	Sludge	Organic compounds represent significant fractions of sewage sludge Sn which are more toxic than inorganic forms	[26]

Specifically, negative impacts include the gradual accumulation of heavy metals in cultivated soils due to repeated application of activated sludge wastewater [108], and therefore toxicity through the trophic chain in water sources, vegetation and livestock to humans [104]. Vigilance of the water industry is required to ensure the environmental sustainability of this resource in processing industrial effluents [94]. Ensuring these metals are not in the form of salts is desirable, as these are typically more soluble and therefore more available to plants; however even at higher loadings, where solubility plateaus, sludge borne metals represent a significant source of metals in agriculture [26, 104].

Insecticides
Insecticide use represents a direct application of exogenous material to the above-ground plant surfaces and tissues. In UK agriculture, there are now few permitted heavy metal containing insecticides since those containing As and Hg were prohibited [94]. For instance, As containing insecticides in plant production leads to substantial As levels in tobacco, and reduction of arsenical sprays correlates with a reduction in tobacco As level [77]. It is recognised that breeding for pest resistance is the most effective way to reduce environmental harm and promote production of safer tobacco [14].

11.3.1.4 Bulk localization of heavy metals in the tobacco plant

The botany and physiology of the tobacco plant must be taken into account when describing the distribution of various elements and compounds within the plant. For instance, the development of the large leaf area can only be supported by an incredibly active system of roots (see section 11.2.2.1), and the position of the stem nodes demarcating patterns of relative nicotine, total nitrogen or reducing sugars distribution [14]. This, in combination with local effects in the rhizosphere, has a greater effect than bulk soil characteristics and chemistry in dictating the uptake of toxic elements across the 'soil-plant barrier'; ranked Pb, Cr, Hg> Cu>Ni, Zn, Cd>Mo, Tl, with regard to relative effectiveness [26].

The process of negotiating the 'soil-plant barrier' (see section 11.3) and subsequent pervasion of metal ions to the above ground plants involves sequestration by root cells, transportation through the symplasm to the stele and mediation into the xylem via highly specific membrane protein [57]. From the xylem sap, transition metals can be scavenged and redistributed through the leaf cells via apoplastic and symplastic pathways, then to organelles according to the required specific physiological parameters for metal-containing proteins [57].

The concentration of metals in plant tissues is usually inversely proportional to uptake resistance. Generally root crops have stronger barriers than crops harvested for leaves, and the lowest concentrations found in fruit or edible seeds, compared to leaves, roots and stems, although there are exceptions for different crops, metals and environments [26]. The tobacco plant is known to accumulate species of heavy metals (see section 11.3.1.2) from the soil into leaves [91], and with an effectiveness that makes it amenable for application as a soil remediation biotechnology [33], especially for Cd [43]. Specifically, bulk concentrations of elements in leaves are generally concentrated according to stalk position, in a gradient of highest in the lowest, older leaves and lowest in the young, topmost leaves [32]. There are exceptions and extenuating circumstances, for example concentrations of the essential elements Cu and Zn have been found higher in upper leaves compared to lower, except when high concentrations of fertilizer are applied in which case the opposite is observed [107].

Cadmium
It is generally observed that plants will accumulate Cd into the root system, with a proportion translocated to above ground plant parts with an efficiency that is specific to genus and species (lettuce and tomato are particularly efficient, whereas crops such as maize are as low as 25% translocated to leaves) [85]. Also, higher concentrations are frequently observed in leaves compared to grains and fruiting bodies [48].

Tobacco has been established as an efficient accumulator of Cd from the soil [61] (see section 11.3), although different species have different behaviours for localised sequestering of the metal; N. rustica and N. rutifolia accumulate Cd very efficiently in roots and poorly in leaves, whereas N. tabacum accumulates equally efficiently in both [51].

When Cd, and an analogue Zn, is absorbed into the tobacco plant and translocated to the above-ground plant parts via the stalk, the concentrations found in the leaves decrease with increasing height [109], which is equivalent to leaf age. Wagner reported that Cd distribution in leaf laminae is uniform, with lower relative concentrations in stems and leaf midribs [19].

Distributions of elements within the differentiated cells of the leaf laminae, the epidermis and mesophyll cells, have been determined in other plant species [57], but not investigated to any significant extent in tobacco plants although this may be fundamentally important in excretions mechanisms. At the cellular level, elements such as Ca have been discovered in crystalline oxalate form in the vacuoles (specialized idioblast cells within leaves) [31].

Trichome excretion mechanism
Tiny leaf hairs (trichomes) are thought to be highly influential in many plants for the detoxification and storage of many elements [57]. An important property of the tobacco leaf that influences insect resistance and susceptibility, and ultimately the aroma and flavour of the final product, are the compounds on the leaf surface derived from viscous fluids exuded at trichomes [14].

Trichomes are glandular structures capable of excreting various alkaloids. Tobacco has two distinct varieties, namely long trichomes that exude nicotine, and short trichomes that exude defensive proteins, resins and terpenoids [31]. The accumulation rate of metals in trichomes is governed more by physiological requirements than potential toxicity [57]. The tolerance of tobacco to high levels of Ca, Cd and Zn can be attributed to a cooperative mechanism leading to the proliferation of trichomes and precipitation of grains (20 – 150 µm) containing those elements [31, 57]. Mineralogical analysis of these grains indicates the presence of stable and metastable calcium carbonates (calcite, vaterite and aragonite) with some Zn substitution, as well as calcium oxalate [31]. Cd has also been found to be substituted in carbonate grains, which is an important property that could be exploited to reduce metal exposure through smoking, and therefore harm, if a process can be adapted to remove them before cigarette manufacture [31].

11.3.1.5 Conclusion

The quality of agricultural soils, microbial processes, phytotoxicity, and transfer of zootoxic elements through direct ingestion or through the trophic chain to human diet are all important considerations in crop production [94]. The availability of information, translation to policy, and implementation through governance is important to regulate the inputs of specific elements to soils through applications of low quality, toxic heavy metal containing fertilisers, sewage sludge, insecticides, pesticides, and atmospheric deposition from myriad emission sources [94]. Optimisation of the growing media, environment and practices, selection or breeding of tobacco for low uptake or enhanced excretion

of target elements, and inclusion of processes intended to exclude or remove as much of the undesired elements could potentially be adopted as a strategy in cigarette manufacture to reduce final bulk elemental concentrations. However, focusing on concentrations alone is an over-simplification of the potential for harm to humans of some elements and the relationships between speciation and health are fundamental.

11.3.2 *Species, complexes and ligands*

11.3.2.1 Species

From the outset, it has been made clear that total elemental concentration is not the single determining factor expressing toxicity in organisms. The relationships are, as yet, poorly understood for many elemental species [20].

11.3.2.2 Species bioavailability

Bioavailability is defined as "the measure of the proportion of the total amount of a nutrient that is utilised for normal body function" [64], however the proportion not utilised could therefore alter or negate that normal function resulting in toxicity.

In terms of total bulk concentrations of nutrient elements that a plant may absorb there is a general consensus that the trace elements Cr, Fe and Mn are the most poorly absorbed (estimated as 15%), that Ca, Mg and Zn are reasonably well absorbed (50%), that Cu, P and Se are taken up at approximately 70%, and that >90% of K is accumulated [64]. Though the aforementioned elements essential for nutrition or as co-factors can become deleterious to plant health in deficit or excess, consideration must be given to the fact that the chemical form (or species) of these, or non-essential elements, dictate bioavailability and toxicity [108]. These characteristics are in turn predicted by physiological barriers to uptake within the plant, microbial processes conditions in the environmental in which the plant is grown (see section 11.3), and the source, species and relative concentration (Table 5).

In species analysis within biological tissues understanding and identifying the analytical target amongst naturally occurring species can be obfuscated due lack of research, or limitations of techniques for chemical species remaining undiscovered at lower and ultra-trace levels [4].

11.3.2.3 Elemental species interaction and substitution

Divalent, dicationic species such as Cu^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+} etc. are essential plant micronutrients, which can become toxic in excessive concentrations [103], and have a high affinity for amines and thiolates, or as cofactors in the case of Ni^{2+}; thus in excess or substitution, uncontrolled binding to DNA and inactivation of proteins can occur [57].

Other redox active and oxidative stresses inducing metal toxicity add into these increasingly complicated multifactorial mechanisms, such as reactive oxygen species in Fenton-like reactions [35]. Ni carbonyl has been reported not to transfer to tobacco smoke (Table 4), however Co and Fe also form carbonyl complexes at the temperatures at which tobacco burns, and therefore may present a risk to smokers [29].

Table 5. Potential species of metals in tobacco and their properties

ELEMENT	VALENCE STATES	SPECIES		COMMENTS	REF.
Antimony (Sb)	Sb(0)			Occurs naturally oxidation states (0), (-III), (III) and (V)	[56]
	Sb(-III)				
	Sb(III)			Most common and stable Sb oxidation state, and absorbed by mammalian cells equally well as As(III)	[56]
		Antimony trioxide	Sb_2O_3	Commercially significant Sb compound worldwide as a constituent of adhesives, paper, pigments, and textiles and as a flame retardant in rubber. There is sufficient evidence for carcinogenicity in laboratory animals has been identified by the IARC	[56]
		Antimony trisulphide	Sb_2S_3	Limited evidence for the carcinogenicity of Sb2S3 in laboratory animals	[56]
	Sb(V)				
Arsenic (As)				Human exposure is generally to inorganic; arsenite (As(III) or arsenate (As(V))	[37]
				Human intake mainly via of food and drinking water (water usually <10µg L-1)	[22]
				Inorganic As compounds present in drinking groundwater in several countries	[35]
				Organic arsenic compounds are primarily found in fish (human exposure route)	[22]
	As(III)			Associated diseases diabetes, hypertension, tumours (skin, bladder, liver and lung)	[35]
				Predominant form in drinking water from deep (anaerobic) wells, which when imbibed is actively transported into cells via aquaglycoporins 7 and 9, remaining unchanged at physioloigical pH, and so passes through the cell membrane quickly, resulting in increased toxicity compared to As(V) which is transported relatively slowly	[37]
				Observations in epidemiological studies associates this species with increased cancer risk	[37]
				Enzymatic methylation in cells, catalysed by methyltransferases that utilise an S-adenosyl-methionine (SAM) cofactor, produces monomethylarsonic (MA) and dimethylarsinic acids (DMA)	[36]
		Arsenite	As_2O_3 or H_2AsO_3-	Most humans excrete 10-30%	[37]
		Methylarsonous Acid (MA(III))	$H_2AsO_2CH_3$	Humans metabolise As compounds by methylation; a major detoxification pathway, as excretion in urine is faster in methyl-As species than inorganic species. The process is also observed in bacteria, fish and rats, though large inter-individual variations are observed in humans	[36, 37]

				More toxic than As(III) both *in vitro* and *in vivo*; exposed humans excrete 10-20%	[37]
				Due to the associated toxicity of MA(III), methylation may be also be considered an activation pathway; some humans individuals express high As methylation rates, and compared to those excreting less methyl-As species and excreting more inorganic As, will experience more toxic effects	[36]
		Dimethylarsinous Acid (DMA(III))	$HAsO(CH_3)_2$	More toxic than As(III) both *in vitro* and *in vivo*; exposed humans excrete 60-80%	[37]
		Dimethylarsine	$HAs(CH_3)_2$		
		Trimethylarsine	$As(CH_3)_3$		
		Arsenate	AsO_4 or H_2AsO_4		
		Monomethylarsonic Acid (MA)	CH_5AsO_3	Less toxic than As(III) or As(V); most humans exposed excrete 10-20% but some populations differ	[37]
		Trimethyl Arsine Oxide (TMAO)	$AsO(CH_3)_3$		
		Beryllium oxide	BeO	Often used in ceramics, structural materials in the aerospace and electronics industries, sports equipment and aircraft	[39]
	Cd(II)			Forms soluble salts and does not undergo redox cycling	[96]
		Cadmium sulphide	CdS	Used in electroplating, pigment manufacture, stabilization of plastics, NiCd batteries currently, and potentially solar collectors in the future	[19]
		Cadmium sulphate	$CdSO_4$	Has lower solubility and free Cd^{2+} activity applied to soils in this form than residual Cd concentrations in sludge amended soils; important for predicting short-term crop uptake or leachability of toxic metals	[26]
			CdCl	Stays relatively mobile in soils due to the chloride ions higher affinity for Cd^{2+} than for absorbent surface in soils	[101]
				Under physiological conditions, Cr(III) are Cr(VI) are no longer considered to be thermodynamically stable	[113]
				Plants favour uptake of Cr(VI) due to its higher mobility in soils relative to Cr(III)	[55]
		Chromate	CrO_4^{2-}	The CrO_4^{2-} ion is the dominant form of Cr(VI) in neutral aqueous solutions, can readily cross cellular membranes via non-specific anion carriers	[96]
		Potassium Dichromate	$K_2Cr_2O_4$	Most soluble chromates were the least carcinogenic	[96, 97]
	Co(0)			Forms active oxygen that exhibit genotoxic activity	[56]

		Cobalt dichloride	$CoCl_2$	Can form active oxygen species resulting in in vitro DNA damage in the presence of H2O2 (chelators can alter this capacity)	[56]
		Cobalt sulphide	CoS		
	Co(III)				
	Co(IV)				
Copper (Cu)	Cu(I)			Shows an extremely high affinity to various organic molecules; redox-activity can lead to the generation of oxygen radicals	[57]
	Cu(II)			Shows an extremely high affinity to various organic molecules, and a potent catalyst in Fenton reactions (radical are produced) which indicate increased and 'functional' Cu activity in smokers	[33, 57]
	Fe(0)			Found in elemental metallic form in tobacco aerosol particulate	[2]
		Iron carbonyl	$Fe(CO)_5$	Complex exhibits high toxicity in human even at very low levels of intake	[40]
		Iron Sulphate	$FeSO_4$	Aspiration exposure injury has been reported; acute bronchial damage and early histological change in the biopsy specimens resulted	[60]
	Fe(III)			Ferric ion is a weak oxidant	[60]
				Forms insoluble hydroxides with limited bioavailability because of low solubility in oxygenated water and strong binding to soil particles	[57]
Lead (Pb)				Does not readily undergo valence changes; mechanisms enabling Pb to induce oxidative stress are not clear	[35]
		Tetraethyl Lead	$(CH_3CH_2)4Pb$	Previously a common additive to petrol (use has decreased dramatically in developed countries	[22]
		Methylmercury	$HgCH_3$	Most common and the most toxic environmental form of Hg. Algae and bacteria methylate Hg entering the waterways	[35]
				An organic compound converted from inorganic Hg, methyl-Hg is very stable and commonly accumulated in the food chain until the 1970s due to its use as a fungicide on seed grain. More recently, exposure is via consumption of fish, or dental amalgam. Poisoning has a latency sometimes in excess of 1 after acute exposure; symptoms relate to nervous system damage	[22]
	Hg(II)			3% to 6% of the organic Hg accumulated in a brain is converted to Hg2+, so mercury-induced oxidative damage is possible	[35]
	Ni(0)			Produces relatively low, but measureable levels of ROS in cells compared to other redox-active metals	[69]
		Nickel tetracarbonyl	$Ni(CO)_4$	Carcinogenic, but reported not to be present in cigarette smoke as a metal carbonyl	[32, 68]
	Ni(II)			Formation of the Ni(III)/Ni(II) redox couple around pH 7.4 dictates oxidative effects which is only possible when Ni(II) is complexed by natural ligands	[69]

				(including peptides and proteins); however soluble Ni(II) was found to be non-carcinogenic	
		Nickel dichloride	$NiCl_2$	Found to be a weaker carcinogens than NiS, NiO and Ni_3S_2 in animal experiments	
		Nickel acetate	$Ni(H3CO_2)_2$	Found to be a weaker carcinogens than NiS, NiO and Ni_3S_2 in animal experiments	[69]
		Nickel oxide	NiO	Low solubility/ higher carcinogenicity in animal experiments compared to the more Ni acetate, chloride or sulphate	[69]
		Nickel sulphide	NiS	Low solubility/ higher carcinogenicity in animal experiments	[69]
		Trinickel disulfide	Ni_3S_2	Low solubility/ higher carcinogenicity in animal experiments	[69]
		Nickel sulphate	$NiSO_4$	Carcinogenicity in humans implicated from evidence in the Ni refining industry. Also found to have relatively high carcinogenicity in animal experiments, predicted by low solubility, compared to the more Ni acetate, chloride or sulphate. Tobacco smoking has been considered as a weak confounder	[69]
Tin (Sn)	Sn(IV)			Organic compounds are more toxic than inorganic	[26]
		Tributyltin		More toxic than inorganic Sn species	[26]
Zinc (Zn)	Zn (II)			A trace inorganic divalent cation, that causes cultured tobacco cells exposed to high concentrations to produce little phytochelatins and accumulate Zn principally in the vacuole	[19]

11.3.3 *Transference and translocation of metals from the environment to humans via tobacco*

As established above, tobacco plants take up and become contaminated by levels of toxic constituents from the environment during growth; some transferred to the plant from the soil through the root system, or physically soil deposited on the above-ground parts from agricultural practices and wind [49]. Anthropogenic activity is also important, via atmospheric precipitation as polluted rains, and can lead to the introduction of a range of unspecified toxins to the plant, though more directly, specific species applied to plants as pesticides such as As or Hg containing compounds, bromate and chlorinated or organic compounds, or fungicidal dithiocarbamates [83]. Therefore the threat of direct ingestion of contaminated soil may be significant; however the bioavailability of any particular element may differ greatly from that in mineral dusts etc., compared to its ultimate plant component form [114]. Over recent decades the number of components identified in tobacco and smoke has increased from about 1200 in 1968 to about 8400 in 2008, reflecting improvements in analytical techniques [17]. However the number of components is not the important factor compared with the toxicity of individual species and to this end it is most important to have the ability to model and predict their fates in physiology.

11.3.3.1 Contamination of harvested material

Harvested tobacco is seldom washed before drying and curing, and atmospheric dusts can contribute high concentrations of Cd and Pb in mineral form [24]. Sb, Br, As, Cd and Co (as Co-carbonyl) contaminants have been found to be present in tobacco leaves, not only due to uptake from the soil increasing industrialisation and pollution or application of fertilizers and pesticides, but also from packing, storage and other domestic processes [34, 40].

Use of fumigant fungicides (or pesticide) in tobacco production representing a toxicological concern, specifically methyl bromide directly [83] and Cd in Zn-containing fungicides which persist into the manufacturing process [107].

11.3.3.2 Manufacturing cigarettes

From the field to the user, there is a continuous chain of events [14]. At the beginning this chain, a proportion of the total elemental concentration of metals may originate from the soil, and much from the manufacture and processing which may include addition of flavouring agents and preservatives [34, 40, 76, 89].

Of course, a cigarette contains more than just cured, processed tobacco (Figure 2); the filter and paper are also sources of trace and other elements [32]. Activated carbon filters may be able to trap several times more Pb than the more commonly used cellulose acetate filters for instance, whereas pure acetate filters trap more Fe [84]; however no filter has yet been demonstrated to remove the greater proportion of potentially harmful metals from the smoke although this is major aim of the manufacturers.

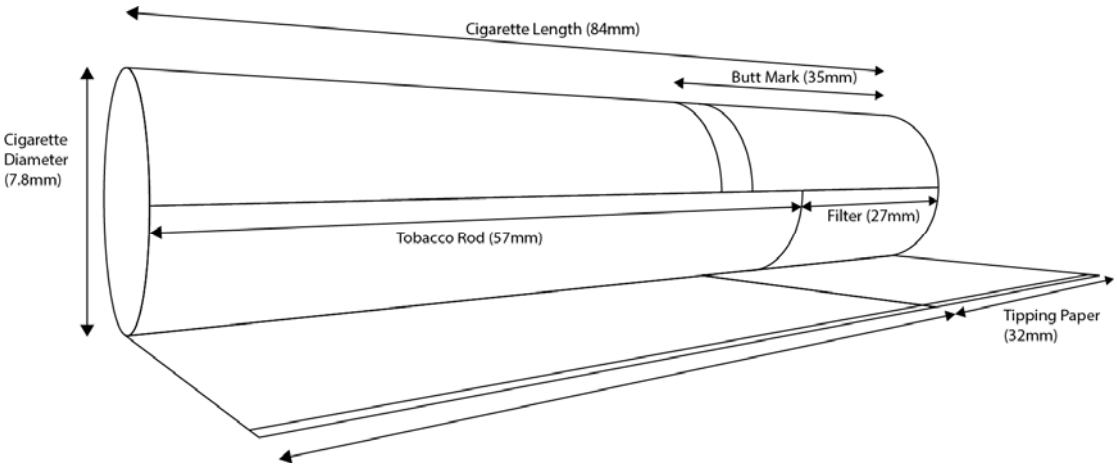

Figure 2. A tear-away diagram of some of the dimensions and components of a king-size cigarette.

There also appears to be a trade-off in terms of economy, such as the situation in which a larger smoking population could access a popular brand at a cheaper price [40]; however it was more likely to negatively affect the smokers' health than a relatively more expensive fine brand. Furthermore, counterfeit tobacco products often contain higher elemental metal concentrations than their genuine equivalents [79, 115].

The nature of the manufacturing processes, as well as the associated outcome of the smoking articles in terms of quality and properties as a delivery system for any toxins potentially contained within, may all have an impact on the species transferred or evolved during pyrolysis, and therefore the fate of those species in the consumer.

11.3.3.3 Cigarette to ash

Like the butt and filter, ash is not a desired or consumed product of smoking, but is relevant to the mass balance of metals. It has been observed that constituents in tobacco such as Ca, Cr, Fe and Sr are almost fully retained in ash, whereas Br, Co, Hg, Na, Sb, Se and Zn transfer in relatively high amounts [16] (Table 3). With regard to species, Cr(VI) has been observed in high concentrations relative to Cr(III); high temperatures during pyrolysis have been implicated in this oxidation [28].

11.3.3.4 Smoke

The quality of raw materials (including additives, filter and paper), curing and manufacturing processes dictate the initial concentrations of chemical constituents in the cigarette as a product, however it is the design of the combustion and smoking environments, and smoking method that dictate the chemical constituents and concentrations within the smoke. The act of pyrolysis is not intended to achieve complete combustion [14].

Al, Cd, Cr, Cu, Hg, Ni, Pb and Zn are toxic metals that have been detected in the filter, paper, tobacco and smoke of cigarettes [33]; the specific nature of the oxidation state or species are yet to be determined fully despite the importance of such data for accurate predictions of toxicological harm.

Both SS released to the immediate environment between puffs, and MS inhaled by the consumer during a puff, differ qualitatively and quantitatively [95], and

more specifically with regard to the metals and toxic substances released [24, 45].

Approximately 15% of the smoke inhaled and exhaled by the smoker is the mainstream component, the remaining 85% comprised of the sidestream which has smaller mean particulate diameter (a tenth of that of MS), being more persistent in air and more difficult to expel from the lung [95]. Toxic metals have been detected in MS which are absorbed into the body of a smoker, damaging the organs [84]. This sidestream component often has higher concentrations of toxins than MS due to the abundance of small particles (pm<1) and lack of filtration [90]. Also included in the codification of cigarette smoke is the environmental tobacco smoke (ETS), also referred to as in terms of involuntary, passive and second-hand smoke, due to the fact it is MS and SS emitted to the environment and inhaled by others than the intended consumer [32, 95].

11.3.3.5 Particulate phase elements

Besides codification for the pyrolysis components in terms of fate for recipients, other codifications exist for the physical components in terms of 'condensate' as the particulate phase, and the 'vapour' or gaseous phase.

Little investigation of the vapour phase has been conducted, due to the difficulty of isolating these highly volatile gases from the remaining particulate during pyrolysis; analyses have historically focused on the condensate due to the relative ease of capture onto filters and other media. Cd, Cr, Ni, Pb, Th, Tl and U, which are known to be carcinogenic, especially synergistically in conjunction with known organic constituents of smoke (benzopyrene, nicotine and so on), have been reported to be inhaled into the bronchial system as part of smoke condensate [102].

Table 6. Notes on some of the properties of smoke components.

Component	Comments	Ref.
From tobacco	Elements transfer from tobacco to the smoke in differing concentration during pyrolysis, the smoke comprising of gaseous, vapour phase and the particulate/ condensate ("tar").	[32]
	Carcinogens have been discovered in both the vapour and condensate components of smoke.	[40]
	Pyrolysis temperatures and volatility characteristics combined, dictate a low concentration of low vapour-pressure compounds (e.g. Methyl-Hg).	[84]
	The majority of the metals in tobacco are transferred to the ash and cigarette butt, some to the total particulate and relatively low proportions in the MS	[77]
	Tobacco Ca, Cr, Fe, and Sr is almost fully retained in the ash.	[16]
	Relatively large proportions of Br, Co, Hg, Na, Sb, Se, Zn are transferred to smoke through volatilization.	[16, 40]
	Tobacco Cd and Pb transfer relatively well to smoke.	[32, 45, 111]
Gas (or vapour) phase	Pyrolysis vaporization of metals accounts for the concentrations in smoke.	[84]
	Volatile Hg and Ni compounds generated during pyrolysis transferred to vapour not collected by electrostatic traps or filters.	[24, 102]
	Hg and Se which reside predominantly in the vapour phase, all other toxic metals are mostly retained in the particle phase.	[66]
	Pb can form volatile compounds in the burning zone of the cigarette (eg. Tetramethyl-Pb and plumbane) which sorb and decompose in the filter.	[46]
	Cd transfers poorly to the vapour phase (CdO is speculated to condense onto Cd rich particulates).	[46]]
Particulate/ Condensate	Toxic heavy metals from tobacco reside in MS particulate (inhaled by the smoker), in SS (between puffs) and in the ash and butt.	[77]]
	Relatively high concentrations of toxic agents and heavy metals can be found in the particulate phase of SS (inhaled by non-smokers/passive smoking).	[32]
	Passive smokers experience deeper penetration of SS particulates (and consequent deposition) into the alveolar spaces of the lung compared to MS, as due to the smaller particle sizes in SS.	[32]
	Very high rates of Cd transfer occur from unburned tobacco to the particulate phase of environmental tobacco smoke (ETS).	[66]
	Smoke particulate can be collected for qualitative analysis using cooled solvent traps (containing organic solvents and dilute inorganic acids); quantitative analysis can be conducted the filters (Cambridge Filter Pads have been associated with unsatisfactory efficiency and high blanks) and electrostatic precipitation techniques	[24, 46, 102]
	The Cd and Pb content in the particulate phase of MS, SS and ETS represent a high concern.	[24]

11.3.3.6 Smoke to filter

As already mentioned, filters do not trap all the potential toxins in cigarette smoke (see section 11.3.3.6), only retaining a portion of the total elemental concentrations [32]. Factors such as polarity, structure and volatility of agents in the smoke dictate to what extent filter tips can reduce concentration of toxins that could be inhaled by the smoked [68]. Principally volatile substances pass through the filter to be absorbed by the consumer, whereas a fraction of the less volatile compounds are captured [24], however heavy metals are transferred largely to the filter with the remainder persisting in the smoke [102].

Models based on experimental data predict the relatively high transference of Cd to MS, while Pb is retained relatively well in the filter [46]. Direct analysis has provided contrary evidence that while burning, Pb retention in the filter is lower than that of Cd [45]. It has been suggested that alterations to filter design could enhance this kind of selective removal of Cd from the vapour phase [68].

There may well be a trade-off, with regards to which element species are absorbed more readily, depending on the design and materials in a filter, and which phase of the smoke these agents are transferred to, depending on the stage of pyrolysis.

11.3.3.7 Smoke to instruments

The data are limited to that can be observed and quantified by the techniques available; classical methods rely on time-averaged concentration determinations which could be vulnerable to contamination and loss during sample preparation [116], and smoking experiments suffer loss of SS to the container or flaws in the seals no matter how apparently negligible [117].

11.3.4 *Potential health implications*

Smoking tobacco is a significant route of exposure to heavy metals in humans [31]; inorganic elements released by smoking may contribute to the alteration of metabolism and tumour growth [16], but it is unlikely that a single agent alone amongst the numerous carcinogens present in cigarette smoke is responsible for health impacts such as lung cancers [111]. More than 4,000 agents have been found in smoke condensate; harmful compounds benzopyrene, carbon monoxide, H2S, nicotine, nitrosamines and NOx may act synergistically with inorganic and metallic cigarette smoke constituents that are already suspected to be carcinogenic or toxic even at very doses [24].

As previously discussed (see sections 11.3.3.4 & Table 6), smoke is not only inhaled by the smoker directly through the MS, but through the SS and ETS which are also inhaled by non-smokers [77]. Of these cigarette smokes types, SS contains the highest relative concentrations of harmful, carcinogenic and toxic agents [32]. Noxious agents present in tobacco smoke are enriched in some organs once inhaled [24].Combinations of agents may exhibit synergistic, antagonistic and neutralising interactions; eg. "If more zinc and iron got into the body by smoke, they would reduce the harmful health effects of cadmium and lead in active and passive smokers' organs" [84]. Below, some of the health effects associated with certain elements found in the components of smoke are discussed, as well as discussion on the mechanisms by which they may cause harm; specific associated risks of exposure are tabulated by element (Table 7).

11.3.4.1 Reactive oxygen species

A significant factor that determines the toxicity of an agent once in the body and cells is that of reduction-oxidation (redox) potential. Metallic elements take on the characteristics of Lewis acids as ions in living systems; with an available lowest unoccupied molecular orbital, a net positive charge allows for the species to accept and electron [20]. Oxidative tissue damage can occur due to the production of reactive oxygen species (ROS) from a diverse range of environmental toxicants and heavy metals [96]. The smoke generated from pyrolysis of tobacco products can result in altered Ca and sulfhydryl homeostasis, altered NADPH redox status (cell energy cycle), increased DNA damage and increased lipid peroxidation [96]. Heavy metal ions such as Ag+, Hg2+ and Cu2+ and Pb2+ are reported to induce hemolysis and lipid peroxidation [35].

Redox processes are fundamental for biogeochemical elements such as Cr, Cu, Fe, Mo and Ni [118]; Fe redox processes are involved, amongst others in the spoilage of meat products and other foodstuffs.

Fenton-like reactions also generate ROS in conjunction with redox active metals, also including Cu, Cr, Fe and V [119]. A simplification of the process published is displayed below [50]:

$M(X) + O2^{-\bullet} \rightarrow M(X-1) + O2$
$2O2^{-} + 2H^{+} \rightarrow H2O2 + O2$
$M(X-1) + H2O \rightarrow M(X) + OH^{\bullet} + OH^{-}$

Free radicals generated by ROS in these redox reaction are generated by the metal constituents of tobacco smoke inhaled into the lung, which causes damage of these tissues [50].

11.3.4.2 Sidestream smoke and health

The list of carcinogens identified in the vapour and condensate of tobacco smoke continues to increase [34], however SS has been consistently proven to contain more carcinogenic substances per unit mass of particulate material than MS [65, 66] although it is more dilute when inhaled. As an example, the majority of the Cd found in cigarettes is reported to pass into the SS on pyrolysis, most of the Cd passes into sidestream smoke, putting passive smokers at risk [32].

11.3.4.3 Environmental tobacco smoke / Second Hand Smoke / Passive smoking and health

It has been established that heavy metals pose a threat to the health of smokers due to inhalation of MS, and passive smokes from inhalation of SS [77], however ETS also represents important air pollutant source, being associated with an increased lung cancer risk [90] being comprised of many toxic organic and inorganic substances associated with deleterious health effects for smokers and non-smokers exposed to it [65, 90, 117], and building on the previous example (see section 11.3.4.2), if the majority of Cd passes into SS, Landsberger et al. impress that 70% of this Cd resides in airborne ETS particles smaller than 2 microns (those that penetrate the deepest in the lung) [86].

11.3.4.4 Chronic exposure and accumulation

Amongst other contributors such as ingestion of ground water contaminated by agricultural and industrial toxins, and the corrosion of dental and medical prostheses, inhalation of cigarette smoke represents a major source leading to the accumulation of heavy metals in humans [44]. Cd and Pb tend to accumulate in vital organs and have a biological half-life of up to 30 years;

absorption through the gastro-intestinal tract being dependent on species and dose as well as age, combined with nutritional status and uptake and interaction of Ca, Mg, Fe, P and Zn [53]. Heavy metals introduced in this way can induce effects similar to radiation with regards to chromosomal aberrations and delayed cell death at concentration that produce no acute symptoms of toxicity [44], leading increase of undiagnosed health problems which may only manifest apparent symptoms [53]. This is of concern as carcinogenic effect develop, principally from chronic exposure [37].

Table 7. Health effects of some elements that are found in cigarette smoke

Element	Note	Ref.
Antimony (Sb)	ETS has been found to contain elevated levels. Chronic inhalation and accumulation in the lungs can result in bronchitis, pharyngitis, rhinitis, tracheitis, and ultimately emphysema and obstructive pulmonary disease.	[50, 65]
Arsenic (As)	There are no significant differences in As levels in the kidney cortex, hair, liver and lungs of smokers compared with non-smokers; higher creatinine levels have been detected in the urine of children with parents that smoke. ETS contains elevated levels and long term inhalation, even at low levels, presents respirable disease risk. High dose exposure differs from low dose exposure with regard to genotoxicity, reactive species formation, signal pathway activation and gene expression of As compounds. Acrocyanosis, carcinogenicity, hepatotoxicity and neurotoxicity can result from chronic exposure to inorganic As compounds. As exposure represents a threat to human health. Inorganic As specifically is acutely toxic; intake of large quantities results in gastrointestinal symptoms, disturbance to cardiovascular and central nervous systems, even death. Bone marrow depression, haemolysis, hepatomegaly, melanosis, polyneuropathy and encephalopathy have also been linked.	[32] [65] [37] [50] [22]
Beryllium (Be)	Respiratory diseases such as acute chemical pneumonitis, chronic beryllium disease (CBD) and cancer have been related to environmental and occupational exposure.	[39]
Cadmium (Cd)	Cd inhibits photosynthesis in plants and is mutagenic and toxic in animals and humans. In plants, accumulation correlates with phytochelatin deficiency (a fungi/plant toxic response). The main exposure routes are dietary (i.e. ingestion) and respiratory (inhalation), involving consumption of Cd containing plant material. Depending on the chemical form, MS smoke represents a major source of Cd and a health hazard to smokers.	[50, 57, 93, 120] [22, 57, 93, 121, 122]

	Unburned tobacco has a particularly high transfer rate to the particulate phase of ETS (70% to particle diameters <1.8 pm), which penetrates deepest in the lung.	[[66, 86]
	For inhalation, the lethal dose for humans has been determined at 2600 mg Cd m^{-3} min^{-1} (a dose only likely to be experienced as occupational exposure). Emphysema and	[65, 120]
	Due to the high toxicity of Cd smoking potentially represents an epidemiological risk even at relatively low levels of exposure.	[22, 32, 45, 92]
	Therapies exist that reduce Cd burdens in humans, but no treatments exist that completely purge the element from the body.	[19, 52]
	A potentially toxic Cd dosage for a smoker could come from an average daily consumption of 1gram (especially in the European diet); exacerbated due to the poor excretion rate observed in humans (0.005% of the body burden).	[19, 35, 48, 123, 124]
	Due to the potential harm posed by Cd, Germany and Australia have set limits of 0.1 and 0.05 mg kg^{-1} f.w. in crops.	[48, 85]
	In non-occupationally exposed persons, tobacco smokers may receive twice the lifetime body burden compared to non-smokers.	[52, 85]
	Levels in urine, the kidneys, liver and prostate of smokers are higher in smokers than	[32, 77]
	Stimulation of metallothionein synthesis, a response that reduces availability of metals for intestinal absorption by binding to them to proteins, on inhalation of Cd in cigarette smoke is suggested.	[32]
	About 90% of inhaled Cd is absorbed, compared to about 5% of that which is ingested.	[32, 52, 95]
	Once in the body the binding protein metallothionein (MT) is thought to detoxicate Cd so that it does not undergo metabolic degradation, however this process significantly	
	The expression of MT on increased blood Cd level increases risk of carcinogenesis, as these proteins also bind Zn (an essential nutrient). In this way Zn is also accumulates	[33, 61, 125]
	Gastrointestinal absorption and subsequent tissue deposition is influenced by the Cd species in ingested plant materials; subsequent bioavailability in humans can be influenced by Ca, Cu, Fe, Se, ascorbic acid, pyridoxine and various concentrations and	[19, 35]

	Cd interferes with Zn-mediated metabolic processes producing toxic effects as both elements favour similar bioligands. In this way Cd also mimics similar divalent cations (Ca2+,Mg2+ etc.) interfering with the metabolism of these essential elements.	[52, 53, 120, 126]
	The WHO/FAO (Food and Agriculture Organisation) established provisional tolerable weekly intake (PTWI) limits of 400-500 µg (or 60/70µg day^{-1}); due to the major contribution of tobacco smoke in combination with dietary intake. `smokers may exceed this limit.	[48, 127]
	Cd is persistent in the environment, and in the human body (especially the lungs and kidneys) it has a biological half-life of about 25 years (ranging 10 to 40 years, depending on age).	[[19, 24, 35, 124, 125]
	Cd is carcinogenic but does not induce lipid peroxidation or produce ROS (which damage DNA) from Fenton-type chemical reactions like other heavy metal(loid)s, though does create oxidative stress which can indirectly activate aberrant genes,	[35, 52]
	The blood cadmium levels of smokers are 4-5 times that of non-smokers (ETS still potentially contributing to total cadmium body burden in non-smokers), and 2-4 fold	[22, 24, 124]
	Cd accumulates in the brain, kidneys, liver, lung, kidneys and testes, resulting in alterations in central nervous system neurochemistry, carcinogenesis, hepatotoxicity and oncogenicity (in the hematopoietic system, lung, prostate and testes).	[33, 96, 122]
	Accumulation correlates with age up to the age of about 50; decreases in the kidney cortex beyond this point are related to alterations in diet and smoking habits, or kidney	[61, 125]
	Due to lower Fe stores and therefore increased intestinal absorption, as well as lower energy consumption rates, the concentration of Cd in women is higher than that in men.	[22, 124, 128]
	Cd affects estrogen, however humans may not experience these effects as smoking protects against estrogen-dependent endometrial cancer. Interactions of other agents present in smoke in this phenomenon could neutralise these effects, demonstrating that Cd toxicity is not straightforward.	[127]
	Evidence for a placental Cd barrier has been reported, however so have correlations between low birth weight in infants and increases in the Cd concentration of milk from	[19, 32]

	Evidence suggests a non-steroidal estrogen potency of Cd in vivo, as an endocrine disrupter. Effects on male reproductive capacity, as well as increased breast cancer	[127, 129]
	Cadmium exposure (from above background ingestion to chronically exposed of workers to Cd dust and fumes) in general is related to development of conditions from slight anaemia and anosmia to both benign and malignant chronic disease; these include cataracts, formation of calcium renal stones, cardiovascular pathologies (hypertension), gastrointestinal and metabolic problems (Fanconi Syndrome, disruption of Ca, P and vitamin D metabolism), lung disturbances and respiratory insufficiency (emphysema, obstructive lung disease, pulmonary fibrosis), nephrotoxicity, osteomalacia and debilitating osteoporosis (combination of which is called itai itai (ouch-ouch) disease), oncogenesis (through aberrant gene activation, suppressed apoptosis, altered DNA mismatch repair (MMR) or gene repair) and cancer (breast, colorectal, kidney, lung, prostate).	[32, 33, 50, 52, 95, 120, 130]
	With regard to Cd exposure through cigarette smoking (complex mixtures of cadmium and other carcinogens through inhalation of tobacco smoke) , possible relationships have been reported for increased tissue Cd levels, cardiovascular diseases, gastroenteritis, hypertension, osteoporosis (decreased estrogen levels leading to fractures and early tooth loss in smoking women), pulmonary emphysema, renal tubular dysfunction, organ-specific carcinogenesis and aggressive prostate oncogenesis.	[19, 52, 130]
Chromium (Cr)	Ubiquitous in the environment, occurring naturally in soils, rocks and living organisms (required for carbohydrate metabolism in humans). Hexavalent chromium from fossil fuel combustion and steel production accounts for two thirds of the Cr found in air, the remainder is in trivalent form.	[96, 97, 131]
	Supplements containing Cr have been shown to improve variables in glucose	[96]
	Cr accumulates well in lung tissue with increasing age (and without decreasing after smoking cessation) where it can impair function (emphysema, pharyngitis, rhinitis and ulceration) and promote carcinogenesis. In the skin, allergic dermatitis has been reported.	[32, 33, 50]

Cobalt (Co)	Co is an essential nutrient component co-factor in vitamin B-12; diet is the main route of exposure for humans. In occupation exposure, inhalation and skin contact to cobalt can result in result in impacts on hemapoietic tissues, the respiratory tract, skin and thyroid gland. There is some evidence for potential generation of malformation of foetuses in pregnant women and carcinogenic effects in humans.	[56]
Iron (Fe)	There is evidence for the carcinogenicity from inhalation of Fe dust (asbestos/silica), as well as for Fe-compounds being vectors for other carcinogens (PAHs, Ni, Cr etc.) Inhalation of Fe could be more cytotoxic to humans, as Fe2+ oxidises readily in pH <7 environments such as the lung, creating ROS. Iron overload plays a role in hepatic carcinogenesis, which is already associated with tobacco consumption. Pb body burden can be indicated by depression in the enyzme 5-aminolevulinic acid dehydratase; cigarette consumption depresses this enzyme.	[60] [32]
Lead (Pb)	The intake of Pb for the UK population has fallen well below the WHO PTWI limit due to measures taken to reduce contamination of the environment (replacement of leaded petrol), however the metal is still a hazard to human health even at relatively low Several countries have set workplace limits of 40-50µg dl-1 (usually 4-5 times the limit for children). Studies have found a correlation between Pb burden and vehicle density near the home for children. In addition to the concentrations still found in many workplace environments, levels still remain unacceptably high in chemicals present in complex mixtures of air pollution and cigarette smoke. Smoking has been found to increase the blood-Pb where other Pb can persist in the blood for about a month and is excreted slowly through the urine, however this slow rate allows for accumulation in the bones (where it takes 20-30 years to eliminate). Higher gastrointestinal uptake and blood-brain permeability rate make children especially at risk of Pb exposure and brain damage. In adults, gastrointestinal dysfunction, hypertension, reproductive impairment and peripheral arterial diseases can be potential health effects. Acute poisoning presents with abdominal pain, headache, irritability, and nervous Evidence for lead carcinogenicity is very weak, but may express as lung and stomach	[22, 45, 62, 63, 132] [53, 63] [32, 63] [22, 33, 50] [22] [22, 63]

	cancer as well as gliomas. Pb oncogenicity manifests through respiratory and renal tumours through exposure concentrations lower than those exhibiting nephrotoxicity. Pb increases hydrogen peroxide production in human cells, leading to oxidative chromosomal DNA damage chromosomal (not genotoxic).	[63]
Manganese (Mn)	An essential element in humans found in food, as well as a potent neurotoxin that has been associated with Parkinson-like disease. However Parkinson Disease is inversely correlated with smoking, and there have previously been no correlations found between blood-Mn levels and smoking habits.	[32, 33]
Mercury (Hg)	The element is a threat to human health, but smoking is only a minor source exposure in humans. In fact smoking has not been found to alter the Hg levels in blood, hair, kidney cortex, liver, lungs, nor influence levels in maternal and cord blood; Hg was	[22, 32, 33]
	Hg is not partitioned into the particulate phase of smoke, only the gas phase.	[66]
	Lung damage is a potential symptom of acute exposure. Neurological and psychological symptoms (anxiety, depression, personality changes, restlessness, sleep disturbance and tremors are chronic exposure symptoms that are reversible after smoking cessation.	[22]
	Mercury compound toxicity can present toxicological characteristics including gastrointestinal, nephro- and neural toxicity, haemorrhages and ulcers.	[50]
Nickel (Ni)	The Ni content of SS may be sufficient to health of smokers and passive smokers.	[121]
	High levels of carbon monoxide in tobacco smoke, in combination with Ni content can allow for synthesis of the toxic and potentially carcinogenic compound Ni-carbonyl compound.	[32]
	Some Ni compounds have been found to be mutagenic (induce sister chromatid exchanges) in the respiratory tract. Cardiovascular and kidney diseases and lung fibrosis are potential effects of long term chronic exposure.	[33, 69]

Polonium (Po)	Po in tobacco is a source of carcinogenic ionizing radiation; it has been found in higher concentrations in bodily tissues and fluids of smokers than in non-smokers that strongly implicate Po in smoking related carcinogenesis. Po-210 contributes to radon-emitted alpha radioactivity in indoor environments by adhering to dust, settling on clothing and surfaces.	[32]
Selenium (Se)	Selenium is described as an anti-carcinogen element in animals possibly humans. By comparing the sum of elemental mass before and after smoking, it was found that most toxic metals were conserved in the particle phase. Mercury and selenium are the exception. It is believed that these elements are released into the gas phase of Se levels are lower in the blood and serum of smokers than in non-smokers, also the developing foetus of a maternal smoker may experience deleterious health effects through altered selenium metabolism.	[70, 132] [66] [32, 33]
Tin (Sn)	The element is relatively non-toxic, though organo-Sn compounds (such as Tetrabutyl-Sn) are much more toxic than inorganic forms.	[26]
Vanadium (V)	Correlations have been found in the UK for V in air pollution and incidence of respiratory disease (bronchitis, pneumonia and lung cancer). Soluble V absorbs in the lung well; once in the body it exhibits insulin-like effect on animals and humans. Acute toxicity can present as irritation of the eyes and the upper respiratory tract, with neurobehavioral impairments suspected in chronic exposure.	[99] [33]

11.3.5 *Instruments and analyses*

There is variation in the ways in which analyses are conducted and reported, and depending on the perspective and intended recipient audience of the result, communication of the concentrations or dose potentially delivered from tobacco, a cigarette or smoke can be reported in terms of relative abundance and concentration, often done by country or origin or manufacture, sometimes by brand or producer, and occasionally within a pack or down the length of a single cigarette.

The way in which the sample is analysed also provides opportunities as well as difficulties; acid digestions can produce high resolution but destroy the sample and potentially alter the state of the species, direct measurement without destructive sample preparation can lead to lack of sensitivity or sample matrix interference.

This section deals with a selection of methods that can be applied to determine the total elemental concentration and species concentrations in samples, and some examples and criticisms of the techniques (Table 8).

11.3.5.1 Direct Sample Introduction

Inductively-coupled plasma mass spectrometry (ICPMS) involves presenting a highly ionised sample to a sensitive detector. However, introduction of gaseous samples containing approximately 80% air (desirable to maintain verisimilitude with smoking behaviour) results in a mixed-gas plasma which differs from standard conditions in ways that can reduce precision and accuracy, or even extinguish the plasma torch [116].

Puff-by-puff, direct sample introduction of smoke into ICPMS has been performed with relatively good success and negligible alteration of the plasma, however a problem with quantification arises because calibration with standard aerosols is based on a very different matrix from tobacco smoke [116]. The method offers the possibility of real time puff-by-puff analysis of metals but hitherto it has not been achieved on a fully quantitative basis. This technique is also limited to determination of total elemental concentration only.

11.3.5.2 Trace element speciation

There are very few instruments that have the ability to perform trace element speciation without sample destruction or alteration, except for national and international co-operative ventures such as synchrotron facilities.

Synchrotrons provide the opportunity to perform qualitative and quantitative X-ray Absorption Near-Edge Spectrometry (XANES) and Extended X-Ray Absorption Fine Structure (eXAFS) analyses using ultra-bright, highly tuneable light sources to determine analytes within a sample without sample augmentation. This kind of analysis requires the analyte to have at least one organic-metal bond, and the sample matrix to be stable enough to be transported to the facility, and positioned in the beam line without altering during the data capture. However analyses of these toxic species in complex samples, with relevance to biochemogenic environmental routes in biological sciences, is becoming increasingly important [133].

11.3.6 *Summary*

This review of the literature on metals in tobacco spans cultivation to smoking. The origin and importance of the toxicological constituents, and their potential fate within the human body have been outlined, although not all elements will be explored further. For example, despite its importance in smoking toxicity Cd has not been subject to detailed speciation analysis by synchrotron because the strong Cd K emission lines lie at energies too high to excite using standard

beamline conditions and the lower energy L lines would be too weak for samples containing low- or sub-ppm concentrations. Thus the focus of the analytical work presented in this study is on primarily on As with some investigation of Cr, both being important and multivalent elements in their natural occurrence.

Table 8. Review of some instrumentation and techniques used for trace element analysis of plant materials.

METHOD		**COMMENT**	**REF**
Non-destructive techniques			
	INAA	*Instrumental neutron activation analysis*: A sensitive and versatile method for multi-elemental analysis (not speciation) including biological matrices such as cigarette components; however elements such as Cd and Pb do not have easily measurable isotopes, and the high instrumentation costs make the technique unattractive for routine analyses.	[16, 29, 91]
	X-ray spectroscopy	Various techniques detailed below to record information on concentration and species within biological samples that contain sufficient concentrations in situ. Solid samples are generally used.	[134]
	ED XRF	*Energy dispersive x-ray fluorescence*: Rapid, reproducible, sensitive, versatile and relatively cheap (when using radioisotope instead of X-ray tubes) and requiring little sample preparation, but may not always be sufficiently sensitive for trace analysis in biological samples.	[34, 76, 91]
		Cellulose matrix interference (which dominate absorption correction, background and scatter) may be a major problem in plant samples (such as tobacco leaf) unless polarized X-ray sources are used	[76]
	EXAFS	*Extended x-ray absorption fine structure*: Provides data on the immediate chemical environment around a target element (inter-atomic distances, indicating which atoms are bound to the target element) for comparison with reference standard compounds. The technique does not require separation or pre-concentration of analyte species, and can be conducted without alteration of the sample (a development over species extraction and/or chromatography). Synchrotron radiation (SR - bright, high energy, tuneable X-ray light sources) is required for this and similar techniques, and beam-time is currently relatively exclusive and highly expensive compared to laboratory based instruments.	[134, 135]
	SR-μCT	*Synchrotron radiation x-ray computed micro-tomography*: determine the variation in elemental concentration along three dimensions, permitting observation of the internal structure or composition of a specimen without sample preparation or need to produce thin-sections (thus avoiding alteration of species composition or generation of artefacts. The technique does however require beam-time at an SR facility, and is therefore currently not ideal for routine analyses.	[135]
	XANES	*X-ray absorption near edge structure:* An in situ solid phase analysis technique that can be used to produce some qualitative and quantitative chemical state information from target elements in plant materials. Requires beam-time at an SR facility, and is therefore currently not ideal for routine analyses.	[134, 135]

Destructive techniques			
	Acid digestion	Organic samples are digested using acids in order to bring the analyte into solution (sometimes called extraction depending on technique and level of analysis), usually with some reaction acceleration (i.e. temperature/ microwave). Severe acids such as hydrogen fluoride (HF) are necessary to dissolve silicates (usually in bulk concentration analyses). Limits of quantification (LOQ) can be very low; however some escape of volatile elements and compounds can occur from high temperatures acid digestion in open vessels (loss of analyte).	[24, 136]
		Digestion and extraction techniques can involve several steps; usually starting with dilute acid digestion followed	[28]
		Ideal digestion would involve total dissolution of a sample, complete extraction into the aqueous phase, with minimum addition of acids and solvents, in a short timescale and without alteration of the redox conditions or species of the analytes.	[29]
	AAS	*Atomic absorption spectrometry*: A chemical flame technique for determination of bulk concentration (some speciation capacity with coupled techniques), that suffers from low sensitivity for trace analysis in biological sample.	[91]
	CPE	*Cloud point extraction:* A technique developed to separated and pre-concentrate trace metal ions; advantage include high efficiency, lower toxicity and simplicity however it has not been widely adopted in trace elemental analyses of plant sample.	[28]
	ETAAS	*Electrothermal atomic absorption spectrometry:* Has been applied successfully in determining a range of elements in many components of cigarette before and after smoking.	[29]
	ICPAES	*Inductively coupled plasma atomic emission spectrometry:* A non-chemical flame technique with good sensitivity for bulk elemental analysis, but with limited capacity for speciation and high running costs.	[91, 137]
	ICPMS	*Inductively coupled plasma mass spectrometry:* A technique with excellent sensitivity for rapid multi-elemental quantification. Complex artefact or saline matrices can affect the LOQ and produce interferences that can affect data quality, therefore complex extraction and digestion procedures may be required before analysis. Often combined with other instruments to produce hyphenated techniques (e.g. HPLC-ICPMS) for speciation analyses. The technique has been applied to 'puff-by-puff', direct introduction of cigarette smoke for several toxic elements, though mixed-gas can disrupt the plasma and reduce sensitivity and confidence in the data (reference gases will lack verisimilitude to complex gas-smoke mixtures).	[91, 116, 137] [116]
	HPLC-ICPMS	*High performance liquid chromatography inductively coupled plasma mass spectrometry:* A technique for qualitative and quantitative speciation that is robust and sensitive for aqueous samples of digested and extracted and biological samples. Chromatography, high resolution mass analysers and reaction/collision cell technology have been developed to tackle spectral interferences, though chromatographic comparison is heavily dependent on reference standards (for retention time matching) as no structural information is generated (as with X-ray techniques) restricting the identification of novel compounds	[134]
	HT-GC-ICPMS	*High temperature gas chromatography inductively coupled plasma mass spectrometry:* Has been used to analyse organometallic compounds in crude oil, but is not widely adopted for trace or speciation analysis in plant sample.	[133]

	RPIP-HPLC	*Reversed-phase ion-pair chromatography high performance liquid chromatography:* An efficient and rapid separation method for qualitative species analyses in complex biological matrices with versatility elution conditions compared to ion-exchange chromatographic techniques.	[113]

11.4 Compound Speciation by Coupled HPLC-ICPMS

11.4.1 *Background*

This study focuses on arsenic, one of the four heavy metals currently proposed for regulation [2] and addresses the variability in chemical speciation of arsenic in 14 tobacco products sampled from a wide range of geographical localities and As levels. Studies of the risks associated with dietary exposure to arsenic tend to emphasise two aspects of its speciation, namely the chemical species (primarily whether present as organic and/or inorganic compounds) and oxidation state (whether mainly As(III) or (As(V)). There is now a large body of evidence that implicates inorganic As(III) species in human toxicity associated with exposure to arsenic in the gastrointestinal tract, however the risks of exposure to the different arsenic species during inhalation are less well characterised [138, 139]. Nevertheless long-term, low-dose exposure to inorganic arsenic by whichever pathway is implicated in increased mutagenesis [138] and habitual smoking may be an example of such exposure. The fraction of total arsenic released into smoke during tobacco combustion is in the range 9-16% [78] suggesting that 100s ng As could be transferred per cigarette although measured quantities in machine smoking using low arsenic products indicate the transference of just a few ng per cigarette [80, 81].

A method for characterising arsenic species in tobacco using HPLC-ICPMS was recently published [8, 9]. These authors focused on method development and produced results for As speciation in the US Reference Cigarette 3R4F. They extracted tobacco from 3R4F and found that 89% of the total water-soluble arsenic is inorganic, dominantly in the As(V) oxidation state with indications of minor quantities of organic species including dimethylarsinic acid (DMA) and monomethylarsonic acid (MA). These studies also investigated the speciation of arsenic in smoke condensate using both HPLC-ICPMS and XANES although speciation results were less clear due to very low levels of arsenic compounds in the condensate. A key question is whether these results for US Reference Cigarette 3R4F can be extrapolated to global cigarette tobaccos and we address this question using tobaccos chosen to represent the typical range of arsenic concentrations sampled from a range of global localities and analysed using a similar HPLC-ICPMS methodology. Another aim is to test whether any predicable relationships exist among the arsenic species that could contribute to risk assessment on a global scale.

11.4.2 *Methods*

11.4.2.1 Samples

14 tobacco samples were selected for study including certified reference materials (CRMs), authentic commercial US, UK and Chinese cigarette brands, and counterfeit cigarettes seized by UK Customs chosen as they were known to be rich in As ([79]. These samples and their geographical (where known) and concentration ranges are shown in Table 9.

11.4.2.2 Quality control and assurance

CTA-OTL-1 and CTA-VTL-2 are certified reference materials for trace elements in Oriental and Virginia tobacco leaves and their As values are given in Table 9 [30, 140, 141]. GBW08514 and GBW08515 are Chinese tobacco reference standards

for elements but not including As. 1R4F and 1R5F are reference standards typical of US low tar (1R4F) and US ultra-low tar (1R5F) cigarettes [142], produced for smoking experiments and do not have certified total As values. No certified reference materials for As speciation in plant materials had been formally validated at the time the analyses were conducted.

Table 9. Cigarette reference materials (CRMs) and samples selected for As species determination with corresponding sample codes and comments on origins. Certified values for the CTA standards [140, 141], others are information values determined for this study by X-ray fluorescence spectrometry [30].

SAMPLE		RATIONALE FOR SELECTION	As(mg kg^{-1})
REFERENCE MATERIALS			
	CTA OTL-1	Bulgarian Oriental tobacco certified standard	0.539±0.060
	CTA VTL-2	Bulgarian Virginia tobacco certified standard	0.969±0.062
	1R4F	Research Cigarette typical of US low tar blends	0.4
	1R5F	Research Cigarette typical of US ultra-low tar blends	0.8
	GBW08514	Chinese tobacco standard	1.2
	GBW08515	Chinese tobacco standard	0.6
COMMERCIAL BRANDS			
	B-1	Major US brand	0.7
	B-2	Major UK brand	0.5
	B-3	Major UK brand	0.5
	B-4	Major UK brand	0.1
	B-5	Major Chinese brand	0.9
ILLICIT (COUNTERFEIT) PRODUCTS			
	B-6	Unusually high arsenic	3.4
	B-7	Unusually high arsenic	3.3
	B-8	Higher than normal arsenic	1.8

11.4.2.3 Elemental and extract arsenic concentrations

Sample preparation was a modification of an established procedure [143]. Sample (0.250 g) was weighed into Teflon vessels (DAP-80s, Berghof GmbH, DE), with 10.0 ml nitric acid (70% v/v) and the reaction accelerated in a pressure- and heat-controlled microwave digestion system (Speedwave MWS-3+, Berghof, DE) programmed to ramp temperature from 120 to 170 °C over a 55-min cycle, pressure limited to 30 bar. Samples were then diluted to 0.250 l (a total dilution factor of 1000) with double de-ionised water (Q-gard 1 Gradient A10, Millipore, FR). An aliquot of 5.0 ml of the sample was pipetted into disposable ICP-MS vials together with 5.0 ml of a standard solution containing 25 µg l-1 germanium (Ge), 5 µg l-1 indium (In) and 50 µg l-1 rhenium (Re) ICP-MS single element standards, Inorganic Ventures Inc., US). Analysis was performed using an ICP-MS (X-Series 2, Thermo Scientific Corp., UK)

quadrupole mass spectrometer with collision cell technology using kinetic energy discrimination (CCTED) to determine As (75 m/z), Ge (72 m/z), In (115 m/z) and Re (185 m/z).

Extract sample preparation involved centrifuging samples and a 5.0 ml aliquot was pipetted together with 5.0 ml of a standard solution containing 25 µg l-1 Ge, 5 µg l-1 In and 50 µg l-1 Re into disposable ICP-MS vials for the analysis.

11.4.2.4 Arsenic species concentrations

Species extraction sample preparation involved weighing 0.200 g of sample for digestion over 24 hours in 10.0 ml nitric acid (1% v/v) in 50.0-ml centrifuge tubes (a dilution factor of 50). A microwave reaction accelerator system (MARS CEM, Matthews Inc., US) was utilised, programmed to ramp temperature from 55 to 95 °C over a 65-min cycle [144]. Samples were then frozen to limit the transformation between species, and defrosted 24 h before analysis to allow the sample to reach room temperature. Samples were centrifuged at 15 kG for 10 min, and 0.50 ml of supernatant was pipetted into HPLC-ICP-MS vials with 0.050 ml hydrogen peroxide (H2O2) for the analysis. Analysis was performed using HPLC (Agilent 1100 series, Agilent Technologies Inc., DE) fitted with an anion-exchange column (250 by 4.6 mm PRP-X100 10 µm, Hamilton Company, CH & US) [144]) with a pH 6.2 balanced ammonium nitrate (NH4NO3)/ammonium di-hydrogen-phosphate (NH4H2PO4) buffer solution, that was connected post-column to an ICP-MS (Agilent 7500) [145] via a Teflon t-piece, directly to the nebulizer to determine As, Rh (103 m/z) and Se (77 & 82 m/z).

Terminology and definitions for chemical species follow established terminology [5, 134].

11.4.3 *Results*

11.4.3.1 Reference standards and quality control

Analytical recoveries for total As in the Certified Reference Materials CTA-OTL-1 (certified values = 539 ± 60 µg As kg-1) and CTA-VTL-2 (certified value = 969 ± 62 µg As kg-1) were 113 ± 7% and 104 ± 4%, respectively (n = 2) (Table 10). The limit of detection for total As by ICP-MS was 21 µg kg-1 determined by mean plus three standard deviations of the blanks (n = 3). There are no certified values for As in reference tobaccos GBW 08514 and GBW 05815, and reference cigarettes 1R4F and 1R5F. Accuracy of the total arsenic determinations is indicated by good agreement with certified values of the reference standards CTA-OTL-1 and CTA-VTL-2 supported by good precision indicated by low standard deviations for these standards (Table 10).

11.4.3.2 Arsenic and extract concentrations

Total arsenic in these 14 samples ranges from 144 to 3914 µg As kg-1 (median = 538; n = 14), with total As concentrations in extracts ranging from 82 to 1791 µg As kg-1 (median = 257; n = 14) (Table 10).

Table 10. Concentrations of total As, DMA, MA, inorganic As, sum of extracted As species and extract total As in six reference tobacco and six commercial samples (legal and illegal), with extraction efficiencies (∑ As sp./ Totals) and column recovery (∑ As sp./ Extracts)

	Total As		Species DMA		MA		Inorganic As		Σ As Spec	Extracts As		Extract efficiency	Column recovery
Sample	**µg kg^{-1}**	**n**	**µg kg-^{1}**	**n**	**µg kg^{-1}**	**n**	**µg kg^{-1}**	**n**	**µg kg^{-1}**	**µg kg^{-1}**	**n**	**%**	**%**
CRMs													
CTA OTL-1	611 ± 41	2	31 ± 1	2	B.D.L.	1	127 ± 8	2	158	304 ± 3	2	25.9	52.0
CTA VTL-2	1008 ± 39	2	54 ± 6	2	B.D.L.	1	256 ± 17	2	310	569 ± 59	2	30.8	54.5
1R4F	465 ± 3	2	24 ± 4	2	B.D.L.	1	96 ± 2	2	120	209 ± 3	2	25.8	57.4
1R5F	318 ± 10	2	37 ± 5	2	B.D.L.	1	78 ± 26	2	115	154 ± 8	2	36.2	74.7
GBW 08514	656	1	26	1	30	1	112	1	168	310	1	25.6	54.2
GBW 08515	429	1	23	1	B.D.L.	1	62	1	85	191	1	19.8	44.5
Samples													
B-1 (legal)	443	1	39	1	B.D.L.	1	79	1	118	192	1	26.6	61.5
B-2 (legal)	191	1	23	1	B.D.L.	1	39	1	62	113	1	32.5	54.9
B-3 (legal)	317	1	21	1	B.D.L.	1	66	1	87	162	1	27.4	53.7
B-4 (legal)	144	1	B.D.L.	1	B.D.L.	1	16	1	16	82	1	11.1	19.5
B-5 (legal)	816	1	49	1	33	1	218	1	300	409	1	36.8	73.3
B-6 (illegal)	3914 ± 90	3	150 ± 15	4	45 ± 12	4	948 ± 36	4	1143	1791 ± 47	4	29.2	63.8
B-7 (illegal)	3504	1	176	1	116	1	846	1	1138	1777	1	32.5	64.0
B-8 (illegal)	2339	1	120	1	42	1	487	1	649	1180	1	27.7	55.0

11.4.3.3 Species concentration analysis

DMA concentrations ranged from 21 to 176 µg kg-1 (median = 37 µg kg-1; n=13), MA from 30 to 116 µg kg-1 (median = 42 µg kg-1; n=5), and inorganic As from 16 to 948 µg kg-1, (median = 104 µg kg-1; n=14) (Table 10, Fig. 3). Across all samples, the proportion of extractable As species ranged from 11.1 to 36.8% (Table 10), and column recoveries ranged from 44.5-74.7%.

Estimating species concentrations using chemical extraction methods suffers from potential inaccuracy due to incomplete extraction recoveries, and this study was hampered by the lack of an accepted speciation reference standard for As in plant material. Notwithstanding, the analytical procedure has been validated and compares favourably with other extraction techniques [146]. Another technical difficulty involving the calculation of species concentrations from the spectra, due to the coelution of the As(III) and DMA elution peaks, was overcome by oxidizing As(III) to As(V) by the addition of H2O2 [144, 147]. The addition of sufficient to excess H2O2 converts all inorganic As(III) in a sample to As(V) with no degradation of organic arsenicals MA and DMA [148]. As(III) elutes at the ejection front, and As(V) much later in a distinct peak; oxidization also enables inorganic As (As(III) and As(V)) to be differentiated from cationic species which, if present, would also elute with the solvent front [149]. Broadly similar results for inorganic As were obtained for 1R4F (this study, Table 10 & Fig.3) and 3R4F made with a similar blend of tobacco types [9].

11.4.3.4 Bulk arsenic concentrations

As concentrations in the reference standards and legal samples of this study range from 144 - 1008 µg kg-1 (median = 443; n = 11) and are similar to those in the literature [79, 150]. They indicate that the tobacco plants were probably cultivated in conditions largely uncontaminated with As (Table 10). In contrast the plants used to make the three illicit (counterfeit) products have much higher As concentrations (2339 – 3914 µg As kg-1; median = 3504, n = 3) and were probably cultivated on soils quite heavily contaminated with arsenic due to natural enrichments in the soil, addition of contaminated fertilisers such as sewage sludge, and/or treatment with arsenical pesticides

11.4.3.5 Arsenic species concentrations

These new data demonstrate that organic As species are present in minor concentrations compared with inorganic As species in all 14 tobacco samples, as shown graphically in Fig.4, extending the earlier finding on 3R4F [9] to a much wider range of reference tobaccos and publicly-consumed products. Also notable is that the same pattern applies in high arsenic samples known to be counterfeit (B6-B8 in Fig.3). Total arsenic in these samples varies by a factor of over 20 yet the relative inorganic:DMA:MA concentrations remain relatively consistent.

While As was found to be present principally in inorganic form with minor DMA and MA contributions, As-thiol complexes may represent a significant proportion of the species but conditions were not setup in advance to extract or detect such complexes [151].

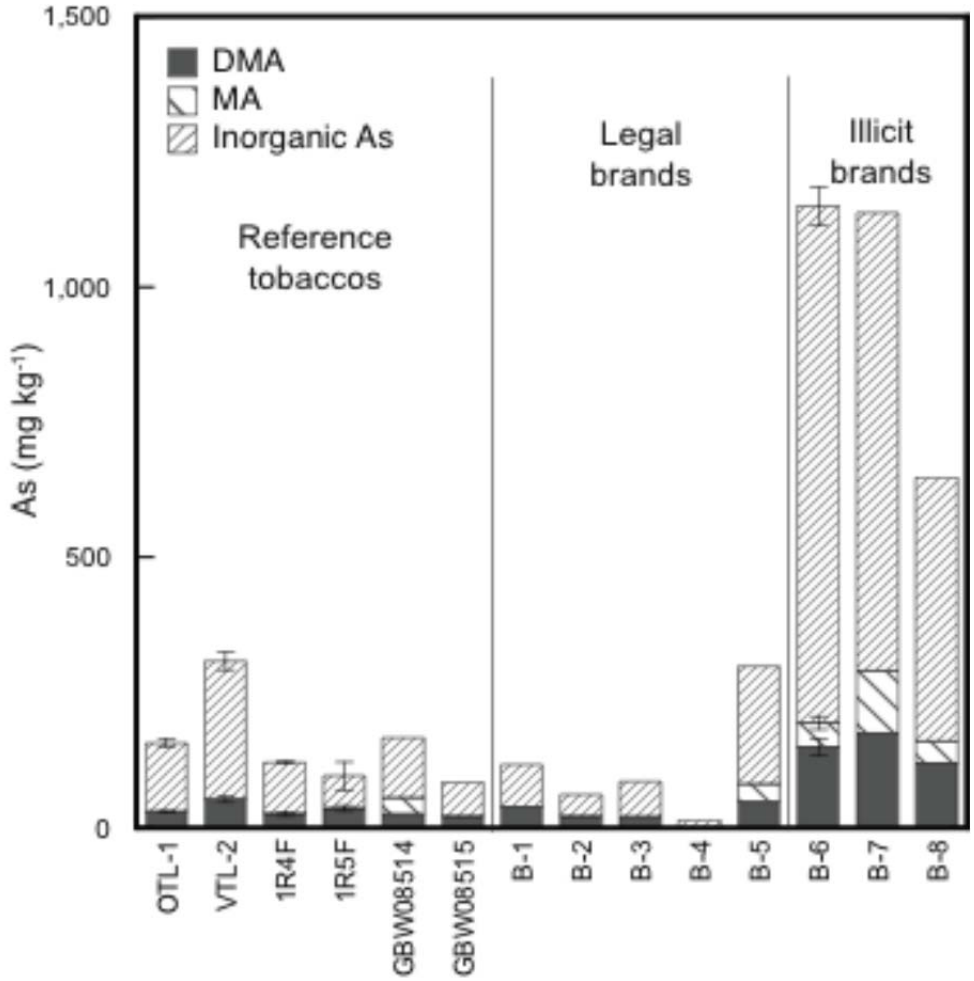

Figure 3. Concentrations of DMA, MA and inorganic arsenic in six reference tobaccos and eight tobacco products (legal and illicit). Error bars set at one standard deviation

11.4.4 *Discussion and Conclusions*

11.4.4.1 Relationships between arsenic species

The 14 samples analysed in this study span the whole concentration range normally encountered in cigarette tobacco [2] and cover a wide geographic range (US, EU, China) yet there is remarkable consistency in the fractions attributable to inorganic and organic (MA+DMA) arsenic species. Fig.4 shows how these vary with total sample As, with the slopes of the regression lines indicating that inorganic As is present at about four times the sum of the measured organic species (MA+DMA), a ratio maintained in samples with greatly elevated levels (as in B6-B8 counterfeit products). This lends confidence to the use of counterfeit samples in the study of As in tobacco in experiments where analytical instrumentation fails to detect As at more "normal" concentrations.

MA concentrations are generally lower in these samples (but not significantly) than those of DMA, or are below detectable limits. Overall the slopes of the regression lines indicate that approximately 80% of the arsenic species detected in these tobaccos is in inorganic form with the remaining 20% present as organic species (DMA and MA)

MA and DMA in plants is thought to be derived from soil rather than from in planta metabolism [152], as plants appear not to methylate inorganic As, unlike animals, bacteria and fungi [153], though methylated As species are readily translocated to the shoot once assimilated through the root system [150]. Low methylated As content in tobacco samples indicates low As methylation rates in the original growing environment. Relative concentrations of methylated species and total inorganic As species within the reference standards are generally stable, potentially due to growing environment conditions that favour inorganic As bioavailability to the plants (treatment with sewage-based nitrate or phosphate fertilisers), or application of arsenicals directly to the plant (perhaps atmospheric deposition of inorganic dust on leaves). As the presence of biological agents can affect the methylation of soil As, and thus passage into the food chain, as well as amount and source of the contaminant, soil properties, and the magnitude and rate of plant uptake and/or extent of absorption by animals [21] it is important to understand these factors in terms of As migration through both food chains and smoking in contributing to human exposure [46]. Soil has the capacity to buffer the effects of contaminants by binding these agents to soil constituents, or chemically converting them to inactive, insoluble or biologically unavailable forms. These factors alone make for complex dose response relationships in crops [46] and may provide a strategy for reducing the total As, and more specifically the inorganic As available to the plant, and therefore to the receptor.

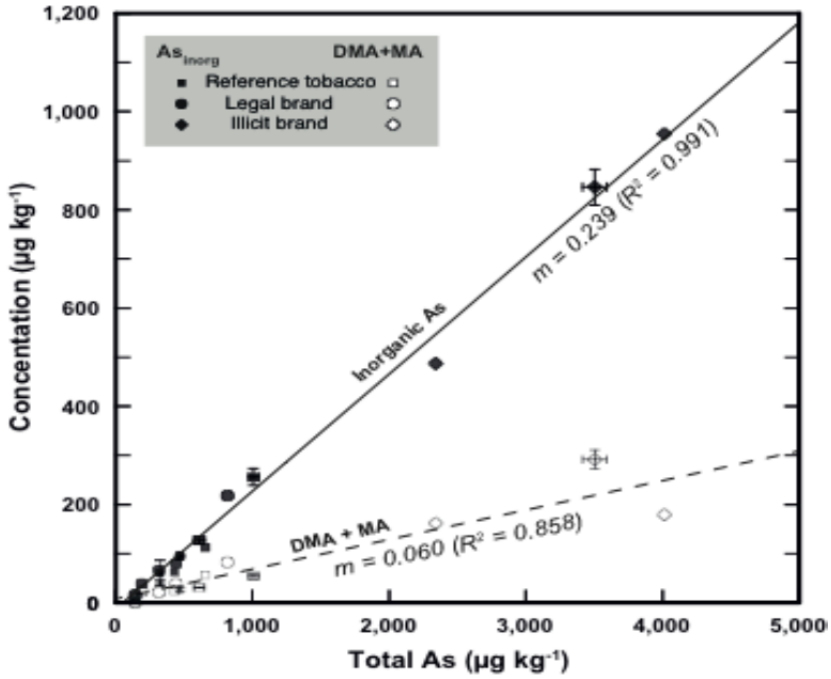

Figure 4. Regressions of extractable inorganic arsenic and DMA+MA against total arsenic in six certified reference materials, five legal cigarette brands from the US, UK and China, and three counterfeit samples. Regression lines are significant at ρ=0.05, and slopes (m) are indicated (both pass through the origin within error).

11.4.4.2 Health implications

Human toxicity symptoms associated with exposure to arsenic include cancers of the lung and skin, and cardiovascular, gastrointestinal, hepatic and renal diseases [2, 138]. Our findings on the prevalence of inorganic As species over organic forms, the former being the more toxic and persistent in the human body, could be directly relevant to gastrointestinal exposure due to the use of oral tobacco. As for smoking, taking the arsenic concentrations present in the tobacco samples used in this study, we calculate that a potential dose ranging from 6 to 505 ng per cigarette (based on 0.7g cut tobacco in a single cigarette) could be transferred to the respiratory system under standard ISO smoking conditions using published transference factors [78]. Smoking is a complex process and the components of tobacco are not necessarily transferred unchanged to the lungs; As being potentially released as particulates or other species generated by the processes of pyrolysis and pyrosynthesis [154]. These new compounds may be more or less toxic than their precursor compounds, depending on redox conditions and other factors during combustion and smoke ageing. A possible insight comes from the combined HPLC-ICPMS and XANES a study of 3R4F [7, 8]. The XANES spectra in this paper were difficult to model due to the low As concentration in their sample, however they were able to demonstrate the presence of As(III) in smoke condensate which indicates that arsenic is being reduced in the burning coal, at least in 3R4F. Further studies are required to identify the compositions and concentrations of As species that are quantitatively transferred into smoke and their persistence.

Tobacco is grown in over 120 countries [155]. The available data suggest that arsenic concentrations in tobacco used to manufacture cigarettes varies with geographical region. Comparing China and United States, the largest producers of tobacco leaf in the developing and developed world respectively [155], a recent study estimated a mean value for arsenic of 0.29 mg kg-1 (standard deviation 0.04) in tobacco extracted from 50 samples of popular US cigarette

brands [156], whereas the mean for 47 samples of popular cigarette brands in China is 0.85 mg kg-1 (standard deviation 0.73) [157]. These arsenic levels are significantly different ($\rho<0.001$) suggesting that smokers in these countries may be exposed to very different levels of arsenic. If, as this study indicates, most of this arsenic will be present as an inorganic species possibly in reduced state, any risk of smoking-related disease due to arsenic exposure would appear to be considerably greater in China, a nation that is home to a quarter of all the world's smokers [158].

If arsenic is to be regulated by concentration in crops and commercial products, as has been recommended [2], then there must be strong scientific evidence that it is likely to expose smokers and other users of tobacco to hazardous levels of toxic species of arsenic. The evidence of the 14 samples in the present study combined with previous studies of reference cigarette 3R4F [7-9] indicate that smoking does involve exposure to arsenic much of which is present in smoke as inorganic arsenite species. These findings provide strong support for regulation although better information is required on the valence states of arsenic in tobacco smoke and the inhalation toxicology of arsenic in humans.

11.4.4.3 Conclusions

The principal organic arsenic species detected in the 14 tobaccos analysed were DMA (detected in 13 samples) and MA (detected in 5 samples). Both organic and inorganic species increase with total arsenic concentration and regression analysis indicates that Inorganic forms of arsenic dominate over all organic arsenic species (DMA+MA) by a factor of 4:1 in tobaccos sampled over a wide range of compositions and geographical regions.

Given that inorganic arsenic is considered to be more toxic to humans than organic forms this has consequences for the health of users of oral tobacco and, depending on changes that may occur during combustion, also for the health of smokers.

A consequence of large regional differences in arsenic concentrations in tobacco may be regional differences in degree of exposure and risk to health, China being notable for the high levels of arsenic in many of its cigarette brands.

The evidence on arsenic speciation in relation to health risks presented in this paper strongly supports the recent proposal to regulate arsenic in tobacco crops and products [2].

11.5 Valence Speciation Using XANES

11.5.1 *Introduction*

According to the World Health Organisation (WHO) tobacco smoke contributes to the deaths of about half its users[23] yet the individual and combined effects of the components of smoke responsible for the various smoking-related diseases are not fully understood. A recent EU-sponsored review identified 98 compounds in tobacco smoke for which risk factors are available [1] among which are seven metals or metalloids including arsenic (As) and chromium (Cr). The US Food and Drug Administration, recently given responsibility for the regulation of tobacco products, included arsenic and chromium in a slightly different group of elements in its list of "harmful and potentially harmful constituents in tobacco products and tobacco smoke" [3]. The Tobacco Product Regulation Study Group (TobReg), an expert panel advising the WHO, reviewed the available scientific evidence of harm caused by metals and metalloids in tobacco smoke before recommending that "tobacco purchased from each new agricultural source [be tested] for levels of arsenic, cadmium, lead and nickel" and that tobacco blends offered for sale be tested for concentrations of these elements [2]. These reviews invariably identify arsenic in tobacco as hazardous, however due to the paucity of available studies none of these reviews takes the speciation of arsenic in smoke into account, whether it is transferred in elemental or compound form, inorganic or organic, and the valence state(s) (trivalent/pentavalent) on combustion and transfer to the respiratory tract. Similarly chromium exists in nature in multiple valence states, notably Cr(III) and Cr(VI) but only the hexavalent form is highly toxic to humans. These factors are fundamental to assessing the hazard given that a large body of toxicological research identifies inorganic As(III) and Cr(VI) species as highly toxic to humans based on ingestion studies [138]. Exposure to arsenic through inhalation is less well studied and the effects of species on the risk to humans is not yet known, although there is evidence that As(III) causes tumours in rats [138].

Here we use a synchrotron approach to establishing the valence state of the two tobacco smoke carcinogens that exist in multiple valence states with very different cancer risks.

The concentration of As in tobacco ranges from less than one µg g-1 to a few µg g-1, while up to a few tens of nanograms per cigarette are present in mainstream smoke [23]. Estimates of mass transference of As from tobacco to smoke fall in the range of 7-18% [78]. Such levels could pose a risk to smokers' health if As is dominantly present at the point of human exposure in its inorganic and/or reduced state.

The aim of the present study is to determine the speciation of As delivered to the smoker, in order to inform those assessing the risks of individual smoke components. More specifically the objective is to characterise the valence of As along its pathway from the harvested plant, through curing to the smoking product, and in particular through the combustion event during smoking that creates the respirable aerosol. The analytical approach uses X-ray Absorption Near Edge Structure (XANES) spectra obtained using synchrotron radiation. Unlike HPLC-ICPMS and most other speciation techniques XANES can be applied in situ without requiring chemical extraction and the associated risk of modifying speciation can be monitored

Past attempts to achieve this aim using XANES have been largely thwarted by insufficient sensitivity of available synchrotrons to characterise the valence state of As in smoke products at low As concentrations. Liu and colleagues in series of papers linked their XANES results on tobacco and smoke condensate with speciation studies using HPLC-ICPMS [7-9]. The weak As response in some of their XANES spectra led to uncertain conclusions concerning the valence of As in

smoke. Notwithstanding, the impression given by the most recent literature is that the reduced and potentially more toxic forms of As are minor and transient species in tobacco smoke products, and that any As(III) present is rapidly oxidised soon after generation to As(V) forms [8]. If this is correct then As in tobacco may not be sufficiently hazardous to engage the interest of regulators, however there is currently sufficient uncertainty to warrant the collection of higher resolution XANES spectra on a much wider variety of samples.

Here we adopt a two-fold approach to overcoming the technical difficulties besetting earlier studies. Firstly, use of a third generation synchrotron (Diamond, the UK's national facility) offers a considerably brighter source of X-rays generating much stronger signals at lower concentrations of As than earlier studies. Secondly tobacco burdened with elevated concentrations of As has been successfully cultivated for use in smoking experiments, taking care to ensure verisimilitude with products available for public consumption.

11.5.2 *Methodology*

11.5.2.1 Materials

KT209 seed was supplied by F.W Rickard Seeds, INC., USA. Toxic chemicals required for burdening and for synchrotron characterisation (As2O3, As2O5, NaAsO2, HNa2AsO4) were supplied by Sigma Aldrich. Arsenopyrite (FeAsS) is a mineral phase often found in soils that nominally has an oxidation state of -1. The standards were pressed into 13 mm pellets using a 6-ton die press.

Tobacco materials analysed include 1R4F (Kentucky University low tar reference cigarette widely used in smoke testing laboratories), CTA-VTL-2 is a trace element reference standard of Virginia tobacco with a recommended value of 0.969 ±0.072 µg/g As, and distributed by the Institute of Nuclear Chemistry and Technology, Warsaw, Poland. STA336 and STA486 are two commercial cigarette samples, STA336 being a major brand purchased in the USA with a low As concentration, and STA486 a brand purchased in China where As levels are sometimes elevated [157].

11.5.2.2 Cultivation of As burdened KT209 tobacco

KT209 tobacco seeds were planted into propagators containing John Innes seeding compost with coatings intact and allowed to germinate and develop to transplanting maturity under controlled greenhouse conditions of 26°C and 60 % relative humidity (relative humidity) over a 12 hour artificial day, and 18 °C and 30 % relative humidity overnight with regular watering. The mature seedlings (approximately 8 weeks) were then transferred into growth bags containing an equal mixture of John Innes No.1 and John Innes Ericaceous composts for the As experiments. Plants were maintained with adequate soil moisture content, removal of senesced leaf material, and removal of flowering heads or removal of non-viable plants, for 120 days when they were harvested. Arsenic was added to the compost in solutions containing various concentrations of sodium arsenate and sodium arsenite, together and separately (Table 11).

Plants stems were cut 2 cm from the base to minimise soil contamination, and washed (except for RCS102 and RCS108) in deionised water. Tobaccos generated as unadulterated powders (i.e. no humectant) were dried at 75 °C (RCS102, RCS108, RCS125) then frozen at -18 °C to limit alteration to species; two tobaccos (RCS120, RCS123) were air-cured at 10 °C, ~50 % relative humidity beforehand. Curing conditions for cigarette tobacco (RCS132, RCS133) were reproduced in a heating cabinet providing a temperature of c. 50 °C and 60 % humidity for 14 days.

Figure 5. Montage of metal-burdened tobacco plants grown under controlled greenhouse conditions at St Andrews University. Plants were harvested after 120 days growth and cured in a drying cabinet.

11.5.2.3 Smoking experiments

Cured tobacco was maintained at 22±2 °C and 60±5 % relative humidity before being shredded with a hand operated, table mounted mechanical device to achieve a strip width of about 1-2 mm, and sprayed with 1,2-Propanediol (99.5 % ACS puriss, Sigma-aldrich, UK) 7 % fresh weight (fresh weight of tobacco conditioned at 22 °C, ~65% rel. hum.). Protocols for making and machine-smoking hand rolled cigarettes have proved difficult to establish but as the purpose was to determine the relative changes in oxidation state rather than measure concentrations in emissions this was not considered a significant problem; no equivalent ISO protocol currently exists.. Approximately 1000±100 mg of the resulting tobacco was loaded into premade cigarette tubes (Make Your Own brand, Imperial Tobacco) using a small commercial device for home fabrication of cigarettes. This machine ensured reasonably consistent packing of tobacco into tubes. The reference cigarette 1R4F was stored under ISO recommended conditions of temperature and humidity leading up to the smoking experiment (ISO 3402) [159].

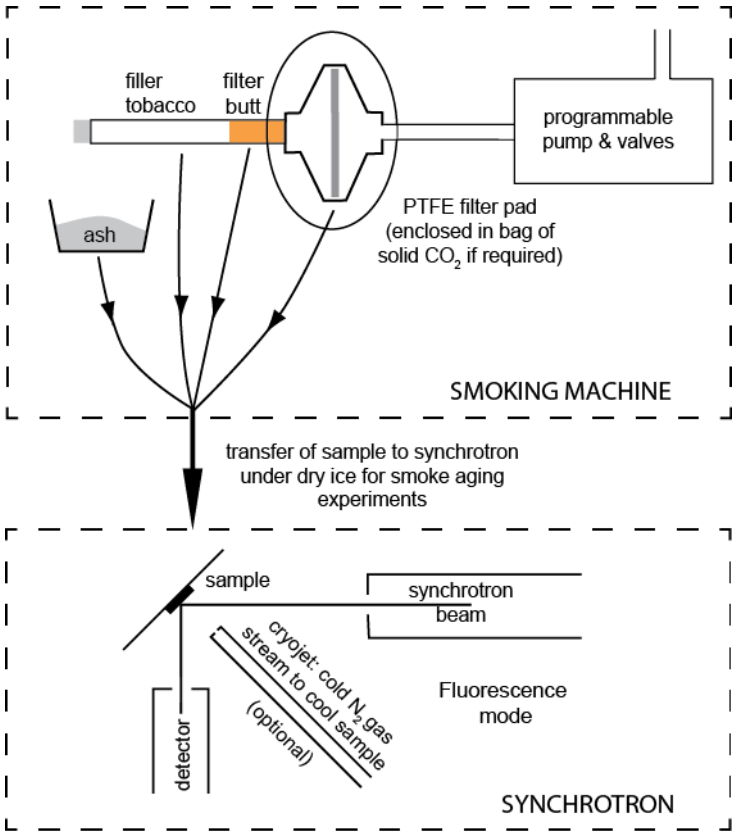

Figure 6. Schematic of the experimental setup.

Smoking experiments were carried out using an in-house smoking machine constructed at RIVM, Bilthoven, Netherlands. This machine was programmable for various machine smoking protocols, adhered to ISO 7210: 1997 where possible (ignition with a standard propane lighter, 47mm. Conventional Cambridge filter pads proved unsuitable as XRF analysis of their bulk composition showed this batch to be contaminated with low levels of As, as well as substantial levels of some other metals [160]. Whatman PTFE 0.45 µm pore filters of 47 mm diameter proved to be below the limit of synchrotron detection (e.g. 100 ppb) and was used to collect smoke particulates. It was found that transmission of smoke was limited because hydrophobic PTFE induced smoke to escape around the outer edge of the filter. To overcome this, minute holes (100-250 µm) were pierced in the filter membrane using As-free optical glass needles that allowed the passage of smoke through all parts of the filter. As was easily detected by the synchrotron in smoke condensate deposited around these perforations.

The smoking machine was sited in a laboratory a few metres from the beamline to minimise the lag time between smoking and XANES spectral acquisition, during which sample modification may occur. In addition there was a facility to collect filter samples for transfer from the smoking machine to the beamline under dry ice in order to limit the potential for atmospheric oxidation. Fig.6 is a schematic of the set-up used for smoking experiments.

11.5.2.4 Synchrotron

All standards and samples were analysed on beamline I18 of the Diamond 3rd Generation Synchrotron facility[161] at Didcot, Oxfordshire, UK (www.diamond.ac.uk).

Diamond (Fig.7) is a 3 GeV synchrotron and measurements were taken with a typical beam current of 250 mA. Beamline I18 receives x-rays from an insertion device and the beamline employs a Si(111) monochromator, the radiation from which was focused by Kirkpatrick-Baez mirrors into a ~3 µm diameter spot. The surface of the sample was 45° to the incoming beam and the fluorescence detector was perpendicular to the beam (see electronic Appendix). XAS data were collected on the As K-edge to include the Near Edge (XANES) region only. The response of the sample was measured as As K-alpha X-ray fluorescence using a VORTEX 4-channel X-ray detector with dedicated fast-counting electronics, and the final response is the sum of all channels. The monochromator energy calibration was checked with an Au foil (11,919 eV) and the measurement of an As_2O_5 standard was repeated at the beginning of every session to check for reproducibility. The final data are the averages of up to 50 cycles (depending on concentration) and each cycle typically took 5-9 minutes to acquire.

The samples were leaf fragments, pelleted powders (ground leaf, shredded leaf and ground ash), condensates and filters. These were presented to the beam on Kapton® film or occasionally on high-purity quartz slides. Analyses of As(III) standards at room temperature showed modification of the spectra with time (20-30 min), inferred to be due to X-ray induced oxidation, therefore experiments were performed at cryogenic temperatures. Initially this was performed in a continuous flow nitrogen cryostat (Oxford Instruments MicrostatHiResII) with the sample mounted in vacuum and cooled by conduction and a Kapton® window for the x-rays. Under such conditions, no sample modification of As(III) standards was observed [162]. Similarly smoke condensate with very low As concentrations showed no observable changes in edge position with exposure to the beam (experiments described later).

Figure 7. Diamond Light Source near Oxford, UK.

We also compared data from the continuous flow cryostat with results of analysis under a Cryojet (Oxford Instruments) in which a flowing jet of cold nitrogen gas close to 77 K is directed at the exposed sample surface. The temperature of the sample dropped to ~90 K under those conditions and the absence of frosting in the analysis area indicated that the continuous nitrogen flow kept air away from the analysis area. Analyses of samples using the cryojet provided for far faster sample changes and, as As(III) standards showed no sample modification, the cryojet became our preferred method. Analysis of a natural diamond (C) procedural blank showed no evidence for As responses from scattered x-rays coming from the beamline table and experimental hutch. The background subtraction and normalisation of data for XANES was performed using ATHENA [163]. The edge position is determined using ATHENA from the maximum of the first derivative of the raw data.

X-ray absorption edge profiles of As were obtained using XANES analyses of tobacco leaf (As burdened and unburdened), manufactured cigarettes, and smoked products (ash, filter and particulates). In summary, As burdened tobacco was grown from KT209 tobacco seeds using seeding compost and various concentrations of sodium arsenate and sodium arsenite in solution, added together and separately. XANES spectra were obtained from inorganic compounds for edge energy calibration (arsenic (III) oxide, arsenic (V) oxide, sodium arsenite, sodium arsenate, and arsenopyrite), tobacco leaf grown from KT209 seed with and without As burdening. Those selected for XANES investigation were unburdened leaf samples RCS115 (in vivo), RCS123 (in vivo), RCS102 (0.7 µg/g As), and RCS133 (0.3 µg/g As), burdened leaf RCS108 (10.3 µg/g As), RCS120 (8.6 µg/g As), RCS123 (13.5 µg/g As), RCS125 (246 µg/g As), and RCS132 (10.1 µg/g As), international reference standards (CTA-VTL-2 (0.97 µg/g As) and 1R4F (0.31 µg/g As), and commercial tobacco products (STA336 (0.7 µg/g As) and STA486 (3.3 µg/g As)). Note that all concentrations are quoted as dry mass.

11.5.2.5 As concentration measurement

Tobacco samples were dried at 72 °C for 48 hours before being powdered in a tungsten carbide swing mill. 32 mm pellets were produced at 20 tonnes and analysed for As concentration using polarised X-ray fluorescence with Gd K-alpha primary radiation (PANalytical Epsilon 5) [30]. The precision of As analysis by XRF is estimated as ± 0.14 µg/g 2σ based on 10 replicates of tobacco standard 1R4F at a mean concentration of 0.31 µg/g.

Table 11. List of compounds used for edge energy calibration with CAS numbers and abbreviations, and the tobacco samples used in this study with their bulk arsenic concentration (in µg/g ±0.14, dry weight) where appropriate.

ANNOT-ATION	**MATERIAL**	**PURPOSE**	**CAS No. or As µg/g**
As_2O_3	Arsenic (III) oxide	Inorganic compound for As(III) edge energy calibration	1327-53-3
As_2O_5	Arsenic (V) oxide	Inorganic compound for As(V) edge energy calibration	12044-50-7
$NaAsO_2$	Sodium arsenite	Inorganic compound for XANES reference calibration	7784-46-5
Na_2HAsO_4	Sodium arsenate	Inorganic compound for XANES reference calibration	10048-95-0
Apy	Arsenopyrite	Inorganic compound (mineral) for XANES reference calibration	1303-18-0
RCS115	Leaf grown from KT209 seed and unfermented (no added As)	Control leaf	
RCS123	Leaf grown from KT209 seed in compost with added 0.53mM $NaAsO_2$ & 0.17mM $Na_2HAsO_4.7(H_2O)$, unfermented	As(III) & As(V) burdened tobacco leaf	11.3 µg/g
RCS102	Leaf grown from KT209 seed, fermented (no added As)	Control (powder)	0.7 µg/g
RCS133	Leaf grown from KT209 seed, fermented, shredded, propylene glycol casing, cigarette smoked for condensate and ash (no added As)	Control (smoked shredded tobacco condensate and ash)	0.3 µg/g
RCS108	Leaf grown from KT209 seed in compost with added 3.89mM $NaAsO_2$, fermented and shredded	As(III) burdened tobacco	10.3 µg/g
RCS120	Leaf grown from KT209 seed in compost with added 0.11mM $NaAsO_2$ & 0.03mM $Na_2HAsO_4.7(H_2O)$, fermented & shredded	As(III) & As(V) burdened tobacco	8.6 µg/g
RCS123	Leaf grown from KT209 seed in compost with added 0.53mM $NaAsO_2$ & 0.17mM $Na_2HAsO_4.7(H_2O)$, unfermented	As(III) & As(V) burdened tobacco	13.5 µg/g
RCS125	Leaf grown from KT209 seed in compost with added 0.40mM $NaAsO_2$ & 0.55mM $Na_2HAsO_4.7(H_2O)$, fermented & shredded	As(III) & As(V) burdened tobacco	246 µg/g
RCS132	Leaf grown from KT209 seed in compost with added 0.11mM $NaAsO_2$ & 0.04mM $Na_2HAsO_4.7(H_2O)$, fermented, shredded, propylene glycol casing	As(III) & As(V) burdened tobacco for smoking experiments	10.1 µg/g
CTA-VTL-2	Powdered tobacco	International tobacco reference standard for trace elements	0.97 µg/g
1R4F	Tobacco from research cigarettes	International tobacco reference standard for smoke components	0.31 µg/g
STA336	Tobacco from commercial cigarettes	Cigarettes with As concentration close to global average	0.7 µg/g
STA486	Tobacco from commercial cigarettes	Cigarettes with As	3.3

		concentration considerably above global mean	µg/g

11.5.3 *Results for arsenic*

11.5.3.1 Cultivation of As burdened tobacco plants

Glasshouse cultivation of As burdened tobacco leaf resulted in up to three orders of magnitude increases in As concentration. The dried leaf of unburdened control sample of KT209 contains 0.7 µg/g As whereas intermediate burdening (RCS132) created tobacco leaf containing 5-15 µg/g As. More substantial burdening led to tobacco leaves with 100-250 µg/g As, although there were many instances where plants failed to develop under burdening conditions, particularly when As was added solely as As(V). Only outwardly healthy plants were preserved through the extent of the growing period (typically 120 days). XANES spectra were collected across this range of burdened tobacco but for smoking experiments sampling was confined to the lower levels of burdening.

11.5.3.2 XANES spectra of standards

Edge energy, defined as the maximum in the first derivative of the data, lies ~11,868 eV for As(III) from arsenic trioxide (As_2O_3) and sodium arsenite ($NaAsO_2$), whereas the As(V) absorption edge lies ~11,873 eV in As pentoxide (As_2O_5) and sodium arsenate ($Na_2HAsO_4.7(H_2O)$) (Fig.8). The edge for arsenopyrite (FeAsS, Apy in Fig,8) is significantly lower at 11,866 eV, in reasonable agreement with interpretations of this feature as indicating the dominance of As(-1) in arsenopyrite [164, 165].

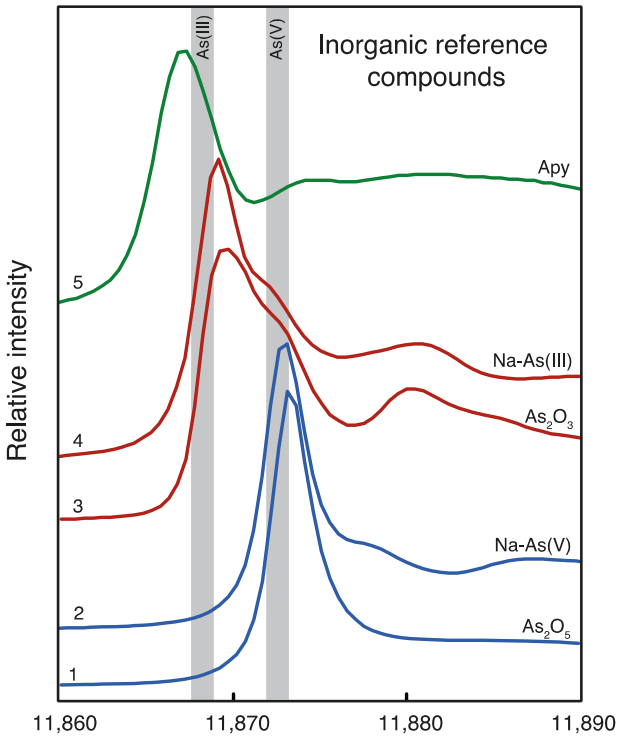

Figure 8. XANES spectra for inorganic reference compounds. Compound abbreviations and spectral interpretations are given in Table 12. Grey bands are centred on the first derivative maxima in As2O3 and As2O5, taken to indicate the edge energies of inorganically bound As(III) and As(V) respectively, and are reproduced in all other XANES spectra for reference. Error is typically ±0.25eV.

11.5.3.3 XANES spectra of fresh and cured tobacco leaf

Control sample RCS115 was inserted as freshly cut green leaf into the beam and its profile represents unburdened live leaf. Its edge energy shows As(III) to be dominant with the hint of a shoulder consistent with minor As(V). RCS102 is a similar sample that has additionally been fermented and it shows the development of a more prominent feature attributable to As(V). Note that these unburdened samples have <1 µg/g As when dried yet credible XANES spectra can be generated using a third generation synchrotron. Burdened sample RCS123IV was inserted as freshly cut green leaf into the beam and its profile represents burdened live leaf. Its edge energy shows As(V) to be dominant with the hint of a shoulder consistent with minor As(III).

The spectra for RCS120, RCS123, RCS125 and RCS108 represent leaf grown in As burdened conditions. In all cases a distinct As(III) edge is present, and is even dominant in the case of RCS120. Of these burdened samples only RCS123 was not fermented and it is noteworthy that As(V) dominates its XANES spectrum. There is no systematic response to As burden, for example RCS108 was cultivated with As(III), yet As(III) in the resultant tobacco is less prominent than As(V) (Fig 9A, spectrum 11). Similarly there is little correlation between valence balance and total As uptake. RCS125 is the most As rich sample and As(III) and As(V) appear to be approximately equivalent in its XANES spectrum. Less burdened samples such as RCS123 (13.5 µg/g) and RCS120 (8.6 µg/g) show very different balances. It should be noted that some of the peaks (particularly RCS120 and RCS1250) occur at higher energies than associated with the As(V) edge in the inorganic reference compounds (Fig.9A) and this shift most probably reflects different organic molecular environments.

In summary these spectra indicate that fresh leaf may be dominated either by As(III) or As(V) and burdening does not lead to a predictable balance between the valence states. Fermentation leads to spectra with both As(III) and As(V); again the relative valence balance is not predictable.

Table 12. List of samples and their XANES spectra with interpretation of valence balance. More details on the samples are provided in Table 11.

XANES spectrum	Figure	Sample	Remarks	Valence balance
1	8	As(V)	Inorganic reference	n/a
2	8	Na- As(V)	Inorganic reference	n/a
3	8	As(III)	Inorganic reference	n/a
4	8	Na-As(III)	Inorganic reference	n/a
5	8	Apy	Arsensopyrite, inorganic reference	n/a
6	9A	RCS102	KT209 tobacco leaf (unburdened control)	III $\prec$ V
7	9A	RCS115 IV	KT209 tobacco leaf (in vivo)	III $\gg$ V
8	9A	RCS120	KT209 tobacco leaf with 8.6 µg/g As	III $\succ$ V
9	9A	RCS123 IV	KT209 tobacco leaf (in vivo) with 13.5 µg/g As (dry weight)	III $\ll$ V
10	9A	RCS123	KT209 tobacco leaf with 13.5 µg/g As	III $\prec$ V
11	9A	RCS108	KT209 tobacco leaf with 103 µg/g As	III $\ll$ V
12	9A	RCS125	KT209 tobacco leaf with 248 µg/g As	III $\approx$ V
13	9B	RCS132	KT209 tobacco leaf, As burdened (10.1 µg/g As), fermented, shredded and cased	III $\succ$ V
14	9B	1R4F	Tobacco from low tar reference cigarette	III $\ll$ V
15	9B	CTA-VTL-2	Trace element reference tobacco	III $\ll$ V
16	9B	STA336	Tobacco from a major US brand	III $\ll$ V
17	9B	STA486	Tobacco from a major Chinese brand	III $\prec$ V
18	10	RCS133	Smoke condensate from 5 cigs made with KT209 fermented, shredded and cased tobacco (0.3 µg/g As)	III $\gg$ V
19	10	RCS132	Smoke condensate from 5 cigarettes made from KT209 fermented, shredded and cased tobacco (10.1 µg/g As)	III $\gg$ V
20	10	1R4F	Smoke condensate from 1 smoked (low tar reference) cigarette	III $\gg$ V
21	10	RCS133	Ash from KT209 fermented, shredded and cased tobacco (0.3 µg/g As)	III $\ll$ V
22	10	RCS132	Ash from KT209 fermented, shredded and cased tobacco (10.1 µg/g As)	III $\ll$ V
23	10	1R4F	Ash from 1 smoked (low tar reference) cigarette	III $\ll$ V
24	10	Filter Butt (proc.blank)	Filter from Roll Your Own brand of tube + filter tip, unsmoked	III < V
25	11	RCS132	5 cigarettes smoked, no $CO_{2(s)}$ cryojet	III $\gg$ V
26	11	RCS132	1 cigarettes smoked, filter stored in $CO_{2(s)}$, no cryojet	III $\gg$ V
27	11	RCS132	3 cigs smoked, filter stored in $CO_{2(s)}$, no cryo	III $\gg$ V
28	11	RCS132	3 cigarettes smoked, 30 minute delay at room T, no $CO_{2(s)}$, cryojet	III $\gg$ V
29	11	1R4F	Smoke condensate analysed with cryojet	III $\gg$ V
30	11	1R4F	Smoke condensate analysed without cryojet	III $\gg$ V

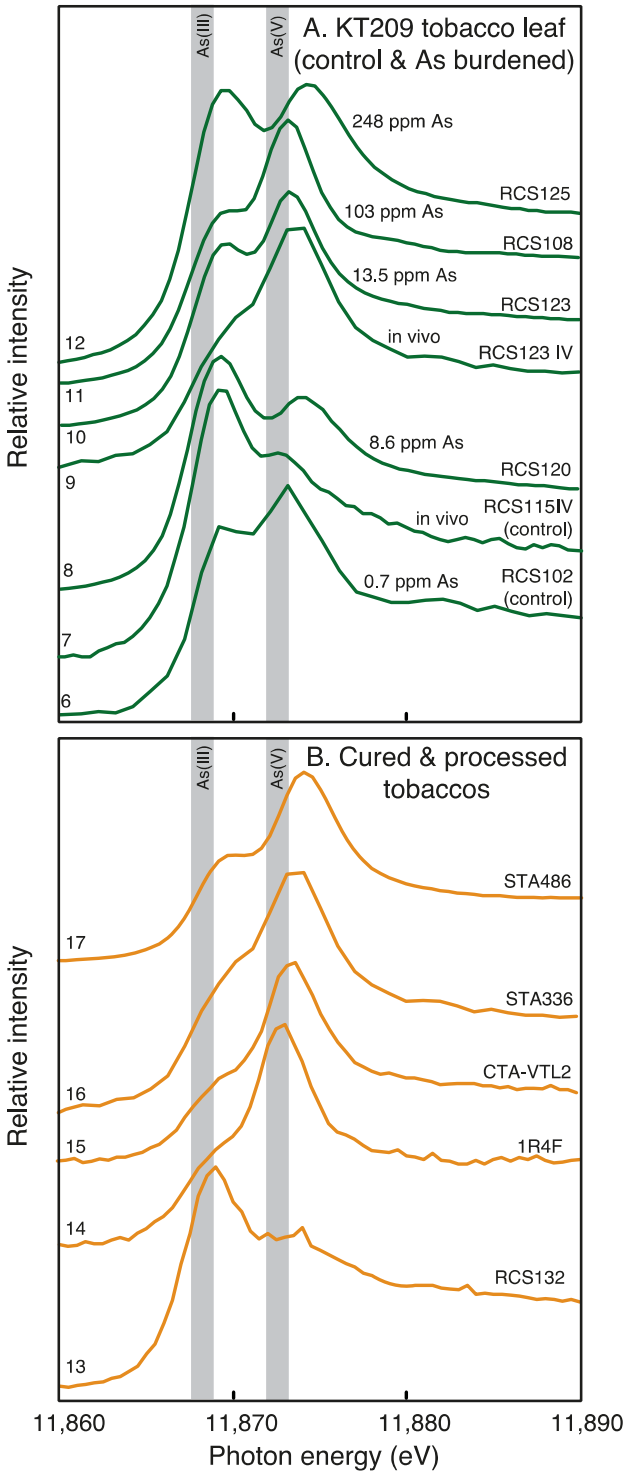

Figure 9. XANES spectra for (A) KT209 control tobacco leaf and KT209 grown with various levels of total As burdening (as annotated), and (B) a selection of cured and processed tobaccos from diverse sources. Sample abbreviations and spectral interpretations for each numbered spectrum are given in Table 12.

11.5.3.4 XANES spectra of cigarette tobacco

As(V) is the dominant valence state in four cigarette tobaccos. Smoking machine reference standard 1R4F, trace element standard CTA-VTL-2, and US popular brand STA336 with a low concentration of As all indicate strong edges corresponding with As(V) with only slight indicators of As(III). A Chinese brand with elevated As (STA486, 3.3 µg/g As) has a greater fraction of As(III) although As(V) still dominates. The only sample to depart from this general pattern is RCS132, tobacco grown under burdened conditions, fermented, cased with propylene glycol and then shredded. This sample has 10.1 µg/g As and shows As(III) to be dominant although a shoulder attributable to As(V) is discernible. These data show that these commercial tobacco products are dominated by the oxidised form with only minor As(III), unless the sample has been grown in an As rich environment.

11.5.3.5 XANES spectra of smoke condensate and ash

Smoke condensate collected around perforations (as described in the methods section) include numerous As rich points even in samples with very low bulk As concentrations. The edge energy of the control tobacco (RCS133 with 0.7 µg/g As), the medium burdened leaf (RCS132 with 10.1 µg/g As) and the reference standard 1R4F are essentially identical with peak positions indicative of As(III) (11,868±1 eV). RCS132 is a much smoother profile since it has a higher concentration. All three samples were created and transferred at room temperature, and analysed under the low temperature conditions of the cryojet.

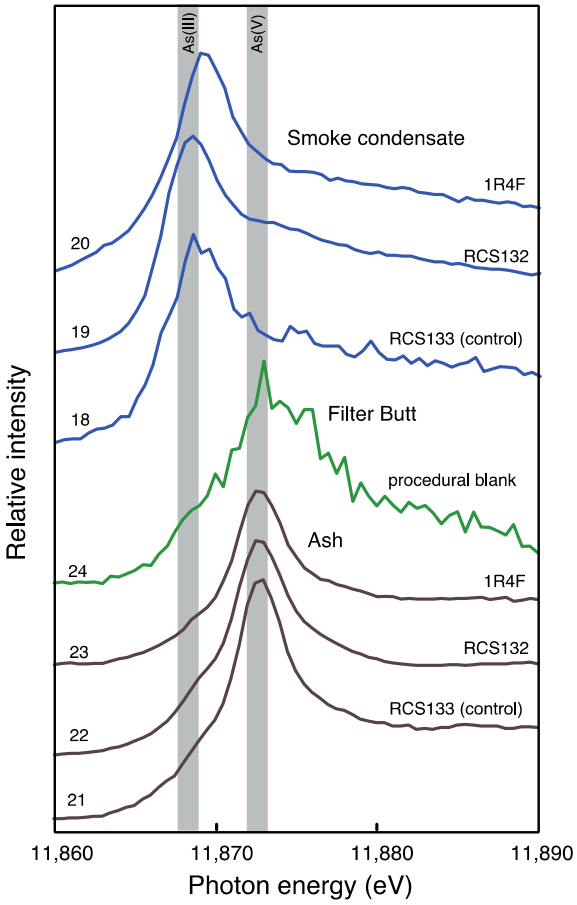

Figure 10. XANES spectra for smoke condensate from the 1R4F reference standard, KT209 control and one As burdened tobacco, a blank filter butt, and ash collected from a selection of experiments. Sample abbreviations and spectral interpretations for each numbered spectrum are given in Table 12.

XANES spectra for ash generated from the same tobaccos are also consistent, composed predominantly of oxidised As(V) species (Fig.10). The edge energies are 11,872 eV (control sample RCS102), 11,875 eV (As burdened samples RCS132), and 11,872 eV (1R4F reference standard), within the As(V) edge energy defined by inorganic standards (11,873 eV). These ash samples contain little or no As(III) and we infer that the combustion processes producing the ash effectively oxidise all the unmobilised As.

11.5.3.6 XANES spectra of ageing experiments on tobacco condensate

Much has been made in earlier papers about the transient character of the As(III) component of tobacco smoke particles [7, 8]. In the present study no change in oxidation state was observed in room temperature experiments on smoke condensate carried out in a normal atmosphere some tens of minutes after completing experiments using the smoking machine (Fig.11). If As(III) is the species posing the greater risk in smoke then the role of smoke ageing could be important in reducing this risk by oxidation. This prompted further investigations into potential changes in As valence state by repeating the experiments on smoke condensate with different time lags and storage conditions prior to acquiring XANES spectra. Although these experiments do not replicate real smoking conditions, particularly within the lungs, any evidence for rapid and complete oxidation of As could potentially be misused in claims that the risk from arsenic exposure is negligible.

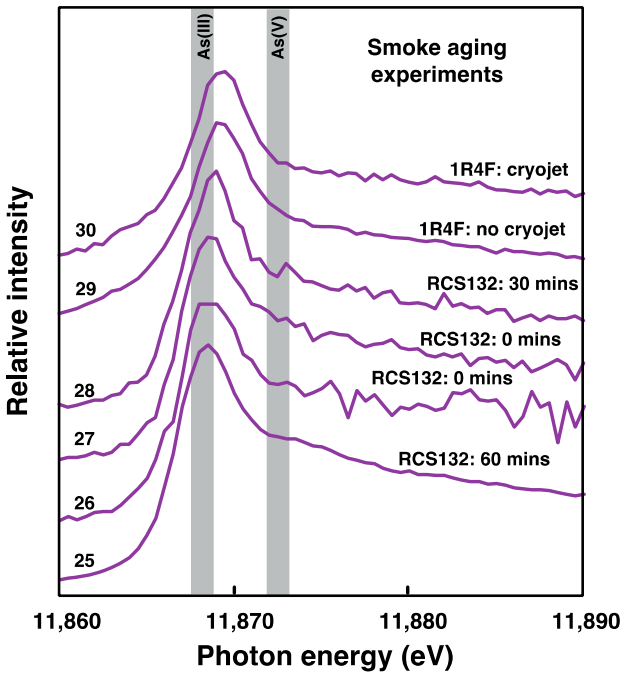

Figure 11. XANES spectra for smoke condensate under different conditions of ageing (lag time after smoking, with or without the cryojet during experiment). Sample abbreviations and spectral interpretations for each numbered spectrum are given in Table 12.

Arsenic burdened tobacco RCS132 was machine smoked with the filter pad encased in dry ice, and the assembly was kept together after the experiment while relocating to the synchrotron beam where it was transferred to the cryojet. The sample was prevented from warming up to room temperature in order to limit alterations in "electrothermal potential" [8]. The XANES spectrum for RCS132 (29 in Fig.11) from the smoke condensate experiment (Fig.10) is included for comparison. The spectra from the smoking of one cigarette (26) and three cigarettes (27) are essentially the same, although the data are noisier in the former given that fewer cigarettes were smoked to avoid ageing effects. No significant shift in edge energy reflecting oxidation is observed. The three cigarettes experiment was repeated and the condensate stored for 30 minutes at room temperature before insertion into the beam. The XANES spectrum (28) shows no significant change and the experiment fails to demonstrate any oxidation of As(III) with ageing. The reference standard 1R4F was smoked and XANES spectra collected without (29) and with (30) the cryojet in order to check the extent of any reduction or oxidation under beam conditions during approximately 30 minutes of exposure to the beam [162, 166]. When compared to un-aged spectra Fig. 10, no such effect is observed over the time taken to transfer the sample to the beamline (a few 10s of minutes).

11.5.4 *Discussion*

11.5.4.1 Summary of experiments

The XANES experiments demonstrate that tobacco combustion generates smoke that condenses on a filter pad with dominantly reduced As(III) species and ash with entirely oxidised As(V) species. This outcome is observed regardless of the balance of As(III):As(V) in the precursor tobacco.

Human exposure to As occurs primarily through ingestion of contaminated food or water. Typical daily intakes from food and beverages in non-occupational settings range between 20-300 µg/day (the higher figures associated with large consumption of seafood). Inhalation may contribute 1 µg/day in a non-smoker but estimates for a smoker's additional exposure range from about 1 to 10 µg/day [138]. Arsenic exposure via tobacco smoke may be even higher in heavy smokers and/or in parts of the world where high concentrations of As are often found in tobacco [157]. Thus smoking can be a significant source of As exposure, although these exposure estimates are based on total As and do not take account of the potentially different toxicities of various As species based on valence state, inorganic and organic compounds. The most recent IARC evaluation of inorganic As compounds implicates arsenic trioxide, As(III) and As(V) in causing cancers of the lung, urinary bladder and skin, and positively associates As and its inorganic compounds with cancers of the kidney, liver and prostate [138]. The evaluation also recognised that As(III) species are more toxic and bioactive than As(V) species as a consequence of greater chemical reactivity of As(III) and because As(III) enters cells more readily. Thus in tobacco smoke, a dominant inorganic As in trivalent state might be expected to be the most harmful, although studies of carcinogenesis in humans due to inhaled As species are lacking [138].

To facilitate interpretation an ordinal sequence of five combinations of valence states is defined, namely As(III)<<As(V), As(III)<As(V), As(III)≈As(V), As(III)>As(V) and As(III)>>As(V), identified by qualitative assessments of the relative contributions of As(III) and As(V) to the XANES spectra based on edge positions and the shape of the spectral profile. These are listed for each sample of tobacco smoke condensate and ash in Table 12. The same data are plotted in Fig.12 summarising all results in context from tobacco leaf to commercial product, through combustion to smoke and ash.

11.5.4.2 As(III)-As(V) variation in tobacco leaf and smoking products

Very high As concentrations in tobacco have been commonplace historically, for example US commercial tobacco averaged 57 µg/g in 1951 when arsenical pesticides were legal [167]. The As burdening experiments reported above resulted in leaf with As concentrations up to 246 µg/g but beyond about 10-15 µg/g plants began to show signs of stress. Only samples with As burdened concentrations at the lower end of this range were used in smoking experiments.

Fig.12 clearly indicates that the tobacco plant has a complex response to As burdening and the As(III)-As(V) relationship does not simply reflect the amount of added As. Indeed a sample to which only As(III) was applied has the strongest As(V) edge indicating that the plant mediates the As oxidation state. However, some general features are apparent. Unburdened and unfermented KT209 tobacco is dominated by As(V) but As(III) appears to increase with fermentation. Addition of different mixes of As(III) and As(V) to KT209 resulted in leaf grown with burdens of 8.6 (RCS120) and 13.5 µg/g As (RCS123) with significant As(III), but again As(V) dominates in the unfermented leaf whereas As(III) dominates in the fermented sample. Exceptional burdening of KT209 with the same As(III)-As(V) mix resulted in leaf with 246 µg/g As and the fermented sample has approximately equivalent As(III) and As(V). Burdening with As(III) only, followed by fermentation, resulted in leaf with 103 µg/g As which is dominated by As(V) but with significant As(III).

Tobacco cultivation occurs in an aerobic environment. The redox conditions in the growth medium may favour As(V) so that higher plants take As(V) via phosphate transporters, though As(III) would be transported through aquaporins [168, 169]. As(V) is reduced to As(III) in the roots, complexed with peptides such as glutathione As(GS)3 then potentially transported to root cells vacuoles, or translocated to the shoot [169, 170]. Reactive oxygen species, generated through reduction of inorganic As introduced within plants initiates a complex constitutive detoxification mechanism in higher plants such as tobacco, involving transcription of phytochelatins or even metallothioneins [162, 166, 169, 171, 172]. The system is even more complex in that the leaves are in a state of senescence once the tobacco plant is harvested; it is known that metallothionein is expressed in the phloem of tobacco for long distance transport of metals [173].

A HPLC-ICPMS study of reference tobacco 3R4F indicated the presence principally of As(V), broadly consistent with our XANES study of 1R4F, a sample prepared from a similar blend [9].

These data indicate that As(V) dominates in fresh tobacco leaf but that fermentation involves the reduction of some (or most) of the arsenic to As(III), a well-established process of detoxification by the plant [174, 175]. This process had not gone to completion in any of the leaf samples studied but an increased relative proportion of As(III) is seen in all fermented samples. Tobacco intended for smoking, including reference materials and commercial products, all indicate that As(V) is prevalent with minor As(III) (Table 12, Fig.12). As(III) dominates only in one burdened and fermented tobacco sample (RCS132, Fig.9B).

Combining all the XANES spectra of unsmoked tobacco it becomes clear that reduction of As(V) to As(III) is a detoxification feature that applies when levels of As are high in the plant. Unburdened leaf samples, reference materials and commercial products all have bulk As concentrations below 1 µg/g and these samples remain dominated by As(V) even after fermentation. Significant quantities of As(III) are found only in burdened tobacco leaf that in our experiments range from 8 to 236 µg/g As. These data suggest that oxidised forms of As will tend to dominate in the product globally, except possibly in

regions where tobacco relatively enriched in As is grown due polluted environments and/or fertilisers [176].

11.5.4.3 Valence state changes due to combustion

Combustion has a marked effect on the valence state of As. This is clear from Fig.12 which emphasises the division of As between As(III) in smoke condensate and As(V) in residual ash. Notable is the absence of obvious mixtures of As(III) and As(V), and this outcome is consistent regardless of the valence balance of As in precursor tobacco. Earlier longer duration, lower resolution XANES studies[7-9] concluded that 3R4F smoke condensate is a mix of As(III) and As(V) but our shorter duration studies found no evidence for significant amounts of As(V) in smoke condensate, although we concur in finding that ash contains arsenic primarily in its oxidised state.

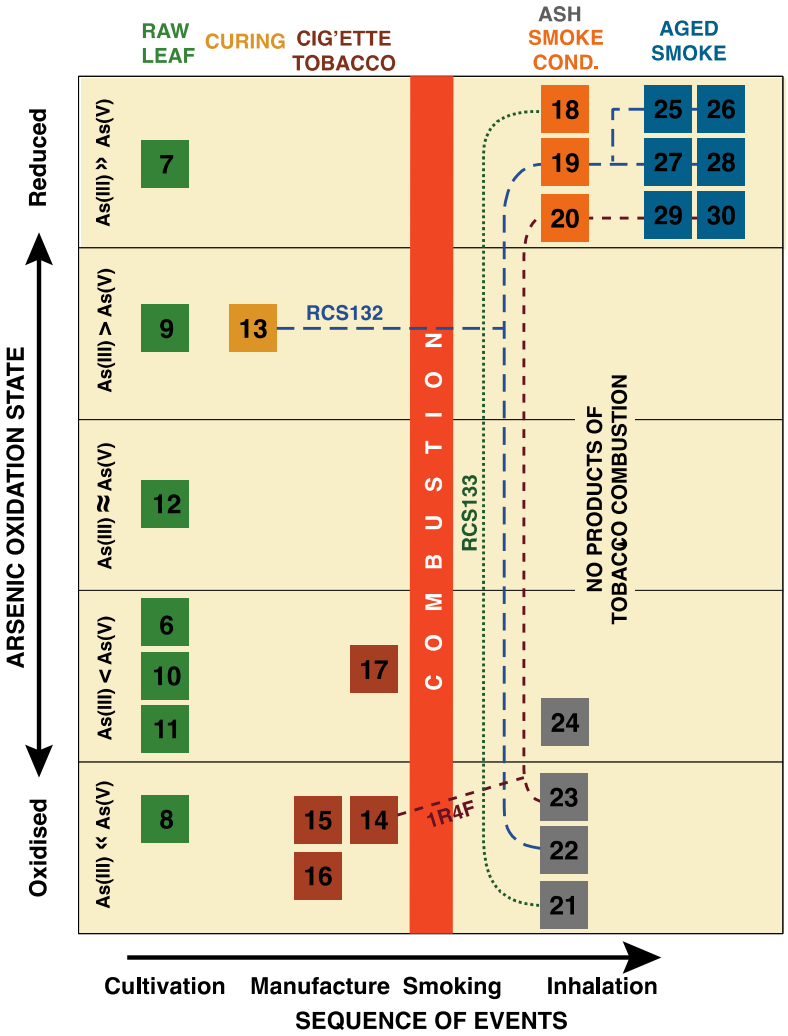

Figure 12. Summary of changes in valence speciation of arsenic along the pathway from raw tobacco through curing and manufacture to combustion and the creation of smoke products (ash and respirable smoke condensate). Numbers refer to the XANES spectra detailed in Table 12 and shown in Figs 9-11. Broken lines join different products of the same sample to emphasise the effect of combustion in effectively partitioning As(III) and As(V) between ash and smoke whatever the valence balance in precursor tobacco.

It is clear that the environment generating the smoke aerosol behind the burning coal is strongly reducing, consistent with findings on the tobacco combustion and pyrolysis microenvironments [154, 166]. Thus smoking As(V)-dominated tobacco generates an As(III)-dominated aerosol along with a residual ash dominated by As(V), as summarised in Fig.12, contrasting strongly with the "mixed" valence findings of Liu and co-workers [7]. Whether some of the ash has been through an intermediate stage of reduction cannot be determined from the XANES data. These results are consistent whether burdened or unburdened tobacco is used in the experiments.

11.5.4.4 Smoke ageing and oxidation

From smoke formation to inhalation takes up to 2 seconds during which temperature falls from >700 °C to ambient. Inhaled and subsequent exhaled smoke undergo further ageing during which redox changes continue over about 15 minutes [166]. It has been argued that any As(III) produced during smoking and preserved in the smoke condensate fraction is transient and will rapidly oxidise during this interval [8]. The XANES spectra of those studies were obtained after 1-7 days and indeed are strongly oxidised except for samples that were stored under dry ice and appear to preserve significant amounts of the reduced species [7, 9]. Here we also use XANES to examine the effects of smoke ageing by observing shifts in edge energy that may indicate oxidation, but over much shorter timescales. No significant shift related to oxidation was observed at room temperature over approximately 60 minutes that it took to smoke the cigarettes and to acquire their XANES spectra, an order of magnitude longer than it normally takes to smoke a cigarette [7, 8].

11.5.4.5 Health implications

Compared with dietary sources smoking tends to expose humans to smaller amounts of arsenic, but this study indicates that it is all essentially in the reduced state and published HPLC studies indicate that the large majority is inorganic [8]. Thus even low concentrations of arsenic in smoke may represent a significant source of exposure to reduced inorganic arsenic species, especially for heavy smokers. Exposure will be magnified in environments, most common in Asia, where As levels in tobacco may be significantly higher than is typical of major western manufacturers [157].

Using volunteers to smoke cigarettes impregnated with a solution of radioactive sodium arsenite (i.e. reduced arsenic) in the form of 74As it has been shown that nearly 90% of inhaled As is absorbed by the body and much of this occurs via the bronchial tree within the first day following exposure [177]. If the smoke condensate used in these XANES experiments faithfully represents the arsenic in the smoke aerosol delivered to the lungs then exposure will initially involve primarily inorganic As(III) species. Further research is required to gain a better understanding of the oxidation rates and the response of speciation to lung fluids and tissue.

11.5.4.6 Regulatory implications

Regardless of precursor species it appears that combustion invariably generates the most hazardous form of As in smoke, and XANES studies of unburdened and As burdened tobacco indicate that the process of reducing arsenic during combustion is effective over a far wider range of concentrations than might be expected in commercial tobacco crops. Thus these findings on As in cigarette smoking (but not oral use) add strong support to TobReg's proposal that regulatory authorities should consider requiring manufacturers to test cured tobacco and commercial products for levels of As. The findings also suggest that As concentration in tobacco, relatively easily determined in most laboratories,

may be a sufficient indication of the risk of exposure to arsenic without requiring the considerably more onerous analysis of speciation.

11.5.5 *Results for chromium*

11.5.5.1 Experiments

Chromium was analysed using the same experimental setup as used for arsenic, except the XANES spectra were collected at Cr K-alpha. See Table 13 for sample details. Tobacco samples were investigated during the 6601 experiments in 2011 and smoke products were analysed during 7744 experiments in 2012. Comparability between the experiments was ensured by running diamond, boron nitride and reference standards at the start of each experiment period as checks on blanks and energy calibration. Both sets of data were unified within the ATHENA data processing package.

Chromium was typically difficult to locate in many of these samples. To facilitate finding chromium "hot spots" maps were produced using X-Y scans and used to locate potential chromium-bearing species.

11.5.5.2 Reference standards

The absorption edges of Cr(0), Cr(III) and Cr(VI) were defined using reference materials of Cr metal, CrCl3 and K2Cr2O7 respectively (Fig.13). Multiple sample runs combined and processed to identify the absorption edge energy. These reference energies are marked on each plot as vertical bands.

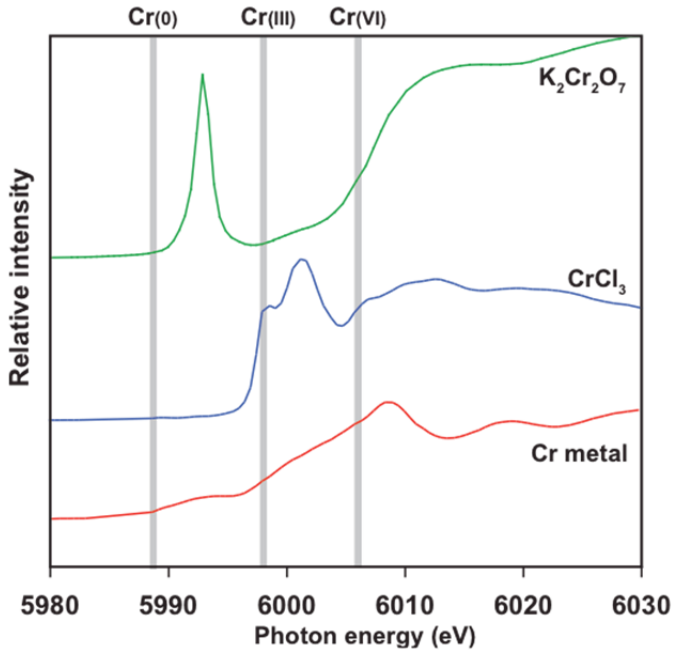

Figure 13. XANES spectra for reference compounds. Grey bands are centred on the first derivative maxima in Cr metal, CrCl3 and K2Cr2O7, taken to indicate the edge energies of Cr(0), Cr(III) and Cr(VI) respectively, and are reproduced in all other XANES spectra for reference. Error is typically ±0.25eV.

11.5.5.3 Tobacco samples

The XANES spectra for several different tobacco samples are shown in Fig.14. Small arrows indicate the energies for any edges detected during processing of the spectrum.

1R4F and CTA-VTL-2 are US and Bulgarian tobacco reference standards used for smoke testing validation and as a trace element standard respectively Table 13. Their spectra (Fig.14) are broadly similar and the modelling identifies edge energies in the range associated with Cr(III) species. No evidence was found for the presence of Cr(0) or Cr(VI).

Table 13. Samples used in the XANES investigation of chromium in tobacco.

Sample	**Material**	**Cr (µg/g)**
CTA-VTL-2	Polish Certified Reference Material for Virginia tobacco leaves	1.9
1R4F	Kentucky University Research Cigarette (standard for tar, nicotine, CO)	1.6
RCS114	KT209 tobacco seed grown in Cr enriched soil	1.4
STA336	Tobacco extracted from popular US brand (Marlboro)	2.9
18/6	Tobacco extracted from seizure of illicit tobacco	67.0
18/21	Tobacco extracted from seizure of illicit tobacco	12.0

Attempts were made to grow tobacco burdened with chromium. This was not as successful as growing As (and Cd) burdened tobacco. RCS114 is a sample of one such attempt and shows no enrichment of Cr (1.4 µg/g). Like the reference standards it has an absorption edge indicating the presence of Cr(III) species (Fig. 14).

Tobacco was extracted from cigarettes of a very popular US brand (Marlboro). This had slightly high Cr (2.9 µg/g, Table 13). Two separate points on the sample STA336 A and B, Fig 14) indicate the presence of both Cr(III) and metallic Cr(0).

18/6 and 18/21 are samples of illicit tobacco known to be enriched in Cr (67 and 12 µg/g respectively, Table 13). These are considerable enrichments over "normal" levels and the products are counterfeits of genuine UK brands. The XANES spectra (Fig.14) show both samples to have locations where the presence of Cr(III) species is indicated ((18/6 A and 18/21 A) but also the presence of Cr(0) (18/6 B and 18/21 B). No Cr(VI) was identified in any of these tobacco samples.

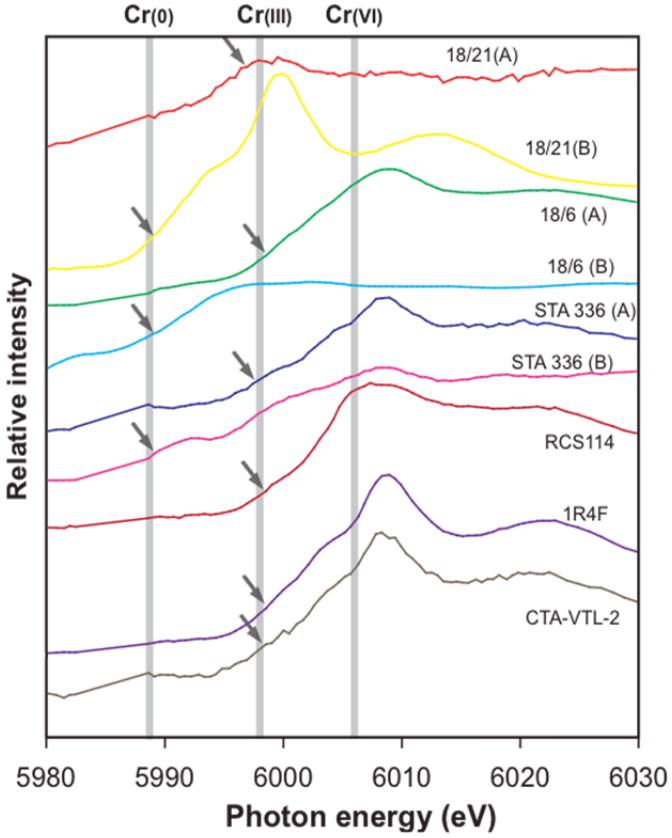

Figure 14. XANES spectra of various samples of reference tobaccos (CTA-VTLS-2, 1R4F), lab cultivated tobacco (RCS114), commercial brands (STA336) and illicit seizures (18/6 & 18/21), some known to be rich in Cr. Arrows indicate edge energies of samples modelled by spectral processing. Grey bands indicate the edge energies of the principal Cr valencies obtained from reference samples (Fig.13).

The presence of Cr(0), especially in those samples with very high Cr, was investigated further. Cr(0) is typical of metal alloys and a XANES spectrum was obtained from a reference sample of steel (not shown) which showed a closely similar absorption edge. These enriched tobacco samples were examined using an optical microscope and small fragments of alloy were detected in some cases (Fig.15). It is assumed that these fragments are related to the wear of machinery used in manufacturing the cigarettes.

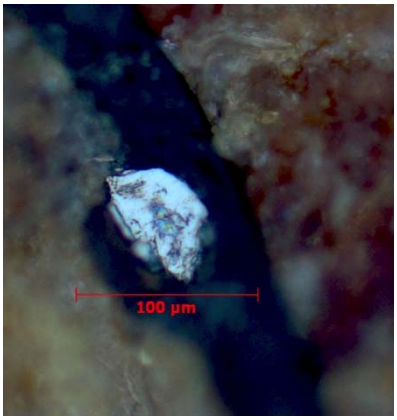

Figure 15. Reflected light microscope image of a fragment of alloy embedded in a tobacco sample. Scale bar 100 µm.

11.5.5.4 Smoke products

It proved exceptionally difficult to locate Cr-rich areas in the tar/smoke condensate fraction of smoking machine experiments, and similarly proved difficult to find in the ash fraction. The only productive area for Cr proved to be the filter tip although there were technical difficulties caused by interference between strong Ti K-lines raising the background around the Cr K absorption edge. Titanium oxide is widely used as a pigment in filter tips.

One Cr-rich point was found in the smoke condensate (RCS133) and this proved to be Cr(0) (Fig.16). It could not be established whether this was a metallic fragment or a deposit condensed from the smoke.

Two points analysed within the filter tips on RCS132 are shown in Fig.16. One is clearly Cr(III) whereas the other is suggestive of Cr(0) but this is tenuous.

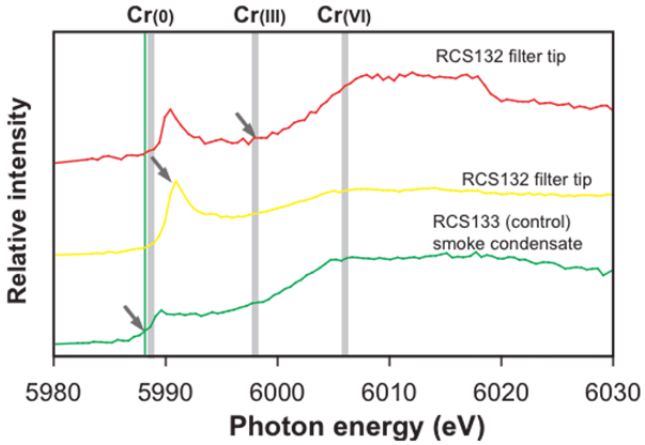

Figure 16. XANES spectra of smoke condensate trapped by a filter in a smoking machine (RCS133) and in filter tips on the cigarette (RCS132). Arrows indicate edge energies of samples modelled by spectral processing. Grey bands indicate the edge energies of the principal Cr valencies obtained from reference samples (Fig.14).

11.5.5.5 Significance of XANES results for Cr

Cr proved less amenable to XANES characterisation but some clear patterns are very significant for the consideration of Cr as a significant smoke carcinogen.

No Cr(VI) was observed in any study of leaf or smoking product. It was possible that Cr(VI) might has been located in ash given that ash is the main repository of oxidised arsenic in smoked products, but no Cr at all was found. The only carcinogenic form of chromium is Cr(VI) and the failure to identify this in any of the materials studied suggests that the smoker may not be exposed to oxidised forms of Cr, and that Cr may not be a significant carcinogen in tobacco smoke.

It is difficult to model the budget of Cr species when it is clear that significant quantities of Cr are derived from the wear of machines using Cr alloys (presumably steels). Cr(0) was found in top brand commercial tobaccos as well counterfeits where worn machinery might be expected. It is possible that a proportion of the Cr(0) in smoke condensate and filters is reduced Cr formed during combustion in a similar way to the creation of As(III), as discussed

above. The present data cannot resolve this. Either way it is not likely to represent a risk to smokers.

11.6 Eh-pH Modelling of Metal Species

11.6.1 *Modelling assumptions*

In this section it is assumed that metals in tobacco smoke can be modelled approximately as simplified aqueous electrochemical systems. The purpose of the following models is to identify the stable phases most likely to exist under varying conditions of redox potential (Eh) and acidity/alkalinity (pH) as the forms in which metals are likely to be transported by smoke at one atmosphere pressure and a temperature representative of smoke exposure (25°C).

Redox potential is the electromotive force between an electrode of noble metal and a reference electrode immersed in a solution. Electromotive potential (E) is expressed in volts (mV), relative to SHE, the standard hydrogen electrode (Eh), which conventionally has zero potential (Eh = 0 V). If other electrodes are used values can be converted to the SHE scale using published factors [178]. High redox potential values (Eh>0) indicate a system that tends to remove electrons thus oxidising the species. In contrast, reducing conditions are indicated when Eh<0, indicating the system's supply electrons to the species. pH is a measure of hydrogen ion activity in solution on a logarithmic scale, to which acid-base reactions are sensitive.

11.6.2 *Pourbaix diagrams*

The Pourbaix diagram (also known as the Eh-pH diagram) spans the typical ranges of Eh and pH in the natural environment, bounded by the stability limits of water, and maps the boundaries between the predominant stable equilibrium phases. The construction is based on the relevant Nernst equations that in turn are derived from thermodynamics, providing information on the stable species at any given Eh and pH [179]. Being thermodynamically based there is an important limitation in that the diagram makes no allowance for reaction rates and kinetics. Eh-pH diagrams are widely used in modelling (mainly inorganic) chemical features of aqueous environments. Use of these diagrams for modelling predicted species in ambient cigarette smoke was recently suggested in studies published by scientists working for the tobacco industry [7, 8]. Here we use a similar modelling approach to predict the most thermodynamically stable form of a metal under specified Eh and pH conditions, its valence and whether it is likely to precipitate or remain in aqueous solution. These factors will strongly influence a metal's capacity for absorption or deposition in the mouth, airways and lungs.

The present study makes use of the Geochemist's Workbench® 10, a set of software tools developed for manipulating chemical reactions and calculating stability diagrams in aqueous solutions using comprehensive thermodynamic datasets [180].

11.6.3 *Eh and pH conditions in smoke*

Smoke from a burning cigarette is an aerosol consisting of particulates dispersed in vapour including air. When considering the chemical environment for modelling it is important to be clear whether we are dealing with whole smoke, the vapour phase only, or the particulate phase only. Also it is important to distinguish mainstream smoke (MS) from sidestream smoke (SS), fresh smoke and aged smoke.

11.6.3.1 Eh and pH conditions in tobacco smoke at ambient temperatures

The literature on Eh in tobacco smoke is sparse and the only one publication that include data has been located [181]. Fig.17 reproduces the data and shows how the vapour phase varies very little in redox potential and remains close to zero. Whole smoke on the other hand shows marked reducing potential with a steady

decline from puff 2 from near zero to -0.09 V (90 mV). These data represent a single US commercial non-filter cigarette brand, and it is not known whether they are broadly representative of global cigarette brands, especially those with filters.

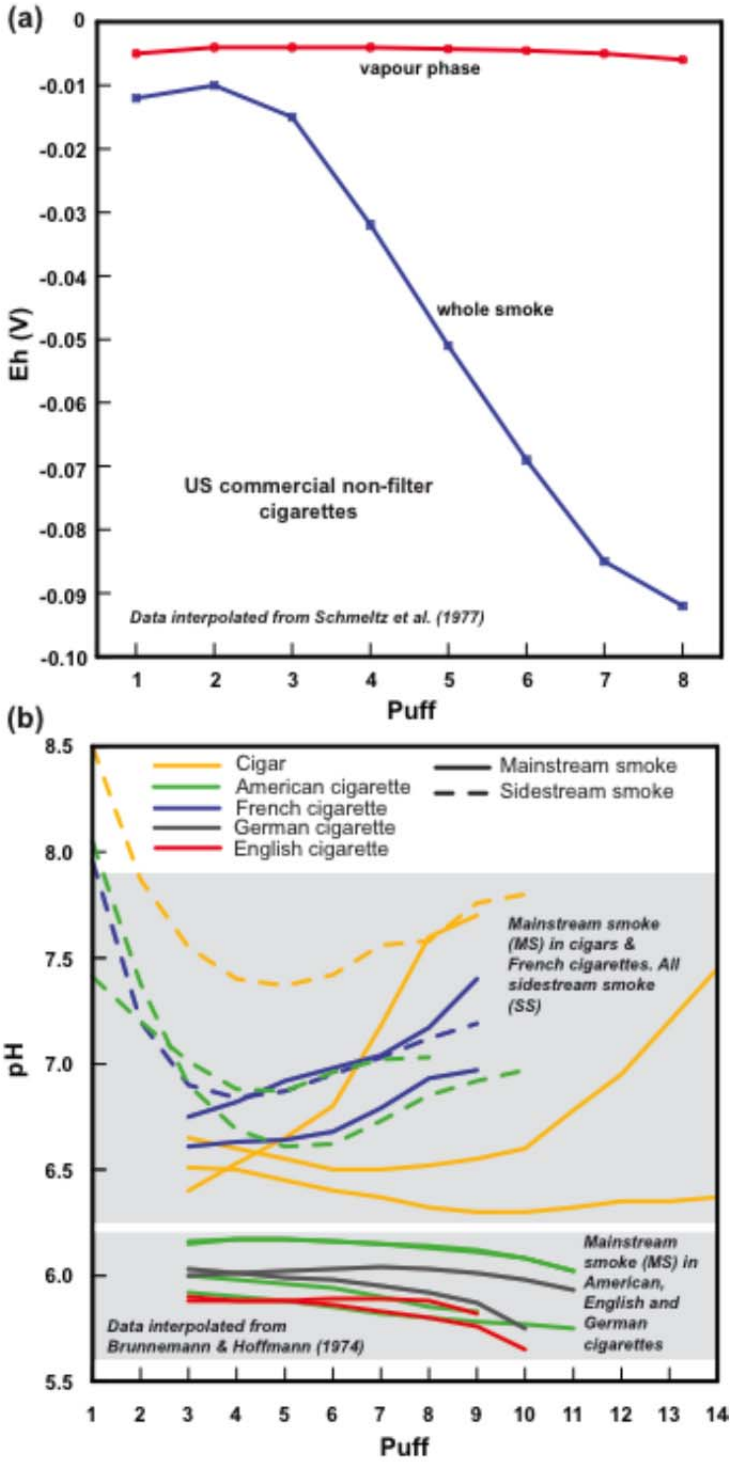

Figure 17. (a) Variation in Eh with puff number in a typical commercial cigarette for the US. (original data taken from graphs in Schmeltz et al., 1977). (b) Variations in pH with puff number in a range of cigarettes and cigars from different regions (original data taken from Brunnemann & Hoffmann, 1974).

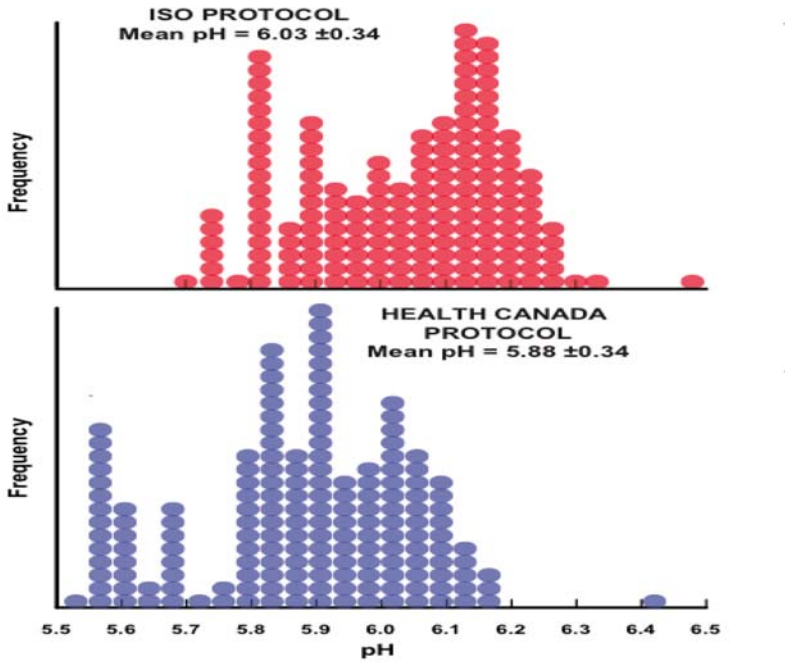

Figure 18. Comparison of the frequency of pH values in mainstream smoke between cigarettes smokes under ISO and Health Canada protocols. See text for details.

More data are available for pH. Fig. 18 reproduces data from a detailed study of pH in mainstream (MS) and sidestream (SS) smoke in cigars and cigarettes from different geographic regions [182]. (No data have been found for pH of the vapour phase alone.) The data suggest that pH in MS in cigarettes from most regions varies between about 5.6 and 6.2 with very little variation with puff number. However, mainstream smoke from cigars and French cigarettes is distinctly more alkaline with values (ignoring puff 1) between about 6.4 and 7.8. Interestingly this study found that sidestream smoke is significantly more alkaline than mainstream smoke.

The role of smoking behaviour on smoke pH is examined in Fig. 18 by comparing the results for the ISO and Health Canada protocols applied to the same brands. Data for pH in MS from a wide variety of cigarette types and geographical regions has been compiled from a survey of Philip Morris brands worldwide [80] and some analyses from Health Canada available on the web.

The histograms (Fig.18) clearly demonstrate that the more intense Health Canada smoking protocol of generates a more acid mainstream smoke with a lower mean pH that the ISO protocol (5.88 compared with 6.03, $2\sigma=0.34$ in both cases). This is a small difference compared with overall variations, and the effect of smoking behaviour on pH is not an important factor in the modelling described below.

Initial Eh and pH conditions of smoke in the burning coal of the cigarette are not well constrained. This is important as these factors may strongly influence the formation of species transferred in fresh smoke. Changes in Eh with smoke ageing after the final puff suggests that the aerosol continues to increase its reducing potential for a few minutes after smoking ceases reaching its lowest value of about -0.07 V, then gradually loses its reducing potential returning to about -0.01 V after about 30 minutes [181].

11.6.3.2 Tobacco smoke and the Eh-pH diagram

Fig.19 is the Eh-pH diagram for Fe with the envelope encompassing tobacco smoke superimposed in green. Solid and aqueous species are coloured differently (see key) and the dominant species for each field on the diagram is labelled. The diagram is bounded at the top by the oxidation of water to O_2 and at the bottom by the reduction of water to H_2.

The field of smoke Eh-pH straddles the boundary between aqueous ionic Fe^{2+} and solid Fe_2O_3. Indeed pH=6, a value typical of most cigarette smoke, is right on this boundary suggesting that Fe is distributed between particulate and vapour phases in tobacco smoke.

The diagram is also useful because it is often argued that Fe is implicated in Fenton reactions that may be damaging to cellular materials. However the large difference between smoke Eh-pH and the Eh-pH conditions at the Fe^{2+}-Fe^{3+} boundary (highly acid and oxidising, Fig.19) suggests that Fenton reaction catalysis of free radicals is unlikely in tobacco smoke without involvement of an additional oxidising agent.

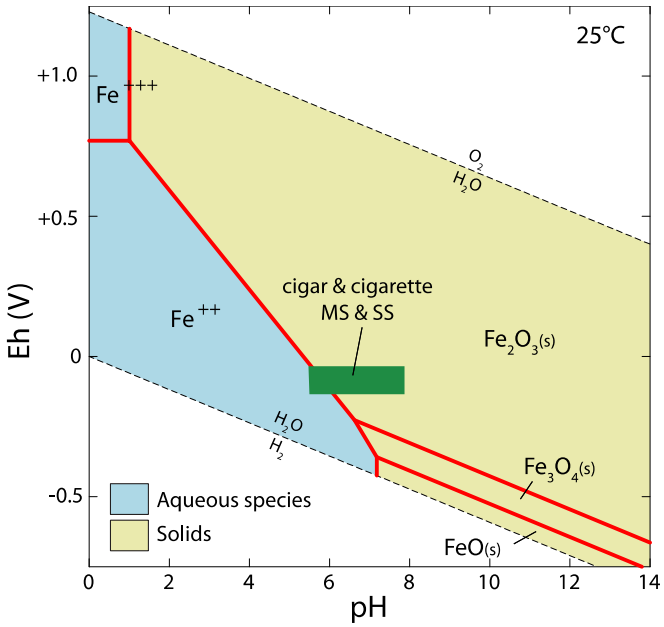

Figure 19. Eh-pH diagram for Fe showing conditions for stable species, solid and aqueous forms, and the range of MS and SS tobacco smoke from cigars and cigarettes.

11.6.4 *Metals of toxicological interest*

Two recent reviews inform the choice of metals and metalloids to include in this review of Eh-pH thermodynamic species stability. A comprehensive review led by RIVM identified 98 hazardous smoke components based on an analysis of all known smoke components and their human health inhalation risks [1]. Of these 98 components 12 are metals or metalloids:

- Arsenic
- Beryllium
- Cadmium
- Chromium
- Cobalt

- Copper
- Lead
- Manganese
- Mercury
- Nickel
- ^{210}Polonium (no thermodynamic data available for modelling)
- Selenium

An analysis of this list by TobReg, an expert panel on tobacco regulation reporting to the WHO, highlighted four elements (underlined in the list above) as potentially warranting regulation in crops and smoking products [2].

The following sections are a series of Eh-pH models of the stable species in ambient smoke of those elements in the list of 12 for which adequate thermodynamic data exist, with a particular focus on the four elements highlighted by TobReg. Modelling conditions are set at activities of 10^{-3} or 10^{-4} M with no additional species using thermodynamic datasets "thermo" or "thermo.com.V8.R6+" at 25 °C and 1.013 bars.

11.6.4.1 Eh-pH model for arsenic

The field for smoke falls almost entirely within the part of the diagram in which As(OH)3 is stable in solution, i.e. inorganic As(III). There is a very slight overlap onto As(V).The XANES study of the previous section concluded that smoke condensate was predominantly in the form of As(III). Arsenic has been studied in more detail than the other elements and this close agreement between a simple thermodynamic model and empirical testing of smoke products lends considerable confidence to the approach. The model predicts that the main arsenic phase in smoke is arsenous acid (As(OH)3) in solution, notably a toxic chemical used as a pesticide and herbicide.

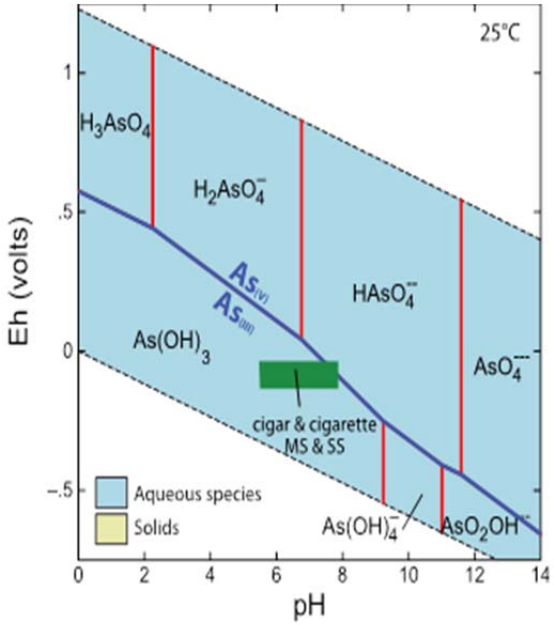

Figure 20. Eh-pH diagram for arsenic. Solid blue line added to emphasise Eh control on oxidation of As(III) to AS(V).

11.6.4.2 Eh-pH model for beryllium

The modelling clearly predicts that if beryllium is transported in tobacco smoke then it will be in the form of an oxide in the solid state. It requires an extremely acid solution (pH<2) to take beryllium into solution, well beyond anything likely in tobacco smoke.

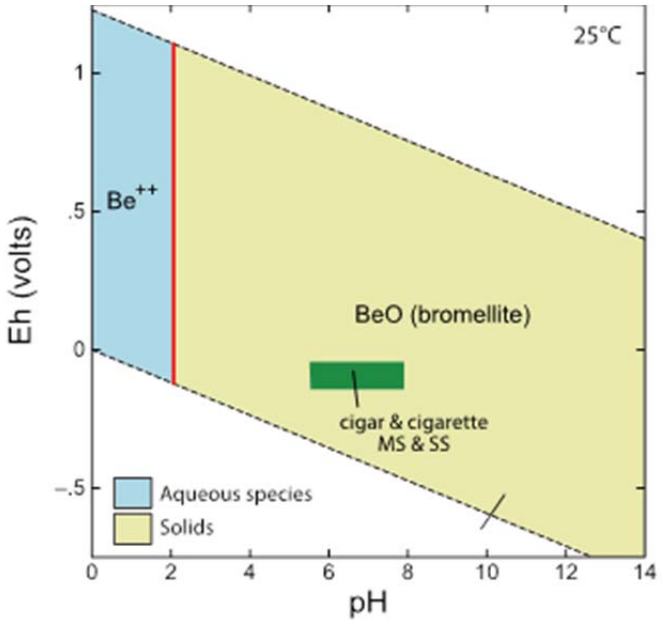

Figure 21. Eh-pH diagram for beryllium.

11.6.4.3 Eh-pH model for cadmium

The model underlines the extent to which cadmium is controlled by pH. Under typical smoking conditions the Eh-pH diagram predicts that cadmium will be present in solution in smoke as the Cd2+ ion. Independently a XANES study of cadmium in tobacco, smoke and ash by scientists in the tobacco industry also concluded that cadmium is present in its Cd2+ (divalent) rather than its metallic state [183]. The resolution of the XANES spectra was not sufficiently good to discriminate ionic Cd in solution from Cd(OH)2.

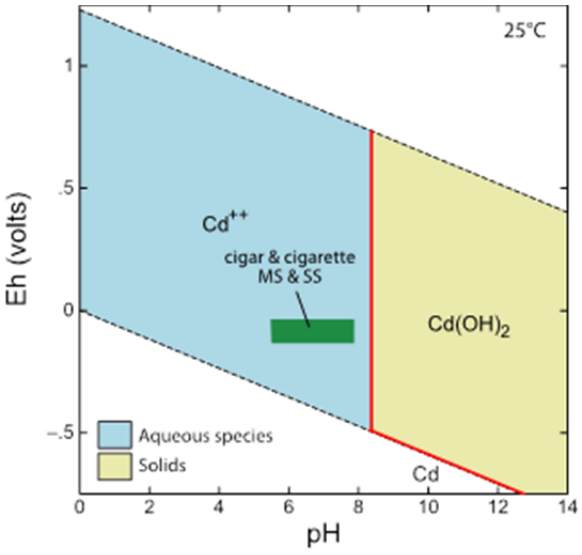

Figure 22. Eh-pH diagram for cadmium.

11.6.4.4 Eh-pH model for chromium

The most toxic form of chromium is Cr(VI) (hexavalent). The Eh-pH model predicts that Cr in smoke will be present as Cr2O3 in the particulate phase, i.e. trivalent. Oxidising Cr to its hexavalent state could conceivably occur during combustion but there is no evidence for this in these models. This conclusion is supported by XANES studies of Cr in smoke condensate (section 11.5.5) where Cr was found only in Cr(III) and metallic (Cr(0)) states, the latter being traced to fragments of steel from machinery. Again EH-pH modelling and XANES characterisation broadly concur.

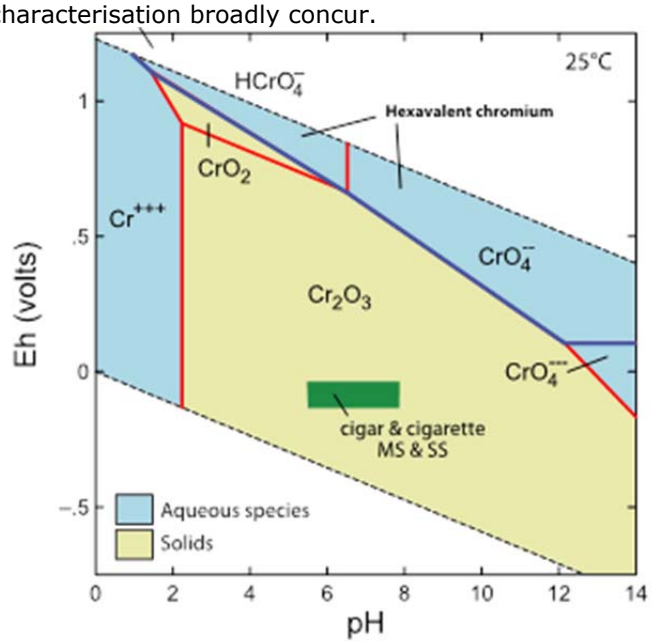

Figure 23. Eh-pH diagram for chromium. Blue line divides hexavalent chromium species above from less toxic species below.

11.6.4.5 Eh-pH model for cobalt

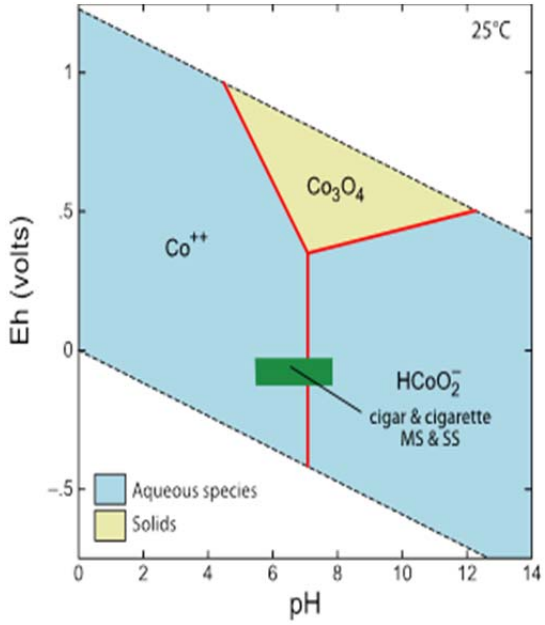

Figure 24. Eh-pH diagram for cobalt.

The model predicts the presence of both the ions Co2+ and/or HCoO2- in solution depending on pH with HCoO2- favoured under only the more alkaline conditions of mainstream smoke from cigars and French cigarettes, and sidestream smoke from all cigarettes. Most mainstream smoke will have cobalt present in the aqueous phase as Co2+.

11.6.4.6 Eh-pH model for copper

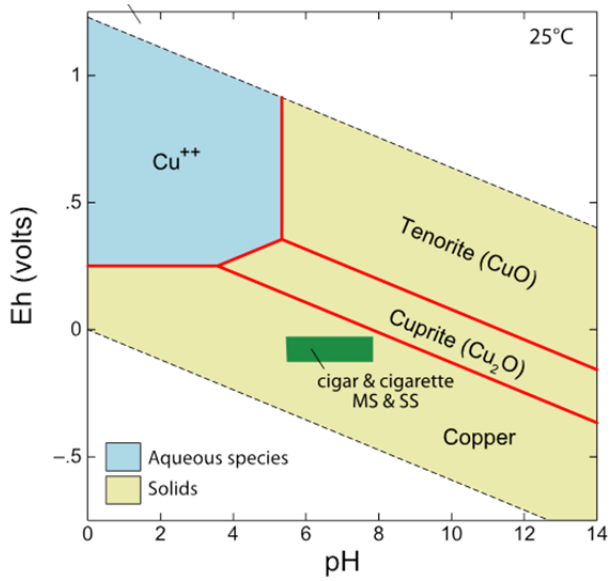

Figure 25. Eh-pH diagram for copper.

The model predicts copper in all forms of smoke (mainstream and sidestream) to be present as metallic copper in the particulate phase.

11.6.4.7 Eh-pH model for mercury

The Eh-pH diagram predicts that any mercury present in tobacco smoke will be present as pure mercury and is likely to be associated with the particulate phase.

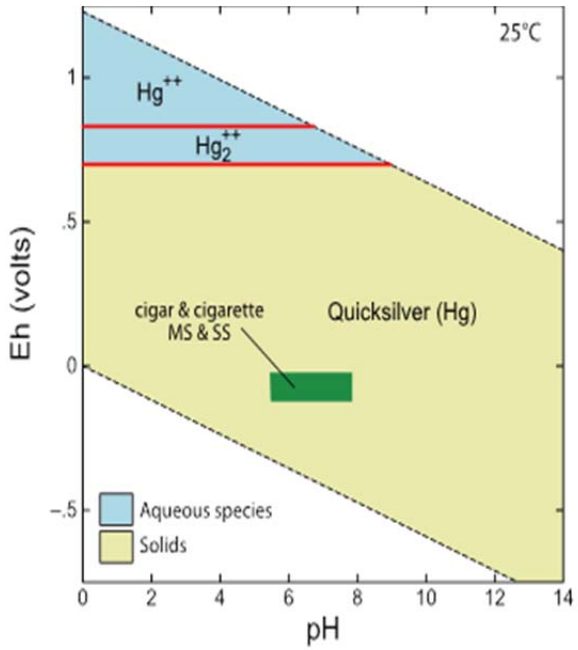

Figure 26. Eh-pH diagram for mercury.

11.6.4.8 Eh-pH model for nickel

The model predicts that nickel in tobacco smoke will be present in solution as Ni2+ although the most alkaline of smoke associated with cigars and sidestream smoke may cross into the stability field of solid phase NiO.

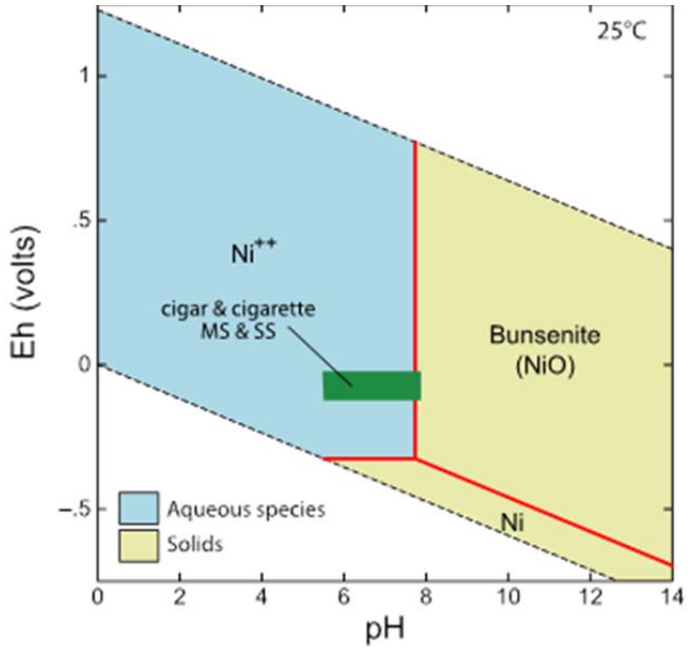

Figure 27. Eh-pH diagram for nickel.

11.6.4.9 Eh-pH model for selenium

Of the wide variety of possible selenium compounds the Eh-pH diagram predicts that selenium will be present in all types of smoke in the solid state as elemental selenium, transported as part of the particulate phase.

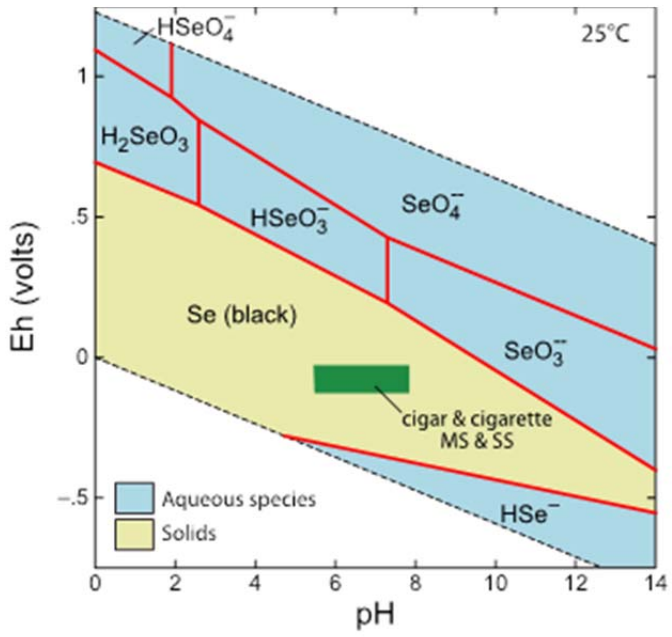

Figure 28. Eh-pH diagram for selenium

11.6.5 *Implications of Eh-pH modelling for toxicity of metals*

Potential toxicity related to these metals will be discussed in sections 11.6.5 and 11.7 in greater detail but it is important to distinguish between those elements that are transported in solution and those that are particulates at 25 °C as this influence their absorption in lung fluids.

Metals As, Cd, Co, Pb, Mn and Ni tend to exist as aqueous species in smoke at 25°C. Water is transported by tobacco smoke both the vapour phase (approximately 20 mg cig-1 in MS) and the particulate phase (approximately 3.5 mg cig-1 in MS) [17]. In contrast Be, Cr, Cu, Hg and Se are modelled as belonging exclusively to the particulate phase.

It is noteworthy that the four metals and metalloids recommended for regulation by TobReg (As, Cd, Ni and Pb) are all predicted to be soluble as aqueous species in smoke at ambient temperatures.

11.6.6 *Cautionary remarks*

Despite apparent agreement between the models and experimental findings there are a number of factors not included in these simple models.

Eh-pH modelling in this section was based on simple aqueous solutions for single metallic species without additional components and dissolved anions. While the composition of Cl, S, P etc are well known in tobacco these elements are rarely measured in tobacco smoke and thus there are few data with which to constrain Eh-pH models. Nevertheless it is likely that additional inorganic components will in some cases have a very large effect, e.g. in the smoking Eh-pH range some metals form ionic complexes with Cl while Hg forms a stable sulphide compounds with S. Analyses of concentrations of the more important anions in tobacco smoke are required before refining these models.

Other components are also omitted in these models. Large numbers of organic compounds are present in smoke, some of which may react with metals and become involved in metal transfer and exposure. Heavy organic molecules probably form the substrates of most particulates onto which some metals may adsorb. Thus models that accurately reflect the dynamic chemistry of smoke exposure will need to take these factors into account.

Also relevant is temperature. The smoke aerosol is initially created in the combustion zone at 700-950°C and pyrolysis products are generated down to 200°C [154]. As oxygen is consumed by combustion with carbonized tobacco the initial smoke conditions are likely to be more reducing than is modelled in the range of smoke Eh used in this study, but no data are available [154]. Eh-pH modelling is possible for higher temperatures (up to 300°C in the GWB package used) and the effects of higher temperatures on the model outcomes should be explored.

The models are based on equilibrium thermodynamics and it is not certain that ambient mainstream smoke reaches equilibrium in the few seconds between creation and delivery to the lungs. Sidestream smoke may have a little longer to equilibrate

11.6.7 *Conclusions*

- Collated literature values for the redox potential and pH of tobacco smoke define a relatively restricted field on a standard Eh-pH diagram
- Cigars, some French cigarette blends and sidestream smoke are more alkaline than mainstream smoke from other (mainly western) cigarette blends.

- Redox potential of tobacco smoke becomes more reduced with increasing numbers of puffs.
- Synchrotron studies have so far determined the valence state of As and Cr in smoke condensate *in situ*, the results being As(III) and Cr(III). A synchrotron study of Cd in the literature indicates Cd is primarily in its Cd(II) state.
- Eh-pH modelling of the same elements predicts the same outcome for smoke at 25°C.
- This agreement between modelling and experiment encourages Eh-pH modelling of other elements identified as possible hazards in tobacco smoke.
- The models predict that As, Cd, Co, Pb, Mn and Ni will tend to exist as aqueous species (all but As in ionic form) in smoke at 25°C whereas Be, Cr, Cu, Hg and Se are modelled as belonging exclusively to the particulate phase of smoke.

11.7 Synthesis and Conclusions

11.7.1 *Pathway modelling and toxicity*

Many studies of metals (and metalloids) in tobacco and smoke have made a direct connection between a metal's concentration and a toxic effect attributable to that metal. While a deficiency of that metal probably indicates that no toxicity will result from that element, the mere presence of an element is usually insufficient to judge its likely contribution to toxicity. A complex mixture like tobacco smoke involving interactions between thousands of components makes judging toxicity on the basis of a single component very risky. Nevertheless, in the absence of practical multicomponent models it still necessary to assess the possible contributions to toxicity of individual metals.

Another weakness of assessing toxic potential on the simple basis of metal concentration is that it does not take into account bioavailability, reactivity and detoxification mechanisms. These are strongly influenced by metal species and exposure to pure metal is rare in the case of smoking. (Note that the modelling in 5.3.6 suggests Cu is transferred as the metal in the smoke particulate phase but this is an unusual case). Thus speciation, i.e. the chemical form in which humans are exposed to metals in smoke is crucial information for toxicological assessment.

Of the metals and metalloids discussed above several exist in the natural environment in multiple valence states and as pure metals. In some case, notably As and Cr, there is a marked difference in toxicity between the valence states of the same element. The reduced form of arsenic (As(III)) is considerably more toxic than the oxidised form (As(V)), at least in dietary exposure, whereas the oxidised form of chromium (Cr(VI)) is highly toxic compared with the other more reduced valence states of this metal. Thus valence state is essential information in predicting the toxic potential.

Smoke is transferred as an aerosol comprising vapour and particulates. The principal solvent of metals is water that in turn is partitioned between vapour (85%) and particulates (15%) [154]. Absorption of metals in the respiratory tract will be strongly influenced by the availability of metals as dissolved ions. In an analogous way the deposition of metals among particulates in the airways will depend on the solid, liquid or solution form of the metal. Again such data are important in predicting the toxicity of a metal.

The foregoing shows that detailed chemical speciation is essential information in assessing the toxicity of a given metal. It is also informative to understand the controls on speciation, whether derived from the original tobacco plant and its cultivation environment, or post-harvesting processing, or combustion and distillation, or possibly to some combination of these. An example is given below

to illustrate how such modelling works for the valence state of arsenic (Fig.29). Details of this example are given in section 11.5.

X-ray Absorption Near Edge Structure (XANES) was used to determine the valence states of As from the tobacco plant to the crucial combustion stage that creates respirable smoke. Samples studied include cultivated plants (some burdened with additional As), reference standards and commercial products, along with smoke condensate and ash from these samples. The relative contributions of As(III) and As(V) to the XANES spectra provide a consistent pattern of redox changes. Tobacco leaf and manufactured products tend to be dominated by As(V) whereas combustion produces respirable smoke invariably in As(III) form and ash invariably as As(V). The valence state of precursor tobacco is not a controlling factor because all the As mobilised in smoke is reduced during combustion. Thus tobacco combustion exposes smokers to potentially the most toxic forms of arsenic, and this exposure is magnified in regions where arsenic is present in tobacco crops at relatively high concentrations.

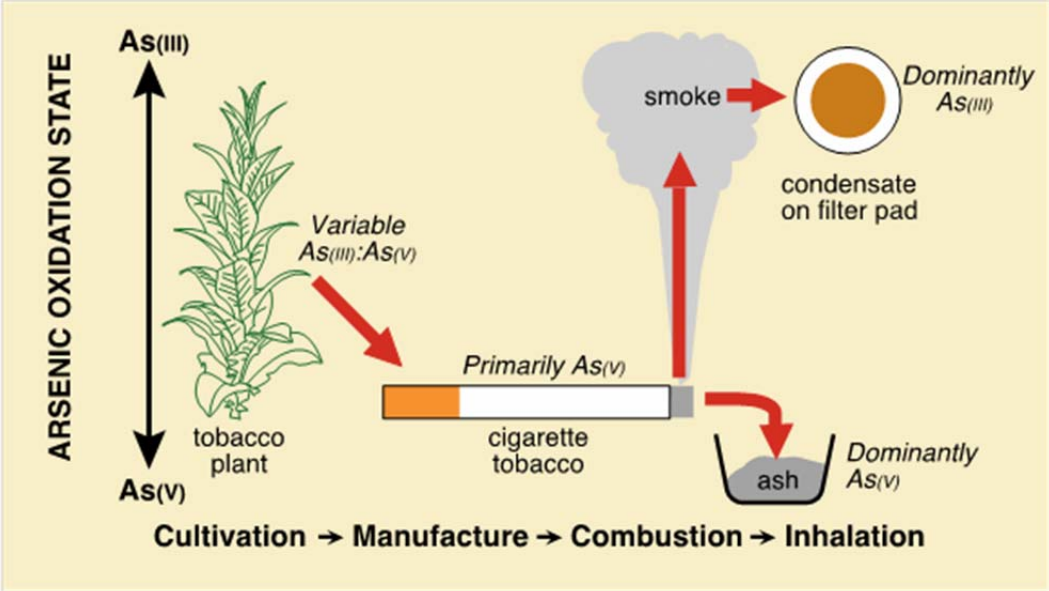

Figure 29. Model pathway of the variation in arsenic valence state (As(III) and AS(V)) from cultivation to exposure. Note that AS(III) is widely considered to be much more toxic than AS(V) and that combustion converts the arsenic involved in the combustion to the As(III) state prior to transfer in smoke.

11.7.2 *Implications for health*

The findings of this study can be interpreted in the context of published studies of the risk of cancer and other diseases due to individual smoke components [82, 184]. While such assessments are simplistic and ignore important factors such as synergistic action the calculated risks of individual hazardous compound span several orders of magnitude and their rank orders at least are informative.

11.7.2.1 Summary of metals in smoke

Table 14 summarises the findings on metal species and valence in the context of health, in particular the IARC classification of carcinogens and other non-cancer adverse health effects. Also important in this context is the concentration of each metal in smoke, and the values listed are derived from various averages presented in the literature [17, 184, 185].

Table 14. Summary of model outcomes in context of the IARC classification of carcinogens and other forms of toxicity, and the concentration range in smoke emissions.

Element	**Predicted major species**	**Predicted form in ambient smoke**	**IARC Classification of carcinogens**		**Other toxicity (list not comprehensive)**	**ISO MS smoke (µg/g)**	
			Group	**Form**		**min**	**max**
Arsenic	$As(OH)_3$	Solution	1	Arsenic and inorganic arsenic compounds	Cardiovascular, gastrointestinal, hepatic and renal diseases.	0.004	0.100
Beryllium	BeO	Solid	1	Beryllium and beryllium compounds	Pulmonary disease	0.001	0.006
Cadmium	Cd^{2+}	Ionic solution	1	Cadmium and cadmium compounds	Stomach irritation (vomiting and diarrhoea); lung damage; kidney diseases	0.031	0.271
Chromium	Cr_2O_3	Solid	1	Cr(VI) compounds	Blood, renal and liver diseases	0.15	1.5
Cobalt	Co^{2+} (major) $HCoO^{2-}$ (minor)	Ionic solution	2B	Cobalt and cobalt compounds	Contact dermatitis mutagenic effects.	0	0.4
Copper	Cu	Solid	Not known to be carcinogenic		Blood, kidney, gastrointestinal disease	0.013	0.013
Lead	Pb^{2+} (major) $Pb_6(OH)_8^{4+}$ (minor)	Ionic solution	2A	Lead compounds (inorganic). Metallic lead (2B)	Neurological damage; renal disease; cardiovascular and reproductive effects	0.032	0.41
Manganese	Mn^{2+}	Ionic solution	Not known to be carcinogenic		Neurological; liver function	0.002	0.003
Mercury	Hg	Solid	3	Mercury and inorganic mercury compounds	Nervous system, kidney damage	0.006	0.292
Nickel	Ni^{2+}	Ionic solution	1	Nickel compounds	Skin disease, allergies	0.001	0.887
Selenium	Se	Solid	3	Selenium and selenium compounds	Gastrointestinal disease, neurological damage	0.002	0.319

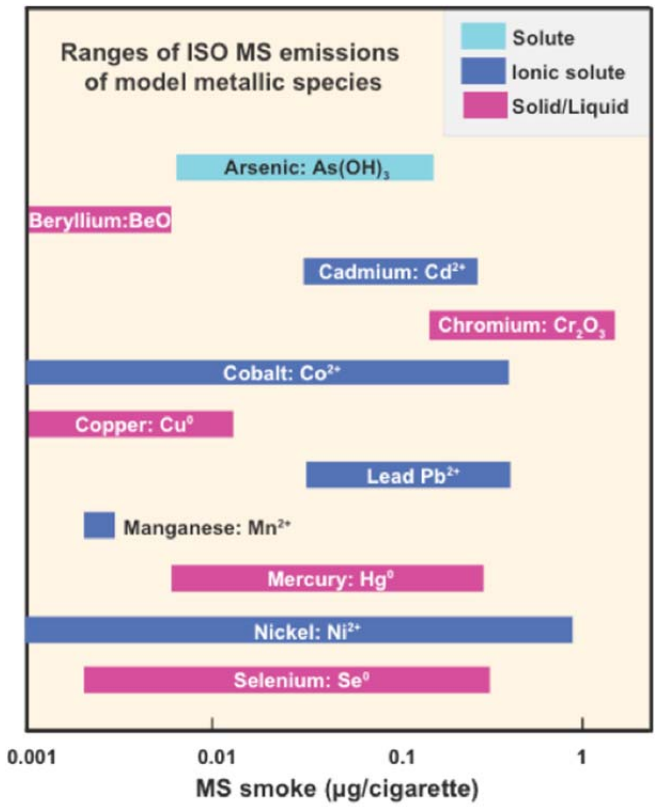

Figure 30.Ranges of mainstream smoke emissions (ISO) quoted in the literature for the metals analysed in this study. The range bars are annotated with the model species of each metal.

The data of Table 14 are graphically portrayed in Fig 30. The important elements in the figure are those that are present in smoke as ions in solution (i.e. the are more bioavailable) and are also emitted in high concentrations. Thus Cd and Pb qualify on this criterion along with Co and Ni at their higher levels of emission. As, though not modelled as an ion, does enter the aqueous phase as a solute and is also present in moderately high quantities. Cr, on the other hand, which is present in smoke in the greatest concentration, does not qualify as it is present as a solid oxide compound. Pure metals such as Hg, Se and Cu also do not qualify, and the oxide of Be also fails on grounds of very low emissions.

This largely qualitative interpretation highlights five metals and metalloids, namely As, Cd, Co, Ni and Pb. Cr is excluded as it may not be as readily bioavailable.

11.7.2.2 Metals and carcinogenesis

The qualitative approach does not take account of any quantitative estimates of toxicity and exposure. This is addressed using published estimates of risk indices for cancer, for which most data are available. Two similar approaches making use of different risk datasets (although some of the data have common roots) and difference emissions values have been published in the peer-reviewed literature. Fowles and Dybing (2003) was the first to show that cancer potency factors derived for air quality risk assessment could be used to estimate the "cancer risk index" of components in mainstream tobacco smoke [184]. Their data are reproduced here with adjustment for a transcriptional error for arsenic concentration in mainstream smoke. Behera *et al.* in a recent paper presented new data for metals in two US and two UK brands purchased in Singapore, and used the data to calculate the "incremental cancer risk" of individual metals [82]. A comparison of the two risk indices is presented in Figure 31, with the cancer risks spanning three orders of magnitude. Perfect agreement would mean all points falling along the dashed 1:1 line.

Figure 31 indicates high risks associated with Cr, Cd, As and Ni with lower risks associated with Be and Pb. Although Cr indicates the highest cancer risk the risk data are specifically associated with exposure to Cr(VI), i.e. hexavalent chromium, trivalent chromium having little or no toxicity in humans. It was established in section 11.5.5 that Cr in smoke condensate is primarily in Cr(III) state with minor particles of Cr(0) from steel fragments of machinery. No Cr(VI) was detected in the synchrotron studies. Thus Cr ought to be omitted from this comparison of cancer risk.

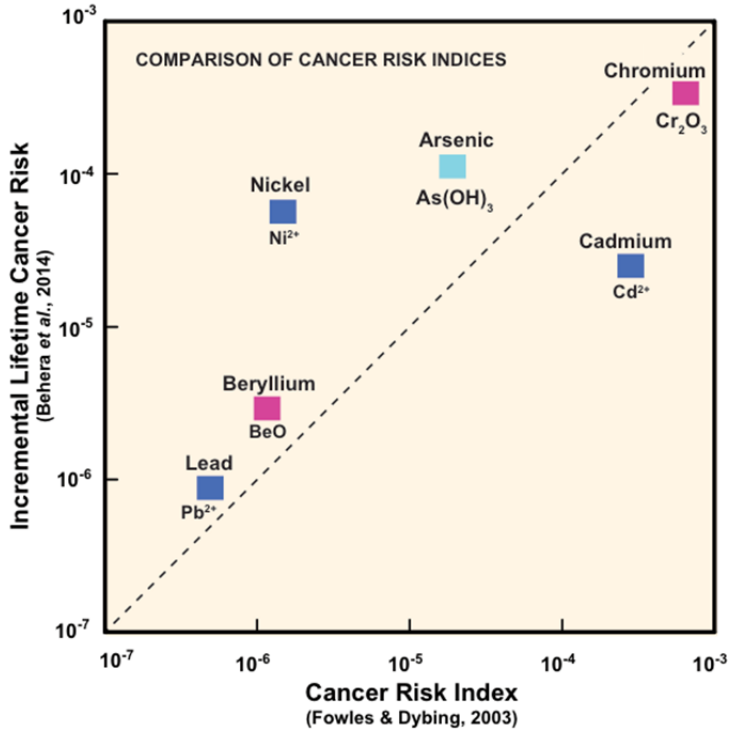

Figure 31. Comparison of values using two different cancer risk indices for various metals found in tobacco smoke. Dashed line indicates 1:1 agreement. Incremental Lifetime Cancer Risk based on the mean analysis of four brands in the paper by Behera et al. (2014). Colour coding as in Fig.30. See text for more details.

In contrast As, an element that also exists in multiple valence states in nature, has been shown by the synchrotron studies (section 11.5.3) to be present in smoke condensate exclusively as As(III). The cancer risk comes entirely from As(III) in inorganic compounds, and section 11.4.4 showed that arsenic in tobacco is approximately 80% inorganic and 20% organic. Thus arsenic should be firmly established as one of the most important metallic carcinogens.

The risk indices in Fig.31 favour Cd and Ni differently, nevertheless both rank highly in the list of metal carcinogens and both are present in smoke as bioavailable ions.

Risk comparison ranks Be and Pb rather lowly. The low rank for beryllium stems from its generally low concentration in emissions. This fact coupled with its modelled presence in smoke as an oxide in the solid state indicate that Be is most probably not a significant carcinogen in tobacco smoke. Pb on the other hand is modelled as being a bioavailable divalent cation in smoke, but its carcinogenic risk factor is relatively low. These data suggest that Pb also should not be regarded as a major tobacco smoke carcinogen.

Cobalt was not evaluated by Behera *et al.* (2014) but data in Fowles & Dybing (2003) suggest that it does not pose a major risk as a carcinogen.

In summary evaluation using data acquired in this study suggests that As, Cd and Ni should be regarded as the most important metal carcinogens in tobacco smoke.

11.7.2.3 Non cancer toxicity of metals

Using different factors Behera *et al.* (2014) developed an analogous risk index for non-cancer diseases and applied their model to four popular international brands purchased in Singapore [82].

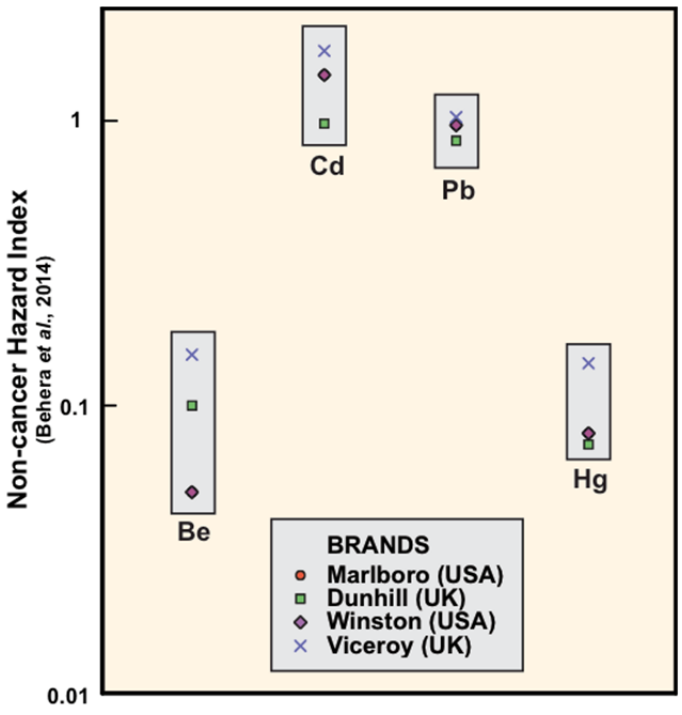

Figure 32. Non-cancer risk index values for Be, Cd, Hg and Pb in four popular cigarette brands. Data from the paper by Behera et al. (2014).

Fig.32 indicates that the risks of non-cancer disease posed by Cd and Pb in these samples are an order of magnitude greater than those for Be and Mg. Modelling results in section 11.6 indicate that neither Be nor Mg are likely to be present in smoke as aqueous species thus limiting their likely bioavailabilities, although metallic mercury is known to rapidly enter the bloodstream.

No data are available for the risks posed by manganese and selenium in smoke. Normally manganese is regarded as a nutrient and neurological toxicity is associated with inhalation of welding fumes and dust or drinking water in which manganese is unusually concentrated. Although Mn^{2+} is likely to be bioavailable in smoke its very low concentration suggests that it is almost certainly not an important contributor to smoking-related disease. Similarly Selenium is an essential nutrient and is only toxic when taken in excess. Modelling predicts selenium to be stable in elemental form in smoke, and the smoke environment is insufficiently oxidising to favour soluble selenite and selenate forms of selenium. Thus it is unlikely to be bioavailable and its low concentration in smoke suggests that selenium is also not an important contributor to smoking-related disease.

11.7.3 *Implications for regulation*

Articles 9 and 10 of the WHO's Framework Convention on Tobacco Control are concerned with the regulation of the contents of tobacco products and their disclosure, requiring the relevant authorities to "adopt and implement effective measures for public disclosure of information about the toxic constituents of the tobacco products and the emissions that they may produce" [186]. Achieving a consensus on these "toxic constituents" is a challenge. As any regulation of toxic constituents will impose burdensome requirements on the tobacco industry they can be expected to challenge each nominated constituent, including the metals identified in this study. There is already evidence for this in challenges to the various "Hoffmann lists" of analytes [185, 187] in reviews published by scientists working for the tobacco industry [17, 188].

One of the most cited lists of carcinogens (Hecht 2003) identifies 9 classes of chemicals in tobacco smoke, four of which are also present in unburned tobacco. Inorganic compounds (essentially the metals) are represented in both smoke and unburned tobacco. Thus metals are one of the most important classes of carcinogens and may well be important in other diseases. Clearly not all metals are implicated in smoking related disease and it is important to identify the subset of metals (and metalloids) that can be robustly defended using the best available scientific data.

A major weakness hitherto is that reviews of metal carcinogenicity and toxicity in smoke have considered only the concentration of the metallic element, and are rarely able to take into account associated compounds and valence state (some important elements vary from being non toxic to highly toxic with valence state). The present study has attempted to address some of these gaps using laboratory determinations of compound and valence species coupled with thermodynamic modelling based on knowledge of pH and redox conditions in smoke.

In 2012 TobReg (see section 1) recommended for regulation a subset of four metals (As, Cd, Ni and Pb, excluding those metals included for their radioactive properties) following very detailed reviews of the available literature on carcinogenicity and toxicity [2]. The present report follows a different approach based on metal (metalloid) speciation. It is noteworthy that the conclusions in section 11.6.7 highlighted As, Cd and Ni as potentially important carcinogens with Cd (again) and Pb as important contributors to other smoking related disease. These are the same elements as those identified by TobReg. This

investigation also finds that some other important IARC group 1 carcinogens such as Be and Cr are probably less important.

The fact that these independent approaches agree on their highest priority carcinogenic and toxic metals is beyond coincidence, adding strength to the scientific case for regulating As, Cd, Ni and Pb in crops and products, as recommended by TobReg.

In terms of practical implementation of regulating metals, the synchrotron studies are very important. As and Cr exist in different valence states with only As(III) and Cr(VI) being carcinogenic. Fig.29 summarises the investigation in this report into As valence state from cultivation to smoke condensate, and shows that combustion reduces all As(V) to As(III). The tobacco industry may argue that it is impractical to analyse crops and products using the most powerful synchrotrons in the world (as would be required for arsenic speciation), but this new evidence suggests that total As concentration, as inexpensively analysed by ICPMS, XRF etc. is sufficient because all As that is transferred by smoke is reduced to As(III) by combustion (at least in this study).

11.7.4 *Research gaps and areas for further research*

In its review of the literature TobReg identified a number of research requirement, many of which relate to the toxicological response to metals (and metalloids) in smoke. Even in the narrower remit of this study many gaps exist, some of which are highlighted below:

- Extend the HPLC-ICPMS studies of As species in tobacco to other elements, especially Cd, Ni and Pb
- Apply a similar HPLC-ICPMS methodology to smoke condensate. This has been done successfully for As [8, 9, 67] and methods should be developed to extend it to other elements.
- Synchrotron studies, hitherto restricted to characterising valence state using XANES, should attempt to use extended X-ray absorption fine structure (eXAFS) in order to constrain the co-ordination chemistry of elements in the neighbourhood of the metal atom. This offers and opportunity to constrain the chemical species of metals in the smoke condensate in situ without the possibility of change due to chemical extraction.
- Thermodynamic modelling should be developed into more complex mixtures including important anions such as S and Cl. The possibility of including kinetic and surface adsorption factors should also be explored.
- Analogous studies on non-smoking tobacco products should be conducted to characterise speciation in saliva, etc.
- Some studies may require metal-burdened samples, either natural or cultivated. Our work showed that cultivation of tobacco in burdened soils produces enrichments in leaf for As and Cd but burdening with Cr proved more difficult. Some other metals and metalloids may prove amenable to this approach.
- Now that methodologies have been developed for determining metal and metalloid species at low concentrations in smoke the logical next step is to characterise these elements in lung tissue following exposure of experimental animals to metal-enriched smoke.

11.7.5 *Conclusions*

This study has aimed to learn more about the nature of metals in tobacco and tobacco smoke. Not all elements could be studied in equal depth and the main focus of the research was arsenic. The principal conclusions from this research are:

11.7.5.1 Methodologies

- An exceedingly powerful third generation synchrotron has proved capable of successfully characterising the valence species of metals and metalloids at sub ppm levels in a range of tobacco materials including *in vivo* tobacco leaf, processed tobacco, filters and ash, and most notably smoke condensate.
- HPLC-ICPMS has also proved a powerful technique for discriminating organic and inorganic species of arsenic in a wide range of tobaccos. The same methodologies should also be applicable to other elements in which organic and inorganic species have variable carcinogenic and toxic effects.
- Simple thermodynamic modelling of metals in smoke produced results consistent with laboratory measurements, although these are currently few in number and more complex modelling may be required as more laboratory constraints become available.

Cultivation of metal burdened tobacco plants was successful for some metals and detailed study of As showed that the chemical speciation of moderately burdened samples is similar to unburdened and commercial tobaccos. This approach makes it possible to apply methods of characterisation that require higher concentrations to function than is normally found in commercial tobacco products.

11.7.5.2 Arsenic

- In tobacco plants arsenic is present at approximately 80% inorganic species and 20% organic species (primarily DMA and MA). Valence varies between largely As(III) and As(V) with most plants containing a mixture of these valencies. There is no relationship between valence balance and the nature of arsenic burdening (or none).
- Arsenic in cigarette tobacco tends to be present in its oxidised form As(V).
- Combustion of tobacco causes arsenic to become reduced entirely to As(III) in smoke condensate, and oxidise to As(V) in ash. Partitioning of oxidation state is complete and no mixture of valencies has been observed in any smoked tobacco product.
- Ageing of tobacco smoke for 30 minutes does not induce reduced As(III)-dominated smoke to oxidise to As(V).
- The data suggest that As may be an important smoke carcinogen.

11.7.5.3 Chromium

- Synchrotron studies show Cr to be present in tobacco as Cr(III) and Cr(0). The metallic Cr appears to be associated with fragments of steel in tobacco presumably from processing machinery.
- Cr was very difficult to detect in smoke products, but one sample of cigarette filter did indicate the presence of Cr(III). No Cr(VI) was detected in any tobacco or smoke products.
- The data suggest that not much Cr is mobilised in smoke and that little or none is in the carcinogenic hexavalent form and thus is unlikely to represent a significant hazard.

11.7.5.4 Other metals and metalloids

- Modelling suggests that Cd (especially) and Ni could be important smoke carcinogens.
- Modelling does not provide evidence for Be as a significant smoke carcinogen.
- There is evidence that Pb may be significant toxic smoke component for non-cancer disease.

- The evidence is not sufficient to include Co, Se, Mn, Cu and Hg among toxic smoke components although more research is warranted, especially on Co.

11.7.5.5 Regulation

- WHO's expert panel TobReg listed four metals recommended for regulation, namely As, Cd, Ni and Pb. This report presents evidence in support of prioritising the same four metals.
- No evidence was found for the presence of Cr(VI) in smoke and thus the inclusion of Cr in the list of elements is not recommended.
- Similarly no evidence was found to support the inclusion of other carcinogens such as Be and highly toxic metals such as Hg. At present the evidence for Co is weak but warrants more research.

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