

Toxic elements in tobacco and in cigarette smoke: inflammation and sensitization†‡

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Biochemically and pathologically, there is strong evidence for both atopic and nonatopic airway sensitization, hyperresponsiveness, and inflammation as a consequence of exposure to tobacco mainstream or sidestream smoke particulate. There is growing evidence for the relation between exposure to mainstream and sidestream smoke and diseases resulting from reactive oxidant challenge and inflammation directly as a consequence of the combined activity of neutrophils, macrophages, dendritic cells, eosinophils, basophils, as a humoral immunological consequence of sensitization, and that the metal components of the particulate play a role in adjuvant effects. As an end consequence, carcinogenicity is a known outcome of chronic inflammation. Smokeless tobacco has been evaluated by the IARC as a group 1 carcinogen. Of the many harmful constituents in smokeless tobacco, oral tissue metallothionein gradients suggest that metals contribute to the toxicity from smokeless tobacco use and possibly sensitization. This work reviews and examines work on probable contributions of toxic metals from tobacco and smoke to pathology observed as a consequence of smoking and the use of smokeless tobacco.

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1. Metals and metalloids in tobacco

Though exposure to substances from tobacco use is obviously a complex exposure, the carcinogens in tobacco smoke have been classified for health risk determinations into five major chemical classes.¹ Some of these have been carefully studied, contributing to a strong weight of evidence for associated health risks.² Toxic metals and metalloids constitute one of the more understudied major carcinogenic chemical classes in smokeless tobacco products and tobacco smoke. Eight of the top forty substances in the Fowles and Dybing table of cancer risk indices are metals or metalloid compounds.¹ In their table of non-cancer risk indices for individual chemical constituents of mainstream cigarette smoke based on a single cigarette per day, three of the top eight listed for respiratory effect health risk, cadmium, hexavalent chromium, and nickel, were metals. Another metalloid, silicon in the form of silicates, poses serious health risks by inhalation, but limited data is available, likely due to analytical difficulties.

Metals and metalloids in smoke from biomass combustion including tobacco are generally considered to be present in ionic form, but may also occur in a gaseous elemental form, as is the case for mercury.³ Whether a tobacco product is consumed by smoking or in a smokeless form, the exposure to toxic metals is directly related to the concentration in the tobacco leaf, assuming no metal containing additives are included during manufacture.^{4–6} The soil (including any amendments to the soil such as sludge, fertilizers, or irrigation with polluted water) has been shown to be the predominant source of characteristic metal content found in tobacco and



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varies with geographical area.^{7–17} Amendments that lower the soil pH increase metal availability.^{8,10,13} Therefore if soil or its amendments have elevated metal concentrations or low pH, this will be reflected in elevated metals concentrations in tobacco crops grown on the soil.

As an example of soil management and effects on tobacco metals concentrations, close to 80% of cropland soil in China was deficient in phosphate in 1980-less than 10 milligrams of phosphate per kilogram of soil. Over the last 30 years, the Chinese government put policies in place to encourage the use of phosphate fertilizer to remediate the phosphate-depleted soil. As a result, the average phosphate content in the soil has nearly tripled.¹⁸ While this increases crop production, phosphate, an excellent chelator of many metal ions, adds metals to the soil also. Fertilization with animal waste acidifies the soil and elevates concentrations from excreted toxic metals. These soil management practices, while founded on good agricultural practices, have increased levels of phosphate and metals in runoff wastewater sometimes used for irrigation as a consequence of excessive application. In unrelated studies, arsenic, cadmium, and lead concentrations in Chinese cigarette tobacco have been found to be two to three times higher than levels found in Canadian cigarette tobacco.⁶

Higher levels of metals in the tobacco likely result in increased exposure for smokers or consumers of smokeless tobacco products. Relating specific chemical intake or “dose” to a particular harmful outcome is difficult due to the chemically complex nature of tobacco products. For single chemical exposures it is much easier to relate specific chemical doses to a particular response or outcome. A dose-response relationship, a mathematical construct useful to characterize the effect on an organism from exposure is often used to estimate harm potential. The characterization may be on the basis of different levels of exposure for a specific duration of time. If the time duration is relatively short, it may be described as an acute exposure. At a constant level of exposure, the dose-response depends on the duration of exposure. If the duration of exposure is relatively long or repeated frequently, it may be described as a chronic exposure. The biological response to exposure to a stressor depends on both the level and duration of exposure.

Two examples of biological responses to both acute and chronic exposures to toxic metals as a consequence of tobacco use, and as a consequence of occupational exposures are included in discussions of metals in the next section. In many cases, the consequences of short duration low level chronic exposures would not be expected to result in the pathological manifestations of an acute high level exposure, nor to the same degree of intensity. Thus, some consequences of acute occupational metal exposures discussed may not be observed as a consequence of lower chronic occupational exposures or chronic exposures from tobacco products. Other factors, however, that should be considered when evaluating the effects of chronic toxic metals exposure are bioaccumulation and sensitization. Although a single acute exposure, or low level chronic exposure may not result in immediate clinical effects, bioaccumulation may result in an increase in pathological consequences over time. Several metals and metalloids described below accumulate in lung and other tissues as a

consequence of tobacco use. Further, if one is sensitized to a metal, then a biological response will often subsequently be observed at much lower concentrations. Several metals described below are potent sensitizers; and some of these also accumulate in the body. While Inhalation Minimum Risk Levels (MRL), when available, have been summarized in Table 4, the American Toxic Substances and Disease Registry cautions on the use of MRLs when sensitization is a consideration. See the Table 4 caption.

The extent to which consumption of a particular tobacco product confers toxic metal exposure risks is an important question. Many factors must be considered such as the form of the product, where and under what conditions the agricultural sources of the product were cultivated, manufacturing treatments prior to marketing, the manner in which the product is consumed, and individual differences in consumption habits. Smokeless tobacco products are consumed in a much different manner than cigarette tobacco or other smoking products. Whether the product is consumed in a smokeless form or by smoking influences overall exposure and subsequent associated health risks. In addition, people who are subjected to exposure in the form of secondhand smoke are often at increased risk. The pathology and associated health risks associated with tobacco products arise from the cumulative exposure to all toxic, irritant, and carcinogenic substances that are biologically available. However, since toxicology and carcinogenesis are complex processes, different toxic substances are usually approached for study individually. This review summarizes available evidence related to selected health risks from metals or metalloid exposures that are classified by the International Agency for Research on Cancer (IARC) carcinogens, or metals which show evidence of sensitization or inflammation as a consequence of exposure in smokeless tobacco products or from cigarette smoke.

2. Selected health impacts from metals and biological availability from tobacco

Exposure to a given toxic metal or metalloid is limited by the concentration of the metal in the tobacco product. Therefore concentrations of metals and metalloids in the tobacco itself are relevant and proportional to the amount transported in smoke from combustion products.^{4–6} Analytical data for metals in tobacco or smoke for estimation of dose, or as dose-limiting values summarized here is from the most recent sources at the time of writing with an effort to consider evidence of analytical accuracy with a few exceptions where well-validated data is sparse. Thus, the data on individual metals or metalloids listed alphabetically should not be construed as an exhaustive or comprehensive compilation. Inhalation Minimum Risk Levels (MRL) where available from ATSDR are included in Table 4.

Aluminum

Occupational inhalation exposures to aluminum in some chemical forms have been reported to result in chronic bronchitis, aluminum pneumoconioses, pulmonary fibroses, granulomatoses, anaphylactic responses, and neurotoxicity.^{19–23} Aluminum has been shown to be absorbed and reach the

Table 1 Metal concentration ranges reported in smokeless tobacco ($\mu\text{g g}^{-1}$ tobacco)

	Ghana ¹⁹⁷	Canada ¹⁹⁸	India ^{199–201}	U.S. ²⁰²	U.S. ³¹	Artificial Saliva ^a U.S. ³¹	Phosphate ^a U.S. ⁷⁰	DTPA, EDTA, or EDDHA ^a 70
Al	3006–5167							
As	0.108–0.256	0.143–0.437	0.1–3.5		0.13–0.29			
Ba	110–203				38–158	3.1–19 (3%–21%)		
Be					0.010–0.038	<0.003–0.010 (21%–32%)		
Cd	1.06–1.11	0.30–1.09	0.1–3.1	0.73–1.58	0.66–1.88	0.302–0.508 (21%–47%)	5%–15%	81%–109%
Co	0.056–0.201				0.26–1.22	0.171–0.739 (30%–65%)		
Cr	0.95–1.41	0.71–2.19	5.25–21.9		0.86–3.20			
Cu	18.5–27.7		9.02–61.5				24%–39%	23%–54%
Fe	2433–6982		354–3213					
Hg	0.007–0.012		0.02–0.11					
Mn	121–139							
Ni		0.84–2.05	1.33–13.1		1.39–2.73	0.370–1.153 (30%–46%)	0%–2.5%	15%–64%
Pb		0.23–1.20	1.76–13	0.27–2.96	0.28–0.85	<0.13–0.153 (8%)		

^a Extractable metals from smokeless tobacco ($\mu\text{g g}^{-1}$ tobacco). Empty spaces represent no reported analysis for the respective analyte.

brain *via* olfactory pathways,²⁴ and accumulates in lungs of smokers. Aluminum has been reported at significantly higher concentrations in the exhaled breath condensate of study subjects with Chronic Obstructive Pulmonary Disorder (COPD), than that of nonsmoking healthy control subjects. When the COPD patients were subdivided into smokers *vs.* ex-smokers and nonsmokers, smokers had significantly higher concentrations of aluminum in exhaled breath condensate.²⁵

Selected results from analysis of aluminum concentrations in smokeless tobacco and cigarette filler tobacco are summarized in Tables 1 and 2.

Arsenic

Arsenic is an IARC group 1 human carcinogen.²⁶ Arsenic is readily absorbed as a consequence of oral or inhalation exposure and has been associated with toxicities related to and causing vasoconstriction and other cardiovascular effects,²⁷ lung cancers, dermal cancers, and dermal sensitization.²⁸ Correlations between arsenic exposure and biomonitoring levels are difficult, since arsenic is rapidly cleared from the blood with a half-life of three to four hours.²⁸

Selected results from analysis of arsenic concentrations in smokeless tobacco and cigarette tobacco are summarized in Tables 1 and 2. Selected results from analysis of arsenic concentrations in cigarette smoke particulate obtained using ISO and Health Canada Intense smoking regimens are summarized in Table 3.

Barium

Barium is a dermal chemical irritant; and may cause dermal lesions.²⁹ When ingested orally or inhaled, barium can cause tachycardia, hypertension, and a benign granulomatous pneumoconiosis.³⁰

Selected results from analysis of barium concentrations in smokeless tobacco cigarette tobacco are summarized in Tables 1 and 2. One study using artificial saliva in an attempt to better mimic human uptake of extractable barium in smokeless tobacco showed that barium was readily extracted.³¹ Though extraction efficiencies from smokeless tobacco shown in parentheses in Table 1 were low in some cases, the net mass of artificial saliva-extractable barium was the highest of all the metals examined.³¹

Beryllium

Beryllium is an IARC group 1 human carcinogen,³² and is known to cause inflammation and sensitization reactions as a result of dermal or inhalation exposure. Pulmonary exposure may result in the granulomatous and fibrotic lung disease, berylliosis, or chronic beryllium disease, which further presents with interstitial edema, and acute obstructive pathology.³³

Because of its low concentration in tobacco and smoke particulate relative to other metals and associated analytical challenges, beryllium in tobacco smoke is generally below

Table 2 Metal concentration ranges reported in cigarette tobacco ($\mu\text{g g}^{-1}$ tobacco)

Citation	Canada ²⁰³	India ²⁰¹	Pakistan ²⁰⁴	U.K. ⁵⁸	U.S. ²⁰⁵	U.S. ²⁰⁶
Al			431–782		699–1200	
As	0.151		0.73–0.86	0.1–0.7		0.250–0.250
Ba					40.7–56.6	68.3–75.1
Be						0.016–0.017
Cd	0.930	0.28–0.87	2.2–4.5	0.5–0.8		
Co					<0.01–0.94	0.348–0.425
Cr	0.353	2.8–5.0		1.3–3.1		0.484–1.27
Cu		9.01–19.2		11.7–16.2		3.49–4.00
Fe		468–1129		293–576		
Hg	0.027					0.020–0.021
Mn					155–400	
Ni	0.250	7.21–10.2	1.2–2.8	1.1–2.7		1.13–1.18
Pb	0.257	0.79–5.79	1.1–1.6	0.4–0.9		0.604–0.607

Empty spaces represent no reported analysis for the respective analyte.

Table 3 Metal concentration ranges reported in cigarette smoke ($\mu\text{g}/\text{cigarette}$)

	Phillip Morris [®] International ISO ²⁰⁷	Phillip Morris [®] International Intense ²⁰⁷	Canada ISO ²⁰³	Canada Intense ²⁰³	U.S. ISO ⁵⁶
As	<LOD-0.0055	<LOD-0.0145			
Cd	0.0016–0.101	0.0435–0.1971	0.0576	0.1608	0.0138–0.0624
Hg	0.0011–0.0063	0.0042–0.0107	0.0032	0.0065	
Pb	0.0039–0.0392	0.0257–0.0932	0.0167	0.0372	0.0071–0.0289

Table 4 Inhalation Minimum Risk Levels derived from reviews of NOAELs and LOAELs reported in the literature. ATSDR cautions that criteria for reported calculations of some observed effect levels are based on serious disease and not appropriate for MRL calculations. In addition, ATSDR cautions that MRLs "... may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis, such as discussed in this manuscript." Where no MRL was calculated, the reasons that the data were rejected for determination of an MRL are cited

Aluminum ²⁰⁸	None	
Arsenic ²⁰⁹	None	
Barium ³⁰	None	
Beryllium ²¹⁰	None	
Cadmium ³⁹		0.01 $\mu\text{g m}^{-3}$ (> 1 yr)
Chromium(vi) ²¹¹		0.005 $\mu\text{g m}^{-3}$ (> 1 yr)
Chromium(iii) ²¹¹		0.1 $\mu\text{g m}^{-3}$ (15–364 days)
Cobalt ²¹²		0.1 $\mu\text{g m}^{-3}$ (> 1 yr)
Copper ⁶⁷	None	
Iron	None	
Lead ⁷⁹	None	
Manganese ²¹³		0.1 $\mu\text{g m}^{-3}$ (> 1 yr)
Mercury ²¹⁴	None	
Silicates	None	

analytical method detection limits. Beryllium concentrations in tobacco smoke were reported below respective method detection limits.³⁴ Thus, it is difficult at present to assess the significance to health consequences of beryllium in tobacco smoke. Beryllium ion in poorly soluble forms, such as the oxide, accumulates in lung up to a concentration plateau when equilibrium is reached between deposition and clearance during continuous exposure. About half is rapidly cleared predominantly *via* the lymphatic system. The more slowly cleared portion may accumulate in the lungs for a longer period and be involved in toxic challenge. In rats, females exhibited less efficient clearance and earlier morbidity and mortality from exposure.³⁵ Rhoades and Sanders³⁶ reported a 400 day half life for clearance of beryllium oxide from rat lung. In a dose study of beryllium sensitization and chronic beryllium disease in a beryllium machining plant, 20 of 235 individuals who had lifetime weighted average airborne exposures between 0.024 $\mu\text{g m}^{-3}$ and 0.6 $\mu\text{g m}^{-3}$, well below the 2 $\mu\text{g m}^{-3}$ occupational exposure limit intended to prevent chronic beryllium disease, nevertheless were found to be sensitized to beryllium.³⁷ Once sensitization is detectable, progression of the obstructive disease often occurs at a rate depending on level of exposure.³⁸ Thus inflammation and sensitization from low beryllium exposure as a result of smoking or use of smokeless tobacco use may be a concern even at low concentrations.

Selected results from analysis of beryllium concentrations in smokeless tobacco and cigarette tobacco are summarized in Tables 1 and 2. Beryllium was extracted from smokeless tobacco using artificial saliva and showed a measurable

extractable beryllium concentration for only one U.S. brand of moist snuff and three samples of leaf tobacco sold for chewing. The other four had less than 0.003 $\mu\text{g g}^{-1}$ extractable beryllium (the method limit of detection).³¹ Extraction efficiencies for beryllium were higher than for barium.

Cadmium

Cadmium is an IARC group 1 human carcinogen³² and is highly toxic to kidney, bone, and the nervous, respiratory, and circulatory systems.³⁹ Blood cadmium levels are strongly associated with increased prevalence of Peripheral Artery Disease.⁴⁰ Associations between increased urine cadmium concentration and periodontal disease,⁴¹ between cadmium exposure, smoking, and pancreatic cancer,⁴² and between cadmium exposure, smoking, and diabetes have been reported.⁴³

Cadmium is typically among the highest concentrations of the toxic and carcinogenic metals found in tobacco. Cadmium has a biological half-life of 13.6 to 23.5 years.⁴⁴ Because of its long biological half-life, cadmium bioaccumulates as a consequence of smoking. Increases in cadmium levels in lung tissue have been correlated with smoking history.⁴⁵ Cadmium concentrations have been reported to be significantly higher in four of five lobes of smokers' lungs examined by Tsuchiyama *et al.*⁴⁶ The mean cadmium concentration was higher in the fifth lobe of smokers than of nonsmokers, but the differences were not significant. Elevated cadmium levels in body fat,⁴⁷ blood,^{48–50} urine,^{48,51,52} and in amniotic fluid of women,^{49,53} indicate systemic absorption from the lungs.

Pulmonary exposure to nebulized cadmium compounds has been demonstrated to induce emphysema⁵⁴ and pulmonary interstitial fibrosis.^{23,55} Cadmium has been reported at higher concentrations in the exhaled breath condensate of study subjects with Chronic Obstructive Pulmonary Disorder (COPD), than that of the nonsmoking healthy control subjects and in current control smokers *vs.* control nonsmokers. When the COPD patients were subdivided into smokers *vs.* ex-smokers and nonsmokers, smokers had significantly higher concentrations of cadmium in exhaled breath condensate. Cadmium in exhaled breath condensate positively correlated with smoking history in pack-years.²⁵

Selected results from analysis of cadmium concentrations in smokeless tobacco and cigarette tobacco are summarized in Tables 1 and 2. One study using artificial saliva in an attempt to better mimic human uptake of extractable cadmium in smokeless tobacco showed that cadmium was readily extracted.³¹ The results of the extractions including extraction efficiencies shown in parentheses are shown in Table 1. Also included in Table 1 are extraction efficiencies from Maier *et al.* using phosphate buffer or 0.001 M chelating agents DHHA, EDTA, and DTPA in phosphate buffer. Selected results from analysis of cadmium concentrations in cigarette smoke particulate

obtained using ISO and Health Canada Intense smoking regimens are summarized in Table 3.

Normalization of the cadmium deliveries from U.S. cigarettes to tar delivery eliminated all significant differences between smoke delivery categories.⁵⁶ Delivery differences could therefore be attributed to differences in filter ventilation levels. Cadmium transported in smoke particulate matter from twenty-one counterfeits of two popular U.S. brands seized in 2003 in six different length and nominal smoke delivery categories were from 2.0 to 6.5 times higher than the authentic U.S. brands purchased in 2003; and the differences were all significant.⁵⁷ Stephens *et al.* reported significantly higher cadmium concentrations in tobacco from counterfeit cigarettes seized in the U.K.⁵⁸

Chromium

Chromium(vi) is known to cause oral and epidermal allergic contact dermatitis as well as pulmonary sensitization.^{59–64} Chromium(vi) is found in cigarette smoke and ash.⁶⁵ There are limited reports that elevated chromium(iii) exposure may also result in contact allergic sensitization.⁶² While it is generally presumed that most of the chromium in tobacco is in the chromium(iii) oxidation state,⁶⁵ manganese oxides are known to oxidize chromium(iii) to chromium(vi) in soil and solutions.⁶⁶ Manganese in one or more oxidation states is transported in smoke particulate, therefore it is possible that this oxidation could also occur in saliva or in smoke moist particulate droplets, and on moist surfaces in the lungs to some degree.

Accumulation of chromium in lung tissue has been correlated with smoking history, confirming that chromium, in some form, reaches the lung.⁴⁵ Chromium concentrations have been reported to be significantly higher in all five lobes of smokers' lungs than in nonsmokers' lungs.⁴⁶ However, it is not clear in what proportions chromium(iii) and chromium(vi) accumulate. Data to date have been based on analyses of chromium(iii) and chromium(vi) by difference in two methods, in cigarette ash, or before and after reduction. Chromium in tobacco smoke is therefore a health concern, but it is currently difficult to assess the full impact of oral, pulmonary, and systemic chromium exposure with regard to health consequences in view of the insufficiently available data to directly and reliably characterize the concentrations and biological consequences associated with the oxidation state of chromium.

Selected results from analysis of chromium concentrations in smokeless tobacco and cigarette tobacco are summarized in Tables 1 and 2.

Copper

Copper is nutritionally required at low concentrations, but inhaled copper is a respiratory irritant, causes alveolar migration of macrophages, eosinophilia, formation of histiocytic and noncaseating granulomas containing inclusions of copper, pulmonary fibrosis, and formation of fibrohyaline nodules very similar to those found in silicosis as a consequence of occupational exposures.⁶⁷ Copper was shown to more strongly induce pulmonary inflammation than other transition metals on a per mass basis when tested in rats.⁶⁸ Copper is an active

oxidation–reduction (redox) metal, as is iron. Since the redox chemistries of iron and copper have similar toxicological consequences, discussion of the relevance of redox activity follow in the discussion of iron.

Although copper has been reported at significantly lower concentrations in the exhaled breath condensate of study subjects with Chronic Obstructive Pulmonary Disorder (COPD) than that of the nonsmoking healthy control subjects,²⁵ copper has been determined at significantly higher concentrations in blood of smokers than of nonsmokers.⁶⁹

Selected results from analysis of copper concentrations in smokeless tobacco and cigarette tobacco are summarized in Tables 1 and 2. Extraction efficiencies using phosphate buffer or 0.001 M chelating agents DHHA, EDTA, and DTPA in phosphate buffer⁷⁰ are also included in Table 1.

Iron

Iron(ii) inhalation was shown to cause pulmonary inflammation in rats, though not as strongly as copper and nickel.⁶⁸ Due to its redox chemistry, iron is also known to catalyze highly reactive hydroxyl radical formation from superoxide ion and hydrogen peroxide by the two-step Fenton reaction,⁷¹ as does copper. As a consequence, inhaled iron and copper could contribute to free radical-induced lung injury.

Iron has been reported at significantly lower concentrations in the exhaled breath condensate of study subjects with COPD compared to nonsmoking healthy control subjects,²⁵ but Padmavathi *et al.* determined iron at significantly higher concentrations in serum of chronic smokers than of nonsmokers⁷² in agreement with other rat and human studies.⁷³ Presence of trace iron with silicates has been shown to augment pulmonary inflammatory response to silica exposure.^{74–77} Selected results from analysis of iron concentrations in smokeless tobacco and cigarette tobacco are summarized in Tables 1 and 2.

Lead

Lead is classified as an IARC group 2A probable human carcinogen.⁷⁸ Lead accumulates over the lifetime in bone. Even at adult blood lead concentrations that are considered to be acceptably low (<10 µg/dL), associations between lead concentration and elevations in systemic blood pressure and decrements in glomerular filtration rate have been reported.⁷⁹ Increased lead accumulation in the blood and in amniotic fluid of women,⁴⁹ and in the cord blood of newborn babies^{80,81} has been associated with smoking. Elevated blood lead levels in U.S. children have also been associated with second-hand smoke exposure.⁸²

Lead concentrations have also been reported to be significantly higher in four of five lobes of smokers' lungs,⁴⁶ indicating accumulation in smokers' lungs also. Lead has been reported at higher concentrations in the exhaled breath condensate of study subjects with COPD, than that of the nonsmoking control subjects and in current normal smokers *vs.* nonsmokers. Further subdivision of COPD patients comparing smokers *vs.* ex-smokers and nonsmokers also showed that smokers had significantly higher concentrations of lead in exhaled breath condensate.²⁵

Selected results from analysis of lead concentrations in smokeless tobacco and cigarette tobacco are summarized in

Tables 1 and 2. One study using artificial saliva in an attempt to better mimic human uptake of extractable lead in smokeless tobacco showed that lead was not readily extracted.³¹ The results of the extractions including extraction efficiencies shown in parentheses are shown in Table 1. Selected results from analysis of lead concentrations in cigarette smoke particulate obtained using ISO and Health Canada Intense smoking regimens are summarized in Table 3. Normalization of the lead deliveries to tar eliminated all significant differences between smoke delivery categories. Delivery differences could therefore be attributed to differences in filter ventilation levels. Lead concentrations transported in identical varieties purchased in 2004 were not significantly different from the comparable 2002 varieties among the brands tested, with only one exception.⁵⁶ However, the lead in mainstream smoke particulate matter from twenty-one counterfeits of two popular U.S. brands seized in 2003 in six different length and nominal smoke delivery categories were from 3.0 to 13.8 times higher than the authentic U.S. brands purchased in 2003; and the differences were all significant.⁵⁷ Stephens *et al.* reported significantly higher lead concentrations in tobacco from counterfeit cigarettes seized in the U.K.⁵⁸

Manganese

Manganese(II) complexes have been studied in tobacco.⁸³ Manganese(III) and (IV) exists in complex-bound forms such as plant photosystem II proteins.⁸⁴ The (III), (IV), (V), (VI), and (VII) oxidation states are generally more toxic in uncomplexed forms. The capacity of manganese oxides to oxidize chromium(III) to chromium(VI)⁶⁶ adds the oxidation–reduction dimension to potentiation of chromium toxicity. U.S. Environmental Protection Agency reports stated that compounds of manganese were suspected of inducing or exacerbating asthma.⁸⁵ Manganese(II) has been shown to cause pulmonary inflammation in rats, though not as strongly as copper or nickel.⁶⁸

Selected results from analysis of manganese concentrations in smokeless tobacco and cigarette tobacco are summarized in Tables 1 and 2.

Mercury

Mercury is highly systemically toxic in a number of forms.⁸⁶ Mercury from dental amalgam is associated with sensitization and intraoral lichenoid lesions in some cases.^{87,88} Metallic mercury and mercury compounds were included among air pollutant compounds of concern due to toxicity and as respiratory tract irritants that may exacerbate asthma.⁸⁵ Selected results from analysis of mercury concentrations in smokeless tobacco and cigarette tobacco are summarized in Tables 1 and 2. Selected results from analysis of mercury concentrations in cigarette smoke particulate obtained using ISO and Health Canada Intense smoking regimens are summarized in Table 3.

Nickel and cobalt

Although both cobalt and nickel are nutritionally required at trace concentrations, nickel is an IARC group 1 carcinogen,⁸⁹ and cobalt is an IARC group 2b possible human carcinogen.^{90,91} Though cobalt is neither considered as potent a carcinogen as

nickel, nor generally present in tobacco at concentrations as high as those of nickel, they are related immunologically in causing metal sensitizations including epidermal and oral allergic contact sensitizations, contact dermatitis inflammations, pulmonary inflammations and pneumoconioses, and asthmatic conditions.^{23,60,63,64,92,93} Once sensitized to one of these metals, immunological cross sensitization to the other is often observed, since they share an endothelial inflammatory activation pathway.^{23,94,95} Though lipopolysaccharide is the natural ligand for human Toll-like receptor 4 (TLR4) that is involved in inflammatory response, nickel(II) has been specifically identified as directly activating proinflammatory signal cascades by binding to this receptor.⁹⁶ Dolovich *et al.* determined an additional mechanism by which nickel sensitization-induced inflammation occurred: binding to the copper binding site of human serum albumin, and causing sensitization to the resulting metal-protein complex.⁹⁷ Cobalt was also able to bind to serum albumin and to the antibody complex. Shirakawa *et al.* also discovered cobalt-conjugated human serum albumin-specific IgE in patients with occupational hard metal asthma.⁹⁸ Like many of the other metals, nickel bioaccumulates. Nickel concentrations have been reported to be significantly higher in all five lobes of smokers' lungs compared to nonsmokers' lungs.⁴⁶ Nickel has been reported as present in significantly higher concentrations in placenta samples of smokers than in placenta of non-smokers,⁵³ affirming systemic absorption from the lungs.

Selected results from analysis of cobalt and nickel concentrations in smokeless tobacco and cigarette tobacco are summarized in Tables 1 and 2. One study using artificial saliva in an attempt to better mimic human uptake of extractable cobalt and nickel in smokeless tobacco showed that cobalt and nickel were readily extracted.³¹ The results of the extractions including extraction efficiencies shown in parentheses are shown in Table 1. Also included in Table 1 are extraction efficiencies for nickel using phosphate buffer or 0.001 M chelating agents DHHA, EDTA, and DTPA in phosphate buffer.⁷⁰ Stephens *et al.* (2005) reported nickel concentrations from 1.1 to 2.7 $\mu\text{g g}^{-1}$ in tobacco from legally purchased cigarettes available in the U.K. (Table 2). They reported nickel concentrations and from 0.9 to 9.2 $\mu\text{g g}^{-1}$ in tobacco filler from counterfeit cigarettes (Table 2).⁵⁸

Silicon

Silicon is taken up from soil by plants in the available silicate form, generally in the form of kaolin (aluminum silicate). Silicates accumulate in higher plants and have structural and stress resistance roles in plant physiology.⁹⁹ The concentrations of silicates in plants exceed solubility and form biogenic “phytoliths”,¹⁰⁰ which are predominantly silica (SiO_2) polymers.

Silica inhaled in the form of quartz or cristobalite (different forms of SiO_2) is an IARC group 1 human carcinogen.¹⁰¹ When tobacco smoke is inhaled, silicates in the forms of metal silicates and silica (SiO_2) particles are transported. Aluminum silicate particles are found in smokers' lungs at elevated concentrations.¹⁰² Lynn *et al.* described bronchoalveolar lavage containing 10^{11} macrophages with prominent lysosomes containing amorphous carbon, round dense particles,

and needle-like crystals of aluminum silicate from a pulmonary patient for whom no source except smoking was found as an explanation for the foreign substances.¹⁰³ Choux *et al.* described the composition of numerous silicate particles in the alveolar macrophages of a patient with tobacco-associated pulmonary fibrosis as fiber-, needle-, or laminar-like inclusions that varied from 0.2 to 2 μm in size, the size range of the major proportion of the total mass of particulate from cigarette smoke.¹⁰⁴ Aluminum and silicon were the major elemental components. Iron and sulfur were additional components. Brody and Craighead described lysosomal “smokers inclusions” in interstitial and alveolar macrophages and lymphocytes as predominantly aluminum silicate with plate-like structures, and suggested their involvement in pulmonary fibrosis.¹⁰⁵ Heckman and Lehman described lung epithelial cells of rats that had received chronic tobacco smoke exposure as containing elongated cytoplasmic inclusions. They stated that macrophages had similar larger inclusions composed of silicon, aluminum, phosphorous, iron and sulfur.¹⁰⁶ Thus silicate metal-bearing particulate is a major component of the particulate found in smokers’ lung. As described above, presence of trace iron has been shown to augment formation of reactive oxygen species in pulmonary inflammatory response to silica exposure.^{74–77} Nonsmokers may also acquire environmental silicate exposure to a much lower extent unless exposed occupationally.¹⁰⁷ Data on silicon (silicates) in tobacco or tobacco smoke is sparse, likely because of analytical difficulties.

3. Transfer of metals in mainstream, sidestream, and secondhand tobacco smoke

Basic smoke chemistry

The temperature of tobacco burning at the tip of a cigarette may reach over 900 $^{\circ}\text{C}$. Smoke inhaled into the mouth (mainstream smoke) is approximately 30 $^{\circ}\text{C}$; and sidestream smoke leaving the burning tip falls below 100 $^{\circ}\text{C}$ about 10 centimetres from the tip.¹⁰⁸ Thus a burning cigarette tip is hot enough to volatilize many metal ions, or to cause them to react with other substances to form volatile compounds and complexes. As a consequence, some of the metals may reside in the gas phase. By the time the smoke is inhaled or rises in a plume from the cigarette as sidestream smoke, most of the metal ions condense with other materials forming particles that comprise much of the particulate matter of the smoke aerosol.¹⁰⁸ Mainstream cigarette smoke, when inhaled, transports many substances through the mouth, throat, and into the lungs, where a substantial portion of the particulate matter and volatiles are deposited. Many of these substances are rapidly absorbed through the lungs, transfer efficiently to the blood stream, and are distributed quickly through the circulatory system. Other smoke constituents including 60% to 80% of particulate are retained, accumulate in the lungs, and gradually partition between lung airways, tissue, and circulatory or lymphatic absorption.¹⁰⁹

Particulate size

Cigarette smoke is a major source of exposure to ultrafine and fine particulate matter. Most of the particulate mass occurs in

particles with diameters between 0.1 and 1.3 μm , the lower half of the fine particle diameter range.^{110,111} Though ultrafine particulate ($<0.1 \mu\text{m}$) from tobacco smoke is not the fraction with the greatest mass, the small size facilitates deeper penetration into the lung, more rapid uptake into cells, and into circulation.^{112,113} Geiser *et al.* found that after an exposure of rat lung to a 4–5 μg dose of insoluble ultrafine TiO_2 particles, the particles were found widely distributed on luminal sides of airways and alveoli, in all tissue compartments and cells, and within capillaries. They concluded that these ultrafine particles were not taken up by endocytic processes, but by diffusion.¹¹⁴ Ferin and Oberdörster¹¹⁵ stated earlier that particles not phagocytized by alveolar macrophages were taken up by endocytosis in alveolar epithelial cells. They stated that an increasing dose in terms of particle number promoted greater interstitialization, associated with inflammation. Whether the systemic dispersion of ultrafine particles was due to diffusion or transcytosis, it is apparent that particle dose, size, and composition impact the response. Smaller particle size increases the potential for oxidative stress per unit mass of particulate matter as a consequence of the greater surface area to mass ratio. Ultrafine or nanosized ultrafine particulate may cause greater neutrophil inflammatory response per unit mass.^{112,113}

Approaches to estimate metals exposures from smoke

Once a metal or metalloid is absorbed in the lung, its biological fate determines much of the resulting health impact. Some metals such as cadmium and chromium may accumulate and remain predominantly in the lung tissue for a very long biological lifetime.⁴⁵

Since pulmonary lavage and biopsy procedures are invasive, analyses of metal concentrations in smoke are used to estimate relative potentials for exposures to metals from cigarettes. Most published reports of metals concentrations in cigarette smoke used standardized machine smoking regimens based on ISO conditions (35 mL puff volume, 2 s puff duration, 60 s puff frequency). Data obtained using Intense smoking conditions (50 mL puff volume, 2 s puff duration, 30 s puff frequency with any ventilation holes blocked) is very appropriate for relative estimations of exposure potentials, given the intentional or unintentional blocking of ventilation holes with smokers’ fingers,¹¹⁶ but data derived from use of the intense regimen is sparse. These regimens have been developed as approximations to a “typical” individual smoking pattern, though individual differences vary widely. Though smoking machine methods are better suited for comparing physical design characteristics or brand to brand differences, they do provide useful information on the presence and typical concentrations of metals in mainstream smoke.

4. Toxicological ramifications of tobacco smoke exposure

Adjuvant effect in sensitization, and inflammation caused by tobacco smoke inhalation

Biochemically and pathologically, there is strong evidence for both atopic and nonatopic airway sensitization, hyperresponsiveness, and inflammation as a consequence of exposure to

tobacco mainstream or sidestream smoke particulate. There is growing evidence for the relation between exposure to mainstream and sidestream smoke and diseases resulting from reactive oxidant challenge and inflammation directly as a consequence of the combined activity of neutrophils, macrophages, dendritic cells, eosinophils, basophils, as a humoral immunological consequence of sensitization, and that the metal components of the particulate play a role in adjuvant effects.

Tobacco smoke exposure has been shown to increase sensitization to organic and biological allergens. Nielsen *et al.* discussed the evidence favoring a sensitization role in occupational exposures to organic and biological substances using smoking as a model for airborne adjuvant effects. They concluded that smoking exhibited an adjuvant effect on the immune response to many potential biological, organic, and inorganic allergens, including platinum metal salts. Development of platinum-specific IgE and sensitization studied with skin prick tests was described.¹¹⁷ Arnson *et al.* discussed data showing that cigarette smoke was able to augment the production of pro-inflammatory cytokines such as TNF- α , IL-1, IL-6, IL-8, GM-CSF (all of which are also associated with sensitization to various substances), and to decrease the levels of interferon- γ (IFN- γ) and anti-inflammatory cytokine IL-10. Data showing that exposure to tobacco smoke leads to elevated IgE concentrations, to the subsequent development of atopic diseases and asthma, and to the known activation of macrophage and dendritic cell activity was also discussed.¹¹⁸ Both alveolar macrophages and dendritic cells may act as antigen-presenting cells that could have induced the IgE production.

Rumold *et al.*¹¹⁹ studied the sensitization of immunological low responder (C57BL/6) mice exposed to nebulized ovalbumin with or without concurrent inhalation of sidestream smoke. They found that sidestream smoke exposure induced sensitization to ovalbumin, evident from antigen-specific IgE compared to no detectable sensitization in those exposed to ovalbumin alone. Upon rechallenge, significantly increased levels of proinflammatory GM-CSF and IL-2 cytokines could be detected in bronchoalveolar lavage even in the secondhand smoke alone-exposed animals.¹¹⁹

Goel *et al.* reported significantly higher serum IgE concentrations in smokers compared to former smokers and non-smokers in a study of 70 individuals. Absolute eosinophil counts from smokers and former smokers were not significantly different between the two groups, but both were significantly higher than those of nonsmokers. No significant airways obstruction was determined in non-smokers, but both smokers and former smokers had significantly greater obstruction than non-smokers. Former smokers showed significantly greater airways obstruction than current smokers.¹²⁰

Regland *et al.* reported a strong relation between smoking and nickel contact allergy in both atopic and nonatopic individuals, though more prevalent in nonatopic smokers than in atopic smokers.¹²¹ In a cross-sectional study, Linneberg *et al.* found with patch test that general contact allergy to any of 23 chemical allergens, specific nickel contact allergy, and allergic nickel contact dermatitis were significantly and dose-dependently associated with smoking history of 15 pack years

or more. Linneberg *et al.* also stated that smoking-associated contact allergy was observed among both atopic and nonatopic subjects; and that no significant association between skin prick test and contact allergy was observed. In these cases, sensitized T cell-mediated cellular immunity may have played a major role.⁶³

Immune cells and metallo-particles: mixed immunological activation

Sanders *et al.* showed that NiO [nickel(II) oxide] and Cr₂O₃ [chromium(III) oxide] dust particles were predominantly found engulfed by alveolar macrophages in hamsters. A smaller fraction was in the alveolar lumen itself. A still smaller fraction was found in neutrophils of hamsters that were also exposed to cigarette smoke and in the type I but not the type II alveolar epithelium. The authors pointed out that neutrophils were rarely observed in alveolar lumens of hamsters exposed to only NiO, and that observed vacuolization was a more common finding in macrophages from animals which were also exposed to cigarette smoke.¹²²

Gilmour *et al.* demonstrated that intratracheal exposure of rats to either high transition metal-containing residual oil fly ash particulate or its major constituent metals alone (nickel or vanadium) caused significant pulmonary inflammation. Increased protein levels and TNF- α , monocyte and granulocyte migration to the site were observed. They reported that the predominantly metals-containing particulate exhibited an adjuvant effect on sensitization to dust mite with IgE production, as several authors cited earlier observed with tobacco smoke particulate inhalation.¹²³ In a separate study, Lambert *et al.* showed that the enhanced sensitization was mediated by the soluble metal constituents of the particulate. Specifically, increased eosinophil numbers in bronchoalveolar lavage were observed in response to either particulate or iron exposure alone during sensitization. Dust mite-specific IgE was higher in groups exposed to particulate, or to nickel or vanadium alone.¹²⁴

Costa and Dreher compared dose response of rats to which oil or coal fly ash particulate was administered intratracheally on the basis of either total particulate mass or on the basis of bioavailable transition metal mass. Their results indicated that it was the lung dose of bioavailable transition metal and not total instilled particulate mass that was the primary determinant for acute inflammatory response.¹²⁵

Walczak-Drzewiecka *et al.* observed allergen-mediated activation of cultured nonsensitized mouse mast cells with only 2% to 5% degranulation in response to 10⁻⁷ M aluminum chloride, nickel(II) sulfate, strontium or cadmium chloride alone. When dinitrophenyl-human serum albumin-sensitized mast cells were exposed to antigen alone, approximately 11% degranulation was observed. Together with aluminum chloride, antigen exposure response more than doubled with 23% degranulation. Similar results were observed for nickel(II) sulfate.¹²⁶

Both airway epithelial cells and alveolar macrophages may phagocytize irritant particles and as a result of the encounters, synthesize pro-inflammatory cytokines that induce airway inflammation and contribute to airway lesions in asthma and

chronic obstructive pulmonary disorder.¹²⁷ Goto *et al.* showed that in response to PM₁₀ particulate, alveolar macrophages released macrophage colony-stimulating factor (M-CSF), macrophage inflammatory protein-1 β , GM-CSF, IL-6, TNF- α , IL-1 β , IL-8, and monocyte chemotactic protein (MCP-1).¹²⁸ Monocytes, which may differentiate into macrophages or dendritic cells, are the predominant inflammatory cells that are recruited from the bone marrow to the alveoli following particulate matter exposure, especially exposure to particulate matter with high metal concentrations.

Carter *et al.*¹²⁹ also studied the inflammatory effects of 5 to 200 $\mu\text{g mL}^{-1}$ environmental particulate matter with low organic and high metals concentrations (2.6% carbon and hydrogen by mass, 18.8% vanadium, 3.75% nickel, 3.55% iron, remainder miscellaneous elements) on normal human bronchial epithelial cells and found transcription induction and expression of pro-inflammatory cytokines TNF- α , IL-6, and IL-8. Cytokine production was inhibited by inclusion of the metal chelator deferoxamine. They concluded that metals present in the particulate matter are predominantly responsible for inducing production and release of inflammatory mediators by the respiratory tract.

Schaumann *et al.*¹³⁰ noted metals-dependent differences in the effects of instillation of suspensions of environmentally relevant concentrations of only 100 μg particulate matter (PM_{2.5}) collected from two different German cities into contralateral lung segments of 12 healthy volunteers. Instillations from both cities increased the numbers of leukocytes in bronchoalveolar lavage after 24 h, but the particulate matter from only one of the two cities, which contained higher concentrations of transition metals, also induced significant increases in monocyte influx, TNF- α , and IL-6 in lavage fluid and increased oxidant radical generation by the lavage cells. It was apparent that the higher concentration of transition metals in the PM_{2.5} from the latter city was responsible for the increased inflammation.

The mRNA for Toll-Like receptors TLR2 and TLR4 mRNA, discussed earlier in relation to inflammation, nickel, and cobalt binding, were respectively upregulated and unaltered, but surface expression of the gene products fell significantly and precipitously when cultured human dendritic cells were exposed to predominantly metal-containing ambient particulate at even the lowest concentrations of 0.1 $\mu\text{g mL}^{-1}$, while pro-inflammatory cytokine (GM-CSF, IL-6, IL-12, TNF- α) mRNA transcription and cytokine secretion increased substantially. Ambient particulate matter directed a nonclassic dendritic cell activation and mixed Th1/Th2-like cytokine response by naïve CD4⁺ T cells. There was no speculation on whether the surface expression decrease for TLR2 and TLR4 was due to internalization with bound ligands (since as discussed earlier, nickel and cobalt bind and activate TLR4). Allergenicity of various metals and the support from the data for an adjuvant-like effect of metals in particulate on dendritic cells (which are antigen-presenting cells) was discussed.¹³¹

Rossi *et al.* reported that the responses of exposure of healthy or asthmatic mice to an acute (10 mg m⁻³) dose of airborne fine TiO₂ particulate or silica-coated nanofine TiO₂ particulate resulted in significant suppression of lymphocyte and eosinophil numbers in lavage and suppressed allergic/asthmatic response

in ovalbumin sensitized mice.¹³² In response to an even higher diesel exhaust particulate dose, suppression of both innate and Th1 cell-mediated responses to *Listeria monocytogenes* (an intracellular pathogen), suppression of IL-1 β TNF- α , and IL-12, but increased IL-10 (anti-inflammatory cytokine) production by alveolar macrophages was observed in rats.¹³³ They also observed downregulation of T cell responses such as suppression of secretion of IL-2, IL-10, and IFN- γ on various days post-infection. Misson *et al.* observed an immediate inflammatory response after acute tracheal instillation of suspended MnO₂ or silica. An “alternative (M2) activation” of murine macrophages presented in the early stages of fibrosis, but returned to classical M1 activation with time and as the fibrosis progressed.¹³⁴ M2 activation phenotypes would be less resistant to intracellular pathogens.

The summary of support for metals involvement in inflammation, sensitization, and immune suppression in this section was based on environmental particulate data because of the greater volume of literature on this particulate and due to the high mass fraction of the transition metal components. This is directly relevant to tobacco smoke particulate, because as participants in the 2001 National Urban Air Toxics Research Center workshop were informed, the main components of environmental tobacco smoke are also urban air toxics.¹³⁵ Specific examples of relations between the contribution of tobacco smoke particulate metals with inflammation and sensitization are discussed below.

Toxicological consequences of metallo-particle inhalation specifically from tobacco smoke

Lin *et al.* studied obstructive lung disorder in 6726 subjects with data obtained from the National Health Assessment Nutrition Examination Survey (III) published by the U.S. Centers for Disease Control and Prevention after exclusions for various conditions. They found adjusted odds of approximately 1.9 for increased prevalence of obstructive lung disorder among those in the lowest zinc-intake tertile *versus* those in the highest tertile regardless of smoking status. They reported relative mean odds ratios for obstructive lung disease of 1.00 for never smokers, 2.60 and 3.37 for former smokers, 4.38 and 7.66 for active smokers in two different regression models. After adjusting for creatinine-corrected urine cadmium concentrations, the effect of smoking on lung disorder risk decreased considerably, suggesting that at least the cadmium intake alone from tobacco smoke was a comparable factor for obstructive lung disorder as smoking itself. Their implication was that metals in tobacco smoke, and specifically cadmium, are major contributors to the risk of obstructive lung disorders.¹³⁶

Using X-ray Microanalysis, Terzakis described particulate compounds from peripheral lung in 18 cases—2 nonsmokers and 1 cigar smoker as autopsy-obtained controls, and 15 with peripheral lung carcinomas (10 of whom were smokers). Of the 15 cancer cases, all cancers were associated with fibrosis. The subjects had neither received occupational exposures nor exposures to asbestos, and no observable asbestos bodies in lung tissue examined. Elevated carbonaceous pigment appeared in fibrotic tissue vicinal to tumors as did particulate material in

the carcinoma cases compared to the control group. The particulate material was composed of silicon, aluminum, phosphorus, vanadium, chromium, iron, nickel, copper, arsenic, cadmium, lead, *etc.* In the 18 total cases examined, silicon was the prominent element in particles. Kaolinite, silica, and other silicates in the particles were present.¹³⁷

Though extensive large aluminum silicate inclusions in alveolar macrophages of smokers were described in earlier citations,^{103–106} Becker *et al.* stated that neutrophils exhibited a stronger oxidative response to silicate exposure, whereas alveolar macrophages exhibited stronger response to oil fly ash particulate (higher in transition metals, lower in silicate,¹³⁸ and consistent with the data from Sanders *et al.*¹²² and suggested that wide variation in macrophage response to metal oxide or silica was likely associated with particle composition. They concluded that reactive oxidant activation as a consequence of various sources of particulate matter is cell specific, and that the inflamed lung is more susceptible to harm from a broader range of particulate size and composition because of the oxidant stress posed.¹³⁸

In response to silica exposure, Beamer and Holian found that large numbers of granulocytes were recruited to the lungs of C57Bl6 mice,¹³⁹ consistent with the findings of Becker *et al.*¹³⁸ They also noted that the alveolar macrophage to dendritic cell ratio was noticeably altered in favor of dendritic cells in response to silica compared to unexposed mice, though a subset population of inflammatory CD11b^{high} alveolar macrophages appeared. Beamer and Holian suggested silica-induced apoptosis of alveolar macrophages as one explanation of their decrease in numbers with time subsequent to silica exposure,¹³⁹ though their observation of macrophage migration to the interstitium (and other observations¹¹⁵) is an additional explanation. The appearance of a new macrophage phenotype, together with other data, indicated recruitment of this population from peripheral sources. In response to silica exposure, portions of both macrophages and dendritic cells migrated to the interstitium, but only the dendritic cells increased the number of CD3⁺ and CD4⁺ lymphocytes, suggesting the dendritic cells as the major antigen presenting cells in this case.¹⁴⁵ Though tobacco smoke transports silicates to the lungs, it has been shown that smoke decreases the number of mouse dendritic cells in the lungs.¹⁴⁰ Hornung *et al.* showed that either silica or aluminum salt crystals were ingested by phagocytosis into peripheral blood mononuclear cells.¹⁴¹ The phagocytosis of the crystals led to destabilization and rupture of the phagosome-lysosomal compartment, releasing contents into the cell cytosol. Lysosomal destabilization triggered activation of the NALP3 inflammasome and induced release of inflammatory cytokine IL-1 β . Dostert *et al.* demonstrated NADPH oxidase activity upon silica particle phagocytosis, which implies generation of reactive oxygen species. They reported that NALP3 inflammasome activation was triggered by the reactive oxygen species.¹⁴² These findings are important links between aluminum silicates and silica and inflammatory response.

TNF- α and IL-1 β inflammatory cytokine signals induced by exposure to tobacco smoke, to particulate, and to metals may also indirectly stimulate fibrotic response to inflammation. TNF- α stimulates production of TGF- β 1. TGF- β 1 in turn

increases production of connective tissue growth factor (CTGF). Both TGF- β 1 and CTGF are major stimulators of collagen production.^{143,144} IL-1 β stimulates macrophages to produce matrix metalloproteinase-9¹⁴⁵ and increases expression of PDGF-AA and of platelet-derived growth factor alpha receptor (PDGFR α) on lung fibroblasts. This hormone system is involved in tissue metal-induced airway fibrosis¹⁴⁶ as the combination of metalloproteinase-catalyzed destruction of tissue and production of fibrous connective tissue by fibroblasts is involved in tissue remodeling observed in COPD development.

Ghio *et al.* found that after exposure of rats to cigarette smoke, lavage concentrations of iron and ferritin, serum ferritin, and nonheme iron concentrations in lung and liver increased.⁷³ The excessive lung particulate iron burden as a consequence of smoking was examined by bronchoalveolar lavage from 27 healthy subjects in three groups of nine nonsmokers, light smokers, and heavy smokers, respectively. More than 3 times the number of macrophages were recovered from light smoker, and more than 8 times the number from heavy smoker lavage compared to lavage from nonsmokers. Zero of nine nonsmokers had iron greater than the 10 ng mL⁻¹ detection limit in lavage, whereas the mean iron concentration in light smoker lavage was 12.5 ng mL⁻¹, and in heavy smoker lavage was 49.7 ng/mL. The authors observed 7.7 times higher ferritin in lavage from light smokers and 31.3 times higher in heavy smokers compared to nonsmokers.¹⁴⁷ Thompson *et al.* found that both the bronchial and alveolar lavage extracellular and intracellular iron burdens of asymptomatic smokers and smokers with chronic bronchitis were very elevated compared with nonsmoking study participants.¹⁴⁸ Moreno *et al.* illustrated the physiological redox availability of iron as a result of ferritin export from alveolar macrophages by demonstrating that aqueous extracts of cigarette smoke could reduce iron(III) and cause its release from ferritin.¹⁴⁹ Boyer *et al.* modeled the role of the effects of polyhydroxy aromatic compounds in cigarette smoke by demonstrating that plant phenolics caused reduction and release of ferritin iron.¹⁵⁰ The latter two studies demonstrated potentiation of iron availability for oxidation–reduction chemistry, supporting its role in generation of reactive hydroxide radical⁷¹ as part of the explanation for the lung oxidative stress and damage caused by smoking. Surface iron has also been shown to increase inflammatory response and increased production of reactive oxygen species from silica exposure in rat lung *versus* silica alone.^{74–77}

The above findings on iron toxicology could be more revealing of the smoking-related lung pathology than superficially appears. Under proinflammatory cytokine stimulus, macrophages differentiate toward the M1 program and produce reactive nitrogen and oxygen species and additional proinflammatory cytokines such as IL-12, IL-23, and TNF α . Though chronic inflammation is a risk factor for carcinogenesis,^{151,152} the M1 phenotype mediates resistance to tumors and is characterized by increased expression of ferritin and suppression of expression of the iron exporter protein, ferroportin (Fpn), favoring sequestration of iron. Differentiation to the M2 phenotype occurs in response to T cell Th2 cytokine IL-4, and is characterized by greater phagocytic activity, Fpn production and iron release, matrix deposition, tissue remodeling,

and immunosuppression,¹⁵³ which decreases resistance to tumors. There is rarely an all-or-none response in any regulated physiological system. Additional macrophage phenotypes have been noted, as well as phenotypes that are somewhat intermediate between the M1 and M2 subsets with characteristics of each.¹⁵⁴ The finding of an increased number of alveolar macrophages in response to toxic insult from tobacco smoke particulate, and both increased intracellular and extracellular ferritin and free iron appears to be an indication that indeed, there is a combined response to particulate metals from both M1 and M2 or mixed macrophage subtypes. This is supported by the finding of both inflammatory response and elevated IgE in smokers as discussed earlier, and of immunosuppression discussed below.

Robbins *et al.* observed that exposure of mice to smoke from 4 unfiltered cigarettes per day decreased the numbers of dendritic cells in lungs, reduced maturation of dendritic cells and expression of MHCII in lymph nodes, and as a consequence, suppressed antigen-specific CD4⁺ T cell proliferation,¹⁵⁵ as did Rossi *et al.* for “lymphocytes” in response to silica-coated TiO₂ particulate.¹³² Robbins *et al.* showed that cigarette smoke compromised antiviral immune responsiveness.¹⁴⁰

Cozen *et al.* studied peripheral monocytic blood cell response to different intensities of chronic cigarette smoking between nonasthmatic monozygotic twins to eliminate genetic factors related to atopy. They determined significant dose responses between heavy smokers (>20 cigarettes/day) and light smokers (<20 cigarettes/day) in monocyte production of IL-5 (166% higher in heavy smokers) and IL-13 (146% higher in heavy smokers) but observed no significant differences in IL-4 production.¹⁵⁶ These cytokines are more consistent with an increased Th2 type immune response in heavier smokers, with consequences that would be in accord with greater susceptibility to intracellular pathogens, as Yin *et al.* observed in response to heavy diesel exhaust particle exposure.¹³³

In the first part of this section, the inflammatory burst-producing cytokine type response was emphasized, whereas in the latter part, discussion emphasizing sensitization, immunological suppression and alternative activation of macrophages was discussed. These roles are not in conflict, but are apparently a continuum of concurrent dose-dependent phenotypic activation and suppression. Basagaña *et al.* reported higher IgE production in smokers, but reported no significant correlations between elevated serum IgE, and atopy, maternal asthma, smoking, and occupational exposure. They described the lack of association between elevated IgE concentrations and atopic diseases as the “healthy smoker effect,”¹⁵⁷ though their study population and data interpretation have been questioned.¹⁵⁸

The findings of Shaykiev *et al.* and Thatcher *et al.* may better explain the reported findings of Basagaña *et al.*¹⁵⁷ Shaykiev *et al.* observed that compared to healthy never-smokers, a relative suppression of the M1 inflammatory macrophage phenotype was associated with smoking, and that the progression toward M2-polarization was observed to increase with development of COPD in smokers.¹⁵⁹ Thatcher *et al.* showed that high dose but not low dose mainstream cigarette smoke suppressed allergic airway inflammation in mice by inhibiting T cell function concurrently with reductions

in eosinophilia, IL-4 and IL-5 reductions in bronchoalveolar lavage, and loss of ovalbumin antigen-specific proliferation and cytokine production by T cells. The authors concluded that although smoking causes systemic inflammatory response, T cell-mediated responses involved in a number of diseases are inhibited by high-dose exposure to smoke.¹⁶⁰ Although progression from M1 fibrosis-initiating oxidative burst response towards sensitization-favoring M2 response appears to progress with dose or chronic exposure to smoke, the M2 response may also be suppressed as a result of loss of T cell function and suppression of IL-4 and IL-5 production. The net result immunologically appears to be initiation of a Th1/M1 inflammatory response with progression toward a more Th2/M2 IgE-producing and tumor-tolerant response but with concurrent suppression of the atopic inflammatory response typically associated with the Th2/M2 phenotypes. Ritter *et al.* found elevated concentrations of chemokine CCL17 and CCL22 (Th2 cell chemoattractants) in both Th1 and Th2 rat inflammation models: smoke-induced inflammation and atopic asthma induced by ovalbumin sensitization. In spite of these elevated chemoattractants, the authors observed no increase in Th2 cell migration to smoke-exposed rat lungs.¹⁶¹ These findings may also help explain earlier data of Goel *et al.* in which reformed smokers showed significantly greater airways obstruction than current smokers.¹²⁰ It may be that after cessation of smoking, the suppression of T cell function is relieved, permitting restoration of either or both of the Th1 inflammatory and Th2 sensitization inflammatory responses. Since pulmonary particle accumulation from smoking requires many years to clear, the atopic or fibrotic consequences of exposure to bioaccumulated metals and silicates may continue to progress after smoking cessation.

Nickel has been shown to dose-dependently suppress immune sensitization as described above. Wu *et al.* showed that mice with “very low” nickel exposure in diet alone could be sensitized to nickel with a single intradermal injection of 50 microlitres 10 mM NiCl₂. “Low” exposure mice could only be sensitized with 50 microlitres 10 mM NiCl₂ in the presence of an adjuvant. “High” exposure mice (chronic oral nickel-supplementation) were not sensitized with this procedure. The authors noted that this dose-dependent nickel tolerance correlated with differences in number and types of nickel-specific T regulatory lymphocytes.¹⁶² Thus some metals may exhibit adjuvant effects, or may be strongly sensitizing at low concentrations, whereas chronic exposure or possibly bioaccumulation as a result of chronic exposure may mediate antigen-specific immune suppression and resulting tolerance.

If this explanation was valid, then it would follow that suppression of the general innate (Th1/M1) immune response would manifest itself in less aggressive resistance to viral and microbial infections. Indeed, as discussed earlier, Yin *et al.* described the “aggravated” infection of rat with *Listeria monocytogenes*, after repeated low doses of diesel exhaust particulate with suppression of Th1 cell-mediated responses, and general downregulation of T cell responses on various days post-infection.¹³⁶ Robbins *et al.* observed that chronic exposure of mice to smoke from 4 unfiltered cigarettes per day decreased the numbers of dendritic cells in lungs, reduced maturation of dendritic cells and expression of MHCII in

lymph nodes, suppressed antigen-specific CD4⁺ T cell proliferation, and compromised antiviral immune responsiveness.¹⁵⁵ Cortés *et al.* reported smoking as a risk factor second only to asthma for a severe case of H1N1 influenza requiring hospitalization during the 2009 pandemic.¹⁶³ Wu *et al.* reported that in human lung organ culture, cigarette smoke extract suppressed the retinoic acid-inducible gene I (RIG-I) initiated innate immune response to influenza virus as well as antiviral cytokine IFN- β .¹⁶⁴ Feng *et al.* reported that exposure of mice to cigarette smoke for two hours twice a day, five days per week inhibited the T-Cell response to influenza virus and *Mycobacterium tuberculosis*.¹⁶⁵ Arnson *et al.* have published an in depth review of effects, not specifically from inorganic, but from tobacco smoke on the immune system including discussion of suppression of both the innate and adaptive immune response.¹¹⁸ Thus, the data bears out the effects on the immune system as impacting both Th1/M1 and Th2/M2, innate and adaptive immune responses as well as impacting the number of cells recruited to the lungs.

Inflammation, sensitization, and pulmonary disease

Asthma is generally increasing in prevalence worldwide.¹⁶⁶ Secondhand tobacco smoke has been associated with development of asthma in children.^{85,167,168} Gavett and Koren reported that environmental airborne particulate matter (PM) promoted allergic sensitization, increased allergic inflammation, and airway hyperresponsiveness. They reported also that exposure of human volunteers to emission source particulate matter samples that had been determined as having high concentrations of iron, nickel and vanadium increased indices of pulmonary oxidant formation. The increased indices of oxidant formation correlated with the quantity of transition metals in the samples. They concluded that PM samples with high concentrations of transition metals may enhance sensitization, promote formation of reactive oxygen species and subsequent lung injury, inflammation, and airway hyperresponsiveness leading to airflow limitation and symptoms of asthma.¹⁶⁹ Mutti *et al.* reported that median nickel concentrations in exhaled breath condensate was higher in asthmatics in their study than in controls and even than smokers who were not otherwise diagnosed with COPD.²⁵ These findings support the possibility of some of the same transition metals from tobacco smoke particulate as having a role in the same sensitization processes as environmental particulate.¹³⁵ Thus metal sensitization must be considered as one of the mechanisms by which atopic asthma and possibly COPD (chronic bronchitis, chronic asthma, and emphysema) are initiated and progress, of course together with other sensitizing compounds such as polycyclic aromatic hydrocarbons. However, previous comments related to dose-dependent immune suppression must also be considered. Exposure to secondhand smoke alone would not be expected to result in the same metalloparticle burden as inhalation of combined mainstream and sidestream smoke to which a smoker is exposed. As a result, the suppression of immune response observed in heavy smokers may not be a consequence of inhalation of secondhand smoke alone, given the discussion of decreased immune suppression in light smokers *versus* heavy smokers above.^{157,159,160} In the absence of suppression of the

Th2/M2 response as a consequence of secondhand smoke exposure, atopic IgE-dependent responses included atopic asthma.

Willers *et al.* investigated associations between environmental tobacco smoke exposure, exposure to “heavy metals,” and nicotine (as the urine cotinine metabolite) in households of 23 children with asthma. They found strong associations between the inquiry data-base tobacco smoke exposure index and urine cotinine, indicative of secondhand smoke inhalation. There were also strong associations between the latter two parameters and nicotine in house dust. Urine cadmium correlated well with urine cotinine, as did lead, though the correlation between cotinine and lead was not significant. The authors concluded that the children with asthma were being exposed to “heavy metals” from sidestream smoke *via* inhalation.¹⁶⁷ An EPA-funded study of air quality in Baltimore city homes of asthmatic children showed that two percent of the PM10 values and seventeen percent of the PM2.5 values exceeded the EPA’s proposed National Ambient Air Quality Standards. The most important indoor contributor to high levels of indoor particulate matter was environmental tobacco smoke (ETS). Average PM2.5 and PM10 concentrations in nonsmoking households were respectively 25.8 ± 14.9 and $37.7 \pm 18.8 \mu\text{g m}^{-3}$. Average PM2.5 and PM10 concentrations in smoking households were respectively 59.1 ± 42.5 and $71.2 \pm 46.7 \mu\text{g m}^{-3}$. This represented a 33 to $54 \mu\text{g m}^{-3}$ increase in PM concentrations in smoking households. It was determined that each cigarette smoked contributed approximately $1 \mu\text{g m}^{-3}$ of airborne particulate matter.¹⁷⁰

Leikauf commented that complex mixtures including fine particulate matter and tobacco smoke are associated with respiratory symptoms and hospital admissions for asthma.⁸⁵ Leikauf further described hazardous air pollutant components of particulate matter as including “occupational asthmagens,” or components that act as adjuncts during sensitization.⁸⁵ Once sensitized, an individual may respond to remarkably low concentrations of such compounds. Irritants may also lower the bronchoconstrictive threshold. Among the 33 hazardous air pollutants of greatest concern for exposure cited from U.S. Environmental Protection Agency reports, were compounds of metals described as suspected of inducing or exacerbating asthma: cadmium, chromium, manganese, and nickel. Cobalt compounds were also listed as a hazardous air pollutant that can exacerbate or induce asthma, though it was not on the list of 33 compounds of greatest concern. Metallic mercury and mercury compounds were included on EPA’s list of 33 compounds of greatest concern due to toxicity but were described as respiratory tract irritants that may exacerbate asthma rather than act as an inducer of asthma.⁸⁵

Chronic Obstructive Pulmonary Disease (COPD) is increasing in prevalence worldwide. It is estimated that COPD in all forms will increase from the fourth leading cause of death in 2004 to the third leading cause of death in 2030.¹⁷¹ As was the case for atopic asthma, allergic sensitization may also be associated with COPD. Itabashi *et al.* showed that though allergen skin test scores were higher in asthmatic patients than those with COPD, serum IgE was significantly higher in elderly COPD patients, as well as in asthmatic patients than in healthy subjects.¹⁷² Though asthma is classified as a distinct disease from COPD, some patients with asthma develop

irreversible airway obstruction characteristic of COPD.¹⁷³ Pacheco *et al.* concluded that at least 17.6% of patients with emphysema associated with smoking had a clear asthmatic profile.¹⁷⁴ Silva *et al.* further found active asthma as conferring a mean risk factor of 10 for developing chronic bronchitis, 17 for developing emphysema, and 12.5 for “fulfilling COPD criteria.”¹⁷⁵ Jang *et al.* reported a study of 843 asthmatic patients. Total IgE was higher in smokers than nonsmokers, but there was no significant difference in atopy. The prevalence of emphysema was higher among smokers; and asthmatic smokers with fixed airway obstruction were significantly higher than asthmatic nonsmokers.¹⁷⁶

The projected increase in COPD is predominantly based on projected increases in tobacco consumption.¹⁷¹ Several metals, aluminum, cadmium, and lead have been reported at higher concentrations in the exhaled breath condensate of study subjects with Chronic Obstructive Pulmonary Disorder (COPD), than that of the nonsmoking healthy control subjects, as well as in current smokers vs. nonsmokers. Smoking COPD patients had significantly higher concentrations of several metals in exhaled breath condensate than ex-smokers and than nonsmokers with or without COPD,¹²³ apparently due to smoking habits. The description of the pulmonary inflammatory cytokine environment described earlier as a consequence of metals or tobacco smoke particulate exposure is a fibrosis-potentiating cytokine environment. The role of metals in inflammation, sensitization, or in exacerbation of existing COPD thus warrants further study. Further investigation on relations between mainstream and sidestream smoke metals, potential for sensitization, inflammation, consequential development of atopic asthma and COPD, irritant-induced nonatopic bronchial hyperresponsiveness, and exacerbation of these diseases are needed.

Both asthma and COPD are inflammatory disorders. Feron *et al.* and Mueller have specifically described the risk of carcinogenesis resulting from chronic inflammation of various epithelial and mucosal tissues, without regard to whether the nature of the cause was irritant, allergic, or other.^{151,152} Thus the participations of various metals in the sensitization processes and inflammatory processes of asthma and COPD, or exacerbation of either disease pose carcinogenic risk beyond the immediate pulmonary pathology.

Several of the metals discussed, especially those that are strongly inflammation-inducing and sensitizing, such as beryllium, chromium(vi), cobalt, nickel, silicates, and those which are toxic chemical irritants that possibly act by causing production of reactive oxygen species (copper, iron, manganese, silicates), are characterized as causing interstitial lung diseases when considered as occupational exposures.²³ The concept of smoking-related interstitial lung disease (ILD, characterized by dyspnea, restrictive pulmonary function, impaired gas exchange, and diffuse lung eosinophilic edematous infiltrates) is relatively recent with regard to tobacco.¹⁷⁷ Though the topic remains controversial, numerous authors have published on the clinical symptoms, underlying pathology, and radiological observations related to smoking-related interstitial lung disease. Selman lists smoking-related diffuse interstitial lung disorders as including respiratory bronchiolitis-associated ILD, desquamative interstitial pneumonia, and pulmonary

Langerhans cell histiocytosis. Selman described the symptomatology and pathology as including dyspnea, cough, restrictive pulmonary function, bronchiole-centered lesions, interstitial and airspace inflammation, and fibrosis extending to the alveoli.¹⁷⁸ Caminati and Harari further describe smoking-related ILD with regard to symptomatology, smoking history, radiology, and pathology.¹⁷⁹ Attili *et al.* presented radiological data describing the pathological manifestations of smoking-related ILD.¹⁸⁰ Washko *et al.* reported that interstitial lung abnormalities were positively correlated with greater exposure to tobacco smoke and current smoking.¹⁸¹ Since metals and silicates independently of tobacco smoke in industrial exposures, environmental particulate exposures as described above cause sensitization, interstitial macrophage and dendritic cell migration and inflammation, increase in proteinaceous lavage, cough, restrictive pulmonary function, eosinophilic infiltrates, and may cause interstitial lung diseases, it is reasonable to consider possibilities of metal and silicate involvement in smoking-related interstitial lung diseases also. Corradi *et al.* reported that concentrations of silicon, nickel, copper, and iron in exhaled breath condensate were significantly higher from patients with idiopathic pulmonary fibrosis (13 of 19 were ex-smokers with a group mean of 24.5 pack-years) and non-specific interstitial pneumonia (10 of 15 were ex-smokers with a group mean of 26.4 pack-years) than healthy nonsmokers.¹⁸² Taskar and Coultas summarized the epidemiological evidence for causal relationships with idiopathic fibrotic lung diseases with the “strongest evidence for cigarette smoking and metal dust.”¹⁸³ Miyake *et al.* reported significant mean odds ratios of 9.55 (metal dust exposure) and 3.23 (20.0 to 39.9 pack years of smoking) for development of idiopathic pulmonary fibrosis.¹⁸⁴ More study should be devoted to metals and silicate involvement in smoking-related ILD and fibrotic disease in general.

As more and more information becomes available, it appears likely that the increasing number of non-cancer inflammatory and fibrotic lung diseases are associated with smoking in general and with metals exposure from smoking and other environmental sources. In some cases, the data on causes related to tobacco smoking overlaps the data on the same or similar diseases caused by the metals alone, or in particulate matter, including predominantly metals-containing environmental and tobacco smoke particulate matter. Chronic inflammatory response may, in turn, increase cancer risk.^{151,152}

Health risks from exposure to metals in smokeless tobacco

Smokeless tobacco has been evaluated by the IARC as a group 1 carcinogen, which is to say, carcinogenic to humans beyond a reasonable doubt.¹⁸⁵ Of the many harmful constituents in smokeless tobacco, potentially metals may constitute a significant health risk.

Toxic metal exposure from smokeless tobacco products and associated health risks have been studied to a very limited degree compared to particulate metal inhalation toxicology. The epithelial tissues of the oral cavity have high proximal transfer potential, which permits absorption and transfer of toxic metals from smokeless tobacco products across the epithelial tissue. Oral exposures are related to solubility in

saliva and transfer by way of direct contact and absorption by oral mucosa. Systemic exposure likely occurs from direct oral absorption or from swallowed saliva or tobacco particulate in the digestive tract.

It is evident from dental studies that oral exposure to individual metals may have an impact on oral health. In particular, oral sensitization to cobalt, nickel, mercury, and other metals from dental materials has been shown to result in allergic contact inflammations, joint pain, positive allergic skin patch tests to the respective metals and other systemic manifestations in some individuals.¹⁸⁶ Amini *et al.* have shown that nickel concentrations of oral mucosal cells of patients with fixed orthodontic appliances were significantly higher than those without the appliances, demonstrating that oral exposure to nickel was not only superficial, but by cellular absorption from saliva.¹⁸⁷ Bolewska *et al.* reported that mucosal contact lesion mercury absorbed from dental amalgam was found predominantly in macrophage lysosomes.¹⁸⁸ Though less work on toxicology from oral metals exposure from smokeless tobacco has been performed, leukoplakia and lichen planus lesions caused by metals alone or by smokeless tobacco are quite similar.

In order to estimate bioavailable or extraction efficiency for toxic and carcinogenic metals from smokeless tobacco, a few studies have reported concentrations of extractable toxic metals in artificial or human saliva. Unfortunately, there is not a standardized saliva formulation for tobacco extraction at the present time. Artificial saliva formulations used for extraction of toxic metals from tobacco have included 0.1 M phosphate buffer and EDTA, EDDHA, or DTPA;⁷⁰ and saturated calcium phosphate, inorganic salts, sugars, enzymes, and mucin,³¹ the latter a closer approximation of natural saliva. Given sufficient extraction time, strong chelating agents almost quantitatively extract some toxic metals into solution from tobacco. The use of water alone or with added salts likely does not reflect saliva conditions. If phosphate is added without proteins and mucin, it may result in undetected metal extraction as a result of coprecipitation of insoluble phosphates when tobacco is centrifuged or filtered from the solution. Since mucin and protein functional groups are capable of chelating metals, a formula including these is more representative of metal bioavailability. Though a formula containing appropriate salts, saturated or supersaturated calcium phosphate, proteins and mucin better represents saliva conditions,³¹ the difficulty of preparation makes the use of saturated or supersaturated calcium phosphate an impractical component of a formula used for frequent analyses. A useful compromise formula might contain calcium and phosphate at 50% saturation to permit longer storage without precipitation.

Kazi *et al.* reported significantly higher blood and scalp hair cadmium and lower zinc concentrations in male oral cancer patients than in "referent" subjects. They further reported that users of chewing tobacco with areca nut or betel quid had higher blood and scalp hair cadmium and lower zinc concentrations than those who did not use chewing tobacco.¹⁸⁹ In a separate study, the same observations were reported for female mouth cancer patients *versus* "referents". The same was true of tobacco smokers, for whom the cadmium/zinc ratios were even higher.¹⁹⁰ Cadmium competes with zinc for

biological binding sites. Elevated cadmium/zinc ratios have been associated with increased tendency for carcinogenesis. For example, Ogunlewe and Osegbe observed that serum and prostate tissue cadmium/zinc ratios in healthy control subjects and those with benign prostatic hypertrophy were always less than those in patients with prostate cancer,¹⁹¹ as was true in the Kazi *et al.* study of oral cancer.¹⁸⁹ The Kazi *et al.* studies implicate oral absorption of cadmium from smokeless tobacco products in order to produce elevated blood and hair cadmium to zinc ratios in smokeless tobacco consumers. These studies were also examples of health risk from the impact of the cumulative absorption of a carcinogenic metal from smokeless tobacco or from tobacco smoke, whereas a one-time exposure alone (sometimes discussed and used as a means of minimizing health risk implications) would probably pose minimal risk.

An epidemiological study of arsenic-induced skin lesions in an area of Bangla Desh where well water has high arsenic concentrations showed that 157 women who chewed tobacco had significantly higher urine methylarsonic acid metabolite than 352 who did not use tobacco. Women with urine methylarsonic acid in the lowest tertile who used chewing tobacco had mean odds ratios of 3.8 for the arsenic-induced skin lesions *versus* those in the same tertile who did not use tobacco. Women with urine methylarsonic acid or inorganic arsenic in the highest tertile who used chewing tobacco had mean odds ratios of 7.3 and 7.5, respectively, compared to those in the same tertile who did not use chewing tobacco.¹⁹² Although one could attribute arsenic from water as the principal etiologic factor for the skin lesions, smokeless tobacco products seemed to potentiate the occurrence of this endpoint.

Additional evidence to support a role for metals in oral inflammatory processes comes from changes in metallothionein concentration and distribution in oral mucosa with development of dysplasia that is characteristically observed with leukoplakia, and commonly observed as a consequence of smokeless tobacco use. Cellular metallothionein concentrations and metallothionein distributions from superficial to basal mucosal layers dramatically differ between non-dysplastic oral mucosa and moderate dysplasia observed with leukoplakia.¹⁹³ Under inflamed conditions, the oral mucosa apparently acts to protect itself from toxic metals that would bind to metallothionein. The risk of carcinogenesis resulting from chronic inflammation of various epithelial and mucosal tissues, without regard to whether the nature of the cause was irritant, allergic, or otherwise has been described.^{151,152} Whether from acute or chronic exposure to metals alone, or from metals together with other tobacco components, the chronic oral inflammations observed as a consequence of smokeless tobacco consumption pose health risks.

Some combination of irritants, toxins, and allergens from smokeless tobacco causes the contact inflammations observed as a consequence of smokeless tobacco use. Davis *et al.* describe the oral cavity as possessing a lining of highly vascular mucosa, parts of which are uniquely sensitive to irritants because they can penetrate the tissue easily.¹⁹⁴ Metal sensitization or toxicity resulting from exposure to metals extracted by saliva from tobacco held close to oral tissues may contribute to the hyperkeratosis, leukoplakia, erythroplakia, and other oral stomatitis inflammatory lesions observed as a

consequence of smokeless tobacco use. Petro and Zhang examined murine T cells in whole splenic mononuclear cell populations and enriched T cells costimulated with anti-CD3 and anti-CD28 to enable activation in culture. They exposed the cells to 1/100 to 1/10 000 dilutions of centrifuged and sterile-filtered smokeless tobacco extract in cell culture medium. The results showed that T cell interferon- γ (IFN- γ) production was decreased at all dilutions of the extract, and that IL-10 was decreased after exposure to the 1/100 dilution. Decreased levels of IL-10 relieves suppression of inflammatory or sensitization responses. IL-2 production was increased after exposure to the 1/100 dilution.¹⁹⁵ Proinflammatory IL-2 synthesized by T4+ helper (Th) lymphocytes is considered classically to promote proliferation of cytotoxic T lymphocytes and activation of B cells,¹⁹⁶ and increases in IFN- γ production that selects for a predominantly Th1 (cell-mediated) lymphocyte response. However, the suppression of IFN- γ may at the same time partially relieve regulatory counteraction of a Th2-mediated response.¹⁹⁰ Interleukin-4 (IL-4) production was unaffected at any dilution. IL-4 is associated with induction of B cell class switching to IgE production, and increases in Major Histocompatibility Complex class II (MHCII) production. Thus the combined suppression of IL-10 and IFN- γ even under influence of elevated IL-2 synthesis, along with unsuppressed IL-4 and MHCII would imply enablement of a state of tissue inflammation and sensitization as a consequence of exposure to soluble tobacco extract, similar to that discussed for tobacco smoke particulate.

Summary

Sufficient evidence exists that suggest exposure to tobacco smoke *via* inhalation or from smokeless tobacco products *via* oral exposure result in significant uptake of many metals and metalloids. These exposures may have significant health ramifications including increased inflammatory and fibrotic lung diseases and cancers, oral inflammatory diseases and cancers, asthma, suppression of immune resistance, and possibly other pathological consequences not discussed in this review.

At present there are no large scale means for reducing the levels of metals in tobacco post harvest. Given the potential for significant health risk associated with metals, cessation is the only proven means to reduce health risks associated with metal and metalloid exposure from tobacco use. Cessation reduces but does not eliminate health risks from tobacco use. Thus, complete avoidance is preferable still. Tobacco products deliver a complex mixture of chemicals to the user. Reduction of a single class of potentially harmful constituents may not reduce overall risk. However, it seems that if reductions of harmful constituents are technically feasible, it would be prudent to do so.

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry.

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