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Household air pollution: a call for studies into biomarkers of exposure and predictors of respiratory disease

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Abstract

Household air pollution (HAP) from indoor burning of biomass or coal is a leading global cause of morbidity and mortality, mostly due to its association with acute respiratory infection in children, chronic respiratory and cardiovascular diseases in adults. Interventions that have significantly reduced exposure to HAP improve health outcomes and may reduce mortality. However, we lack robust, specific and field-ready biomarkers to identify populations at greatest risk, and to monitor the effectiveness of interventions. New scientific approaches are urgently needed to develop biomarkers of human exposure that accurately reflect exposure or effect. In this perspective, we describe the global need for such biomarkers, the aims of biomarker development, and the state of development of tests which have the potential for rapid transition from laboratory bench to field use.

Exposure to household air pollution is a global problem

Household air pollution (HAP) is a daily exposure for nearly half of the world's population and a leading environmental cause of death. HAP related morbidity and mortality predominantly affects people in lower and middle income countries (LMIC), especially women and young children. Almost 4 million deaths per year are attributable to HAP due to childhood respiratory infection, and chronic lung disease, lung cancer and cardiovascular disease in adults(22, 32). HAP exposure in LMIC mostly results from the combustion of biomass fuels (wood, charcoal, dung and crop residues), coal or kerosene within homes for cooking, heating and lighting(49). Biomass fuels have also been implicated in the development of tuberculosis, asthma, cataracts and low birth weight(13).

Even in high income countries such as the US and Canada, HAP is an issue, especially for the rural poor(5). In Southwest Alaska, the Pacific Northwest, tribal lands, and Appalachia as much as 60% of the population burns solid fuels like wood, coal, and/or coke for seasonal heating. In these areas, HAP exceeds the World Health Organization (WHO) air quality guidelines in up to 80% of homes(5). Similar exposures probably exist in other high income countries, although this represents only a fraction of the global burden that occurs in LMIC(36).

Given the effects of exposure, and the large at-risk population, massive effort is being directed globally at HAP exposure reduction programs, mostly centered on the implementation of improved cook stoves. For example, a multi-national public-private partnership between the US government and the United Nations Foundation launched the Global Alliance for Clean Cookstoves (http://www.cleancookstoves.org), which aims to have 100 million homes adopt clean and efficient stoves and fuels by 2020. Increasing the efficiency of combustion in cooking and heating appliances should provide multiple benefits, including reduced expenditure on fuel, local environmental protection leading to increased livelihood security, and potentially wider benefits on climate change. Reduction of health risks associated with HAP by any intervention remains challenging(25), and success is determined by the level of community and household adoption. Cook stove and other intervention programs urgently need to define and monitor personal exposure, and directly evaluate the health benefits of exposure reduction, especially as intervention programs are scaled up. Effective biomarkers used in these studies will allow us to determine which interventions work for meaningful health outcomes, and ensure that the significant resources now available are appropriately directed. This requires the scientific community to develop improved biomarkers of both exposure and health risk, and validate these in human studies in order to help assess the impact of millions of new stoves new types of fuel.

In this perspective, we provide an overview of current approaches to HAP assessment, the aims of biomarker development, and the state of development of tests which have the potential for rapid transition from laboratory bench to field use. This is not a comprehensive review, but should direct the reader to important and exciting areas of current research.

Current approaches to indoor smoke exposure assessment

Current HAP assessment tools include direct quantitative measurement of products of incomplete combustion as well as qualitative methods including use of questionnaires or the categorization of HAP exposure by the type of cookstove or fuel.

Particulate matter

Particulate matter (measured as the concentration of particles less than 2.5 or 10 microns in diameter [PM2.5 and PM10]) is used to describe ambient outdoor and indoor air pollution, in tandem with measurements of ozone and oxides of nitrogen and sulphur. Ultrafine particles (less than 100micron diameter) are also important as they have high toxicity per mass owing to their high surface area. PM is usually monitored at stationary sites and represents emissions from fossil fuel use for energy and transport, agricultural management and forest fires, and HAP. As a major constituent of HAP, black carbon (soot) represents a target for improving health(2). High levels of ambient PM exposure are associated with low birth weight(45), myocardial infarction(34), cardiovascular mortality, lung cancer with poorer lung cancer outcomes(50), reduced lung function(11) and total mortality(38).

Static particulate measurement has been the standard for assessment of outdoor air pollution. Gravimetric sampling is the accepted standard for quantification of airborne particulate mass, and allows both cumulative mass exposure and chemical composition to be retrospectively determined. Photometric detection, by real-time monitoring of changes in light diffraction, generates more refined time-exposure data. Indoors, static measurement can be combined with positioning information to account for exposure variations due to time and proximity to the source, fuel type and condition (water content), burn conditions (completeness of oxidation), and personal behavior such as seasonal outdoor fire use and night time exposure from a partially extinguished source in the sleeping areas. These factors are also likely to determine the correlation between PM and other measures such as carbon monoxide, which can vary widely.

Direct exposure assessment of HAP by personal monitoring is commonly preferred to static monitoring of rooms as it takes into account proximity to the source and other behaviors which modify exposure. Difficulties arise due to the size, portability and recording capacity of equipment, and by acceptability to the user. New devices currently being field tested and scaled up for commercial use, address many of these concerns.

Particulate measurement alone also cannot differentiate the multiple sources of pollution (e.g. mixtures of HAP, tobacco smoke and outdoor pollution.)

Carbon monoxide monitoring

Personal environmental CO monitoring is a helpful proxy of HAP, particularly when black carbon emission is low (e.g. charcoal burning); such situations are especially dangerous to users as the absence of visible smoke permits levels of CO to rise undetected. Electrochemical sensors record levels of CO with high temporal resolution but are expensive. Diffusion tubes represent a simpler method: CO enters a short tube worn on the outer clothing by passive diffusion, and catalyses the reduction of sodium palladoslfite. The resulting palladium discolours a length of tube proportional to total CO exposure. These measurements are associated with important health effects, including the rate of respiratory infections(43) and neurodevelopmental outcomes in young children exposed in utero(10).

Qualitative measures

Epidemiological studies continue to identify populations at risk from HAP, and to define biological and behavioral correlates of risk. Questionnaire studies of exposure are commonly employed in intervention programs as an adjunct to quantitative data. Typically, self-report questionnaires are

employed which detail information on exposure, often related to cooking times. Stove use monitoring systems can be used to objectively describe the pattern of exposures from cooking, and these measures can be compared with environmental monitoring of pollutant concentrations in household air. Time-activity surveys and questionnaires allow time-weighted exposures to be estimated(14). Modeling methods can refine these exposure estimates, and incorporating biomarker data might provide further improvements.

In summary, current HAP exposure measurement methods are expensive, technically challenging, difficult to use with large population studies and have substantial limitations making an urgent case for the development of biomarkers of both exposure and health effects.

Need for improved biomarkers of HAP

The National Institutes of Health defines a biomarker as a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention". In HAP, these may include biomarkers of exposure, susceptibility or effect. Validation of biomarkers by comparison with direct measurement of specific HAP components is a key research need. We focus on biomarkers of exposure as these are most urgently needed and potentially most accessible. However, further development of biomarkers of susceptibility and effect could facilitate large scale studies examining the impact of HAP on health and disease in human populations.

Effective biomarkers of HAP would (a) improve epidemiological accuracy in association studies with health effect, (b) reduce the cost and complexity of monitoring intervention studies, (c) provide data for education of the public and policy makers about risk and (d) inform clinicians and the public health community about human environmental exposures that are not well characterized.

Biomarkers in public health interventions

Public health interventions to reduce the health effects of HAP are well under way. These also address wider concerns about the environment (reducing fuel use), the empowerment of women (by reducing the time spent collecting wood), and community (improving fuel security). Although the stated intent is often to improve health, the complexity and expense of exposure and health measurements have limited good quality data. The RESPIRE study in Guatemala demonstrated for the first time in a randomized controlled trial that improved cook stoves can significantly reduce exposures and reduce risk for severe pneumonia in children under the age of five(42). Adopting validated biomarkers within larger scale studies would be likely to improve the cost and ease of assessing health risks. Widespread adoption of such monitoring could enhance the effectiveness and geographical scope of large scale implementation programs, although this has yet to be tested.

Biomarkers for educational use

Biomarker testing is a useful educational tool when results are communicated to the public and policy makers. Although the adoption of improved/advanced (non-traditional) cook stoves may by mostly driven by economics(30), biomarkers could demonstrate health benefits to policy makers as well as end-users.

Biomarkers of clinical diagnosis and markers of disease progression

While biomarkers can be used for diagnostic testing, HAP biomarkers are unlikely to be useful in this context because: 1) validation against disease outcomes is difficult due to the limited data available

from health systems in developing countries; 2) biomass exposure is associated with common diseases such as lower respiratory tract infection and COPD which have multiple risk factors. Individual biological monitoring would allow more in depth mechanistic studies of harm, however, and would give useful data on inter and intra-individual variation in exposure.

Biomarker development

The Institute of Medicine in a recent report to the Food and Drug Administration suggested a three stage framework for the development of biomarkers(28):

1. "Analytical validation": to ensure reliability, reproducibility, sensitivity and specificity
2. "Qualification": to confirm a strong association with the clinical outcome of concern
3. "Utilization": contextual analysis to determine that the biomarker is appropriate for the proposed use

Specific issues relating to development and field use of HAP biomarkers are summarized in Table 1.

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Biomarkers of exposure to HAP

Biomarkers for environmental particulate exposure are already in use: for example, cigarette smoke exposure can be estimated by concentrations of nicotine and its metabolites, tobacco specific derivatives of nitrosamine, metabolites of pyrene, acrolein and butadiene amongst other markers(40). This model underscores that biomarkers of inhaled substances may be assessed in multiple body compartments, each with different implications for chronicity of exposure. Biomass and coal smoke contain some of these and other constituents which may cause direct toxicity, generate reactive oxygen species and enhance inflammatory pathways. Targets for HAP biomarker development therefore include the gaseous and particulate products of biomass combustion and less specific measures of inflammation, oxidative stress, carcinogenesis and endothelial activation(23). Existing biomarkers and tracers of wood burning in the context of forest fires, and in diesel exhaust and ambient air pollution might also be employed.

No single potential biomarker has been found to be sufficiently precise in both controlled and field conditions. In settings where HAP exposures are very high, single tests could be used in field trials even when their specificity is relatively low. In moderate and lower exposures, combining two or more measurements within a composite endpoint would increase costs, but may be an effective approach.

200 Table 2 Potential lines of investigation for HAP biomarkers – ambient and *ex vivo* measurement

201 Table 2 legend

202 A summary of potential investigations and promising biomarkers: their physiological relevance.

Investigation	**Physiological relevance**
Exhaled CO / transcutaneous COHb: field test in HAP	High CO might explain atherosclerosis and foetal effects (through left shift in O2 dissociation curve and myoglobin binding)
Methoxyphenols, levoglucosan: field test and controlled use in HAP	Unmetabolized urinary product should reflect exposure – physiological determinants of this correlation are not well understood
1-hydroxypyrene (1-OHP): field tests for discrimination of pyrene metabolite levels at low concentration	Polyaromatic hydrocarbons known to be carcinogenic Unknown relevance of CYP enzyme polymorphisms in terms of biomarker
DNA methylation: case control or cohort studies of effect of HAP	Epigenetic effects may explain long term effects (*e.g.* ischaemic heart disease) Known effects of methylation on promoters of genes for inflammatory pathways (*e.g.* iNOS)
Generation of robust animal models for HAP, especially chronic exposures	Chronic exposure effects are likely to be different than experimental acute exposures due to physiological responses (*e.g.* antioxidant upregulation, negative and positive feedback within signalling pathways)
Malondialdehyde, 8-isoprostane, 8-oxo-7,8-dihydro-2'-deoxyguanosine: Investigation of urinary and plasma performance in controlled and field tests	HAP products are known to cause oxidative stress including lipid peroxidation and DNA strand breaks. Measurement within organisms is complicated by buffering and repair mechanisms.

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Figure 1 shows *in vivo* metabolism and excretion of these potential biomarkers. Currently, urinary and plasma biomarkers seem most extensively studied. However other methods provide significant advantages, including: dried blood spots; exhaled breath; induced sputum; transcutaneous and personal area monitoring.

Exhaled CO and carboxyhemoglobin (COHb)

Invariably, CO is a by-product of incomplete combustion even with use of advanced stoves and "cleaner fuels" such as liquefied petroleum gas (LPG). Exhaled CO correlates with environmental CO in highly HAP exposed individuals(21) and can be measured in the field with relatively easy-to-use and inexpensive devices. Blood COHb can also be measured by spectrophotometry. In complex HAP reduction programs, COHb among 20 subjects fell from a pre-intervention range of 1.1 to 13.9% to a post-intervention range of 0.7 to 1.3%(48). Transcutaneous measurement of COHb offers the potential for ease of use without the need for access to peripheral blood and correlates with blood samples, although field testing has shown considerable random error at low levels: the required accuracy is debatable even when used in clinical contexts(24). Further testing is needed to validate whether non-invasive assessment of COHb is accurate, reproducible, relevant to air quality monitoring, and practical in low resource settings.

CO or COHb are already accepted clinical tests supported by a body of literature related to acute and chronic exposures. However, there is a further need for research to assess their validity as biomarkers in the context of HAP.

Methoxyphenols

Methoxylated phenols contribute by weight around one fifth of organic pollutants in urban air. Wood burning results in the pyrolysis of lignins to form polymeric syringyl or guaiacyl propane derivatives. Syringyl derivatives are found in higher proportions in hard wood combustion products(15), have lower background concentrations, and are detectable by gas chromatography-mass spectroscopy (GC-MS) of urine. However metabolism is variable: the usual elimination half-life for syringyls is around 2-3 hours, but some individuals demonstrate late peaks around 30 hours which may represent enterohepatic recirculation(9). Dietary intake of smoked foods and some over the counter cold remedies increase levels of methoxyphenols(9), as might tobacco smoke.

Urinary syringyl methoxyphenols have been demonstrated to correlate with personal CO exposure in cookstove intervention studies(7) and with personal PM2.5 exposure to indoor wood fires(9).

Levoglucosan (1,6-anhydro-β-D-glucopyranose)

Levoglucosan is formed by the burning of cellulose and starches. As the major single organic contributor to wood smoke, it is used as a tracer in ambient monitoring, but also forms in tobacco smoke. Relative quantities depend strongly on wood type, but increased urinary levels can be measured after wood smoke inhalation. By gas chromatography-mass spectrometry, 85% of an intranasal dose was excreted unmetabolized within 4 hours(29). Diesel exhaust exposed mice showed no increase, suggesting specificity of fuel type. Preliminary, uncontrolled human exposure experiments have not demonstrated a correlation between personal PM2.5 or home use of wood stove and urinary levoglucosan(4), perhaps due to dietary contribution from various sources (e.g. caramels). Among wild land firefighters, urinary levoglucosan does not consistently rise after exposure(17, 35).

Measures of oxidative stress

Malondialdehyde / isoprostanes

Malondialdehyde (MDA, formed from the peroxidation of polyunsaturated lipid) and 8-isoprostane (derived from the interaction of arachidonic acid and free radicals) can be used to measure oxidative stress. Despite considerable evidence of increased levels in disease states, few studies have examined lipid peroxidation markers in HAP. 8-Isoprostane may be measured by liquid chromatography/mass spectroscopy or ELISA. Both MDA and 8-isoprostane may be measured in urine, serum and exhaled breath condensate, and may be increased after inhalation of various forms of PM, including that in cigarette smoke(51).

Controlled experimental exposure to wood smoke in humans increases MDA in exhaled breath, and 8-isoprostane urinary excretion(3). A cohort study comparing highly biomass exposed (rural) dwellers with an urban group found that serum levels of MDA in Turkish women were raised in the former group(18). A blinded randomized intervention used residential HEPA filtration as an intervention in a community shown to have high levels of residential wood combustion. Indoor PM2.5 was reduced by approximately two thirds, but with low absolute decreases of 6.6 $\mu g/m^3$. Both MDA and 8-isoprostane concentrations were unchanged(1). So far, there is insufficient evidence to use MDA and 8-isoprostane as biomarkers for clinical studies.

DNA damage/ mutagenesis

DNA damage is a marker of oxidant-mediated injury from exposure to HAP and links such exposures mechanistically to increased rates of lung cancer(33). A simple screening assay for DNA damage is the comet assay, which measures DNA breaks by the differential movement of DNA subjected to electrophoresis on agarose gels. A more precise measure of DNA damage, 8-oxo-7,8-dihydro-2´-deoxyguanosine (8-oxodG), is formed after oxidation of the guanine nucleotide in DNA. It may be detected in plasma or urine by HPLC, liquid chromatography or ELISA. 8-oxodG is increased in the urine of mice exposed to ultrafine particles(47), but the usefulness of this assay as a biomarker of HAP is extrapolated from the literature on atmospheric PM. Using more than one measure of DNA damage, or combining with tests of oxidative stress should be considered to give a more accurate assessment of genotoxicity and carcinogenicity in humans.

A recent meta-analysis of the usefulness of these potential biomarkers of exposure to air pollution(31) suggested that 8-isoprostanes, MDA and 8-oxodG show promise but require testing in large scale prospective studies. It should be noted, however, that only one study(18) of the more than 30 studies included specifically related to HAP.

Polycyclic aromatic hydrocarbons and their metabolites

Incomplete combustion of biomass or fossil fuels may result in the formation of polycyclic aromatic hydrocarbons (PAH, three or more fused benzene rings). Systemic absorption of pyrenes (4 ring structures) from traffic related air pollution and occupational exposure can be measured by the metabolite urinary 1-hydroxypyrene (1-OHP) by HPLC. Urinary 1-OHP increases in those exposed to gas cookers compared with electric ones(12) and reflects environmental exposure even at low levels, although it is increased in passive cigarette smoke exposure and with some foods. In communities using wood as a fuel for cooking, 1-OHP is increased(39), but in only 1 of 3 Mexican communities examined did concentrations exceed those suggested as occupational exposure limits (1.4µmol/mol creatinine). A study in Burundi compared urban and rural indoor environments: in the latter PAH

mean concentrations were 43µg/m^3. In rural dwellers, urinary 1-OHP levels were 30 times higher than in urban dwellers (1.5 vs. 0.05µmol/mol creatinine), and independent of cigarette smoking(52). Similar results are reported from Afghanistan (2.1 vs. 0.75 µmol/mol creatinine in adults and children from rural vs. urban communities)(16). These studies were uncorrected for vehicle emissions.

In the context of clean stove interventions in Mexico, 1-OHP levels fell from 6.7 to 4.8 µmol/mol creatinine when associated with a fall in mean COHb from 4.9% to 1.0%(48). Similar results were obtained at a separate site, despite a 3 fold decrease in CO exposure, suggesting that overall exposures remained high. 1-OHP measurement is complicated by polymorphisms in the metabolic enzyme CYP1A1 which are associated with 2 fold higher urinary levels(53).

Aldehyde-protein compounds

Acrolein, an unsaturated aldehyde, can be produced by burning almost any hydrocarbon, and is both ingested and inhaled. It has been extensively investigated for its toxic effects that include lipid peroxidation and binding of cytoplasmic and nuclear nucleophiles. Downstream effects of acrolein include activation of pro-inflammatory signalling through NF-κB and AP-1 transcription factors. Specific metabolites, such as 3-hydroxypropylmercapturic acid can be measured in the urine by liquid chromatography-tandem mass spectroscopy and gas chromatography. Levels are raised in cigarette smokers(6), but are unknown in HAP.

Epigenetic modifications

A recent report notes DNA methylation changes in newborns whose mothers were active tobacco smokers during pregnancy(19). Differential methylation across the genome occurs mostly, in early life. It may explain HAP-associated adverse outcomes such as low birth weight, impaired lung function, childhood infections and, potentially, the increased risk of non-communicable diseases such as asthma and COPD. Postnatal cord blood specimen, together with exposure assessments during pregnancy, may provide insights into future disease risk of children exposed to HAP during vulnerable windows of organ development.

Biomarkers of health effect from HAP

Biomarkers of health effects may overlap with the anticipated physiological effects of exposure. A number of such associations have been noted, which are commonly early pathological events, and subclinical effects. Although these tend to lack specificity, they may provide a useful proxy measure of exposure in well-defined populations. Further development could improve our current understanding of the physiological effects of HAP exposure, which is to date not well understood.

Pulmonary

Cross sectional surveys suggest a reduction in FEV_1 in household members using biomass fuel compared with LPG. This correlates with PM_{10} concentrations and duration and intensity of exposure(8). Long lag times between exposure and disease, and considerable confounding make these measurements alone unlikely to be useful as a HAP biomarker. Bronchial hyper-responsiveness is a feature of HAP related lung disease, but has not been examined in a HAP-exposed asymptomatic population.

As a marker of airway inflammation, the fraction of exhaled nitric oxide (FeNO) possibly increases after experimental and ambient wood smoke exposure, although the data are somewhat unclear(46,

51). FeNO has been expensive and complex, precluding its widespread use in field studies in LMIC, but continued improvements in the technology may allow its wider use. Experimental exposure to wood smoke causes systemic inflammation and increases a marker of alveolar epithelial permeability (CC16), which has relevance to cardiovascular effects.

Chest radiography is not sensitive enough to demonstrate effects of HAP. However, frequent interstitial changes in lungs of smokers suggest that it is plausible that similar changes may be present in those exposed to HAP.

Pulmonary macrophages may be isolated from spontaneously coughed or induced sputum, or more directly by bronchoalveolar lavage. Such measurements assess PM2.5 exposure, are increased with biomass exposure(20) and correlate inversely with lung function test results, but require technical skill and resources that may be beyond the capabilities of many institutions in LMIC. Similarly, effects at the level of the immune system such as alveolar macrophage dysfunction are measurable, but invasive and technologically demanding.

Cardiovascular

Exposure to $PM_{2.5}$ is associated with increased inflammatory markers (IL-6, IL-8, TNFα, CRP), oxidative stress, reduced heart rate variability and increased arterial stiffness. HAP exposure reduction decreases blood pressure(27) and ECG abnormalities(26). None of these measures are specific to air pollutant exposures, although they are often sensitive enough to detect physiologic and biologic responses to the exposures.

Avenues for developing the potential assays

Potential biomarkers for HAP exist but few if any have been validated for use in clinical studies. Without a gold standard test, external validation is challenging, but should involve other accepted measures such as PM and CO concentrations. Disease pathogenesis that may be well understood in acute exposure is likely to be altered in chronic exposures(41). Animal models, especially for chronic exposures, could be useful for pharmacological and toxicological validation, and for comparing effects across different fuel types and conditions.

Longitudinal studies of HAP health effects are likely to be confounded by multiple associations with poverty. They may be complicated by the effects of genetic predisposition, diet, recurrent infections, other exposures such as tobacco smoke, outdoor air pollution, etc. Developers of biomarker must keep these in mind as the biomarker itself may be subject to alteration by unexpected confounding factors. When biomarkers are to be studied in human populations, these are ideally embedded within an intervention study. This allows an assessment of performance at “real world” concentrations, and external validation. Additionally, physiologic responses to chronic exposure are not well described, but may induce protective phenotypes which alter the expected dose-response relationship. As no single measurement describes HAP perfectly, multiple associations are required, and normal ranges are yet to be established. Some potential avenues of investigation, and their physiological basis, are given in Table 2.

Current challenges and recommendations

There is a need to document worldwide exposures and health effects due to HAP, particularly in LMIC where billions of people continue to use solid fuels such as biomass or coal. The biology of exposure to fine particulate matter and the induction of oxidative stress have been well documented

in other areas. However, few of the biomarkers discussed here have been used to assess the impact of HAP on these markers human health and disease. Virtually none meet usability criteria in the context of field use in low resource settings (see Table 1). The grand challenge to the research community is to produce simple and validated tests to identify populations at risk from HAP and individual responses to exposure reduction strategies.

Development of biomarkers is time-pressured. Large scale implementation programs are already underway. Field researchers, public health officials and advocacy groups need quick, simple and robust HAP exposure biomarkers. Improvements in point-of-care screening and diagnostics are enhancing global healthcare delivery in hard-to-reach populations by using uncomplicated equipment that can be deployed at lower cost due to economies of scale. The current global campaign to promote clean cooking solutions provides an opportunity to produce a biomarker of HAP exposure that could enhance intervention programs that are being applied to one of the most urgent and widespread public health issues of our time.

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545 # Table 1 Characteristics of ideal HAP biomarkers

546 **Table 1 legend**

547 In order to be useful and meaningfully implemented, HAP biomarkers should pass tests of field use
548 and applicability. This table summarises key characteristics.

Issue	Requirement
Field readiness	
Rural population Lack of cold storage	Stable to temperature, storage and transport conditions.
Limited laboratory equipment	Single point of care test is ideal(37), such as those sought in the Gates' Foundation "Grand Challenges in Global Health".
Marker of exposure	
Time lag from exposure to outcome	Chronic complications, such as COPD result from long exposures. Measuring exposure rather than health outcome is more immediately relevant for monitoring and intervention purposes.
Heterogeneity of effect by age	Children most susceptible to infection, and adults to chronic respiratory complications. Biomarker of exposure more likely to be widely relevant.
Rapid fluctuations in exposure levels	Reflect cumulative exposure over days rather than hours
Validity / applicability	
External validity: Consistency with current measurements	Strong correlation between currently used metrics of exposure to ensure continuity within research and intervention programmes. Correlation with disease risk or outcome is ideal, but of secondary importance.
Specificity	Discriminate from other particulate exposures (*e.g.* tobacco, traffic pollution.)
Sensitivity	Be sensitive to multiple fuel sources (*e.g.* wood, charcoal, dung, paraffin.)
Pre-test likelihood of outcome unknown	Interpretable independent of background data. Unlike clinical biomarkers, there is no known "pre-test probability".
Use in diverse populations	Consistent throughout the population at risk. Heterogeneity of response due to genetic polymorphisms (*e.g.* in metabolic pathways can be a problem(44))

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Table 2 Potential lines of investigation for HAP biomarkers – ambient and *ex vivo* measurement

Table 2 legend

A summary of potential investigations and promising biomarkers: their physiological relevance.

Investigation	Physiological relevance
Exhaled CO / transcutaneous COHb: field test in HAP	High CO might explain atherosclerosis and foetal effects (through left shift in O2 dissociation curve and myoglobin binding)
Methoxyphenols, levoglucosan: field test and controlled use in HAP	Unmetabolized urinary product should reflect exposure – physiological determinants of this correlation are not well understood
1-hydroxypyrene (1-OHP): field tests for discrimination of pyrene metabolite levels at low concentration	Polyaromatic hydrocarbons known to be carcinogenic Unknown relevance of CYP enzyme polymorphisms in terms of biomarker
DNA methylation: case control or cohort studies of effect of HAP	Epigenetic effects may explain long term effects (*e.g.* ischaemic heart disease) Known effects of methylation on promoters of genes for inflammatory pathways (*e.g.* iNOS)
Generation of robust animal models for HAP, especially chronic exposures	Chronic exposure effects are likely to be different than experimental acute exposures due to physiological responses (*e.g.* antioxidant upregulation, negative and positive feedback within signalling pathways)
Malondialdehyde, 8-isoprostane, 8-oxo-7,8-dihydro-2'-deoxyguanosine: Investigation of urinary and plasma performance in controlled and field tests	HAP products are known to cause oxidative stress including lipid peroxidation and DNA strand breaks. Measurement within organisms is complicated by buffering and repair mechanisms.

Figure 1 Potential biomarkers of biomass exposure, metabolism and excretion

Figure 1 legend

Biomarkers may be derived from primary products of combustion, from their metabolites or from products of their interaction with body proteins, DNA and lipids. Biomarkers may measurable from one or more body compartment, as summarised here.