Urinary biomarkers of smokers’ exposure to tobacco smoke constituents in tobacco products assessment: a fit for purpose approach

Evan O. Gregg¹, Emmanuel Minet², and Michael McEwan²

¹ENI Limited, Towcester, United Kingdom and ²British American Tobacco, Group Research & Development, Southampton, United Kingdom

Abstract

There are established guidelines for bioanalytical assay validation and qualification of biomarkers. In this review, they were applied to a panel of urinary biomarkers of tobacco smoke exposure as part of a “fit for purpose” approach to the assessment of smoke constituents exposure in groups of tobacco product smokers. Clinical studies have allowed the identification of a group of tobacco exposure biomarkers demonstrating a good doseresponse relationship whilst others such as dihydroxybutyl mercapturic acid and 2-carboxy-1-methylethylmercapturic acid – did not reproducibly discriminate smokers and non-smokers. Furthermore, there are currently no agreed common reference standards to measure absolute concentrations and few inter-laboratory trials have been performed to establish consensus values for interim standards. Thus, we also discuss in this review additional requirements for the generation of robust data on urinary biomarkers, including toxicant metabolism and disposition, method validation and qualification for use in tobacco products comparison studies.

Introduction

One of the most effective ways to estimate smokers’ exposure to tobacco smoke constituents is the measurement of biomarkers. This approach has the advantage of estimating integrated exposure over a period of time, without having to take into account smoking behaviour or counting the number of tobacco articles smoked (Gregg et al., 2006; Hatzukami et al., 2003; Scherer, 1999). Body fluids such as blood (Foulds et al., 1992), saliva (Jarvis et al., 1992) and excreted products such as exhaled breath (Wald et al., 1981) and urine (Carmella et al., 1997) have been used to measure biomarkers of smoke exposure but the collection of urine is potentially the least invasive of these approaches. Further, sufficient urine sample may be collected on a frequent basis, allowing typical analytical assay validation, including linearity, accuracy, precision, repeatability and reproducibility of measurements, to be conducted.

After the publication of the report from the Institute of Medicine (IOM) on tobacco harm reduction (Institute of Medicine, 2001), there has been resurgence in interest in the measurement of biomarkers obtained from smokers. Additionally, the World Health Organization (WHO) Study Group on Tobacco and Product Regulation (TobReg) suggested that the yields of some smoke toxicants should be regulated and lowered (Burns et al., 2008). More recently, Hecht and colleagues discussed the use of tobacco toxicant biomarkers for potential product regulation and cancer prevention and they concluded that “the methods are now sufficiently routine that their application in large studies is feasible” (Hecht et al., 2010). This conclusion has received some endorsement for a more recent IOM report concerning scientific standards for evaluating modified risk tobacco products (Institute of Medicine, 2012).

Along with chemical characterization of the product and estimation of the yield of toxicants from the product in use, measurement of biomarkers of exposure to tobacco toxicants will play an important role in the evaluation of any new types of tobacco product aimed at reducing smokers’ exposure to toxicants (Ashley et al., 2007; Hatzukami et al., 2006; Institute of Medicine, 2001). The IOM originally introduced the term “potential reduced-exposure product” (PREP) for such products (Institute of Medicine, 2001) and, more recently, the US Food and Drug Administration (FDA) and also the IOM have used “modified risk tobacco product” (MRTP) to describe them (Institute of Medicine, 2012; O’Connor, 2012). Throughout this article, the term “reduced toxicant prototype” (RTP) is used to designate novel products that are being evaluated because it is recognized that claims about the potential for risk modification cannot be made until the actual human exposure to toxicants from these products and long-term data from their use in populations becomes available. Partial data informing such an assessment may be obtained by using suitable biomarkers.
of exposure. Biomarkers other than exposure (effect, potential harm, risk, susceptibility, etc.) are not considered in this review.

In other non-tobacco use situations, guidelines for the evaluation of biological measurements, e.g. those of the FDA (Food and Drug Administration, 2001) or the International Organization for Standardization (ISO) (International Organization for Standardization, 2005, 2007), are applied before their widespread use in diagnostic or clinical settings, and so it is of interest to apply existing guidelines to tobacco exposure biomarkers. This article reviews the application of urinary biomarkers in comparisons between groups of smokers of different products and groups of non-smokers. Monitoring environmental exposure to tobacco smoke is not considered. To make these comparisons, currently available data on urinary biomarkers of exposure to tobacco smoke toxicants, taken from the lists published by WHO TobReg (Burns et al., 2008) and Hecht and colleagues (Hecht et al., 2010), is reviewed. The alignment of these data with existing guidelines is summarized and, when available, the key data are presented to demonstrate how well each criterion is met and to show where expected data are not available. Assuming the purpose of measuring biomarkers is to evaluate groups of smokers of RTPs in comparison with conventional cigarette smokers and with non-smokers, this comparative approach allows biomarker validation, qualification and “fitness for purpose” to be assessed. Any gaps in the data are highlighted as high-priority activities for tobacco biomarkers research, and for RTP assessment. Completion of these activities would enhance science-based manufacturing stewardship and regulatory scrutiny of RTPs.

Methods

A list of urinary biomarkers for smoke constituents taken from those provided by TobReg (Burns et al., 2008) and by Hecht and colleagues (2010) was compiled and assessed against general guidelines on biomarker and bioanalysis: those outlined by the FDA (Food and Drug Administration, 2001), ISO (International Organization for Standardization, 2005, 2007), the IOM (Institute of Medicine, 2010), Scherer (2005) and Chau & colleagues (2008). The approach taken was to gather information on the analytical techniques from recently published studies and to cross-check these data against the guidelines. For most biomarkers examined the recently applied analytical techniques typically use gas chromatography (GC) or liquid chromatography (LC) followed by mass spectrometry (MS) or tandem mass spectrometry (MS/MS). Thus, the literature reviewed was not exhaustive and was based on these recent publications and those cited within the biomarker list publications that used the same techniques for biomarker analysis. For each potential biomarker, the method of analysis, limit of detection (LOD), lower limit of quantification (LLOQ), precision, accuracy, recovery and sample stability under assay and storage conditions were recorded (Food and Drug Administration, 2001). This subset of the published guidelines is highlighted because, if this information is not available, it is unlikely that other data such as upper limit of quantification and assay linearity could be determined from the published literature. Thus, any laboratory wishing to use the biomarker might expect more difficulty in validating the assay before performing a study. Furthermore, in the absence of a standard reference material, inter-laboratory comparisons are required to assign absolute values to specific analyte measurements (International Organization for Standardization, 2005). Unless reference standards or a consensus value for a standard has been assigned by appropriate inter-laboratory comparisons, then biomarker values should only be used with caution; for example, in within laboratory relative comparisons rather than absolute value assignment or after taking into consideration the methods, size, design and overall quality of the studies being compared.

Assay validation is a necessary but not sufficient step to determine whether a biomarker is qualified for use in particular circumstances. For biomarker qualification, an approach has been outlined by others to assess overall “fitness for purpose” in a pharmaceutical environment (Chau et al., 2008; Lee et al., 2006). In a tobacco context, several distinct uses of biomarkers for RTP evaluation can be envisaged: (1) a small study of short duration (up to a few days) in which RTP users are directly compared with conventional cigarette smokers under conditions of clinical confinement and all other variables (diet, etc.) are controlled; (2) short-term evaluation (1–12 weeks) of RTP users compared to conventional cigarette smokers and to non-smokers, with periodic episodes of clinical confinement; (3) long-term assessment (>12 weeks) of RTP users compared to conventional cigarette smokers and to non-smokers, with periodic episodes of clinical confinement; (4) cross-sectional and population studies of several groups including RTP users. The specific requirement to qualify a biomarker as fit for purpose would be different in each set of circumstances and depends on the objectives of the study. For example, biomarkers of exposure to smoke constituents with other known dietary or environmental sources could be suitable for use under controlled conditions, like those in clinical confinement, but may be unsuitable in study designs where such variables are not controlled, such as cross-sectional studies.

A comparison of specific biomarker concentrations in smokers and non-smokers should give the greatest magnitude of change that might be found in an RTP study, and could be used for power calculations. However, if a smoking cessation study has been conducted and the biomarker evaluated, then both a practical degree of change and the kinetics of that change can be used to inform study design. Further information about a biomarker’s performance can be obtained from product switching studies in which individuals or groups have experimentally changed their tobacco product use for defined periods of time. All such data were sought for the biomarkers included in this review.

Other biological considerations may affect biomarker performance and influence the practical aspects of a study design; e.g. diurnal variation in enzyme activity may affect smoke constituent metabolism and the biomarker elimination half-life. This could be of importance in studies where an early morning spot urine sample, but not the first void, is collected for a biomarker with a short elimination half-life. Thus, a combination of all of these properties, and the
objectives of a study, should be considered to ascribe overall fitness for intended purpose.

Results

The data gathered on a series of urinary biomarkers of exposure to tobacco smoke constituents are summarized in Tables 1–3. In Table 1, features of the analytical techniques (limits of detection and quantification, accuracy and precision) and sample handling, namely stability on storage and freezing, are presented. In Table 2 the reported ranges in groups of smokers and non-smokers and other characteristics that address expected uncertainty in these measurements are shown. Typically, at least a 2-fold change in biomarker concentrations between groups of smokers and non-smokers would be expected for practical application. However, this level of difference can be affected by the design of the study (controlled versus non-controlled), the study setting (clinical confinement versus unrestricted subject movement) and the reproducibility characteristics of the assay over the short and long term. In some circumstances, a difference of less than 2-fold might be suitable; whereas, for uncontrolled, cross-sectional studies a difference of 5- or even 10-fold might be required. Other parameters that can affect the interpretation of biomarker data are captured in Table 3 and, together, these data are all used in determining fitness for purpose. This approach was not intended to capture every urinary biomarker assay that has been performed for smoke constituent exposure but to focus on the types of assays typically being used by current bioanalytical laboratories, often using chromatography followed by MS or MS/MS. For ease of reading, the tables are presented in the same general format and order, with the first column listing the smoke constituent and the second column the relevant biomarkers. When data are available, a summary value from the published study is given along with a reference to that publication. Blank cells indicate that relevant data were not found in the published literature. There is no definitive manner in which to split these data and an overall judgment about a specific biomarker requires data from all tables, as well as a consideration of the intended study application.

Tobacco-specific nitrosamines (TSNAs) are a group of N'-nitrosamines derived from tobacco alkaloids such as nicotine, nornicotine, anabasine and anatabine during the curing and processing of tobacco as well as during the pyrolysis process (Scherer & Richter, 1997; Stepanov & Hecht, 2005). There are few or no other known sources. There are four main TSNAs, 4-(methyleneamino)-1-(3-pyridyl)-1-butanone (NNK), N-nitrosonornicotine (NNN), N'-nitrosodibenzylamine (NAB) and N'-nitrosodimethylaniline (NAT) are not as well-characterized as NNAL but assay validation data (Kavvadias et al., 2009b; Stepanov & Hecht, 2005) and ranges in smokers and non-smokers are available (Kavvadias et al., 2009b; Sarkar et al., 2008; Stepanov & Hecht, 2005) as well as some smoking product switching studies, which give comparative data using the same laboratory for analysis (Sarkar et al., 2008). However, data from long-term studies with product switching or smoking cessation, the elimination half-life in humans and inter-laboratory comparisons were not found.

Polycyclic aromatic hydrocarbon (PAHs) are chemically diverse and they are formed during the incomplete combustion of organic materials, such as tobacco. They are found in ambient air, cooked foods, and in numerous occupational settings. For PAH exposure, two biomarkers are widely used in smoking studies: 1-hydroxypyrene (1-OHP) and 3-hydroxybenzo[a]pyrene (3-OHBaP). Assay validation data are available (Carmella et al., 2004; Feng et al., 2006; Jongeneelen et al., 1986; Lafontaine et al., 2006; Scherer et al., 2007a; Suwan-ampai et al., 2009), but the qualification data for these biomarkers is equivocal. Both 1-OHP and 3-OHBaP give approximately a twofold difference between smokers and non-smokers in some studies (Lindner et al., 2011; Sarkar et al., 2010), but the ranges of values for smokers and non-smokers overlapped in other studies (Lafontaine et al., 2006; Scherer et al., 2007a; Suwan-ampai et al., 2009). For 1-OHP there was variability in the longer term data (Carmella et al., 2004), but this was not found in a more recent study of smokers over a 6-month follow up (Sarkar et al., 2008). Also for 1-OHP, a reduction in mean values in groups using an electrically heated cigarette compared to a group continuing to smoke conventional cigarettes was reported (Feng et al., 2006; Frost-Pineda et al., 2008a, b; Roethig et al., 2007), but another study did not report an alteration in mean values on smoking cessation (Carmella et al., 2009). For 3-OHBaP a reduction on switching from conventional cigarettes to snus oral tobacco consumption and on smoking cessation was observed (Sarkar et al., 2010), but long-term data comparisons in tobacco product switching or smoking cessation studies and other properties like urinary accumulation kinetics were not found.

Aromatic amines, also known as arylamines, are usually encountered industrially in the manufacture of dyes but also in rubber processing and pesticide production and biomarkers of exposure to aromatic amines have been widely studied in occupational settings, but less so in tobacco exposure studies. Biomarker assays based on their measurement in urine samples are available, along with precision, accuracy, recovery and stability in sample matrix (Grimmer et al., 2000; Riedel et al., 2006; Weiss & Angerer, 2002). Differences in
Table 1. Smoke constituents and their biomarkers in urine: assay validation parameters summary.

| Smoke constituent | Biomarker     | Analytical technique* | Limit of detection [in matrix] (Ref) | Lower limit of quantification (Ref) | Storage studies in matrix temp. (Ref) | Precision\(\%\) (Ref) | Accuracy (Ref) | Recovery (Ref) |
|-------------------|--------------|----------------------|--------------------------------------|-------------------------------------|--------------------------------------|------------------------|----------------|----------------|----------------|
| NNK               | Total NNAL†  | GC-TEA               | 0.04 pmol/ml [8.4 pg/ml] (Yuan et al., 2009) 0.1 pmol/ml [20.9 pg/ml] (Church et al., 2010a) | -20°C 4 years (Yuan et al., 2009) | 10.9% intra-day RSD (Yuan et al., 2009) 6.4% (Church et al., 2010a) | >95% (Church et al., 2010a) | <85% (Kavvadias et al., 2009b) | 30.3–31.7% (Shah et al., 2009) | 32.1–45.5% (Kavvadias et al., 2009b) |
| NNK               | NNN          | GC-TEA               | 2.0 pg/ml (Kavvadias et al., 2009b) | -21°C 6 days, -20°C 8 months (Kavvadias et al., 2009b) Freeze–thaw 3 cycles (Kavvadias et al., 2009b) | 6.0–11.9% (Kavvadias et al., 2009b) | 94.1–103.0% (Bhat et al., 2011) 100.4–110.7% (Kavvadias et al., 2009b) | 19.4–4.4% (Kavvadias et al., 2009b) | 90.3–91.1% (Kavvadias et al., 2009b) | 37.9–56.8% (Kavvadias et al., 2009b) |
| NAT               | Total NAT    | GC-TEA               | 2.65 pg/ml (Stepanov & Hecht, 2005) | -21°C 6 days, -20°C 8 months (Kavvadias et al., 2009b) Freeze–thaw 3 cycles (Kavvadias et al., 2009b) | 1.4–13.2% (Kavvadias et al., 2009b) | 93.5–103.9% (Kavvadias et al., 2009b) | 46.3–77.4% (Kavvadias et al., 2009b) | <85% (Kavvadias et al., 2009b) | <85% (Kavvadias et al., 2009b) |
| NAB               | Total NAB    | GC-TEA               | 3.44 pg/ml (Stepanov & Hecht, 2005) | -21°C 6 days, -20°C 8 months (Kavvadias et al., 2009b) Freeze–thaw 3 cycles (Kavvadias et al., 2009b) | 1.1–11.7% (Kavvadias et al., 2009b) | 88.2–97.2% (Kavvadias et al., 2009b) | 42.0–76.3% (Kavvadias et al., 2009b) | <85% (Kavvadias et al., 2009b) | <85% (Kavvadias et al., 2009b) |
| Pyrene            | 1-OHP†       | HPLC-FD              | 5 pg/ml (Lafontaine et al., 2006) 0.05 pmol/ml (Carmella et al., 2004) | -20°C >12 month (Feng et al., 2006) | 4.6% (Feng et al., 2006) 4.2% 30 replicates RSD (Carmella et al., 2004) | 88.6–91.7% (Feng et al., 2006) 90% (Scherrer et al., 2007a) | 50% (Carmella et al., 2004) | <85% (Kavvadias et al., 2009b) | <85% (Kavvadias et al., 2009b) |
| Benzo[a]pyrene    | 3-OHβaP†     | HPLC-FD              | 134 pg/ml (Jongeneelen et al., 1986) | -20°C >12 month (Feng et al., 2006) | 1.5–5.7% (n> 2500) (Sawan-ampai et al., 2009) | <85% (Kavvadias et al., 2009b) | <85% (Kavvadias et al., 2009b) | <85% (Kavvadias et al., 2009b) | <85% (Kavvadias et al., 2009b) |
| Aromatic amines   | 1-AN         | GC-MS                | 0.05 pg/ml (Grimmer et al., 2000) 150 pg/ml (Weiss & Angerer, 2002) | stable on >1 freeze-thaw cycles | 2.4–7.9% Inter-assay CoV (n> 2500) (Sawan-ampai et al., 2009) | <85% (Kavvadias et al., 2009b) | <85% (Kavvadias et al., 2009b) | <85% (Kavvadias et al., 2009b) | <85% (Kavvadias et al., 2009b) |
|                  | 2-AN         | GC-MS                | 0.05 pg/ml (Grimmer et al., 2000) 75 pg/ml (Weiss & Angerer, 2002) 4 pg/ml (Riedel et al., 2006) | stable on >1 freeze-thaw cycles | 7.9% (Grimmer et al., 2000) 2.8–8.2% Within series RSD (Weiss & Angerer, 2002) | 96.4–100.2% (Sarkar et al., 2010) 70–90% (Weiss & Angerer, 2002) 93.9% (Grimmer et al., 2000) | 90% (Weiss & Angerer, 2002) 92.5% (Grimmer et al., 2000) | 90% (Weiss & Angerer, 2002) 90% (Weiss & Angerer, 2002) | 90% (Weiss & Angerer, 2002) 90% (Weiss & Angerer, 2002) |
|                  | 4-ABP        | GC-MS                | 0.05 pg/ml (Grimmer et al., 2000) 50 pg/ml (Weiss & Angerer, 2002) 2 pg/ml (Riedel et al., 2006) | stable on >1 freeze-thaw cycles | 3.3% (Grimmer et al., 2000) 2.7–3.9% Within series RSD (Weiss & Angerer, 2002) | 100–108% (Weiss & Angerer, 2002) 96.8% (Grimmer et al., 2000) | 90% (Weiss & Angerer, 2002) 90% (Weiss & Angerer, 2002) | 90% (Weiss & Angerer, 2002) 90% (Weiss & Angerer, 2002) | 90% (Weiss & Angerer, 2002) 90% (Weiss & Angerer, 2002) |
|                  | o-tol        | GC-MS                | 50 pg/ml (Weiss & Angerer, 2002) 1 pg/ml (Riedel et al., 2006) | stable on >1 freeze-thaw cycles | 7.0–7.3% Within series RSD (Weiss & Angerer, 2002) | <85% (Kavvadias et al., 2009b) | <85% (Kavvadias et al., 2009b) | <85% (Kavvadias et al., 2009b) | <85% (Kavvadias et al., 2009b) |
| 1,3-Butadiene     | DHBMA†       | GC-MS/MS             | 0.9 pg/ml (Seyler & Bernert, 2011) | stable on >1 freeze-thaw cycles | 7.0–7.3% Within series RSD (Weiss & Angerer, 2002) | <85% (Kavvadias et al., 2009b) | <85% (Kavvadias et al., 2009b) | <85% (Kavvadias et al., 2009b) | <85% (Kavvadias et al., 2009b) |
| Substance          | Analyte | Method       | Detection Limit | Accuracy          |
|--------------------|---------|--------------|-----------------|-------------------|
| Benzene            | tMA     | GC-MS        | 3 ng/ml         | 2.5%              |
|                    | SPMA    | GC-MS        | 0.03 ng/ml      | 3%                |
|                    | HPLC-MS/MS | GC-MS        | 0.03 ng/ml      | 4%                |
|                    |         | HPLC-MS/MS   | 0.03 ng/ml      | 4%                |
|                    |         | HPLC-MS/MS   | 0.013 ng/ml     | 3%                |
|                    |         | HPLC-MS/MS   | 0.01 ng/ml      | 3%                |
| Catechol           | catechol| GC-MS        | 9 ng/ml         | 10.5%             |
|                    |         | HPLC-MS/MS   | 9.9 ng/ml       | 10%               |
| Acrolein           | 3-HPMA  | LC-MS/MS     | 50 ng/ml        | 1.6%              |
|                    |         | HPLC-MS/MS   | 2.3 ng/ml       | 9.4%              |
| Crotonaldehyde     | HMPMA   | LC-MS/MS     | 28 ng/ml        | 1.6%              |
|                    |         | LC-MS/MS     | 28 ng/ml        | 1.6%              |
|                    |         | CMEMA        | 32 ng/ml        | 1.6%              |
| Acetaldehyde       |         | Mainly DNA adducts | 0.02 ng/ml       | 1.6%              |
| Formaldehyde       |         | Mainly DNA adducts | 0.004 ng/ml        | 1.6%              |
| Ethylene Oxide     | HEMA    | HPLC-MS/MS   | 0.024 ng/ml     | 1.6%              |
|                    |         | HPLC-MS/MS   | 0.03 ng/ml      | 1.6%              |
| Acrylamide         | Acrylamide | HPLC-MS  | 0.5 ng/ml       | 1.6%              |
|                    | Glycidamide | HPLC-MS  | 2.5 ng/ml       | 1.6%              |
| AAMA               | AAMA    | HPLC-MS/MS   | 0.8 ng/ml       | 1.6%              |
|                   | GAMA    | HPLC-MS/MS   | 0.5 ng/ml       | 1.6%              |
| Acrylonitrile      | CEMA    | LC-MS/MS     | 0.5 ng/ml       | 1.6%              |
|                    |         | HPLC-MS/MS   | 0.06 ng/ml      | 1.6%              |
| Cadmium            | Cadmium | ICP-MS       | 0.01 ng/ml      | 1.6%              |
| Storage studies in matrix temp. duration (Ref) | Precision (intra-day coefficient of variation, unless stated) | Accuracy (Ref) | Recovery (Ref) | Lower limit of quantification (Ref) | Limit of detection (in matrix) | Analytical technique* | Biomarker |
|-----------------------------------------------|-----------------------------------------------------------|----------------|---------------|----------------------------------|-----------------------------|----------------------|------------|
| 1–18% relative measurement                    | 100 ± 5 (Pocshal et al., 2000)                             | <10% difference | (Paschal et al., 2000) | 0.05 ng/ml (Hoffmann et al., 2000) | 0.1 ng/ml (Paschal et al., 2000) | AAS                  | Smoke |

Abbreviations: 1-AN, 1-aminonaphthalene; 1-OHP, 1-hydroxypyrene; 2-AN, 2-aminonaphthalene; 3-OHBaP, 3-hydroxy-benzo[a]pyrene; 3-HPMA, 3-hydroxypropylmercapturic acid; 4-ABP, 4-aminobiphenyl; AAMA, N-acetyl-S-(2-carbamoyl-ethyl)-L-cysteine; CEMA, N-acetyl-S-(2-carboxyethyl)-L-cysteine; CMEMA, 2-carboxy-1-methylethylmercapturic acid; DHBMA, dihydroxybutyl mercapturic acid; GAMA, N-(R,S)-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine; HEMA, N-acetyl-S-(2-hydroxyethyl)-L-cysteine; HMPMA, 3-hydroxyl-methylpropylmercapturic acid; MHBMA, monohydroxybutenyl mercapturic acid; <trans,trans>THBMA, trihydroxybutyl mercapturic acid; tt-MA, beta-muconic acid; Total NAB, NAB+ NAT-N-glucuronide, measured after sample glucuronidase treatment; Total NNAL, NNAL-glucuronide measured after sample glucuronidase treatment.

Precision and Accuracy are as defined by the FDA (Food and Drug Administration, 2001) as the closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogeneous volume of biological matrix. Generally, precision was measured using pooled urine samples sometimes spiked and several replicates were measured to achieve this. Whereas accuracy was generally measured in most papers using spiked samples at several levels. However, in some paper it is merely stated that these guidelines were followed and no information on how samples were prepared for accuracy and precision measures were described.

urinary concentrations between smokers and non-smokers were not always found (Grimmer et al., 2000), but at least three studies did report differences for 2-aminonaphthalene (2-AN), 4-amino-phenyl (4-ABP) and <i>ortho</i>-toluidine (o-Tol) (Lindner et al., 2011; Riedel et al., 2006; Seyler & Bernert, 2011). Data from product switching studies are becoming available (Frost-Pineda et al., 2008a; Sarkar et al., 2010). However, additional work on stability under assay conditions and further characterization of half-life of elimination for these chemicals is required. For 1-aminonaphthalene (1-AN), in addition to these criteria, use in products switching or smoking cessation studies were not found. Further, for all these aromatic amine biomarkers, no reports in long-term smoking studies and no inter-laboratory comparisons were found. Thus, additional data on urinary aromatic amine measurements, especially from long-term studies, are required to establish their utility as biomarker of exposure in smoker studies. It has to be noted that DNA and haemoglobin adducts of aromatic amines have been used in a number of studies prior to the development of urinary methods. The adducts were sensitive enough to distinguish between smokers and non-smokers but not non-smokers environmentally exposed to tobacco smoke and not between low ‘tar’ and higher ‘tar’ cigarette smokers (Bartsch et al., 1990; Bernert et al., 2005; Bryant et al., 1988; Vines et al., 1996). Confounding factors from food and chemicals such as hair dyes are limiting factors for the detection of discrete differences between non-smokers with and without environmental exposure to tobacco smoke (Ambrosone et al., 2007).

1,3-Butadiene is an industrial petrochemical used in the production of polymers, polybutadiene, styrene-butadiene rubbers and nitrile-butadiene rubbers (International Agency for Research on Cancer, 1992). It is also a product of incomplete combustion of wood and vegetable matter and is a component of vehicle exhaust fumes. 1,3-butadiene is in IARC Group 1 (‘‘carcinogenic to humans”) (International Agency for Research on Cancer, 2012). For exposure to 1,3-butadiene exposure, the assay for the biomarker monohydroxybutenyl mercapturic acid (MHBMA) has been validated (Carmella et al., 2009; Ding et al., 2009; Schettgen et al., 2009; Sterz et al., 2012; Urban et al., 2003; van Sittert et al., 2000) and used in smoker product switching and cessation studies (Carmella et al., 2009; Sarkar et al., 2008). With the exception of inter-laboratory comparison studies, the data support MHBMA as qualified for use in tobacco studies. Furthermore, a recent study has described a method to quantify two isomers of MHBMA (Sterz et al., 2012). In that study, 2-MHBMA was the more abundant isomer but the 1-MHBMA isomer could be detected with higher sensitivity, specificity and accuracy. The biomarker trihydroxybutyl mercapturic acid (THBMA) has been used in one study and the assay characteristics showed that it would be suitable for use as a biomarker of tobacco smoke exposure (Kotapati et al., 2011). An assay for the biomarker dihydroxybutyl mercapturic acid (DHBMA) is available and has characteristics that validate its use (Carmella et al., 2009; Ding et al., 2009; Schettgen et al., 2009; Urban et al., 2003; van Sittert et al., 2000). However, DHBMA did not differentiate between smokers and non-smokers (Ding et al., 2009; Urban et al., 2003) and did not change on smoking cessation.
| Smoke constituent | Biomarker | Analytical technique | Ranges in smokers (Ref) | Ranges in non-smokers (Ref) | Short-term repeatability (Ref) | Long-term study (Ref) | Inter-laboratory proficiency test (Ref) |
|-------------------|-----------|----------------------|-------------------------|----------------------------|-------------------------------|----------------------|-------------------------------------|
| NNK               | Total NNAL| GC-TEA               | 99.8–1420.8 ng/24 h urine (Hecht et al., 1999) | 0.0016–0.0094 ng/mg creatinine (Meger et al., 2000), (Anderson et al., 2003) | 13.7% inter-day RSD (Yuan et al., 2009) 7.8% (Church et al., 2010a) | Statistically non-significant changes over 6 months in control group smokers (Sarkar et al., 2008) | G-EQUAS** |
|                  | Total NNK | GC-TEA               | 0.382 ± 0.12 pmol/mg creatinine mean ± SD (Stepanov & Hecht, 2005) | < LOD (Kavvadias et al., 2009) 0.49 ng/24 h mean (Sarkar et al., 2008) | 5.1–11.4% (Kavvadias et al., 2009b) | Non-seasonal effect detected (Carmella et al., 1997) 6-weeks individual RSDs 8.6–71% (n = 13) (Carmella et al., 2004) | G-EQUAS** |
|                  | Total NAT | GC-TEA               | 0.187 ± 0.11 pmol/mg creatinine mean ± SD (Stepanov & Hecht, 2005) | < LOD (Kavvadias et al., 2009) 0.04 ng/24 h mean (Sarkar et al., 2008) | 2.5–8.8% (Kavvadias et al., 2009b) | Non-significant changes over 6 months in control group smokers (Sarkar et al., 2008) | G-EQUAS** |
|                  | Total NAB | GC-TEA               | 0.041 ± 0.04 pmol/mg creatinine Mean ± SD (5) | < LOD (4) 1.21 ng/24 h mean ± SD (Sarkar et al., 2008) | 3.5–6.5% (Kavvadias et al., 2009b) | No seasonal effect detected (Carmella et al., 1997) 6-weeks individual RSDs 8.6–71% (n = 13) (Carmella et al., 2004) | G-EQUAS** |
|                  | Pyrene   | 1-OHP                | 0.346 µg/g 24 h (Scherer et al., 2000) | 0.157 µg/g 24 h (Scherer et al., 2000) | 4.6% (Scherer et al., 2007a) 23–30% 7-day variability (n = 13) (Carmella et al., 2004) | No seasonal effect detected (Carmella et al., 1997) 6-weeks individual RSDs 8.6–71% (n = 13) (Carmella et al., 2004) | G-EQUAS** |
|                  |          | HPLC-FD              | 0.196 ± 0.94 µg/g 24 h (Scherer et al., 2000) | 0.130 ± 0.076 µg/g 24 h (Scherer et al., 2007a) 0.024–0.532 µg/g 24 h (Lafontaine et al., 2006) | 1.30–1.5% 7-day variability (n = 13) (Carmella et al., 2004) | Non-significant changes over 6 months in control group smokers (Sarkar et al., 2008) | G-EQUAS** |
|                  |          | HPLC-MS/MS           | 291 ± 123 ng/24 h mean ± SD (Lindner et al., 2011) | 123 ± 101 ng/24 h mean ± SD (Lindner et al., 2011) [LC-MS/MS] | 15–5.7% inter-assay CoV (n > 2500) (Scherer et al., 2007a) | Non-significant changes over 6 months in control group smokers (Sarkar et al., 2008) | G-EQUAS** |
|                  |          | GC-MS                | circa 150 ± 30 pg/ml mean ± SD (Suwan-ampai et al., 2009) | circa 50 ± 30 pg/ml mean ± SD (Suwan-ampai et al., 2009) | 1.5–5.7% inter-assay CoV (n > 2500) (Suwan-ampai et al., 2009) | No seasonal effect detected (Carmella et al., 1997) 6-weeks individual RSDs 8.6–71% (n = 13) (Carmella et al., 2004) | G-EQUAS** |
|                  | 3-OHHAaP | HPLC-FD              | < 40–1267 ng/24 h (Lafontaine et al., 2006) | < 44–227.2 ng/24 h (Lafontaine et al., 2006) | 2.4–7.9% inter-assay CoV (n > 2500) (Suwan-ampai et al., 2009) | No seasonal effect detected (Carmella et al., 1997) 6-weeks individual RSDs 8.6–71% (n = 13) (Carmella et al., 2004) | G-EQUAS** |
|                  |          | GC-MS                | >50% sample LOD (smokers & non-smokers) (Suwan-ampai et al., 2009) | >50% sample LOD (smokers & non-smokers) (Suwan-ampai et al., 2009) | 3.6–10.9% (Scherer et al., 2010) | No seasonal effect detected (Carmella et al., 1997) 6-weeks individual RSDs 8.6–71% (n = 13) (Carmella et al., 2004) | G-EQUAS** |
| Aromatic amines   | 1-AN     | GC-MS                | 506–707 ng/24 h (Grimmer et al., 2000) mean ± SD | 68.9 ± 105.4 ng/24 h (Grimmer et al., 2000) | 12.6–18.6% inter-day RSD (Weiss & Angerer, 2002) | 12.6–18.6% inter-day RSD (Weiss & Angerer, 2002) | G-EQUAS** |
|                  | 2-AN     | GC-MS                | 84.5–1027.2 ng/24 h mean ± SD (Grimmer et al., 2000) 17.5 ± 30.9 ng/24 h mean ± SD (Lindner et al., 2011) | 120 ± 272.2 ng/24 h mean ± SD (Grimmer et al., 2000) 1.70 ± 3.6 ng/24 h mean ± SD (Lindner et al., 2011) [LC-MS/MS] | 12.9–13.0% inter-day RSD (Weiss & Angerer, 2002) | 12.6–18.6% inter-day RSD (Weiss & Angerer, 2002) | G-EQUAS** |
|                  | 4-ABP    | GC-MS                | 78.6 ± 85.2 ng/24 h mean ± SD (Grimmer et al., 2000) 24.7 ± 75.4 ng/24 h mean ± SD (Lindner et al., 2011) [LC-MS/MS] | 68 ± 91.5 ng/24 h mean ± SD (Grimmer et al., 2000) 2.77 ± 11.8 ng/24 h mean ± SD (Lindner et al., 2011) [LC-MS/MS] | 9.8–15.1% inter-day RSD (Weiss & Angerer, 2002) | 12.6–18.6% inter-day RSD (Weiss & Angerer, 2002) | G-EQUAS** |

(continued)
| Smoke constituent | Biomarker | Analytical technique | Ranges in smokers (Ref) | Ranges in non-smokers (Ref) | Short-term repeatability‡ (Ref) | Long-term study (Ref) | Inter-laboratory proficiency test (Ref) |
|-------------------|-----------|----------------------|-------------------------|-----------------------------|-------------------------------|-----------------------|-------------------------------------|
| o-tol            | GC-MS/MS  | 8.69 (7.43–10.16) pg/mg creatinine geo mean (95% CI) (Seyler & Bernert, 2011) | 1.64 (1.30–2.07) pg/mg creatinine geo mean (95% CI) (Seyler & Bernert, 2011) | 10.6–14.1% Inter-day RSD (Weiss & Angerer, 2002) |
| 1,3-Butadiene    | DHBMA     | 179 ± 49.1 ng/24h mean ± SD (Lindner et al., 2011) | 63.5 ± 128 ng/24h mean ± SD (Lindner et al., 2011) | 8.7% (Carmella et al., 2009) |
|                  | LC-MS/MS  | 1038 ± 514 nmol/24 h mean ± SD (Carmella et al., 2009) | 662 ± 248 nmol/24 h mean ± SD (Carmella et al., 2009) | 7 month QC inter-day 12–13% (Ding et al., 2009) |
|                  | LC-MS/MS  | 113–1830 ng/ml (Ding et al., 2009) | ND-73.4 ng/ml (Ding et al., 2009) | G-EQUAS |
|                  | MHBMA     | ND-132 ng/ml (Ding et al., 2009) | ND-0.26 ng/ml (Ding et al., 2009) | G-EQUAS |
|                  | LC-MS/MS  | 66.1 ± 69.4 nmol/24 h mean ± SD (Carmella et al., 2009) | 3.66 ± 2.41 nmol/24 h mean ± SD (Carmella et al., 2009) | 16.0% (Carmella et al., 2009) |
| Benzenne         | SPMA      | 2.0 ± 3.80 nmol/24 h mean ± SD (Carmella et al., 2009) | 0.21 ± 0.21 nmol/24 h mean ± SD (Carmella et al., 2009) | <5% (Scherer et al., 2007a) |
|                  | HPLC-MS/MS| 3.20 ± 3.57 mg/24h urine mean (Carmella et al., 2009) | 0.8 mg/24 h urine mean (Mascher et al., 2001) | 4 labs (Minet et al., 2011b) |
| Catechol         | catechol  | 2.07 µg/ml mean (Waidyanatha et al., 2004) | 1.69 µg/ml mean (Waidyanatha et al., 2004) | 0.3%–11.9 µg/ml median (range) (59) [All subjects inc smokers] |
| Acrolein         | 3-HPMA    | 2.8 mg/24 h urine mean (Mascher et al., 2001) | 0.8 mg/24 h urine mean (Mascher et al., 2001) | <2.5% (Scherer et al., 2007a) |
Urinary tobacco smoke exposure biomarkers

| Biomarker | Unit | Concentration Range | Method | Control Group (Scherer et al., 2008) |
|-----------|------|---------------------|--------|-------------------------------------|
| Acrylamide | g/g creatinine | 0.59–0.73 | ICP-MS | G-EQUAS |
| Cadmium   | g/g creatinine | 0.30 | AA | 1.8% measurement error (Hoffmann et al., 2000) |
| Ethylene Oxide | ng/ml | 1.7–4.1 | GAMA HPLC-MS/MS | G-EQUAS |
| Acrylonitrile | ng/ml | 3.6–7.3 | CEMA LC-MS/MS | G-EQUAS |
| Crotonaldehyde | ng/ml | 1.4–6.1 | HMPMA LC-MS/MS | G-EQUAS |

†Abbreviations: as for Table 1.
‡This parameter is sometimes described as ‘inter-day precision’ and we have captured many variations in its presentation. The inter-day coefficient of variation, unless stated.
§Value from Smokers 56 days after cessation (≥100 half-lives).
++G-EQUAS, German External Quality Assessment Scheme (see www.g-equas.de).
Table 3. Other biomarker parameters.

| Smoke constituent | Biomarker | Direct comparison of tobacco products or user groups? | Half-life[ (Ref) | Metabolic enzymes (Ref) |
|-------------------|-----------|------------------------------------------------------|-----------------|-----------------------|
| NNK               | total NNAL| Switching study (Sarkar et al., 2008) Product yield study (Kavvadias et al., 2009a) Reduction on smoking cessation (Carmella et al., 2009) | 40–45 d (Hecht et al., 1999, Hecht et al., 2004) 10–18 d (Goniewicz et al., 2009) | CYP1B1, CYP2A13, CYP1A2, HSD11D1, AKR1B10, AKR1C1, AKR1C2, AKR1C4, CB1R, DCXR, UGT1A9, UGT1A4, UGT2B7, UGT2B10, UGT2B17, MRPI, MRPR (Leslie et al., 2001, Carmella et al., 2002, Breyer-Pfaff et al., 2004, Wiener et al., 2004, Bao et al., 2005, Lazarus et al., 2005, Martin et al., 2006, Church et al., 2010b, Chiang et al., 2011, Ter-Minassian et al., 2012) |
| NNN               | NNN       | Switching study (Sarkar et al., 2008) Product yield study (Kavvadias et al., 2009a) | 45 min (Upadhyaya et al., 2002), patas monkey | CYP2A6, CYP2A13, CYP2E1, CYP3A4, UGT2B10 (Chen et al., 1980, Patten et al., 1997, Wong et al., 2005) |
| NAT               | total NAT | Switching study (Sarkar et al., 2008) Product yield study (Kavvadias et al., 2009a) | 90 min (Li et al., 2006), rabbit | |
| NAB               | total NAB | Switching study (Sarkar et al., 2008) Product yield study (Kavvadias et al., 2009a) | 30 min (Li et al., 2006), rabbit | |
| Pyrene            | 1-OHP     | Reduction in EHC vs CC (Feng et al., 2006, Roethig et al., 2007, Frost-Pineda et al., 2008a) No statistically significant reduction on smoking cessation (Carmella et al., 2009) | 6 h in 8 US smokers (St Helen et al., 2012) | CYP1A1, CYP1B1, GSTM1, GSTP1, UGT1A6, UGT1A7, UGT1A9 (Nerurkar et al., 2000, Pal et al., 2000, Lamikanra et al., 2001, Chuang & Chang, 2007) |
| Benzo[a]pyrene    | 3-OHBaP   | Reduction on switching to snus versus CC and on smoking cessation (Sarkar et al., 2010) | | CYP1A1, CYP1B1, CYP1A2, CYP3A4, UGT1A1, UGT1A7, UGT1A10, UGT1A8, UGT2A1, SULT1B1, EPHX1 (Kim et al., 1998, Shimada et al., 1999, Zheng et al., 2002, Lodovici et al., 2004, Shimada & Fujii-Kuriyama, 2004, Bushey et al., 2011) |
| Aromatic amines   | 1-AN      | | | NAT1, NAT2, CYP1A2, SULTs, UGTs (Frederickson et al., 1992, Badawi et al., 1995, Kimura et al., 1999, Finel et al., 2005, Al-Zoughool & Talaska, 2006, Sarkar et al., 2005, Butler et al., 2011) |
|                   | 2-AN      | Reduction in EHC versus CC (Frost-Pineda et al., 2008a) | | |
|                   | 4-ABP     | Reduction on smoking restriction and on switching to smokeless product (Sarkar et al., 2010) and EHC (Frost-Pineda et al., 2008a) | 15 h (Frederickson et al., 1992) | |
|                   | o-tol     | Reduction in EHC versus CC (Frost-Pineda et al., 2008a) | | |
| 1,3-Butadiene     | DHBMA     | No statistically significant reduction on smoking cessation (Carmella et al., 2009) | 5–9 h (van Welie et al., 1992) | CYP2E1, CYP3A4, CYP2A6, EPHX1, GSTT1, GSTM1, MPO (Seaton et al., 1995, Himmelstein et al., 1996, Nieuwma et al., 1998, Fustinoni et al., 2002, Abdel-Rahman et al., 2005, Tan et al., 2010) |
|                   | MHBMA     | Reduction in Test versus CC (Sarkar et al., 2008) Reduction on smoking cessation (Carmella et al., 2009) | 5–9 h (van Welie et al., 1992) | |
|                   | THBMA     | Reduction at 56 days following smoking cessation (Kotapati et al., 2011) | | |
| Substance          | Abbreviation | Effect                                                                 | Time     | Enzymes/Proteins                                                                 |
|--------------------|--------------|----------------------------------------------------------------------|----------|----------------------------------------------------------------------------------|
| Benzene            | tt-MA        | Reduction in EHC versus CC and non-smoker versus smoker (Kim et al., 2006) | 6–12 h   | CYP2E1, CYP2F1, GSTT1, GSTM1, COMT, NQO1, EPHX1, AKR1C1, SULT1A1, UGT1A6, MPO (Rossi et al., 1999, Dougherty et al., 2008, Manini et al., 2010, Angelini et al., 2011, Mansi et al., 2012) |
| SPMA               | Reduction in EHC versus CC and non-smoker versus smoker (Feng et al., 2006, Roethig et al., 2007) Reduction on smoking cessation (Carmella et al., 2009) | 6–12 h   | Kim et al., 2006                                                                 |
| Catechol           | Catechol     | Reduction in EHC versus CC and nonsmoker versus smoker (Roethig et al., 2007) Reduction on smoking cessation (Carmella et al., 2009) | 3–7 h    | Metabolite of benzene [See benzene] ALDH, ALR, GSTM1, GSTP1, GSTA1, GGT1, GGT2, GGT3, NAT1, NAT2 (Berhane et al., 1994, Pal et al., 2000, Mascher et al., 2001) |
| Acrolein           | 3-HPMA       | Reduction in EHC versus CC and nonsmoker versus smoker (Roethig et al., 2007) Reduction on smoking cessation (Carmella et al., 2009) | 5–9 h    | ALDH, ALR, GSTM1, GSTP1, GSTA1, GGT1, GGT2, GGT3, NAT1, NAT2 (Berhane et al., 1994, Pal et al., 2000, Mascher et al., 2001) |
| Crotonaldehyde     | HMPMA        | Significantly reduced on cessation or switch to lower toxicant cigarette (Scherer, 2005, Scherer et al., 2007b) Reduction on smoking cessation (Carmella et al., 2009) | 5–9 h    | GSTP1 (Pal et al., 2000) |
| COMEMA             | No significant change on cessation or lower toxicant cigarette (Scherer, 2005, Scherer et al., 2007b) | 5–9 h    | Van Welie et al., 1992, Scherer et al., 2006                                    |
| Ethylene oxide     | HEMA         | Reduction on smoking cessation (Carmella et al., 2009) Significantly reduced on cessation or switch to lower toxicant cigarette (Scherer, 2005, Scherer et al., 2010) | <5 h     | GSHT1 (Muller et al., 1998, Fennell et al., 2000) ALDH2, ALDH1B1 (Weiner & Wang, 1994, Stagos et al., 2010) ALDH2, ADH5 (Hedberg et al., 2001, Wang et al., 2002b, Thompson et al., 2010) |
| Acetaldehyde       | Formaldehyde | Reduction on smoking cessation (Scherer et al., 2007b) | | ADH5 (Hedberg et al., 2001, Wang et al., 2002b, Thompson et al., 2010) |
| Acrylamide         | Acrylamide   | 2.4 h (Fuhr et al., 2006) | | CYP2E1, CYP2F1, CYPs (Doroshyenko et al., 2009, Huang et al., 2011a, Huang et al., 2011b) |
| Glycidamide, AAMA, | 17.4 h (Fuhr et al., 2006) | | | |
| GAMA               | 25.4 h (Fuhr et al., 2006) | | | |
| Acrylonitrile      | CEMA         | Significantly reduced on cessation or switch to lower toxicant cigarette (Scherer, 2005, Scherer et al., 2010) | 2.4 h    | CYP2E1, GSTP1 (Kedderis et al., 1993, Thier et al., 2001, Thier et al., 2002, Wang et al., 2002a, Suhua et al., 2010) |
| Metals             | Cadmium      | | | Months (Huang & Yang, 1997, Hoffmann et al., 2000, Paschal et al., 2000, McElroy et al., 2007a, McElroy et al., 2007b) |

†Abbreviations as in Table 1. CC, conventional cigarette; EHC, electrically heated cigarette.
‡Approximate half-life based on elimination from body fluid or measurement in urine.
Benzene is used as a reagent for the polymer industry. It also occurs ubiquitously in the environment with petrochemical, vehicle and combustion processes being important sources. Benzene is in IARC Group 1 (“carcinogenic to humans”) (International Agency for Research on Cancer, 2012). For exposure to benzene, two biomarkers, \textit{trans}, \textit{trans}-muconic acid (tt-MA) and S-phenyl mercapturic acid (SPMA) have been widely used. Both assays have been validated (Carmella et al., 2009; Ding et al., 2009; Feng et al., 2006; Kim et al., 2006; Ruppert et al., 1995; Scherer et al., 2007a) and used in a variety of tobacco product studies (Feng et al., 2006; Kim et al., 2006; Roethig et al., 2007) but only SPMA differentiated between groups of smokers and non-smokers with a consistently greater than 2-fold difference between group means across studies (Scherer et al., 2007a) and showed a difference between groups on smoking cessation (Carmella et al., 2009). However, a review on tt-MA by Scherer et al. (1998) reported significant differences between smokers and non-smokers with seven of the 14 studies reviewed showing a greater than 2-fold difference. This article also indicated that it is known that tt-MA is also formed from the metabolism of sorbic acid, which is widely used in foods, possibly interfering with studies in smokers and that if this urinary biomarker is used it is recommended that ingestion of sorbic acid should be taken into account (Scherer et al., 1998). Additionally, SPMA assay precision has been characterized in long-term studies (Ding et al., 2009). Catechol is also a metabolite of benzene; however, while it has been used as a biomarker of benzene exposure (Kerzic et al., 2010; Waidyanatha et al., 2004), a 2-fold separation between smokers and non-smokers was not observed (Waidyanatha et al., 2004). Additional data would be required to qualify this biomarker for use in tobacco smoke exposure studies.

Acrolein, also known as propenal, is the simplest unsaturated aldehyde. It is a chemically reactive compound found in the environment as a by-product of overheated organic matter (oils), plastics, and fossil fuel combustion and can also be formed by lipid peroxidation and oxidative stress in normal mammalian tissues (Chung et al., 1996; Esterbauer et al., 1991). For biomarker assays of exposure to acrolein, 3-hydroxypropylmercapturic acid (3-HPMA) has been validated (Carmella et al., 2007, 2009; Ding et al., 2009; Mascher et al., 2001; Scherer et al., 2007a) and showed a difference in group mean concentration between smokers and non-smokers with a ratio of greater than 2 (Lindner et al., 2011; Mascher et al., 2001; Minet et al., 2011a). 3-HPMA has been used in product switching studies and smoking cessation studies (Carmella et al., 2009; Roethig et al., 2007). Furthermore, it has been used in short-term and long-term studies in groups of smokers (Lindner et al., 2011; Sarkar et al., 2008) and an inter-laboratory comparison study has been published (Minet et al., 2011b; Shepperd et al., 2009).

Crotonaldehyde, like acrolein, is an unsaturated aldehyde which is produced through the combustion of carbon-containing fuels and is therefore an important environmental pollutant (Budiawan, 2001). In addition, it is commonly found in foodstuffs such as fish, meat, fruit and vegetables and alcoholic beverages including wine and whisky (Budiawan, 2001). It is also reported to be produced endogenously through lipid peroxidation (Chung et al., 1996; Hecht, 2001). The assays for the biomarkers of crotonaldehyde exposure, 3-hydroxyl-methylpropylmercapturic acid (HMPMA) and 2-carboxy-1-methylthylmercapturic acid (CEMA), have been characterized to a similar extent (Scherer et al., 2007b), but data on sample storage stability and long-term evaluation in studies of smokers were not found. However, while HMPMA could differentiate groups of smokers from non-smokers CEMA could not (Scherer et al., 2007b). These data show that HMPMA would be suitable for exposure assessment in smoker studies but that CEMA would not; although further work on HMPMA is still required.

Ethylene oxide is used as an intermediate in the production of several industrial chemicals and is used as a fumigant or sterilizing agent. Exposure to ethylene oxide in the general population is through medical, food, clothing and cosmetics that have been sterilized with the compound and it has also been detected in tobacco smoke and automotive exhaust fumes (U.S. Department of Health and Human Services Public Health Service National Toxicology Program, 2011). The compiled data on N-acetyl-S-(2-hydroxyethyl)-l-cysteine (HEMA) as a biomarker for ethylene oxide exposure show a validated assay (Carmella et al., 2009; Ding et al., 2009), differences between groups of smokers and non-smokers (Ding et al., 2009; Eckert et al., 2011) and reductions in group mean values in tobacco products switching studies (Scherer, 2005; Scherer et al., 2010). An elimination half-life of \(<5\) h (Haufroid et al., 2007) could be problematic in study design (e.g. time since last product use would be a critical datum) and, although the sample is stable to repeated freeze–thaw cycles (Ding et al., 2009), extended storage at assay temperature in the urine matrix was not reported.

Acrylamide is an industrial chemical used in a wide range of applications including water treatment, oil extraction, biotechnology and paper manufacturing. It is also formed in the heating process of starch-containing/carbohydrate-rich food (Tareke et al., 2002). For acylamide exposure, more data are available on the mercapturic acid metabolites, N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-l-cysteine (AAMA) and N-(R,S)-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-l-cysteine (GAMA) than the parent molecule or its metabolite glycidamide (Fuhr et al., 2006; Urban et al., 2006). Glycidamide was included in one study because the investigators considered it to be a marker of the toxicity pathway based on rodent studies (Fuhr et al., 2006). An approximate 2-fold difference between group means in smokers and non-smokers for AAMA and GAMA was reported (Urban et al., 2006). Nonetheless, sample stability data and long-term studies were not found and further work would be required to qualify AAMA and GAMA for use as tobacco smoke exposure biomarkers.

The main source of exposure to acrylonitrile is occupational, since it is primarily used in industry, where it is used to make other chemicals such as plastics, synthetic rubber and acrylic fibers. It has also been detected in food which has been stored in containers manufactured from plastics constructed with acrylonitrile, such as acrylonitrilebutadiene-styrene (ABS). For acrylonitrile, data on the biomarker N-acetyl-S-(2-carboxyethyl)-l-cysteine (CEMA)
assay validation and some comparisons of smokers and non-smokers are available (Minet et al., 2011a; Schettgen et al., 2009), but sample storage stability, use in long-term studies of smokers and inter-laboratory comparisons were not found.

The main exposure to cadmium, in people, occurs through the consumption of foods and drinking water, the inhalation of cadmium particles from ambient air or cigarette smoke, and the incidental ingestion of contaminated dust or soil. Urinary cadmium has been measured in several large studies conducted over different time periods and much of the assay validation and biomarker qualification data are available (Hoffmann et al., 2000; McElroy et al., 2007a, b; Paschal et al., 2000). However, the cross-sectional studies reported that urinary cadmium concentration increased as the subjects’ age increased in both smokers and non-smokers (McElroy et al., 2007a) and this observation suggests that longitudinal studies of tobacco products use would require data to be presented as individual changes over time rather than group mean changes. Of course, statistical techniques such as age-adjustment may also be applied to cross-sectional data, to facilitate inter-group comparisons.

Discussion

The intended study application is fundamental when considering whether a biomarker is fit for purpose. Aspects of the assay validation, such as linearity, accuracy, precision, repeatability and reproducibility of measurements (Food and Drug Administration, 2001), may be considered as basic required information, but such data alone do not qualify a biomarker for use in a particular application. Lee et al. (2006) described fitness for purpose as “[the] notion that assay validation should be tailored to meet the intended purpose of the biomarker study, with a level of rigor commensurate with the intended use of the data” and, although their description was for a pharmaceutical application, we contend that this description applies to considerations of different biomarker measurement study designs that might be applied to tobacco products. For example, if the purpose of a study is to compare the absolute amount of a biomarker in a sample across several different testing laboratories, then a reference standard should be available or, at the least, a consensus value for a standard material based on an inter-laboratory trial (Food and Drug Administration, 2001, International Organization for Standardization, 2005, 2007). Further, if a single spot urine sample is to be collected for biomarker measurement in a cross-sectional study, then the metabolic pathway leading to the biomarker formation or destruction and the kinetics of its appearance in urine should be known, along with a measure of time since subjects’ last exposure. Clearly, a situation with rapid elimination of a biomarker into urine, a short urinary half-life and a long time, or even a variable time, between exposure and urine collection would compromise any data collected.

The approach taken to collect urine samples is also an important consideration for study design. While many studies have used a 24 h collection period, this is difficult to achieve in studies that are conducted without subject confinement. If a spot sample is taken, then the time of sample collection and the approach taken to correct biomarker concentration for subjects’ hydration and urine volume output over any defined period will also affect the biomarker measurement variability. The time of collection of spot urine samples was found to affect the variability in studies of sex hormones, with a morning spot sample being less variable than an overnight collection in a group of normotensive women (Muti et al., 2000). However, in studies of urinary electrolyte concentrations, overnight samples were better predictors of 24-h calcium excretion than were daytime collections (Cirillo et al., 1993), and afternoon spot urine samples, adjusted for creatinine concentration, correlated better with 24-h sodium excretion than did morning spot samples (Mann & Gerber, 2010). Correction of spot urine samples for creatinine concentration is an adjustment that is widely used throughout the biomedical scientific literature (Arndt, 2009; Cote et al., 2008). Previous studies of smokers reported that adjustment of urinary biomarkers for creatinine concentration was itself highly variable and could be improved further by correction for urinary specific gravity (Heavner et al., 2006). From this, it appears that the use of any spot urine sample for biomarker measurement in tobacco product comparison studies would require a separate investigation to qualify the biomarker for use.

In many tobacco studies, nicotine exposure biomarkers are considered to give the best objective measure of tobacco exposure but they were not included in this review because another recent summary is available (Tricker, 2006). However, it is appropriate to summarize the characteristics of nicotine as a biomarker here, to allow comparison with other putative tobacco smoke exposure biomarkers. Nicotine is present in milligram per gram quantities in tobacco and approximately 10% transfers to mainstream smoke. Upon inhalation of mainstream smoke, nicotine is rapidly absorbed into the bloodstream and rapidly metabolized by several enzyme systems. Nicotine elimination from the plasma has a half-life of approximately 2 h in man and little unchanged nicotine is recovered from the urine of smokers. The major metabolites, cotinine and trans-3'-hydroxycotinine are eliminated into urine more slowly and, together with nicotine and all their glucuronide conjugates (nicotine + 5), urinary measurements account for approximately 80% of the initial mass of nicotine absorbed into the body. When another four metabolites (nornicotine, norcotinine, nicotine N-oxide and cotinine N-oxide) are considered (nicotine + 9), urinary measurements account for approximately 90–95% of the initial mass of nicotine absorbed. Collectively, these metabolites have elimination half-lives of <24h and so their measurement mainly reflects very recent and the previous 2–3 days smoking activity. Nicotine metabolites are often expressed as “total nicotine equivalents” based on calculations allowing for the molecular mass of each metabolite converted back to nicotine and usually expressed in milligrams. Numerous studies have used nicotine metabolites as biomarkers of smoke exposure for both products switching and smoking cessation. Despite this extensive characterisation, nicotine would not be fit for purpose as a biomarker of toxicant exposure, in studies comparing RTPs in which nicotine levels were maintained, while other toxicants were reduced, an approach suggested more than 30 years ago (Russell, 1976). More recently, it has been proposed that the
toxicant to nicotine ratio could be used as one measure for the potential harm reduction of RTPs use (Burns et al., 2008). However, the use of this ratio would require an understanding of the other toxicant biomarker elimination kinetics, in relation to nicotine, if urinary biomarkers were the means of assessment.

In considering other putative biomarkers of smoke constituent exposure, few are as well characterized and as widely used as nicotine metabolites. From this review, the biomarkers DHBMA (Urban et al., 2003), catechol (Waidyanatha et al., 2004) and CMEMA (Scherer et al., 2007b) were not able to distinguish between groups of smokers and non-smokers and thus are not fit for purpose in studies of smoking cessation or tobacco products switching, such as in RTP assessment. Further, while urinary cadmium concentration was elevated in cross-sectional and long-term studies of smokers compared to non-smokers (Hoffmann et al., 2000), all subjects showed an increased urinary cadmium concentration with age (McElroy et al., 2007a). Therefore, careful study design considerations would be needed to allow this putative biomarker to be used for RTP assessment, as it would be important to differentiate between product use and bioaccumulation with age.

For the great majority of biomarkers considered, it is clear that assay validation and biomarker qualification has been performed for only short-term studies, typically of less than one-week duration. Thus, the TSNAs other than NNK (NNN, NAB and NAT), 3-OHBaP, 2-AN, 4-ABP, o-tol, THBMA, HMPMA, AAMA, GAMA and CEMA may only be described as fit for purpose, as urinary biomarkers of tobacco smoke exposure, if the study duration does not exceed 1 week. With this group of putative tobacco smoke exposure biomarkers, several details required for assay validation and biomarker qualification were not found in the literature. It is likely that some of these data, such as assay linearity and recovery after extraction from the biological matrix, may exist within the originating laboratory but were not reported.

Long-term studies (typically of 6–7 months duration) were only found in the literature for NNAL (Sarkar et al., 2008), 1-OHP (Carmella et al., 1997, 2004; Sarkar et al., 2008), MHBMA (Ding et al., 2009; Sarkar et al., 2008), SPMA (Ding et al., 2009; Sarkar et al., 2008), 3-HPMA (Ding et al., 2009; Sarkar et al., 2008) and HEMA (Ding et al., 2009) but, even then, sample storage data and other basic assay characteristics were often not reported. This would be a concern if, for example, the analysis plan called for storage of all samples so that they could be analysed as a single batch within the laboratory. The FDA guidance on full validation of bioanalytical method recommends that freeze and thaw stability, short-term stability and long-term stability should be established for the analytes (Food and Drug Administration, 2001). In particular, the short-term temperature stability test should be conducted for a time period reflecting the expected duration at which a sample will be kept at room temperature and at 4 °C, for instance holding time in the autosampler. In the panel of studies we have reviewed, some laboratories have conducted a stability assessment at least at room temperature and for frozen samples, some have only conducted the stability assessment for frozen samples, and others did not report stability testing (Table 1).

As a PAH biomarker, 1-OHP has been widely studied and it has been used in several smoking studies, with mixed results. While it can be used to show differences between the type of smoking product used in a controlled study (Feng et al., 2006; Roethig et al., 2007), the magnitude of change and potential confounding by external influences such as diet and vehicle exhaust exposure (Chuang & Chang, 2007; Menzie et al., 1992) limit its use. A greater concern is that pyrene is not a carcinogen and it is more hydrophilic than most other PAH. Therefore, 1-OHP is unlikely to be a good surrogate biomarker of other, carcinogenic, PAH exposure. Some investigators have used 3-OHBaP as a biomarker of benzo[a]pyrene (LaFontaine et al., 2006; Sarkar et al., 2010) although, as noted above, some of the assay validation and biomarker qualification data for the long-term use of 3-OHBaP were not found. Recent data have suggested that urinary concentrations of other PAH may also give clear and statistically significant differences between smokers and non-smoker groups. For example, in one study of 622 spot urine samples taken in the USA and Poland, 1-hydroxyfluorone and 2-naphthol were reported to be more selective of tobacco smoke exposure than 1-hydroxypyrene and hydroxynaphthalenes (St Helen et al., 2012). In addition, a further study by St Helen et al. (2013) showed that racial differences in urinary naphthal and total PAH levels indicating regional, international and racial variations are also significant considerations for these and other biomarkers proposed for possible RTP assessment. Further studies may qualify these PAH metabolites as useful biomarkers for use in tobacco products switching and in smoking cessation studies.

For several of the biomarkers included in this review, other methods of analysis such as DNA and haemoglobin adducts are available. 4-ABP is typically measured as haemoglobin adducts (Bartsch et al., 1990; Bernert et al., 2005; Bryant et al., 1988) and formaldehyde and acetaldehyde are typically measured as DNA adducts in leukocytes (Chen et al., 2007; Lu et al., 2009; Wang et al., 2000, 2009). A recent study quantified NNK-derived DNA adducts in the oral mucosa of smokers and non-smokers (Stepanov et al., 2013). DNA-adduct from tissue biopsies, white blood cells, and haemoglobin adducts biomarkers, accumulate over a prolonged period of time, depending on the specific matrix used. For instance T-lymphocytes have a half-life of a few months to a few years (Vrisekoop et al., 2008) whilst red blood cells have a half-life of 60 days (Berlin et al., 1959). Hair and nails have also been proposed as matrices to measure the cumulative exposure to smoke toxicants over months (Avila-Tang et al., 2013). Those matrices also have their specific limitations, for instance the NNK-DNA adducts are well correlated with smoking in mouth epithelial cells (Stepanov et al., 2013), however in other studies conducted in tissue biopsies, myosmine, which is found in tobacco and food was suspected to be a significant confounding factor (Schlöbe et al., 2008). Acrolein DNA adducts were also well correlated with smoking status when DNA was extracted from mouth epithelial cells (Nath et al., 1998) but not from white blood cells (Zhang et al., 2011). One explanation is that the formation of mercapturic acid from the reaction of acrolein...
with blood glutathione subsequently excreted in urine is sufficiently efficient to protect leukocytes from DNA damage (Zhang et al., 2011). Hair contamination by sweat and environmental toxicants and hair pigmentation also have an impact on the reliability of the data collected from this matrix (Avila-Tang et al., 2013). These examples illustrate that biomarkers should be carefully selected according to the purpose of the study to take into account half-life and matrix.

The difficulties in validating, qualifying and establishing adduct assays as fit for purpose should not be overlooked. Indeed, it could be expected that such assays would be inherently less reproducible and give greater intra- and inter-assay imprecision and greater variability in long-term sample storage stability than the urinary assays described here (Angerer et al., 2007; Chen et al., 2007; Lu et al., 2009; Wang et al., 2000, 2009).

Some investigators have used urinary mutagenicity as a biomarker of exposure to genotoxic chemicals. Studies have confirmed that cigarette smokers have higher levels of urinary mutagenicity than non-smokers, even when both groups were maintained on a low-mutagenic, boiled food diet (Doolittle et al., 1990). Since then, several studies have confirmed that urinary mutagenicity was decreased in cigarette smokers who switched to using an electrically heated cigarette (Frost-Pineda et al., 2008a; Rahn et al., 1991; Roethig et al., 2005, 2007, 2008; Smith et al., 1996) or the use of oral snus (Sarkar et al., 2010). However, the urinary mutagenicity assay is not based on chemical standards and its characteristics, such as linearity, precision, accuracy, limits of detection, etc., cannot be compared with the other assays discussed in this review. It gives a relative assessment of the mutagenicity of samples made in a direct comparison test and its use as a regulatory action standard would be problematic, requiring reference cultures, standards and frequent inter-laboratory comparisons to ensure robust data. Further, the output of urinary mutagenicity testing is a combination between the exposure dose to the mutagenic agent and the metabolism of those toxicants within the exposed subject. Metabolic inter-individual differences are a source of assay variation and specific genotypes have been associated with an increase risk of tobacco related diseases (Daly et al., 1994). However our current understanding of the metabolic pathway variations limits our ability to factor genotype and phenotype in the interpretation of urine mutagenicity studies.

Nonetheless, well-controlled data generated with such assays could help to inform the process of RTP assessment.

In compiling these data on putative tobacco smoke exposure biomarkers, we considered that the metabolic pathways and kinetic of the pathways leading to the biomarker appearance in the urine were also of importance. Although much information on the metabolic pathways was available, fewer formal calculations of the mass balance for specific metabolites were found. Also, little information on diurnal variation in metabolic enzymes was retrieved; although this is reported not to affect the mercapturic acids (van Welie et al., 1992), which are the biomarkers for several of the smoke constituents considered in this review. Also, from the elimination half-lives of the biomarkers examined, most exposures, with the exception of NNAL and cadmium, would be expected to change in relatively short-term studies. While this is helpful to confirm a toxicant reduction in a controlled RTP assessment study, a biomarker with a short elimination half-life would be of less use in cross-sectional or population studies, where an occasional cigarette smoker could give high readings based on smoking one or two cigarettes shortly before urine sample collection. There remains a need for the availability of biomarkers other than NNAL that have half-lives in the days to weeks range, to give better estimates of long-term exposure to smoke toxicants.

Overall, if biomarkers measurements are intended for the long-term assessment of RTPs or for the setting of regulatory action levels, as proposed by some groups (Ashley et al., 2007; Burns et al., 2008; Hecht et al., 2010), then this would require measurements to be made across bio-analytical laboratories. It is not clear that sufficient information is available for any biomarkers on the proposed lists, for these purposes. Indeed the only published data on an inter-laboratory comparison that were found was for 3-HPMA across four testing laboratories (Minet et al., 2011b) and a study by Biber et al. (1987) on nicotine and cotinine in serum and urine conducted in 11 laboratories. A larger inter-laboratory study for this biomarker has been conducted recently through CORESTA but this was a laboratory proficiency trial and was not intended to set a consensus value for a standard material, and the data have not been submitted for publication yet. Some proficiency testing schemes, such as the German External Quality Assessment Scheme (G-EQUAS: see, http://www.g-equas.de), are available. This scheme includes occupation and environmental levels of many biomarkers discussed in this review but it does not include several, such as total nicotine equivalents and TSNAs. Thus, by registering with such a scheme, testing laboratories should be able to determine whether their measurements for specific biomarkers fall within a group consensus, which would facilitate obtaining reproducible results across laboratories. Ideally, any schemes would include the ranges of concentrations of biomarkers found in smokers, to establish assay validity as one of the criteria of fitness for purpose.

A key characteristic for any candidate biomarker is its specificity for tobacco-related exposures, and the investigator’s ability to discriminate between the contributions of tobacco smoke exposure from those of non-tobacco exposures. Common dietary and environmental exposures to precursor compounds, such as PAH (Chuang & Chang, 2007; Scherer et al., 2000), benzene (Scherer et al., 1998) or acrylamide (Fuhr et al., 2006) pose a continuing challenge for otherwise promising urinary biomarkers with regard to the design and interpretation of investigations intended to address the exposures that result from the smoking of tobacco in all of its diverse forms.

As a final remark, we need to consider that one of the key purpose of a biomarker in the context of tobacco smoke exposure and product risk assessment is the predictive nature of such biomarkers for the tobacco-related diseases. Unfortunately very little data are currently available regarding the disease predictivity of biomarkers of exposure, but some interesting results are starting to emerge from prospective clinical studies. Dose-dependent association between urinary biomarkers of cotinine, total NNAL, and
tetrahydrophenanthrene were associated with risk of lung cancer in a Chinese cohort (Yuan et al., 2011). A similar association was found for NNAL in the serum of smokers (Church et al., 2009). In contrast, urinary metabolites of 1,3-butadiene, ethylene oxide, benzene, and acrolein, were not independent risk predictors for lung cancer (Yuan et al., 2012). Finally the haemoglobin adduct of 4-amino-phenylalanine has also been identified as a good risk predictor for bladder cancer but this has only been shown in non-smokers (Tao et al., 2013). Since prospective clinical studies are conducted over many years, it is likely that the ultimate goal of a comprehensive understanding of the predictivity of urinary biomarkers for the tobacco-related disease will require a significant effort over a prolonged period of time.

Acknowledgements

The authors acknowledge the critical review and discussion of this manuscript by all members of the CORESTA Biomarkers Sub-Group.

Declaration of interest

Emmanuel Minet and Michael McEwan are employees of British American Tobacco. Evan Gregg was paid as a consultant by British American Tobacco for time spent working on this review. This review was requested by the CORESTA Biomarkers Sub-Group. [CORESTA is the Cooperation Centre for Scientific Research Relative to Tobacco. See: www.CORESTA.org].

References

Abdel-Rahman SZ, Ammenheuser MM, Omiecinski CJ, et al. (2005). Variability in human sensitivity to 1,3-butadiene: influence of polymorphisms in the 5′-flanking region of the microsomal epoxide hydrolase gene (epx1). Toxicol Sci 85:624–31.

Al-Zoughool M, Talaska G. (2006). 4-Aminobiphenyl n-glucuronidation by liver microsomes: optimization of the reaction conditions and characterization of the udp-glucuronosyltransferase isoforms. J Appl Toxicol 26:524–32.

Ambrose CB, Abrams SM, Gorlewskia-Roberts K, Kadlubar FF. (2007). Hair dye use, meat intake, and tobacco exposure and presence of carcinogen-DNA adducts in exfoliated breast ductal epithelial cells. Arch Biochem Biophys 464:169–75.

Anderson KE, Klijirs J, Murphy L, et al. (2003). Metabolites of a tobacco-specific lung carcinogen in nonsmoking casino patrons. Cancer Epidemiol Biomarkers Prev 12:1544–6.

Angelini S, Kumar R, Bermejo JL, et al. (2011). Exposure to low environmental levels of benzene: evaluation of micrornucleus frequencies and s-phenylmercapturic acid excretion in relation to polymorphisms in genes encoding metabolic enzymes. Mutat Res 719:7–13.

Angerer J, Ewers U, Wilhelm M. (2007). Human biomonitoring: state of the art. Int J Hyg Environ Health 210:201–28.

Ardnt T. (2009). Urine-creatinine concentration as a marker of urine dilution: reflections using a cohort of 45,000 samples. Forensic Sci Int 186:48–51.

Ashley DL, Burns DM, Djordjevic M, et al. (2007). The scientific basis of tobacco product regulation: report of a who study group. Geneva: World Health Organization.

Avila-Tang E, Al-Delaimy WK, Ashley DL, et al. (2013). Assessing secondhand smoke using biological markers. Tob Control 22:164–71.

Badawi AF, Hirvonen A, Bell DA, et al. (1995). Role of aromatic amine acetyltransferases, nat1 and nat2, in carcinogen-DNA adduct formation in the human urinary bladder. Cancer Res 55:5230–7.

Bao Z, He X-Y, Ding X, et al. (2005). Metabolism of nicotine and cotinine by human cytochrome p450 2a13. Drug Metab Dispos 33:258–61.

Bartsch H, Caporaso N, Coda M, et al. (1990). Carcinogen hemoglobin adducts, urinary mutagenicity, and metabolic phenotype in active and passive cigarette smokers. J Natl Cancer Inst 82:1826–31.

Berhane K, Widersten M, Engstrom A, et al. (1994). Detoxification of base propenals and other alpha, beta-unsaturated aldehyde products of radical reactions and lipid peroxidation by human glutathione transferases. Proc Natl Acad Sci USA 91:1480–4.

Berlin NI, Waldmann TA, Weissman SM. (1959). Life span of red blood cell. Physiol Rev 39:577–616.

Bennett JT, Jain RB, Pirkle JL, et al. (2005). Urinary tobacco-specific nitrosamines and 4-amino-phenylalanine hemoglobin adducts measured in smokers of either regular or light cigarettes. Nicotine Tob Res 7:729–38.

Bennett JT, Pirkle JL, Xia Y, et al. (2010). Urine concentrations of a tobacco-specific nitrosamine carcinogen in the U.S. population from secondhand smoke exposure. Cancer Epidemiol Biomarkers Prev 19:969–77.

Bhat SH, Gelhaus SL, Mesaros C, et al. (2011). A new liquid chromatography/mass spectrometry method for 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (nmal) in urine. Rapid Commun Mass Spectrum 25:1115–21.

Biber A, Scherer G, Hoepfner I, et al. (1987). Determination of nicotine and cotinine in human serum and urine: an interlaboratory study. Toxicol Lett 35:45–52.

Breyer-Pfaiff U, Martin H-J, Ernst M, Maser E. (2004). Enantioselectivity of carbonyl reduction of 4-methyl-nitrosamino-1-(3-pyridyl)-1-butanone by tissue fractions from human and rat and by enzymes isolated from human liver. Drug Metab Dispos 32:915–22.

Bryant MS, Vineis P, Skipper PL, Tannenbaum SR. (1988). Hemoglobin adducts of aromatic amines: associations with smoking status and type of tobacco. Proc Natl Acad Sci USA 85:9788–91.

Budiawan, EE. (2001). Cancer risk assessment for the environmental mutagen and carcinogen crotonaldehyde on the basis of td(50) and comparison with 1.n(2)-propanodeoxyguanosine adduct levels. Cancer Epidemiol Biomarkers Prev 10:883–8.

Burns DM, Dybing E, Gray N, et al. (2008). Mandated lowering of toxicants in cigarette smoke: a description of the world health organization tobreg proposal. Tob Control 17:132–41.

Bushby RT, Chen G, Blevins-Primeau AS, et al. (2011). Characterization of udp-glucuronosyltransferase 2a1 (ugt2a1) variants and their potential role in tobacco carcinogenesis. Pharmacogenet Genomics 21:55–65.

Butler MW, Fukui T, Salit J, et al. (2011). Modulation of cystatin c expression in human airway epithelium related to genotype, smoking, COPD, and lung cancer. Cancer Res 71:2572–81.

Carmella SG, Borukhova A, Akerkar SA, Hecht SS. (1997). Analysis of human urine for pyridine-n-oxide metabolites of 4-(methyl-nitrosa-mine)-1-(3-pyridyl)-1-butanone, a tobacco-specific lung carcinogen. Cancer Epidemiol Biomarkers Prev 6:113–20.

Carmella SG, Chen M, Han S, et al. (2009). Effect of smoking cessation on eight urinary tobacco carcinogen and toxicant biomarkers. Chem Res Toxicol 22:734–41.

Carmella SG, Chen M, Zhang Y, et al. (2007). Quantitation of acrolein-derived (3-hydroxypropyl)mercapturic acid in human urine by liquid chromatography-atmospheric pressure chemical ionization tandem mass spectrometry: effects of cigarette smoking. Chem Res Toxicol 20:986–90.

Carmella SG, Le K-A, Hecht SS. (2004). Improved method for determination of 1-hydropyropane in human urine. Cancer Epidemiol Biomarkers Prev 13:1261–4.

Carmella SG, Le K-A, Upadhyaya P, Hecht SS. (2002). Analysis of n- and o-glucuronides of 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (nmal) in human urine. Chem Res Toxicol 15:545–50.

Chau CH, Rixe O, McLeod H, Figg WD. (2008). Validation of analytic methods for biomarkers used in drug development. Clin Cancer Res 14:5967–76.

Chen CB, Hecht SS, McCoy GD, Hoffmann D. (1980). Assays for metabolic alpha-hydroxylation of n-nitrosomorocitine and n-nitrosopyrrolidine and the influence of modifying factors. IARC Sci Publ 31:349–59.
Chen L, Wang M, Villalta PW, et al. (2007). Quantitation of an acetaldehyde adduct in human leukocyte DNA and the effect of smoking cessation. Chem Res Toxicol 20:108–13.

Chiang HC, Wang CY, Lee HL, Tsou TC. (2011). Metabolic effects of cyp2a6 and cyp2a13 on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (nnk)-induced gene mutation – a mammalian cell-based mutagenesis approach. Toxicol Appl Pharmacol 253:145–52.

Chuang C-Y, Chang C-C. (2007). Urinary 1-hydroxypyrene level relative to vehicle exhaust exposure mediated by metabolic enzyme polymorphisms. J Occup Health 49:140–51.

Chung FL, Chen HS, Nang RG. (1996). Lipid peroxidation as a potential endogenous source for the formation of exocytic DNA adducts. Carcinogenesis 17:2105–11.

Church TR, Anderson KE, Le C, et al. (2010a). Temporal stability of urinary and plasma biomarkers of tobacco smoke exposure among cigarette smokers. Biomarkers 15:345–52.

Church TR, Haznadar M, Geisser MS, et al. (2010b). Interaction of cyp1b1, cigarette-smoke carcinogen metabolism, and lung cancer risk. Int J Mol Epidemiol Genet 1:295–309.

Church TR, Anderson KE, Caporaso NE, et al. (2009). A prospectively measured serum biomarker for a tobacco-specific carcinogen and lung cancer in smokers. Cancer Epidemiol Biomarkers Prev 18:260–6.

Cirillo M, Mellone M, De Santo NG. (1993). Can overnight urine replace 24-hour urine collection to measure urinary calcium in epidemiologic studies? Miner Electrolyte Metab 19:385–8.

Cote AM, Brown MA, Lam E, et al. (2008). Diagnostic accuracy of urinary spot protein:creatinine ratio for proteinuria in hypertensive pregnant women: systematic review. Br Med J 336:1003–6.

Daly AK, Cholerton S, Armstrong M, Idle JR. (1994). Genotyping for polymorphisms in xenobiotic metabolism as a predictor of disease susceptibility. Environ Health Perspect 102:55–61.

Ding YS, Blount BC, Valentin-Blasini L, et al. (2009). Simultaneous determination of six mercapturic acid metabolites of volatile organic compounds in human urine. Chem Res Toxicol 22:1018–25.

Doolittle DJ, Rahn CA, Riccio E, et al. (1990). Comparative studies of the mutagenicity of urine from smokers and non-smokers on a controlled non-mutagenic diet. Food Chem Toxicol 28:639–46.

Doroshynenko O, Fuhr U, Kunz D, et al. (2009). In vivo role of cytochrome p450 2e1 and glutathione-s-transferase activity for acrylamide toxicokinetics in humans. Cancer Epidemiol Biomarkers Biomarkers Prev 18:433–43.

Dougherty D, Garte S, Barchowsky A, et al. (2008). Nqo1, mpo, cyp2e1, 3-methyladenine, 3-ethyladenine, 8-hydroxy-2'-deoxyguanosine and 1-hydroxypyrene, s-phenylmercapturic acid, trans,trans-muconic acid, adducts from acrylonitrile and ethylene oxide in cigarette smokers: a mammalian cell-based mutagenesis approach. Toxicol Appl Pharmacol 253:145–52.

Eckert E, Schmid K, Schaller B, et al. (2011). Mercapturic acids as biomarkers of exposure to 1,3-butanediol in humans. Cancer Epidemiol Biomarkers Biomarkers Prev 11:1082–90.

Ekdaw P, Havel CM, Peng MW, et al. (2009). Elimination kinetics of the tobacco-specific biomarker and lung cancerogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol. Cancer Epidemiol Biomarkers Biomarkers Prev 18:3421–5.

Hecht SS, Murphy SE, Carmella SG, et al. (1999). Quantitation of urinary metabolites of a tobacco-specific lung carcinogen after smoking cessation. Cancer Res 59:590–6.

Frost-Pineda K, Zedler BK, Oliveri D, et al. (2008a). Short-term clinical exposure evaluation of a third-generation electrically heated cigarette smoking system (EHCSS) in adult smokers. Regul Toxicol Pharmacol 52:104–10.

Frost-Pineda K, Zedler BK, Oliveri D, et al. (2008b). 12-week clinical exposure evaluation of a third-generation electrically heated cigarette smoking system (EHCSS) in adult smokers. Regul Toxicol Pharmacol 52:111–17.

Goniewicz ML, Havel CM, Peng MW, et al. (2009). Elimination kinetics of the tobacco-specific biomarker and lung cancerogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol. Cancer Epidemiol Biomarkers Biomarkers Prev 18:3421–5.

Hecht SS, Yuan JM, Hatsukami D. (2010). Applying tobacco carcinogen biomarkers to assessment of reduced exposure tobacco products. Nicotine Tob Res 8:169–91.

Hatsukami DK, Benowitz NL, Rennard SI, et al. (2006). Biomarkers to assess the utility of potential reduced exposure tobacco products. Nicotine Tob Res 8:169–91.

Hatsukami DK, Hecht SS, Henrikus DJ, et al. (2003). Biomarkers of tobacco exposure or harm: application to clinical and epidemiological studies. Nicotine Tob Res 5:387–96.

Hoffmann K, Becker K, Friedrich C, et al. (2000). The German smoking system (EHCSS) in adult smokers. Regul Toxicol Pharmacol 44:262–7.

Grimmer G, Dettbarn G, Seidel A, Jacob J. (2000). Detection of carcinogenic aromatic amines in the urine of non-smokers. Sci Total Environ 247:81–90.

Hatsukami DK, Benowitz NL, Rennard SI, et al. (2006). Biomarkers to assess the utility of potential reduced exposure tobacco products. Nicotine Tob Res 8:169–91.

Hatsukami DK, Hecht SS, Henrikus DJ, et al. (2003). Biomarkers of tobacco exposure or harm: application to clinical and epidemiological studies. Nicotine Tob Res 5:387–96.

Hedberg JJ, Backlund M, Stromberg P, et al. (2006). Biomarkers to assess the utility of potential reduced exposure tobacco products. Nicotine Tob Res 8:169–91.

Hatsukami DK, Hecht SS, Carmella SG, Chen M, et al. (1999). Quantitation of urinary metabolites of a tobacco-specific lung carcinogen after smoking cessation. Cancer Res 59:590–6.

Hecht SS, Murphy SE, Carmella SG, et al. (2004). Effects of reduced cigarette smoking on the uptake of a tobacco-specific lung carcinogen. J Natl Cancer Inst 96:107–15.

Hecht SS, Yuan JM, Hatsukami D. (2010). Applying tobacco carcinogen and toxicant biomarkers in product regulation and cancer prevention. Chem Res Toxicol 23:1001–8.

Hedberg JJ, Backlund M, Stromberg P, et al. (2001). Functional polymorphism in the alcohol dehydrogenase 3 (adh3) promoter. Pharmacogenetics 11:815–24.

Himmelstein MW, Turner MJ, Asgharian B, Bond JA. (1996). Toxicokinetics of 1,3-butadiene: inhalation pharmacokinetics and toxicant biomarkers in product regulation and cancer prevention. Cancer Epidemiol Biomarkers Prev 11:1082–90.

Hatsukami DK, Hecht SS, Henrikus DJ, et al. (2003). Biomarkers of tobacco exposure or harm: application to clinical and epidemiological studies. Nicotine Tob Res 5:387–96.

Hatsukami DK, Hecht SS, Carmella SG, Chen M, et al. (1999). Quantitation of urinary metabolites of a tobacco-specific lung carcinogen after smoking cessation. Cancer Res 59:590–6.
Institute of Medicine. (2010). Evaluation of biomarkers and surrogate endpoints in chronic disease. Washington, DC: National Academies Press.

Institute of Medicine. (2012). Scientific standards for studies on modified risk tobacco products. Washington, DC: The National Academies Press.

International Agency for Research on Cancer. (2012). IARC monographs on the evaluation of carcinogenic risk to humans. In: Agents classified by the IARC monographs, Vols. 1–104. Available from: http://monographs.iarc.fr/ENG/Classification/index.php and http://monographs.iarc.fr/ENG/Classification/index.php: World Health Organization [last accessed 23 Apr 2013].

International Agency for Research on Cancer. (1992). IARC monographs on the evaluation of carcinogenic risk to humans: occupational exposures to mists and vapours from strong inorganic acids; and other industrial chemicals. Lyon: World Health Organization, IARC Publications.

International Organization for Standardization. (2005). ISO/IEC17025. General requirements for the competence of testing and calibration laboratories. Geneva: International Organization for Standardization. www.iso.org

International Organization for Standardization. (2007). ISO 15189. Medical laboratories – particular requirements for quality and competence. Geneva: International Organization for Standardization.

Jarvis MJ, Foulds J, Feyerabend C. (1992). Exposure to passive smoking among bar staff. Br J Addict 87:111–13.

Jongeneelen FJ, Bos RP, Anzion RB, et al. (1986). Biological monitoring of polycyclic aromatic hydrocarbons. Metabolites in urine. Scand J Work Environ Health 12:137–43.

Kavvadias D, Scherer G, Cheung F, et al. (2009a). Determination of tobacco-specific nitrates in urine of smokers and non-smokers. Biomarkers 14:547–53.

Kavvadias D, Scherer G, Urban M, et al. (2009b). Simultaneous determination of four tobacco-specific nitrates (TSNA) in human urine. J Chromatogr B Analyt Technol Biomed Life Sci 877:1185–92.

Kedderis GL, Batra R, Koop DR. (1993). Epoxidation of acrylonitrile by rat and human cytochromes P450. Chem Res Toxicol. 6:866–71.

Kerzic PJ, Liu WS, Pan MT, et al. (2010). Analysis of hydroquinone and catechol in peripheral blood of benzene-exposed workers. Chemico-Biological Interactions 184:182–8.

Kim JH, Breyer-Pfaff U, Wso V, et al. (2006). Purification and characterization of AKR1B10 from human liver: role in carbonyl reduction of xenobiotics. Drug Metab Dispos 34:464–70.

McElroy JA, Shafer MM, Hampton JM, Newcomb PA. (2007a). Predictors of urinary cadmium levels in adult females. Sci Total Environ 382:214–23.

McElroy JA, Shafer MM, Trentham-Dietz A, et al. (2007b). Urinary cadmium levels and tobacco smoke exposure in women age 20–69 years in the United States. J Toxicol Environ Health A 70:1779–82.

Mann SJ, Gerber LM. (2010). Estimation of 24-hour sodium excretion from spot urine samples. J Clin Hypertens (Greenwich) 12:174–80.

Mansi A, Bruni R, Capone P, et al. (2012). Low occupational exposure to benzene in a petrochemical plant: modulating effect of genetic polymorphisms and smoking habit on the urinary T.T-MAS/PMA ratio. Toxicol Lett 213:57–62.

Martin H-J, Mager JS, Scherer G, Schmid ER. (2001). High-performance liquid chromatographic-tandem mass spectrometric determination of 3-hydroxypropylmercapturic acid in human urine. J Chromatogr B Biomed Sci Appl 750:163–9.

Menzie CA, Potocoki BB, Santodonato J. (1992). Exposure to carcinogenic PAHs in the environment. Environ Sci Technol 26:1278–84.

Minten E, Cheung F, Errington G, et al. (2011a). Urinary excretion of the acrylonitrile metabolite 2-cyanoethymercapturic acid is correlated with a variety of biomarkers of tobacco smoke exposure and consumption. Biomarkers 16:89–96.

Minten E, Errington G, Scherer G, et al. (2011b). An inter-laboratory comparison of urinary 3-hydroxypropylmercapturic acid measurement demonstrates good reproducibility between laboratories. BMC Res Notes 4:391–6.

Muller M, Kramer A, Angerer J, Hallier E. (1998). Ethylene oxide-protein adduct formation in humans: influence of glutathione-s-transferase polymorphisms. Int Arch Occup Environ Health 71:499–502.

N. Deutsch A, Freudenheim J, et al. (2000). Why and how to measure urinary sex steroid metabolites in epidemiological studies in women. Nutr Metab Cardiovasc Dis 10:85–91.

Nath RG, Olando JE, Guttenplan JB, Chung FL. (1998). 1,N2-propanodeoxyguanosine adds: potential new biomarkers of smoking-induced DNA damage in human oral tissue. Cancer Res 58:581–4.

Nerurkar PV, Okinaka L, Aoki C, et al. (2000). CYP1A1, GSTM1, and GSTP1 genetic polymorphisms and urinary 1-hydroxyurea excretion in non-occupationally exposed individuals. Cancer Epidemiol Biomarkers Prev 9:1119–22.

Niewska JL, Claflfy DJ, Koop DR, et al. (1998). Oxidation of 1,3-butadiene to (R)- and (S)-butadiene monoxide by purified recombinant cytochrome P450 2E1 from rabbit, rat and human. Toxicol Lett 95:123–9.

O’Connor RJ. (2012). Postmarketing surveillance for ‘modified-risk’ tobacco products. Nicotine Tob Res 14:29–42.

Pal A, Hu X, Zimmik S, Singh SV. (2000). Catalytic efficiencies of allelic variants of human glutathione S-transferase Pi in the glutathione conjugation of alpha, beta-unsaturated aldehydes. Cancer Lett 154:39–43.
Paschal DC, Burt V, Caudill SP, et al. (2000). Exposure of the U.S. population aged 6 years and older to cadmium: 1998–1994. Arch Environ Contam Toxicol 38:377–83.

Patten CJ, Smith TJ, Friesen MJ, et al. (1997). Evidence for cytochrome p450 2A6 and 3A4 as major catalysts for n’-nitrosonornicotine alpha-hydroxylation by human liver microsomes. Carcinogenesis 18: 1623–30.

Rahn CA, Howard G, Riccio E, Doolittle DJ. (1991). Correlations between urinary nicotine or cotinine and urinary mutagenicity in smokers on controlled diets. Environ Mol Mutagen 17:244–52.

Riedel K, Scherer G, Engl J, et al. (2006). Determination of three carcinogenic aromatic amines in urine of smokers and nonsmokers. J Anal Toxicol 30:187–95.

Roethig HJ, Feng S, Liang Q, et al. (2008). A 12-month, randomized, controlled study to evaluate exposure and cardiovascular risk factors in adult smokers switching from conventional cigarettes to a second-generation electrically heated cigarette smoking system. J Clin Pharmacol 48:580–91.

Roethig HJ, Kinser RD, Lau RW, et al. (2005). Short-term exposure evaluation of adult smokers switching from conventional to first-generation electrically heated cigarettes during controlled smoking. J Clin Pharmacol 45:133–45.

Roethig HJ, Zedler BK, Kinser RD, et al. (2007). Short-term clinical exposure evaluation of a second-generation electrically heated cigarette smoking system. J Clin Pharmacol 47:518–30.

Rossi AM, Guarnieri C, Rovesti S, et al. (1999). Genetic polymorphisms influence variability in benzene metabolism in humans. Pharmacogenetics 9:445–51.

Ruppert T, Scherer G, Tricker AR, et al. (1995). Determination of urinary trans-4-muconic acid by gas chromatography-mass spectrometry. J Chromatogr B Biomed Sci Appl 666:71–81.

Russell MAH. (1976). Low-tar medium-nicotine cigarettes: a new approach to safer smoking. BMJ 1:1430–3.

Sarkar M, Kapur S, Frost-Pineda K, et al. (2008). Evaluation of biomarkers of exposure to selected cigarette smoke constituents in adult smokers switched to carbon-filtered cigarettes in short-term and long-term clinical studies. Nicotine Tob Res 10: 1761–72.

Sarkar M, Liu J, Koval T, et al. (2010). Evaluation of biomarkers of exposure in adult cigarette smokers using marlboro snus. Nicotine Tob Res 12:105–16.

Sarkar M, Stabbert R, Kinser RD, et al. (2006). CYP1A2 and NAT2 phenotyping and 3-aminobiphenyl and 4-aminobiphenyl hemoglobin adduct levels in smokers and non-smokers. Toxicol Appl Pharmacol 213:198–206.

Scherer G. (1999). Smoking behaviour and compensation: a review of the literature. Psychopharmacology (Berl) 145:1–20.

Scherer G, Richter E. (1997). Biomonitoring exposure to environmental tobacco smoke (ETS): a critical reappraisal. Hum Exp Toxicol 16: 107–20.

Scherer G. (2005). Biomonitoring of inhaled complex mixtures – ambient air, diesel exhaust and cigarette smoke. Exp Toxicol Pathol 57:75–110.

Scherer G, Engl J, Urban M, et al. (2007a). Relationship between machine-derived smoke yields and biomarkers in cigarette smokers in Germany. Regul Toxicol Pharmacol 47:171–83.

Scherer G, Frank S, Riedel K, et al. (2000). Biomonitoring of exposure to polycyclic aromatic hydrocarbons of nonoccupationally exposed persons. Cancer Epidemiol Biomarkers Prev 9:373–80.

Scherer G, Renner T, Meier M. (1998). Analysis and evaluation of trans, trans-muconic acid as a biomarker for benzene exposure. J Chromatogr B Biomed Sci Appl 717:179–99.

Scherer G, Urban M, Engl J, et al. (2006). Influence of smoking charcoal filter tipped cigarettes on various biomarkers of exposure. Inhal Toxicol 18:821–9.

Scherer G, Urban M, Hagedorn HW, et al. (2007b). Determination of two mercapturic acids related to crotonaldehyde in human urine: influence of smoking. Hum Exp Toxicol 26:37–47.

Scherer G, Urban M, Hagedorn HW, et al. (2010). Determination of methyl-, 2-hydroxyethyl- and 2-cyanoethymercapturic acids as biomarkers of exposure to alkylation agents in cigarette smoke. J Chromatogr B Analyst Technol Biomed Life Sci 878:2520–8.

Schettgen T, Musiol A, Alt A, et al. (2009). A method for the quantification of biomarkers of exposure to acrylonitrile and 1,3-butadiene in human urine by column-switching liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem 393:969–81.

Schlöde D, Hölsche D, Hatz D, et al. (2008). 4-Hydroxy-1-(3-pyridyl)-1-butanone-releasing DNA adducts in lung, lower esophagus and cardia of sudden death victims. Toxicology 245:154–61.

Seaton MJ, Follansbee MH, Bond JA. (1995). Oxidation of 1,2-epoxy-3-butene to 1,2,3,4-tetraoxybutane by CDNAG-expressed human cytochromes p450 2E1 and 3A4 and human, mouse and rat liver microsomes. Carcinogenesis 16:2287–93.

Seyler TH, Bernert JT. (2011). Analysis of 4-aminobiphenyl in smoker’s and nonsmoker’s urine by tandem mass spectrometry. Biomarkers 16: 212–21.

Shah KA, Halquist MS, Karnes HT. (2009). A modified method for the determination of tobacco specific nitrosamine 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanol in human urine by solid phase extraction using a molecularly imprinted polymer and liquid chromatography tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 877:1575–82.

Shepperd JD, Eldridge AC, Mariner DC, et al. (2009). A study to estimate and correlate cigarette smoke exposure in smokers in Germany as determined by filter analysis and biomarkers of exposure. Regul Toxicol Pharmacol 55:97–109.

Shimada T, Fuji-Kuriyama Y. (2004). Metabolic activation of polycyclic aromatic hydrocarbons to carcinogens by cytochromes p450 1A1 and 1B1. Cancer Sci 95:1–6.

Shimada T, Gillam EM, Oda Y, et al. (1999). Metabolism of benz[a]pyrene to trans-7,8-dihydroxy-7,8-dihydrobenz[a]pyrene by recombinant human cytochrome p450 1B1 and purified liver epoxide hydroxylase. Chem Res Toxicol 12:623–9.

Smith CI, Mckarns KV, Davis RA, et al. (1996). Human urine mutagenity study comparing cigarettes which burn or primarily heat tobacco. Mutat Res 361:1–9.

St Helen G, Goniewicz ML, Dempsey D, et al. (2012). Exposure and kinetics of polycyclic aromatic hydrocarbons (PAHs) in cigarette smokers. Chem Res Toxicol 25:952–64.

St Helen G, Dempsey D, Wilson M, et al. (2013). Racial differences in the relationship between tobacco dependence and nicotine and carcinogen exposure. Addiction 108:607–17.

Stagos D, Chen Y, Brocker C, et al. (2010). Aldehydes (3,4-dihydrogen-1B1; molecular cloning and characterization of a novel mitochondrial acetaldehyde-metabolizing enzyme. Drug Metab Dispos 38:1679–87.

Stepanov I, Hecht SS. (2005). Tobacco-specific nitrosamines and their pyridine-N-glucuronides in the urine of smokers and smokeless tobacco users. Cancer Epidemiol Biomarkers Prev 14:885–91.

Stepanov I, Muzic J, Le CT, et al. (2013). Analysis of 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB)-releasing DNA adducts in human exfoliated oral mucosa cells by liquid chromatography-electrospray ionization-tandem mass spectrometry, Chem Res Toxicol. [Epub ahead of print]. doi: 10.1021/tx300262k.

Sterk K, Scherer G, Krumova I, et al. (2012). Identification and quantification of 1-hydroxybutyrate-2-y1 mercapturic acid in human urine by UPLC-HILIC-MS/MS as a novel biomarker for 1,3-butanediol exposure. Chem Res Toxicol 25:1565–7.

Suhua W, Rongzhu L, Wenrong X, et al. (2010). Induction or inhibition of cytochrome p450 2E1 modifies the acute toxicity of acrylonitrile in rats: biochemical evidence. Arch Toxicol 84:461–9.

Suwan-Amplai P, Navas-Acien A, Strickland PT, Agnew J. (2009). Involuntary tobacco smoke exposure and urinary levels of polycyclic aromatic hydrocarbons. In the United States, 1999 to 2002. Cancer Epidemiol Biomarkers Prev 18:884–893.

Tan H, Wang Q, Wang A, et al. (2010). Influence of GSTs, CYP2E1 and MEH polymorphisms on 1,3-butanediene-induced micronuclear frequency in Chinese workers. Toxicol Appl Pharmacol 247:198–203.

Tao L, Day BW, Hu B, et al. (2013). Elevated 4-aminobiphenyl and 2,6-dimethylamine hemoglobin adducts and increased risk of bladder cancer among lifelong nonsmokers – the Shanghai bladder cancer study. Cancer Epidemiol Biomarkers Prev 22:937–45.

Tareke E, Rydberg P, Karlsson P, et al. (2002). Analysis of acrylamide, a carcinogen formed in heated foodstuffs. J Agric Food Chem 50: 4998–5006.

Ter-Minassian M, Asomaning K, Zhao Y, et al. (2012). Genetic variability in the metabolism of the tobacco-specific nitrosoamine 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanone (nnk) to 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanol (nnal). Int J Cancer 130:1338–46.
Thier R, Balkenhol H, Lewalter J, et al. (2001). Influence of polymorphisms of the human glutathione transferases and cytochrome p450 2E1 enzyme on the metabolism and toxicity of ethylene oxide and acrylonitrile. Mutat Res 482:41–6.

Thier R, Lewalter J, Selinski S, Bolt HM. (2002). Possible impact of human CYP2E1 polymorphisms on the metabolism of acrylonitrile. Toxicol Lett 128:249–55.

Thompson CM, Ceder R, Graafstrom RC. (2010). Formaldehyde dehydrogenase: beyond phase I metabolism. Toxicol Lett 193:1–3.

Tricker AR. (2006). Biomarkers derived from nicotine and its metabolites: a review. Beiträge zur Tabakforschung International [Contributions to Tobacco Research] 22:147–75.

Upadhyaya P, Zimmerman CL, Hecht SS. (2002). Metabolism and pharmacokinetics of N-nitrosonornicotine in the Patas monkey. Drug Metab Dispos 30:1115–22.

Urban M, Gilch G, Schepers G, et al. (2003). Determination of the major mercapturic acids of 1,3-butadiene in human and rat urine using liquid chromatography with tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 796:131–40.

Urban M, Kavvavidas D, Riedel K, et al. (2006). Urinary mercapturic acids and a hemoglobin adduct for the dosimetry of acrylamide exposure in smokers and nonsmokers. Inhal Toxicol 18:831–9.

US Department of Health and Human Services, Public Health Service. National Toxicology Program (2011). In: Report on carcinogens. 12th ed. Available from: http://ntp.niehs.nih.gov/ntp/roc/twelfth/roc12.pdf [last accessed 23 Apr 2013].

van Sittert NJ, Megens JJJ, Watson WP, Boogaard PJ. (2000). Biomarkers of exposure to 1,3-butadiene as a basis for cancer risk assessment. Toxicol Sci 56:189–202.

van Welie RT, van Dijck RG, Vermeulen NP, van Sittert NJ. (1992). Mercapturic acids, protein adducts, and DNA adducts as biomarkers of electrophilic chemicals. Crit Rev Toxicol 22:271–306.

Vineis P, Talaska G, Malaveille C, et al. (1996). DNA adducts in urothelial cells: relationship with biomarkers of exposure to amines and polycyclic aromatic hydrocarbons from tobacco smoke. Int J Cancer 65:314–16.

Vrискoоп N, de Boer AB, et al. (2008). Sparse production but preferential incorporation of recently produced naive T cells in the human peripheral pool. Proc Natl Acad Sci USA 105:6115–20.

Waidyanatha S, Rothman N, Li G, et al. (2004). Rapid determination of urinary benzene metabolites in occupationally exposed and unexposed subjects. Anal Biochem 327:184–99.

Wald NJ, Idle M, Boreham J, Bailey A. (1981). Carbon monoxide in breath in relation to smoking and carboxyhaemoglobin levels. Thorax 36:366–9.

Wang H, Chanas B, Ghannayem BL. (2002a). Cytochrome p450 2E1 (CYP2E1) is essential for acrylonitrile metabolism to cyanide: comparative studies using CYP2E1-null and wild-type mice. Drug Metab Dispos 30:911–17.

Wang M, McIntee EJ, Cheng G, et al. (2000). Identification of DNA adducts of acetaldehyde. Chem Res Toxicol 13:1149–57.

Wang M, Cheng G, Balbo S, et al. (2009). Clear differences in levels of a formaldehyde-DNA adduct in leukocytes of smokers and nonsmokers. Cancer Res 69:7170–4.

Wang R-S, Nakajima T, Kawamoto T, Honma T. (2002b). Effects of aldehyde dehydrogenase-2 genetic polymorphisms on metabolism of structurally different aldehydes in human liver. Drug Metab Dispos 30:69–75.

Weiner H, Wang X. (1994). Aldehyde dehydrogenase and acetaldehyde metabolism. Alcohol Alcohol 2:141–5.

Weiss T, Angerer J. (2002). Simultaneous determination of various aromatic amines and metabolites of aromatic nitro compounds in urine for low level exposure using gas chromatography–mass spectrometry. J Chromatogr B 778:179–92.

Wiener D, Doerge DR, Fang J-L, et al. (2004). Characterization of N-glucuronidation of the lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in human liver: importance of UDP-glucuronosyltransferase 1A4. Drug Metab Dispos 32:72–9.

Wong HL, Murphy SE, Hecht SS. (2005). Cytochrome p450 2A-catalyzed metabolic activation of structurally similar carcinogenic nitrosamines: N'-nitrosonornicotine enantiomers, N-nitrosopiperidine, and N-nitrosopyrrolidine. Chem Res Toxicol 18:61–9.

Xia Y, McGuffey JE, Bhattacharyya S, et al. (2005). Analysis of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol in urine by extraction on a molecularly imprinted polymer column and liquid chromatography/atmospheric pressure ionization tandem mass spectrometry. Anal Chem 77:7639–45.

Yuan JM, Koh WP, Murphy SE, et al. (2009). Urinary levels of tobacco-specific nitrosamine metabolites in relation to lung cancer development in two prospective cohorts of cigarette smokers. Cancer Res 69:2990–5.

Yuan JM, Gao YT, Murphy SE, et al. (2011). Urinary levels of cigarette smoke constituent metabolites are prospectively associated with lung cancer development in smokers. Cancer Res 71:6749–57.

Yuan JM, Gao YT, Wang R, et al. (2012). Urinary levels of volatile organic carcinogen and toxicant biomarkers in relation to lung cancer development in smokers. Carcinogenesis 33:804–9.

Zhang S, Balbo S, Wang M, Hecht SS. (2011). Analysis of acrolein-derived 1,N2-propanodeoxyguanosine adducts in human leukocyte DNA from smokers and nonsmokers. Chem Res Toxicol 24:119–24.

Zheng Z, Fang J-L, Lazarus P. (2002). Glucuronidation: an important mechanism for detoxification of benzo[a]pyrene metabolites in aerodigestive tract tissues. Drug Metab Dispos 30:397–403.