CHAPTER 9.

Environmental and occupational toxicants

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Summary

Biological monitoring is the analysis of human biological materials for a substance of interest and/or its metabolites (biomarkers) or a biochemical change that occurs as a result of an exposure to provide a quantitative measure of exposure or dose. These measures can be used in epidemiological studies either directly as estimates of exposure or indirectly in the calibration of other exposure assessment methods, such as questionnaires. This chapter will discuss important methodological considerations for the implementation of biomarkers of exogenous exposure in epidemiology by focusing on biomarker characteristics (e.g. variability, half-life) and their application in different study designs.

Exposure assessment in environmental and occupational epidemiology

In general, the goal in environmental and occupational epidemiology is to estimate the association between levels of exposure and their impact on health in human populations in a valid and precise manner. (The analytical and technical aspects of measuring specific biomarkers of exposure will not be discussed here; see chapters 4 and 11 on biological monitoring of chemicals and nutrients, respectively). In these studies, 'exposure' is described as a substance or factor affecting human health, either adversely or beneficially, which in practice is usually regarded as an estimate of the 'true' exposure a subject under study might receive (3). Exposure might originate from environmental or occupational sources, which, within the context of this chapter, are included within environmental epidemiology. Exposure to humans can be considered a dose when a distinction is made between the available dose, which is the dose that is available for uptake in the human body; the administered dose (or intake); the absorbed dose, which actually enters the body (uptake); and the biologically effective dose, which reaches the target cells in the body. The objective of exposure measurements in any environmental or occupational epidemiological study is to provide an unbiased measure of the actual exposure or dose that an individual receives. To optimize the quantification of

the association between exposure and health effects, these estimates of exposure should be accurate, precise, biologically relevant, apply to the etiological important exposure period, and show a range of exposure levels in the population under study (3).

Exposure is generally characterized by the physical and chemical properties of the agent, its intensity, and temporal variability (4,5). There can be considerable variability in all of these factors, temporal as well as between study subjects, which allow them to be used as metrics for exposure. Several distinct exposure metrics are used in epidemiological studies: cumulative exposure (total accumulated dose), average exposure (total accumulated dose divided by time), and peak exposure (highest exposure level experienced by a subject in a given time period). Each of these exposure metrics can be derived for the whole lifetime of each study subject or just for a particular etiologically relevant time period. Whereas cumulative exposure, average exposure, and peak exposure are basically interchangeable for short time periods, they might not be for long-term exposures due to complex exposure patterns over time (4).

Epidemiological studies generally deal with large population sizes. This makes estimating exposure for all individual study subjects difficult, as often not all subjects' exposure can be measured. Researchers therefore have to rely on some form of modelled or surrogate measure for true exposure. In general, there are two study types for exposure assignment: individual-based studies, in which exposure levels and health outcomes are measured for all persons; and group-based studies, in which samples of persons are measured in each of several groups and group-specific mean values of exposure levels are used to estimate the exposure-response association (6). In the group-based approach, it is important that measurements are made on a random selection of the population; often, however, they are based on convenience samples. In environmental epidemiological studies, these groups are generally defined on the basis of the presence or absence of an exposure source and the distance from it, while in occupational studies, exposure groups are often defined by factories, departments, or job titles (3). The underlying assumption when using this strategy for grouping is that subjects within each group are exposed to comparable exposure characteristics (e.g. intensity, cumulative exposure).

Environmental studies tend to have larger within-subject variability and smaller between-person and between-group variability than occupational studies. Therefore, group-based designs will generally be more appropriate to investigate exposure-response associations in the general population, but to a lesser extent for occupational studies (6). However, in both individual- and group-based designs, the relatively large within-subject variability in environmental and occupational exposures, emphasizes the importance of collecting multiple exposure measurements for each subject in the study (3,5). To assess the relative impact of temporal, between-subject, and between-group variability, studies using a repeated measures design should be conducted. This study design uses multiple exposure measurements for study subjects or groups in time to estimate these variance components by means of advanced statistical techniques, including (hierarchical) mixed effects models (7).

If the intensity or duration of exposure is poorly characterized, due to random measurement or misclassification error, the resulting estimated exposure-response associations will often underestimate the true risk for a given exposure level. This is known as attenuation bias. The expected attenuation in the risk estimate β in a common regression model ($Y_i \sim \alpha + \beta_1 x_1 + e_i$) to assess an exposure-response association, can, for group-based studies, be estimated by (8):

$$\hat{E}(\beta_1) = \frac{\beta_1}{1 + \frac{\sigma^2_{wg}}{kn\sigma^2_{bg} + n\sigma^2_{bh}}}$$

where $\hat{E}(\beta_1)$ is the expected risk estimate (β_1) adjusted for attenuation bias, σ^2_{wg} is the within-group variance (i.e. between-subject), σ^2_{bg} is the between-group variance, σ^2_{bh} is the within-subject variance, k is the number of randomly selected subjects, and n is the number of repeated measurements per subject.

While for individual-based studies this can be described by:

$$\hat{E}(\beta_1) = \frac{\beta_1}{\frac{\sigma^2_{ws}}{\sigma^2_{bs}}}$$

where σ^2_{ws} is the within-subject variance and σ^2_{bs} is the between-subject variance.

Non-random or differential measurement or misclassification, which can result from errors in the design of the study or the measurement technique, can both over- or underestimate an exposure-response association depending on the magnitude and direction of the bias.

Application of exposure markers in environmental epidemiology

Biomarkers of exposure generally aim at measuring the level of an external agent, or its metabolites, in either the free-state or bound to macromolecules. The range of biological samples that can be obtained and analysed includes: blood, urine, exhaled breath, hair, nails, milk, feces, sweat, saliva, semen, and cerebrospinal fluid. The choice of biological sample depends on the substance of interest, its characteristics (e.g. solubility, metabolism, transformation, and excretion), and how invasive the method to obtain it is. As such, several biomarkers can be available to represent the same exposure, including the parent compound itself, a metabolite, or a macromolecular DNA or protein adduct (9).

Whereas 'classical' methods of exposure assessment provide an estimate for exposure from one route of exposure only (e.g. inhalation through the respiratory system, ingestion through the gastrointestinal system, or absorption through the skin) (3), biological monitoring has the theoretical advantage of integrating exposures from all exposure routes. In addition, it also covers unexpected or accidental exposures and reflects interindividual differences in uptake, metabolism, genetic susceptibility, and excretion (10–12). However, some exposure biomarkers can also be formed endogenously and levels may then reflect both endogenous formation and exogenous exposures (13). Nonetheless, the use of exposure markers in epidemiology could potentially lead to a more accurate and/or more biologically relevant exposure estimate than 'classical' methods. For instance, biomarkers for tobacco specific N-nitrosamines, such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), might be more relevant for certain research questions than self-reported smoking habits, as NNK is a known carcinogen and urinary levels of its reduction product 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) reflect differences in smoking habits, the type of tobacco, and individual metabolism (see the example below on tobacco smoke).

Biomarker characteristics

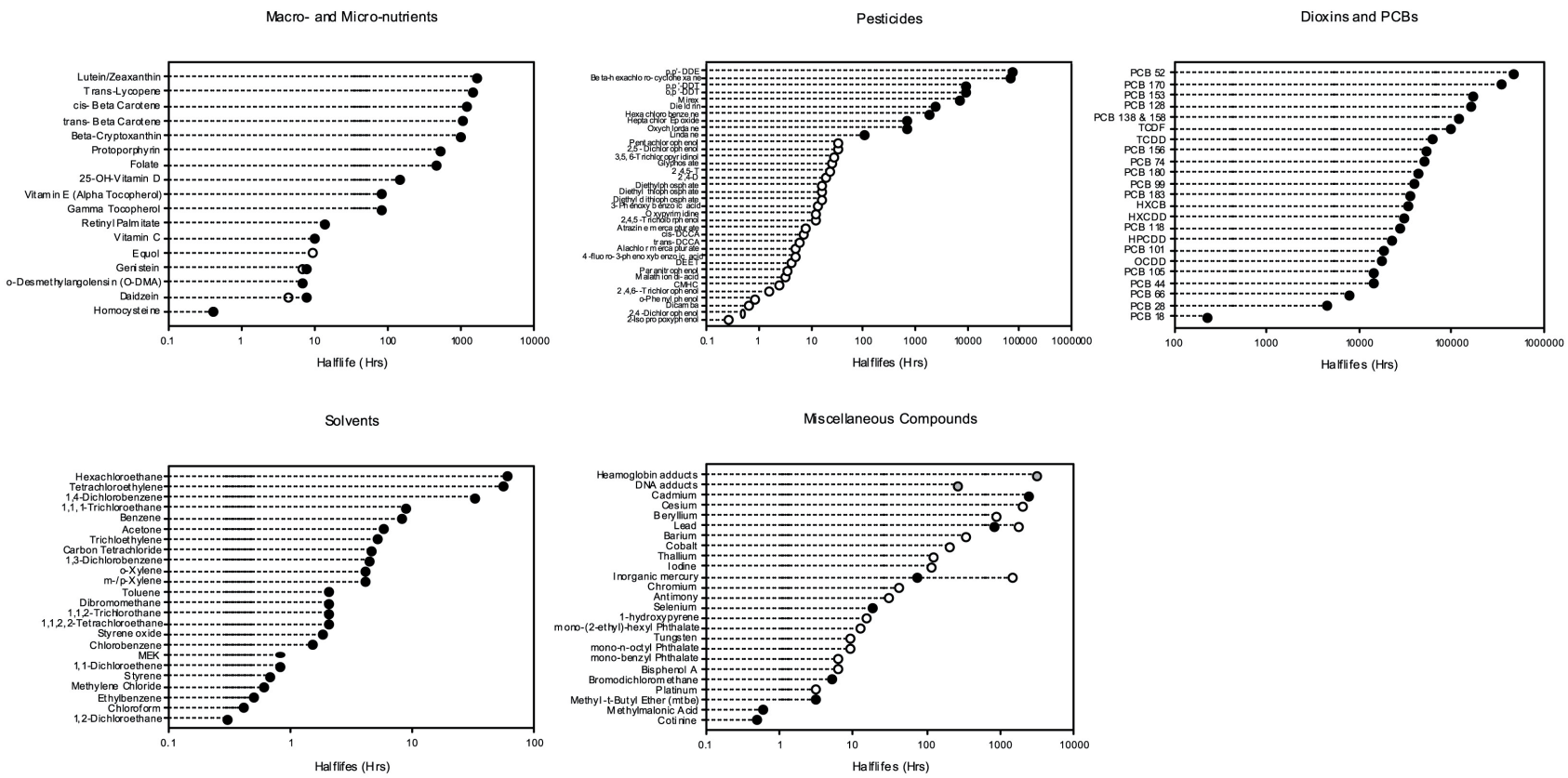
The choice of a biomarker will depend on several considerations, but the main issues are its kinetic parameters and the knowledge of the mechanistic basis of the adverse effects (9). Of these, the biological relevance (i.e. association with 'true' exposure at the site of action) is generally considered the most important selection criterion (14). However, although it is usually assumed that the biomarker is in some way associated with the exposure and the disease, limited information is often available on where the markers are located along the multistep pathway from exposure to human disease (14). Furthermore, to date, only a few biomarkers have been properly validated (15), which limits their application. The National Health and Nutrition Examination Survey (NHANES), conducted by the US Centers for Disease Control and Prevention (CDC), provides a good overview of exposure biomarkers and reference values in the normal population for many environmental exposures (http://www.cdc.gov/nchs/nhanes.htm).

In addition to the biological relevance of the biomarker, its biological half-life is a critical characteristic. 'Biological half-life' refers to the biological clearance of the biomarker from the target tissue. It can be derived from several sources, including empirical modeling of experimental data, compartment models incorporating experimentally determined rate constants, or from simulations based on physiologically and metabolically based parameters (16). Biological half-lives vary substantially between biomarkers. The half-lives of some compounds measured in the NHANES survey are presented in Figure 9.1.

The interpretation of a biomarker measurement depends on the sampling time, as each biomarker has a specific half-life. The analysis may reflect the amount of chemical absorbed shortly before the sample was taken, in the case of a biomarker with a short half-life (e.g. nicotine in blood); it may reflect exposure occurring during the preceding days for markers with intermediate half-lives (e.g. cotinine in blood); or it may reflect the dose integrated over a period of months for biomarkers with long half-lives (e.g. 3- and 4-aminobiphenyl-haemoglobin adducts). Additionally, some chemicals accumulate in specific tissues or organs; thus the biomarker value may reflect cumulative exposure over a period of years (16). However, most existing exposure markers have relatively short half-lives, with exceptions like some metals, and persistent organic pollutants like polychlorinated biphenyls and dioxins (Figure 9.1).

In general, biomarkers with relatively long half-lives are preferred, reflecting weeks, months, or even years of exposure when studying chronic health effects. This does not automatically mean that biomarkers with relatively short half-lives cannot be used in epidemiological studies; they are useful in studies of acute biological or health effects or where exposure is relatively constant over time.

Figure 9.1. Examples of biological indicators and their half-lives measured as part of NHANES. Solid dots indicate measurements in blood. Open circles indicate measurements in urine



Analytical variability

One source of variability in biomarker studies is laboratory, or analytical, variability. Before a new, promising biomarker can be used in population studies, transitional studies should first be conducted to characterize the biomarker in terms of accuracy, reliability in the laboratory, and optimal conditions for use (17). These studies should make certain that the analytical results are sufficiently accurate to ensure correct interpretation of the biomarker results in population studies and that the results will be reproducible.

At present, the contribution of analytical variability to total variability is, in general, much lower than biological variability because of improved techniques and quality assurance procedures in biomarker assessment (9,18). This variability can be further reduced by sharing methods and techniques and exchange of reference materials between laboratories (9).

Individual and temporal variability

Variability in biomarker responses, for continuous, non-fixed biomarkers, has two dimensions: an individual dimension and time. Individual variability in biomarker responses will depend on external exposure variability and on interindividual differences as to how an individual metabolizes the agent of interest. The temporal variability in biomarker response depends primarily on the half-life and on the temporal variability in exposure. Driven by financial or logistic motivations, the assumption is often made in epidemiologic studies that biomarker levels (and other traditional measures of exposure) are a fixed attribute of an individual, rather than being time-dependent, and as such are measured at only one single point in time. However, for this to be valid, biological steady-state conditions are required. In practice, these are not likely to occur since they require stable biokinetics, a constant rate of exposure, dynamic equilibrium among body tissues, and a sufficiently long period of time for the biomarker to stabilize in all relevant tissues (19).

In general, ignoring the temporal variability in biomarker response leads to additional classic measurement error, which results in the attenuation of the biomarker-disease association (17). Biomarkers with relatively short half-lives generally display more temporal variability than biomarkers with relatively long half-lives, which is related to the dampening of the temporal variance in exposure over time (17,20). It has been shown that whereas less than 50% of the temporal variance in exposure is transmitted for many biological markers with a half-life of more than 40 hours, the dampening effect is negligible for markers with a half-life of less than five hours (21). In Figure 9.2, examples of constant and variable occupational and environmental exposure circumstances are given for biomarkers with different half-lives (i.e. five, 20, and 100 hours). These examples suggest that because biomarkers with a relatively short half-life are more sensitive to fluctuations of exposure from hour-to-hour and day-to-day, that timing of sample collection becomes increasingly important. The exception to this is when exposures are constant over time. Therefore, the use of biomarkers with relatively long half-lives is generally more appropriate for epidemiological studies, especially when they can be measured only at a single point in time and not necessarily in the optimal etiological time window. This also depends on the health effect under investigation, since, in principle, biomarkers with a short half-life are needed when (semi-) acute biological and health effects are studied.

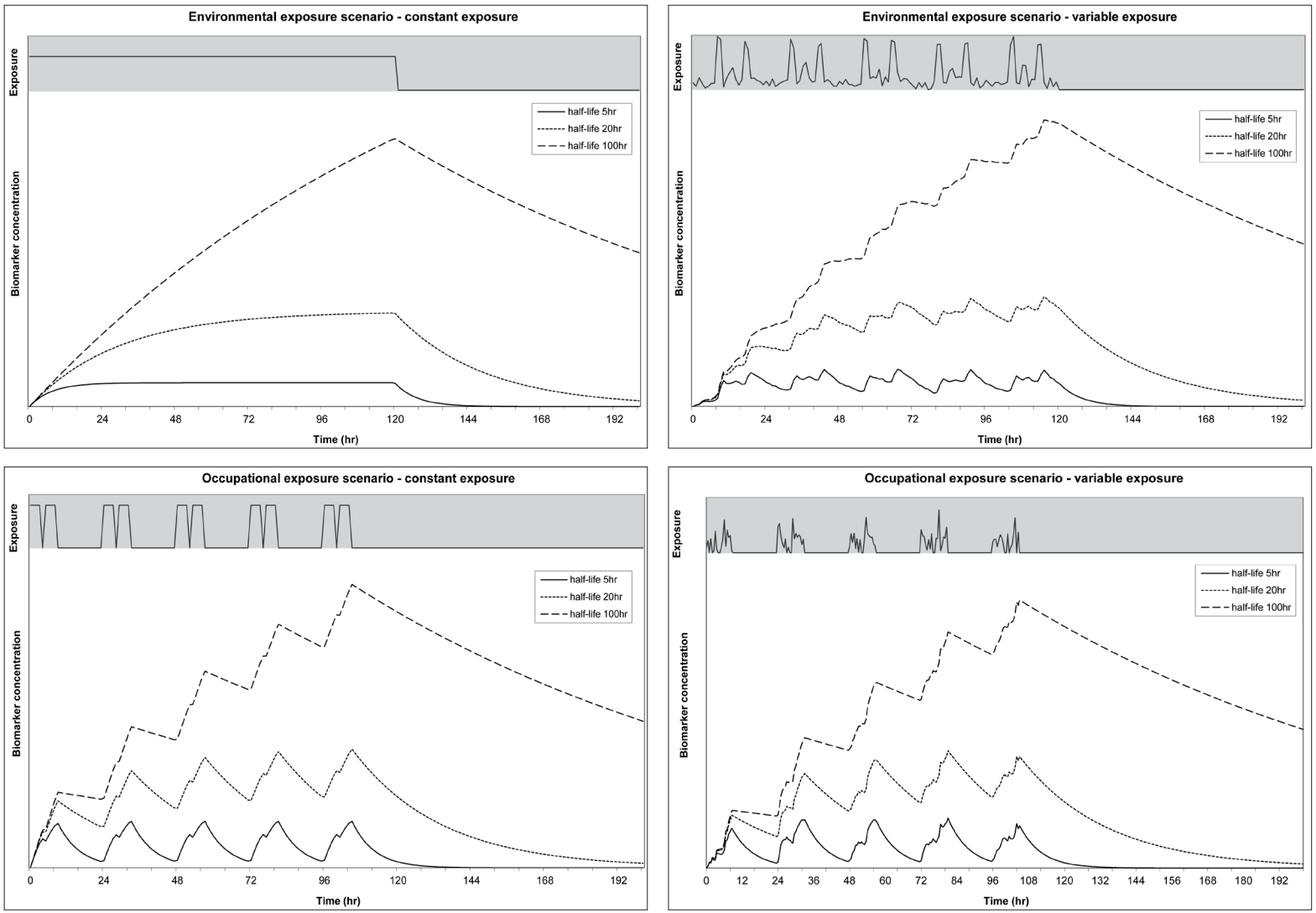
Biomarker validity

Ideally, before starting a study involving biomarker measurements, information on the variation in exposure patterns between individuals, as well as over time, should be known to determine whether a specific biomarker of exposure will be appropriate for the particular study. If this information is not available at the start of the study and it is not feasible to conduct a pilot study to estimate the variability of exposure, the intraindividual variation in the biomarker response can also be evaluated. At the same time, all sources of unwanted variation (e.g. laboratory variation) should be taken into account, by conducting a repeated measures design in the main study.

Several methods are available to assess variability when using biomarkers with a continuous outcome. The coefficient of variation (CV), which is defined as the ratio of the standard deviation (σ) to the mean (μ), is generally used as a measure of the extent of variation between different batches, and/or duplicate samples within batches, and can be used to identify 'bad' sample batches. It does not, however, provide insight into the impact of the observed variance on the biomarker-disease association and it cannot be used to correct measures of association to account for measurement error (17,21). The intraclass correlation coefficient (ICC), described by:

$$ICC = \frac{\sigma^2_{bs}}{\sigma^2_{bs} + \frac{\sigma^2_{ws}}{N}}$$

Figure 9.2. Effects of exposure variability on three biological indicators with half-lives of five, 20, and 100 hours. In all examples, the exposure scenario stopped after 120 hours. Ordinate is an arbitrary scale. Graph A1: Constant continuous environmental exposure. Graph A2: Constant occupational exposure. Graph B1: Variable environmental exposure with three high periods during morning (8–10), afternoon (12–14), and evening rush hour (17–19). Graph B2: Variable occupational exposure (eight hour day-1), one hour break, five days/week



(where σ^2_{bs} is the between-subject variance, σ^2_{ws} is the within-subject variance, and N is the number of repeated measures on an individual), is a more useful measure for evaluating the impact of the total measurement error (temporal plus analytical error).

In addition, the ICC can be used to adjust measures of association to account for measurement error. However, in the absence of a 'gold standard,' the results of such adjustments should not be interpreted as true associations, but instead as indicators for the degree of bias in the observed risk estimates (22; for more details, see Chapter 8).

Study designs

There is a spectrum of epidemiological study designs that make use of biological exposure markers. The choice of design depends on the specific research question and disease under study (e.g. rare versus common; acute versus chronic), and has implications for the use of biological exposure markers. The strengths and limitations of using exposure markers in relation to the major study designs are discussed below (for a more in depth discussion on study designs, see Chapters 14 and 15).

Cross-sectional studies

Cross-sectional studies are often initiated to assess whether a subset of a population has been exposed to a particular exposure, or to validate the exposure assessment from other sources, such as environmental monitoring or data obtained from questionnaires. For example, toenail nicotine levels, together with self-reported smoking habits and exposure to environmental tobacco smoke, were collected from 2485 women to assess the validity of toenail nicotine levels as a marker of tobacco smoke exposure, and to provide insight into its ability to capture non-reported exposure (23).

A distinct advantage of cross-sectional studies over alternative study designs is that detailed and accurate information can be collected on current exposure patterns and on determinants of exposure or potential confounders. To further improve the accuracy of the biomarker assessment, repeated measures should be considered, especially if the temporal variability is relatively large. However, one of the disadvantages of this study design is that current exposure patterns or determinants do not necessarily reflect historic levels, which might be more relevant to the exposure-disease pathway.

Case-control studies

The main goal of case-control studies is to compare exposure patterns in cases and in carefully matched controls during the etiologically relevant time period. One of the important advantages of case-control studies compared to prospective cohort studies, especially for biomarker studies, is their ability to enrol large numbers of cases relatively quickly and the potential to study uncommon diseases (17). A problem inherent to the way cases are recruited is that biological samples, exposure data, and other information is collected after diagnosis and even sometimes after commencing treatment of the disease. This makes these studies susceptible to differential misclassification and may lead to problems in the assessment of the temporal association between the disease and the biomarker under study (17).

For example, in a study on blood levels of organochlorines before and after chemotherapy among Non-Hodgkin lymphoma (NHL) patients, a marked decrease (25–30%) in serum levels of these compounds was found after treatment (24). This could lead to large exposure biases if cases are not enrolled before the start of chemotherapy, as blood levels of organochlorines among controls would not be influenced by therapy.

Prospective cohort studies

Prospective cohort studies are considered the only study design that allows researchers to look at biomarkers that are directly or indirectly affected by the exposure-disease mechanism, since biological specimens and exposure information are collected before disease diagnosis and, ideally, before the beginning of the disease process (25). It can be difficult to recruit enough subjects in the cohort and/or follow-up enough people for the duration of the study, therefore the study can be enriched with cases in subsequent nested case-control or case-cohort studies, which will improve the study efficiency (25). Unfortunately, larger prospective cohort studies have been able to collect only one biological sample at one point in time for individuals enrolled in the cohort. As discussed, this can cause problems for most types of biomarkers of exposure; especially short-term exposure markers which may vary substantially from day-to-day. It has further been discussed that although biomarkers can be collected in a variety of media, and that sometimes more media are available to assess exposure to the same chemical, most studies have only collected blood samples and only a few have collected urine.

An example of environmental exposure markers - tobacco smoke

Exposure to tobacco smoke represents one of the most prominent risk factors for cancer, cardiovascular diseases, and chronic respiratory diseases (26). Environmental tobacco smoke has also been implicated in the etiology of these diseases (27). The immense impact on public health of tobacco smoking and exposure to tobacco smoke has stimulated the development of tobacco-related biomarkers (Table 9.1).

Carbon monoxide and thiocyanate. Carbon monoxide (CO) and thiocyanate are considered the oldest biomarkers used as indicators of tobacco smoke exposure. CO is the product of incomplete combustion of organic materials. Inhaled CO is absorbed through the lungs and binds to haemoglobin (Hb) forming carboxyhaemoglobin (COHb). As the absorption is by the lung alveoli, levels of exhaled CO (COex) or COHb measured in blood are useful biomarkers of exposure, as CO does not undergo metabolic activation. CO has a short half-life

Table 9.1. Overview of tobacco exposure related biomarkers

Biomarker	Specimen	Reflected Exposure to Tobacco Smoke Product	Specificity	Half- Life	Detection Method
COex COHb	Breath Blood	Carbon monoxide	Low	2-3 hours	Infrared spectroscopy and GC
Thiocyanate	Saliva Blood Urine	Hydrogen cyanide	Low	1-2 weeks	Photometry, Ion exchange chromatography followed by UV detection, GC coupled with MS after derivatization
Nicotine	Saliva Blood Urine Toenail Hair	Nicotine	High	2 hours Several months	HPLC with UV detection
Cotinine	Saliva Blood Urine	Nicotine	High	3-4 days	HPLC with UV detection
NNAL and NNAL-Gluc	Urine	NNK uptake	High	Several months	GC
1-hydroxy-pyrene	Urine	Pyrene uptake	Low because of PAHs sources of exposure other than tobacco	Around 15 hours	HPLC
Benzo[a]pyrene diol epoxide DNA adducts	DNA	Benzo[a]pyrene biological effective dose	Low because of PAHs sources of exposure other than tobacco	In general, DNA adducts are considered to provide estimates of exposure for several half-lives of the adduct depending on adduct stability and repair capacity	
3- and 4- aminobiphenyl haemoglobin adducts	Blood	Aromatic amines uptake plus metabolic activation	Low because of aromatic amines sources of exposure other than tobacco	Around 120 days (haemoglobin life-span)	
Trans-trans-muconic acid	Urine	Benzene uptake	Low - influenced by food intake of sorbic acid from food	13 hours	LC/UV
S-Phenylmer-capturic acid	Urine	Benzene uptake	Low because of benzene sources of exposure other than tobacco	14 hours	LC/MS
Anabasine, anatabine and myosine	Saliva Urine	Tobacco products	High	Few hours	HPLC/MS GC/MS

(2–3 hours) making it a marker of recent exposure. However, COex and COHb levels can be affected by physical activity, sex, and the presence of lung or airway diseases.

Hydrogen cyanide. A chemical present in tobacco smoke, hydrogen cyanide (HCN) is formed in tobacco combustion mainly from proteins and nitrates. It is metabolized into thiocyanate (SCN) that can be measured in saliva, blood, and urine. Due to its relatively long half-life (1–2 weeks), SCN reflects at least several weeks of exposure (see section on *Temporal Variability)*. However, both these biomarkers are considered non-specific. Levels of CO and SCN, can be affected by numerous sources other than tobacco smoke, such as air pollution and diet for CO and SCN, respectively (28).

Nicotine. Nicotine is a chemical found in all tobacco products and is the major addictive component. Levels of nicotine can be measured in blood, saliva, and urine, providing a specific biomarker of exposure. However, since this chemical has a short half-life (a few hours), the results are very dependent on time of sampling. Furthermore, urine levels are highly influenced by urine volume and pH, reducing the use of this biomarker. The development of methods for the detection of nicotine in hair and nails has recently been suggested as a promising marker for long-term exposure (29).

Cotinine. Cotinine is the major proximate metabolite of nicotine, but with a longer half-life in the blood (3–4 days) (30). The presence of cotinine in a biologic fluid indicates exposure to nicotine. There is some individual variation in the quantitative relationship between cotinine levels in blood, saliva, and urine and the intake of nicotine, due to the fact that people metabolize nicotine and cotinine differently. Still this metabolite has been widely used as a very specific biomarker of tobacco exposure. Cotinine is also of particular interest as a biomarker for the evaluation of exposure to environmental tobacco smoke (ETS). Cotinine concentrations in plasma, urine, and saliva of non-smokers have been used in assessing population exposure to ETS for developing risk estimates for ETS-related lung cancer (31).

N-nitrosamines. Tobacco smoke contains volatile N-nitrosamines, such as N-nitrosodimethylamine and N-nitrosopyrrolidine, as well as tobacco specific N-nitrosamines, such as N'-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (32). In particular, nitrosamines in tobacco are chemically related to nicotine, and other tobacco alkaloids, and therefore specific to tobacco products. For this reason, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) (the major metabolite of NNK) together with its glucuronide derivative (NNAL-Glucs), which can be detected in urine, provide a particularly valuable biomarker due to their specificity. Moreover, both NNAL and NNAL-Glucs have a relatively long half-life compared to other measurable urinary metabolites. This biomarker has been used to quantify levels of NNK uptake in smokers and smokeless-tobacco users (33) to examine ethnic differences in NNK metabolism (34), and to study the effects of diet and potential cancer chemopreventive agents on NNK metabolism (35,36). There is a consistent correlation between levels of cotinine, NNAL, and NNAL-Glucs in urine (37). The measurement of NNAL and NNAL-Glucs in urine has been particularly useful in studies of ETS. Uptake of NNK by non-smokers exposed to ETS has been shown in several settings, including the detection of these biomarkers in amniotic fluid, indicating that NNK or NNAL are present in fetuses of mothers who smoke (38).

NNK and NNN can also lead to the formation of specific haemoglobin and DNA adducts, which can potentially measure uptake plus metabolic activation and the biological effective dose of these carcinogens, respectively. Methods for the detection of these biomarkers have been developed; however, their levels are frequently low and, in some cases, undetectable in many active smokers.

As for the N-nitrosamines, aromatic amines can undergo metabolic activation leading to the formation of DNA or protein adducts. 4-Aminobiphenyl (4-ABP) undergoes P450 catalysed N-oxidation to a hydroxylamine. O-Acetylation, catalysed by N-acetyltransferases (NATs), produces an O-acetoxy compound that reacts with DNA. Other esterification reactions of the hydroxylamine lead to related intermediates that can also react with DNA. However, since the levels of DNA adducts in humans are generally low (once every 106-108 normal bases), large amounts of DNA and sensitive methods are needed for the analysis. Moreover, little is known about their persistence in human tissue. Animal studies have shown a great variability in this respect depending on the different chemical structures formed and on the repairing systems, which might remove some adducts but not others (32). In general, studies on DNA adducts have reported higher levels in smokers compared to non-smokers and higher levels in tissue samples (from oral, lung, and bladder cancers) from cases than controls.

Aromatic amines, polycyclic aromatic hydrocarbons, and benzene. Aromatic amines (arylamines), such as o-toluidine,

2-aminonaphthalene, and 4-aminobiphenyl, occur in the environment and are constituents of tobacco smoke. A method for measuring these in cigarette smokers was developed, using the acid hydrolysis of the arylamine conjugates in urine. Urinary arylamine excretion in smokers was associated with the extent of smoking as assessed by daily cigarette consumption, urinary excretion of nicotine, cotinine in saliva, and carbon monoxide in exhaled breath. This analytical method is suitable for measuring short-term exposure to arylamines in urine of non-occupationally exposed smokers and non-smokers (39).

Haemoglobin adducts of aromatic amines are an informative type of carcinogen biomarker. Large amounts of haemoglobin are readily available in the blood and protein has a long half-life (120 days), which allows the adducts to accumulate and thus reflect a relatively long-term exposure. Levels of these adducts are consistently higher in smokers than in non-smokers (40). In a recent study, the relative risk of bladder cancer in women who smoked was found to be significantly higher than in men who smoked a comparable number of cigarettes. Consistent with this gender difference, levels of 3- and 4-aminobiphenyl-haemoglobin adducts, in relation to the number of cigarettes smoked per day, was statistically higher in women than in men (41).

Polycyclic aromatic hydrocarbons (PAHs), which cause lung cancer and other smoking-related cancers, are present in tobacco smoke. One of the main metabolites, 1-hydroxypyrene in urine, is the biomarker used to study the uptake of PAHs in smokers. Levels of 1-hydroxypyrene are 2–3 times higher in smokers than in non-smokers and decrease with smoking cessation (42). Benzo(a) pyrene, another main constituent of the PAHs mixture, is metabolized to Benzo(a)pyrene diol epoxides, which reacts with Hb and DNA forming adducts. However, since these adducts are difficult to detect even with highly sensitive methods, levels have been undetectable in many active smokers.

Benzene is another chemical present in tobacco smoke. Its metabolites trans-trans-muconic acid and S-phenylmercapturic acid can be detected in urine to measure benzene uptake; both biomarkers have been found elevated in smokers compared to non-smokers (42).

Aromatic amines, PAHs, and benzene are not exclusively contained in tobacco smoke: they also exist in environmental pollution, diesel exhaust, and as an outcome of many industrial productions. Thus their biomarkers are lacking in specificity towards exposure to tobacco smoke.

Minor tobacco alkaloids. Tobacco contains small amounts of minor alkaloids, such as anabasine, anatabine, and myosmine. As for nicotine, the main tobacco alkaloid, these minor alkaloids are absorbed systemically and can be measured in the urine of smokers and users of smokeless tobacco. The measurement of minor alkaloids is important as a way to quantitate tobacco use when a person is also receiving nicotine from other sources, such as nicotine medications or a non-tobacco nicotine delivery system, for instance, in smoking cessation studies (43).

The above example on tobacco smoke clearly demonstrates that a single environmental exposure can be represented by several biological exposure markers. Choosing the appropriate biomarker depends on several factors including chemical and biological characteristics of the biomarker itself, sources of variation (analytical, population, temporal), and the study design in which the biomarker is to be used.

The future of biomarkers of exposure – the exposome

The term ‘exposome,’ which encompasses all life-course environmental exposures, was coined to draw attention to the need for methodological developments in exposure assessment (44). It is known that environmental exposures play an important role in many common chronic diseases, yet the advances with regard to molecular epidemiology have been focused mostly on the genome. To some extent this can be explained by the complexity of measuring the exposome, as compared to the genome, due to its highly variable nature. However, more recently omics technologies, including transcriptomics, proteomics, metabolomics, and adductomics, are being applied to detect signatures of environmental exposures and to identify novel exposure markers. For instance, human metabolic phenotype diversity was found to be associated with dietary habits across different ethnic populations (45). This promising result suggests that in the future metabolomics might provide new leads to better individual exposure assessment. The development of adductomics, which measures the full complement of protein adducts, might, however, be more relevant for improving exposure assessment in epidemiological studies, as signals can be highly specific for certain (electrophilic) environmental exposures. Furthermore, given the relatively long half-lives of, for

instance, haemoglobin adducts (~3000 hours if adducts are chemically stable), these markers would reflect months of exposure.

Conclusions

Given the potential issues associated with the use of biomarkers in epidemiological studies, it is certainly not a given that biomarkers of exposure always provide the most accurate and precise estimates of true exposure. Although the use of biological markers of exposure can improve the assessment of exposure in epidemiological studies, either by complementing other methods of assessment or by serving as the best method when other methods are absent or less valid, these are not always the most appropriate or valid assessment methods. As such, in addition to assessment of the use of biomarkers, it should be part of the design of any study to also consider 'classic' alternatives for exposure assessment, like personal external exposure measurements and advanced exposure modeling.

In summary, before deciding on a specific biological marker to assess exogenous exposures to investigate a specific hypothesis, there are several factors that should be considered. One should verify that the marker is indeed detectable in human populations and that its kinetics are known. A repeated measures design should be created to evaluate interindividual variation relative to intraindividual variation. In addition, duplicate samples should be included in the design to assess laboratory variation. Furthermore, the timing of sample collection in combination with the biological half-life of the biomarker should be optimized. The effect modifiers should be known and all major sources of variance quantified.

References

1. Lauwerys RR, Hoet P. Industrial chemical exposure: guideline for biological monitoring. 3rd ed. Boca Raton (FL): CRC Press, 2001.

2. Gropper SS, Smith JL, Groff JL. Advanced nutrition and human metabolism. 5th ed. Florence (KY): Wadsworth Publishing Company, 2008.

3. Nieuwenhuijsen MJ. Exposure assessment. In: Wild C, Vineis P, Garte S, editors. Molecular epidemiology of chronic diseases. Hoboken (NJ): John Wiley & Sons, Ltd.; 2008. p. 83–96.

4. Armstrong BK, White E, Saracci R. Principles of exposure measurements in epidemiology. Oxford: Oxford University Press; 1992.

5. White E, Armstrong BK, Saracci R. Principles of exposure measurement in epidemiology: collecting, evaluating and improving measures of disease risk factors. 2nd ed. Oxford: Oxford University Press; 2008.

6. Rappaport SM, Kupper LL. Some implications of random exposure measurement errors in occupational and environmental epidemiology. In: Wild C, Vineis P, Garte S, editors. Molecular epidemiology of chronic diseases. Hoboken (NJ): John Wiley & Sons, Ltd.; 2008. p. 223–231.

7. Snijders T, Bosker R. Multilevel analysis. London: SAGE Publications Ltd; 1999.

8. Tielemans E, Kupper LL, Kromhout H *et al.* (1998). Individual-based and group-based occupational exposure assessment: some equations to evaluate different strategies. *Ann Occup Hyg,* 42:115–119. PMID:9559571

9. Manini P, De Palma G, Mutti A (2007). Exposure assessment at the workplace: implications of biological variability. *Toxicol Lett,* 168:210–218.doi:10.1016/j.toxlet.2006.09.014 PMID:17157456

10. Bartsch H (2000). Studies on biomarkers in cancer etiology and prevention: a summary and challenge of 20 years of interdisciplinary research. *Mutat Res,* 462:255–279.doi:10.1016/S1383-5742(00)00008-9 PMID:10767637

11. Lin YS, Kupper LL, Rappaport SM (2005). Air samples versus biomarkers for epidemiology. *Occup Environ Med,* 62:750–760.doi:10.1136/oem.2004.013102 PMID:16234400

12. Rappaport SM, Symanski E, Yager JW, Kupper LL (1995). The relationship between environmental monitoring and biological markers in exposure assessment. *Environ Health Perspect,* 103 Suppl 3;49–53.doi:10.2307/3432560 PMID:7635112

13. De Bont R, van Larebeke N (2004). Endogenous DNA damage in humans: a review of quantitative data. *Mutagenesis*, 19:169–185.doi:10.1093/mutage/geh025 PMID:15123782

14. Kang D, Lee KH, Lee KM *et al.* (2005). Design issues in cross-sectional biomarkers studies: urinary biomarkers of PAH exposure and oxidative stress. *Mutat Res,* 592:138–146. PMID:16102785

15. Aitio A (2006). Guidance values for the biomonitoring of occupational exposure. State of the art. *Med Lav,* 97:324–331. PMID:17017366

16. Watson WP, Mutti A (2004). Role of biomarkers in monitoring exposures to chemicals: present position, future prospects. *Biomarkers*, 9:211–242.doi:10.1080/13547500400015642 PMID:15764289

17. Garcia-Closas M, Vermeulen R, Sherman ME *et al.* Application of biomarkers in cancer epidemiology. In: Schottenfeld D, Fraumeni JF Jr, editors. Cancer epidemiology and prevention. 3rd ed. New York (NY): Oxford University Press; 2006. p. 70–88.

18. Jakubowski M, Trzcinka-Ochocka M (2005). Biological monitoring of exposure: trends and key developments. *J Occup Health,* 47:22–48.doi:10.1539/joh.47.22 PMID:15703450

19. Bartell SM, Griffith WC, Faustman EM (2004). Temporal error in biomarker-based mean exposure estimates for individuals. *J Expo Anal Environ Epidemiol,* 14:173–179. doi:10.1038/sj.jea.7500311 PMID:15014548

20. Symanski E, Greeson NM (2002). Assessment of variability in biomonitoring data using a large database of biological measures of exposure. *AIHA J (Fairfax, Va),* 63:390–401.doi:10.1080/15428110208984727 PMID: 12486772

21. Nieuwenhuijsen MJ, Droz P. Biological monitoring. In: Nieuwenhuijsen MJ, editor. Exposure assessment in occupational and environmental epidemiology. Oxford: Oxford University Press; 2008. p. 167–80.

22. White E. Effects of biomarker measurement error on epidemiological studies. In: Toniolo P, Boffetta P, Shuker DEG *et al.*, editors. Application of biomarkers in cancer epidemiology. Lyon: IARC Scientific Publication; 1997. p. 73–93.

23. Al-Delaimy WK, Willett WC (2008). Measurement of tobacco smoke exposure: comparison of toenail nicotine biomarkers and self-reports. *Cancer Epidemiol Biomarkers Prev,* 17:1255–1261.doi:10.1158/1055-9965.EPI-07-2695 PMID:18483348

24. Baris D, Kwak LW, Rothman N *et al.* (2000). Blood levels of organochlorines before and after chemotherapy among non-Hodgkin's lymphoma patients. *Cancer Epidemiol Biomarkers Prev,* 9:193–197. PMID:10698481

25. Wacholder S (1991). Practical considerations in choosing between the case-cohort and nested case-control designs. *Epidemiology*, 2:155–158.doi:10.1097/00001648-199103000-00013 PMID:1932316

26. U.S. Department of Health and Human Services. The health consequences of smoking: a report of the Surgeon General. [Atlanta, GA]. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; 2004.

27. U.S. Department of Health and Human Services. The health consequences of involuntary exposure to tobacco smoke: a report of the Surgeon General. [Atlanta, GA]. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; 2006.

28. Scherer G (2006). Carboxyhemoglobin and thiocyanate as biomarkers of exposure to carbon monoxide and hydrogen cyanide in tobacco smoke. *Exp Toxicol Pathol,* 58:101–124.doi:10.1016/j.etp.2006.07.001 PMID:16973339

29. Stepanov I, Hecht SS, Lindgren B *et al.* (2007). Relationship of human toenail nicotine, cotinine, and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol to levels of these biomarkers in plasma and urine. *Cancer Epidemiol Biomarkers Prev,* 16:1382–1386. doi:10.1158/1055-9965.EPI-07-0145 PMID:17627002

30. Benowitz NL (1999). Biomarkers of environmental tobacco smoke exposure. *Environ Health Perspect,* 107 Suppl 2;349–355.doi:10.2307/3434427 PMID:10350520

31. Boffetta P, Clark S, Shen M *et al.* (2006). Serum cotinine level as predictor of lung cancer risk. *Cancer Epidemiol Biomarkers Prev,* 15:1184–1188.doi:10.1158/1055-9965.EPI-06-0032 PMID:16775179

32. Hecht SS (2004). Tobacco carcinogens, their biomarkers and tobacco-induced cancer. Nat Rev Cancer 2003;3(10):733–44. Erratum: Nat Rev Cancer, 4:84.

33. Carmella SG, Akerkar S, Hecht SS (1993). Metabolites of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in smokers' urine. *Cancer Res*, 53:721–724. PMID:8428352

34. Richie JP Jr, Carmella SG, Muscat JE *et al.* (1997). Differences in the urinary metabolites of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in black and white smokers. *Cancer Epidemiol Biomarkers Prev,* 6:783–790. PMID:9332760

35. Hecht SS, Chung FL, Richie JP Jr *et al.* (1995). Effects of watercress consumption on metabolism of a tobacco-specific lung carcinogen in smokers. *Cancer Epidemiol Biomarkers Prev,* 4:877–884. PMID:8634661

36. Taioli E, Garbers S (1997). Bradlow, Carmella SG, Akerkar S, Hecht SS. Effects of indole-3-carbinol on the metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in smokers. *Cancer Epidemiol Biomarkers Prev,* 6:517–522. PMID:9232339

37. Hecht SS (2002). Human urinary carcinogen metabolites: biomarkers for investigating tobacco and cancer. *Carcinogenesis*, 23:907–922.doi:10.1093/carcin/23.6.907 PMID:12082012

38. Milunsky A, Carmella SG, Ye M, Hecht SS (2000). A tobacco-specific carcinogen in the fetus. *Prenat Diagn*, 20:307–310.doi:10.1002/(SICI)1097-0223(200004)20:4<307::AID-PD797>3.0.CO;2-M PMID:10740203

39. Riedel K, Scherer G, Engl J *et al.* (2006). Determination of three carcinogenic aromatic amines in urine of smokers and nonsmokers. *J Anal Toxicol,* 30:187–195. PMID:16803653

40. Phillips DH (2002). Smoking-related DNA and protein adducts in human tissues. *Carcinogenesis,* 23:1979–2004.doi:10.1093/carcin/23.12.1979 PMID:12507921

41. Castelao JE, Yuan JM, Skipper PL *et al.* (2001). Gender- and smoking-related bladder cancer risk. *J Natl Cancer Inst,* 93:538–545. doi:10.1093/jnci/93.7.538 PMID:11287448

42. Hatsukami DK, Hecht SS, Hennrikus DJ *et al.* (2003). Biomarkers of tobacco exposure or harm: application to clinical and epidemiological studies. 25–26 October 2001, Minneapolis, Minnesota. *Nicotine Tob Res,* 5:387–396.doi:10.1080/1462220031000094222 PMID:12791522

43. Benowitz NL, Hukkanen J, Jacob P 3rd (2009). Nicotine chemistry, metabolism, kinetics and biomarkers. *Handb Exp Pharmacol,* 192:29–60.doi:10.1007/978-3-540-69248-5_2 PMID:19184645

44. Wild CP (2005). Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology. *Cancer Epidemiol Biomarkers Prev,* 14:1847–1850.doi:10.1158/1055-9965.EPI-05-0456 PMID:16103423

45. Holmes E, Loo RL, Stamler J *et al.* (2008). Human metabolic phenotype diversity and its association with diet and blood pressure. *Nature*, 453:396–400.doi:10.1038/nature06882 PMID:18425110