Air Toxics: Biomarkers in Environmental Applications—Overview and Summary of Recommendations

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On April 27–28, 1995, the National Urban Air Toxics Research Center hosted a Symposium on Air Toxics: Biomarkers in Environmental Applications. The purpose of the symposium was to define the current state of the art in the application of biomarkers for environmental exposures to benzene, toluene, styrene, 1,3-butadiene, polycyclic aromatic hydrocarbons, manganese, and chromium. Sensitive, specific, and cost-effective biomarkers of exposure, effect, and susceptibility may greatly improve our knowledge of the human health impacts of air toxics exposures. Presentations were made on the first day that provided state-of-the-art background for the need, use, and ethical considerations in biomarker research and applications. In workshops held during the symposium, a number of recommendations were made regarding the use and need for additional research with biomarkers. In general, the sensitivity and specificity of biomarkers for environmental exposures, need to be improved. Reliable effect and susceptibility biomarkers are not available for these compounds. More research needs to be done to establish and evaluate biomarkers, linked to health effects through mechanistic studies, at environmentally relevant exposure levels. — Environ Health Perspect 104(Suppl 5):851–855 (1996)

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Introduction

The Symposium on Air Toxics: Biomarkers in Environmental Applications, held on April 27–28, 1995, in Houston, Texas, was the second in a series of annual symposia hosted by the National Urban Air Toxics Research Center (NUATRC) on important issues associated with exposures to air toxics (defined by 1990 Clean Air Act Amendments). The topic for this year’s symposium was selected by the Center’s Scientific Advisory Panel for the purpose of defining the current state of the art of biomarkers for selected air toxics and their use in population studies in urban settings as they would contribute to NUATRC’s strategic research plan.

NUATRC’s strategic plan is focused on assessing the role of air toxics in causing or exacerbating respiratory and immunological diseases as well as reproductive and neurological health effects. Assessing the public health impacts of human exposures to ambient air toxics is complex due to several factors. These factors include the low atmospheric concentrations of specific air toxics, the myriad of confounding factors in identifying causes of morbidity and mortality in urban populations, and the severe analytical limitations to establishing causal linkages between exposures and health effects. Increased research and experience with biomarkers offer the potential to identify any such causal linkages in a definitive manner.

The first day of the symposium provided overview information on biomarkers, including utilization in risk assessment, specimen banking, and ethical issues. In addition, state-of-the-art presentations on different types of biomarkers were given. The focus shifted on the second day to four classes or groups of air toxics that were selected for discussion within the symposium: aromatic compounds (benzene, toluene, and styrene), a conjugated diene (1,3-butadiene), polycyclic aromatic hydrocarbons, and metals (chromium and manganese). These air toxics were selected on the basis of two criteria: a) they ranked high on NUATRC’s priority list of air toxics and appear on other priority lists (e.g., U.S. Environmental Protection Agency); and b) there is published biomarker research experience for these air toxics. In addition, although some of the compounds are considered carcinogens/leukemogens, they also have immunotoxic potential, addressing the focus of the center’s plan. On the second day, formal presentations were given in each of the four groups by pairs of investigators and were followed by formation of working groups to address specific questions in each of the four groups of air toxics. These questions were designed to identify the status of the different types of biomarkers in each group and recommend future research. Summaries of the working groups follow the “Biomarker Overview.”

Biomarker Overview

The use of biomarkers in the assessment of environmental health has been the topic of a number of symposia and monographs (1–7). In broad terms, a biomarker can be any measurement in or from biological material that defines an exposure or response to that exposure. Typically, biomarkers have been classified into three subtypes: exposure (measurement of the parent compound, metabolite, or unique response attributable to a compound or group of compounds), effect (a quantifiable response of an organism that can be directly linked to exposure), and susceptibility (any factor that can vary from individual to individual that could alter the formation or metabolism of the compound or any intermediate and biological response). Figure 1 illustrates the relationship between personal exposure and potential human health outcome and intervening biomarkers.

The majority of biomarker studies have focused on exposure. This is important because the absence of exposure would preclude the need for other biomarkers. However, this can be misleading. For environmental exposures, it could simply reflect the lack of assay sensitivity. Alternatively, detection of a biomarker of exposure does not connote effect or health outcome, unless some prior association has been established. The major gaps of knowledge...
Occur on the right side of Figure 1. These gaps will be best filled by concerted efforts to link different biomarkers to exposure and health effects, rather than by studies that focus on only one biomarker. Large population studies will only be feasible with the development of cost-effective analyses. Therefore, it may be useful to consider specimen banking when appropriate. Finally, there is the broad issue of biomarker validation in humans and the low levels of exposures that occur in the general population. Some biomarkers are only formed at high exposure concentrations because of different metabolic pathways operating at different exposure concentrations, and controlled human studies with most of the air toxics are not possible. Consequently, creative approaches need to be developed to address these and the research needs identified by the working groups during the symposium, as described below.

Summary of Working Groups

Given today’s technology, what biomarkers are available for use in human population studies?

Biomarkers of Exposure

Aromatics. Most of the biomarkers available for human studies have been tested in occupational or other settings where relatively high levels of exposures can occur. However, in an environmental setting, ambient levels of these same air toxics are lower, and some of these same biomarkers may not be sufficiently sensitive.

Analysis of expired breath is noninvasive and sensitive. Limitations of this procedure include availability of equipment for field collection and the relatively high cost for each collection and analysis. In a similar manner, measurements of toluene, benzene, and styrene in blood have been done with high precision and reliability. The major limitations are that only two laboratories in the world have the required technology to do these assays and thus the cost is high. Both of these biomarkers indicate recent exposures (2 hr).

A number of potential metabolite assays and adducts were discussed. Some metabolites were eliminated because of limited sensitivity and specificity. Mercapturic acid in blood and urine was recommended as a biomarker of exposure for benzene. In addition, muconaldehyde and S-phenylcysteine albumin adducts may be promising biomarkers for benzene. The most promising biomarkers for styrene exposure include mandelic and phenylglycolxilic acids in urine and styrene–hemoglobin or albumin adducts in blood.

1,3-Butadiene. Currently useful biomarkers of exposure are those based on biological matrices. The presence of metabolites in urine, specifically of 1,2-dihydroxy-4-(N-acetylcytamineyl-S)butane has been sufficiently documented to indicate that this is likely to be a useful biomarker of recent (up to 6–8 hr) butadiene exposure. The formation of reaction products of butadiene metabolites with macromolecules is also likely to be useful. Adducts formed by the reaction of the monoperoxide metabolite of butadiene with the N-terminal valine of hemoglobin have been characterized. The measurement of DNA adducts of butadiene metabolites may also be feasible.

As noted above, urinary metabolites should be useful biomarkers for determining very recent (i.e., same-day) exposure to butadiene and would be useful for documenting accidental overexposures. Single samples could be useful for cross-sectional screening of populations for exposure, while 24-hr collections would be more appropriate for assessment of individual exposures. Lymphocyte DNA and protein (e.g., albumin) adduct levels would provide a more useful assessment of average exposure over a period of days or weeks.

Assessments of butadiene or metabolites in exhaled breath or levels of butadiene or its metabolites in blood will probably not prove useful as a tool for biomonitoring. This is because of the poor solubility of butadiene in blood, which results in rapid exhalation of butadiene following termination of exposure. Similarly, blood levels of butadiene are expected to be low because of its rapid exhalation and rapid metabolism.

Polycyclic Aromatic Hydrocarbons. Internal dose markers are available to monitor polycyclic aromatic hydrocarbon (PAH) metabolites in urine. These markers are specific for the compounds they measure, but in many cases the compound measured is not carcinogenic and therefore not related to risk. Measurement of 1-hydroxyphenyrene has been validated in many exposure situations and is currently the internal dose marker of choice. Because 1-hydroxyphenyrene is a noncarcinogenic metabolite, it makes risk assessment, with cancer as an end point, problematic. However, numerous studies have shown a good correlation between total PAHs, carcinogenic PAHs, and 1-hydroxyphenyrene in urine. Methods are being developed to measure metabolites of benzo[a]pyrene and other carcinogenic PAHs although they may not provide any advantages over 1-hydroxyphenyrene measurements. The current focus is on metabolites that reflect the activating metabolism.

The measurement of PAH–hemoglobin adducts is currently in its infancy. Useful techniques are available for small alkylating carcinogens and aromatic amines, but measurement of PAH–hemoglobin adducts has been more difficult.

Metals. The best biomarkers of exposure for metals are determinations of the metals themselves. However, two factors need to be considered. First, many metals are essential in the diet, in fact both chromium and manganese are essential and dietary deficiencies should be treated. Therefore, presence of the metal should not be considered an adverse human health risk, but rather amounts above a certain value (as yet undetermined) would be suspect. Second, the valency of the metal is important. Chromium can exist in multiple valence states, with +3 and +6 being the most important. Furthermore, the +6 state is the form associated with an increased human health risk, because it can cross membranes to enter cells and exert toxicity, whereas the +3 form is membrane impermeable.
Therefore, metal measurements need to take the valency into consideration. Atomic absorption spectroscopy using a graphite furnace is the most sensitive and practical approach for assaying metals. Chromium in red blood cells is the best marker for Cr⁶⁺ exposure, but pure oxygen ashing with a graphite furnace is essential to eliminate interference of iron and other con foundations in red blood cells. Manganese measurements can be done in either red blood cells or urine.

Biomarkers of Effect

Aromatics. There are no established biomarkers of effect for any of the aromatics at environmental levels of exposure. DNA adducts have not been investigated and do not necessarily translate into effect markers. Cytogenetic markers that could be evaluated include sister chromatid exchange, formation of micronuclei, and HPRT mutant lymphocyte assay. The glycoporphin A assay may be useful for benzene but may not be sensitive enough for lower exposures. Toluene does not form adducts and has little known health risks at environmental levels.

1,3-Butadiene. The only effect biomarker that currently appears to be useful for 1,3-butadiene is the HPRT mutant lymphocyte assay which appears to be responsive to chronic exposures of about 1 ppm. However, because various air toxics can cause HPRT mutants, the specificity of association of 1,3-butadiene with HPRT mutations would be a concern in an environmental setting where mixed exposures occur.

Polycyclic Aromatic Hydrocarbons. Carcinogen–DNA adduct measurements have been made in workers and in the general population for a variety of PAH exposures. The data indicating that these markers will be useful in environmentally exposed populations are limited; most studies have focused on groups exposed occupationally. As these markers are used in population studies, associations and correlations must be made with markers of internal dose and health effects. Measurement of markers of mutagenesis such as HPRT and glycoporphin A mutations are in the developmental stage regarding PAH, although initial studies have been done.

Metals. Studies on biomarkers of chromium exposure have focused on DNA–protein cross-links. Considerably more studies need to be done on validation and health effects risk assessment associated with these cross-links. Little is known for manganese. The effect biomarker and clinical outcome are the same, i.e., using magnetic resonance imaging (MRI) to establish neurological symptoms. However, the mechanism to account for this effect needs investigation.

Biomarkers of Susceptibility

Aromatics. No susceptibility biomarkers have been established for aromatics. A number of these biomarkers are possible and provide avenues for future investigation. Aromatics are metabolized by cytochrome P4502E1; polymorphisms may be associated with altered susceptibility. Similarly, the reactive metabolite is inactivated by epoxide hydrolase, which can be expressed in different amounts. Polymorphic differences in DNA repair enzymes might also be important predictors of susceptibility.

1,3-Butadiene. No susceptibility biomarkers for butadiene have been identified; however, there are several potential ones that could be investigated. Butadiene is metabolized primarily by cytochrome P4502E1 and its epoxide metabolites are metabolized by epoxide hydrolase and glutathione S-transferase. It is therefore likely that expression polymorphisms for these enzymes might be associated with altered susceptibility to butadiene toxicity. The most likely to be important are polymorphisms in epoxide hydrolase. In addition, because butadiene metabolites are genotoxic, polymorphic differences in DNA repair capacity, particularly the ability to recognize and remove alkyl products or repair DNA cross-links, might be important predictors of susceptibility.

Polycyclic Aromatic Hydrocarbons. Although progress has been made on the development of susceptibility markers potentially useful in PAH exposures, given the current level of information, none is useful. When possible, DNA should be stored for the analysis of markers such as P4501A1 and glutathione S-transferase. In the application of these studies, the working group emphasized that exposure and outcome (effect) measures are critically needed to assess the impact of these markers on various levels of exposures. The current data indicate that susceptibility markers are extremely specific regarding compound and dose, so information on susceptibility obtained without these data may be difficult to interpret, if not misleading.

Metals. There are no specific biomarkers of susceptibility for metals, but anion transport systems and DNA repair enzymes might be under genetic control. Animal data support the hypothesis that there is genetic variation in susceptibility to manganese, but specific mechanisms are not certain. Nongenetic factors that may also contribute to susceptibility are anemia, high transferrin levels, and acute inflammatory disease.

Recommended Biomarkers

Aromatics. Blood benzene is the most sensitive biomarker for recent, short-term exposure (2 hr). Breath levels also correspond to recent, short-term exposure (2 hr) and are better for subject compliance. Mercapturic acid in urine reflects whole body metabolism and integrates exposures over a longer period (a few hours). Blood protein adducts integrate exposures over a much longer period (weeks to months).

1,3-Butadiene. Air concentrations of butadiene should be used for routine assessment of exposure. Charcoal tubes and air pumps, or passive absorbent technologies are fairly well established for this purpose. For unanticipated accidental exposures and to determine the relationships between external exposure and internal dose, use exposure markers. To address the relationship between internal dose and effect of dose, to investigate mechanistic issues, and for risk assessment, use effect biomarkers.

Polycyclic Aromatic Hydrocarbons. Assays for 1-hydroxypyrene should be incorporated into studies to acquire information about the degree of exposure to PAHs.

Metals. Protein–DNA cross-links should be used to assess chromium exposure. For manganese, the presence of the metal in urine and red blood cells is diagnostic, but magnetic resonance imaging (neurological exam) should be included for suspect populations of manganese exposure.

Current Knowledge

Aromatics. The biomarkers of effect in exposure to aromatics are unclear. To use protein adducts as biomarkers, there is a need for more sensitive and specific assays and knowledge of the time of integration of exposure. There is also a need for more simultaneous measurements of different biomarkers. On the whole, more effects and susceptibility biomarkers are needed (Figure 1, right side). There is insufficient information on the mechanism by which benzene causes leukemia. Development of immunostaining for adduct biomarkers would be useful.

1,3-Butadiene. The relationship between exposure level and biological effect
is poorly defined. More detailed studies in which exposures are determined with more precision will be required to address this problem. The predictive value of biomarkers for toxicity related to butadiene is not known. This cannot be determined unless the human health risks of butadiene exposure are identified. Studies to accomplish this may prove to be difficult.

Appropriate target tissue-specific biomarkers for butadiene are not available. As the major health effects appear to be hematopoietic cancers, target tissues would include the bone marrow and other sites of hematopoiesis. In addition, animal studies implicate the lungs as possible targets. The most practical way to address this problem would be to conduct detailed pharmacokinetic studies and develop pharmacokinetic models to assess dosimetry in humans exposed to 1,3-butadiene.

The influence of other factors on the response of biomarkers is not well understood. Co-exposures to other workplace chemicals and substances related to lifestyle (e.g., smoking, diet) or medications, might influence the response to specific biomarkers. This problem could be addressed with large-scale studies of exposed populations designed to permit multivariate analysis of the effects of factors on the response of biomarkers.

**Polycyclic Aromatic Hydrocarbons.** Sensitive assays for metabolites of carcinogenic PAH need to developed. Associations need to be developed between markers of internal dose and later effects must be determined.

**Metals.** Normal values and reference ranges are not established for metals. In addition there is insufficient information on the mechanism of manganese-induced neurological disease.

**Drawbacks of Current Methodology in Conducting Biomarker Studies in Human Populations**

**Aromatics.** Only two laboratories worldwide do blood assays for aromatics. In addition, there is insufficient information from longitudinal studies on individual variation to predict and interpret single measurements. For benzene there is much greater inter- than intra-individual variation.

**1,3-Butadiene.** In addition to the problems described above (relationship of exposure to effect, predictive value, confounding factors), several additional pitfalls exist for butadiene. The weaknesses and hazards of any observational study design for biomarker studies apply to butadiene. Issues of sample size, ascertainment of exposure, correct assignment to exposure groups, proper selection of end points, and control of confounding factors are all important. Currently available techniques for biomonitoring are relatively complex and expensive to perform, making them too costly for routine use. Simpler, more reliable, and less expensive tests need to be developed. A final and significant pitfall to be resolved is the manner in which the results of biomarker studies are interpreted and communicated. As more experience is gained and the issues are resolved, the interpretation of results will become clearer. During the developmental phase of biomarker use it is important to pay particular attention to the manner in which results are interpreted and communicated to exposed workers and the general public. Although, it is important to be factual and not to ignore the implications of positive findings for future disease risk, it is also necessary to be honest in describing the limitations in current knowledge and the uncertainties they create for interpretation of results. The successful application of biomarkers to the evaluation of health risks in butadiene-exposed workers will require cooperation of industry.

**Polycyclic Aromatic Hydrocarbons.** The technology and methodologies are available using HPLC, GC, postlabeling, and radioimmunoassays for monitoring PAH exposure. PAHs are a mixture and monitoring requires an integrated approach. None of the methods currently available can fingerprint exposure to a specific compound.

Major problems in monitoring PAH exposure are the cost of each assay, the quantity of specimen needed, and availability and accessibility of defined study populations with different levels of exposure to be able to evaluate the dose-response relationships of exposure and markers.

**Metals.** While Cr\(^{3+}\) is an essential element, no amount of Cr\(^{6+}\) is desirable, and all Cr\(^{6+}\) is converted to Cr\(^{3+}\) in cells. Better markers of effect for manganese exposure need to be developed to replace MRI. Manganese is an essential factor in metabolism and deficiency needs treatment.

**Priority of Research Needs**

All groups had key research needs that were similar:

1. develop an understanding of the mechanistic linkage between the response of a biomarker, chemical exposure, and the potential for future disease (i.e., develop an association between biomarkers and risk assessment and etiological studies rather than descriptive studies using a single exposure marker);
2. characterize the behavior of specific biomarkers, the relationships between responses of different biomarkers and the response of the biomarkers to other factors (other chemicals, smoking, diet, etc.); and
3. develop more sensitive and specific assays and knowledge of the time of integration of exposure. Develop immunosassays for adducts.

**Aromatics.** Biomarkers of effect, and more biomarkers of susceptibility should be developed for aromatics.

**1,3-Butadiene.** The exposure of workers in representative workplaces in the United States must be characterized to determine whether current levels of butadiene exposure produce significant responses in exposure or effect biomarkers.

Also, we must determine whether subsets of individuals with unusual susceptibilities to the toxic effects of butadiene exist.

**Polycyclic Aromatic Hydrocarbons.** Integrated studies involving a variety of health professionals should be conducted. Field and laboratory studies should be undertaken not only to understand the exposure in the population, but also the interaction between markers of exposure, effect, and susceptibility (e.g., PAH-metabolites, PAH-hemoglobin and DNA adducts, mutation, P4501A1, and glutathione S-transferase). The study of markers of susceptibility should not be a top priority until the interrelationships of exposure and the markers are established.

**Metals.** Chromium-induced protein-DNA cross-links and association with disease (not gene specific) must be validated.

We must search for other biomarkers of effect for manganese, define the mechanism of action in toxicity, and define non-genetic factors of susceptibility.

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