# Measurement of Metabolites as Indicators of Exposure to Chemicals

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## 1 INTRODUCTION

Many chemicals to which animals or plants are exposed undergo metabolism prior to storage in, or excretion by, the organism. In some cases, measurement of the levels of metabolites in tissues or excretion products can be used as an indicator of exposure to such chemicals. Measurement of metabolites as a means of monitoring exposure may be especially valuable when practical difficulties exist in determining exposure to a specific chemical. Such a situation can arise either when a chemical is metabolised very rapidly or when it is difficult to assess exposure, especially long-term exposure, by environmental monitoring, e.g., when dosing is by inhalation during which air concentrations of a chemical fluctuate widely. Assessing long-term exposure to illustrative air pollutants, such as passive smoking or industrial exposure to volatile organic compounds, exemplifies an area where metabolite measurements may provide an accurate indication of exposure. However, for metabolite measurement to be useful as an indicator of exposure to chemicals, several criteria should be fulfilled:

(1) A metabolite should be specific for the chemical concerned, unless isotope studies are contemplated. By contrast, metabolite determination is likely to be of little value if the chemical is metabolised completely to "non-specific" metabolites, such as water, carbon dioxide, acetate, chloride, or urea.

(2) The metabolism of a chemical in the organism of interest should be known. Studies on the metabolism of drugs and other xenobiotics have shown that for many compounds there are large inter- and intra-species differences in metabolism. There may also be differences in metabolism in the same individual, depending on age, and hormonal and nutritional status. All these factors complicate the use of metabolite determinations in assessing exposure to chemicals.

(3) As with all monitoring, reliable methods of sampling and analysis must be used and analytical quality assurance must be carried out to obtain valid results.

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A wide range of tissues, body fluids, and excretion products are potentially useful for the determination of metabolites *in vivo*: for example, (a) blood or fractions thereof, saliva, and milk; (b) adipose tissue, cerumen, teeth, hair, and nails; and (c) urine, feces, and expired air. A much wider range of tissues can be used in studies of lifetime exposure to chemicals which accumulate in the body, if tissues can be collected from cadavers at autopsy.

Metals present in tissues or body fluids (e.g., lead in blood, bone or teeth, cadmium in kidney cortex, and mercury in hair) may be in a form other than that in which they were introduced into the body, i.e., they may be present as metabolites. However, relatively little is known about the actual chemical form in which these metals are present in the tissues concerned and, for exposure monitoring purposes, they are usually determined as the metal, and not as metabolites, and thus they will not be discussed here.

Examples are given below of how determination of metabolites can be used to assess exposure to a wide range of chemicals, including nicotine (as an indicator of environmental exposure to tobacco smoke), industrial solvents (e.g., benzene, *m*-xylene, and styrene) and persistent pesticides (e.g., *p,p'*-DDT).

## 2 COTININE IN URINE AS A MEASURE OF ENVIRONMENTAL EXPOSURE TO NICOTINE FROM TOBACCO SMOKE

Cotinine is the major metabolite of nicotine found in the urine. The measurement of cotinine has advantages over determination of the parent compound, nicotine, because the circulating half-life of cotinine in adults is 19 to 40 hours (Langone *et al.*, 1973; Benowitz *et al.*, 1983), compared to less than 30 minutes to 110 minutes for nicotine (Isaac and Rand, 1972; Rosenberg *et al.*, 1980). Cotinine can be measured at concentrations of less than 500 pg per millilitre by a sensitive radioimmunoassay (Haley *et al.*, 1983; Hill and Marquardt, 1980). Thus, the presence of cotinine can be used as an indicator of chronic exposure to tobacco smoke.

Greenberg *et al.* (1984) measured the concentrations of nicotine and cotinine in the saliva and urine of 32 infants with household exposure to tobacco smoke and 19 unexposed infants. The concentrations were significantly higher in the exposed group, the best indicator of exposure being urinary cotinine/creatinine ratio (median in the exposed group 351 ng per mg, median in the unexposed group 4 ng per mg ($P<0.0001$)). There was a direct relation between cotinine excretion by infants and the self-reported smoking behaviour of the mothers during the previous 24 hours ($r=0.67$, $P<0.0001$). Thus, the results indicated that infants exposed to tobacco smoke absorbed nicotine from it and that urinary excretion of cotinine in infants is a relatable measure of such exposure.

Jarvis *et al.* (1984) studied the relationship between several biochemical markers of smoke absorption and self-reported exposure to passive smoking. One hundred nonsmoking patients attending hospital outpatient clinics reported their degree of passive exposure to tobacco smoke over the preceding three days and provided samples of blood, expired air, saliva, and urine. Although the absolute levels were low, the concentrations of cotinine in all body compartments surveyed were systematically related to self-reported exposure. Salivary nicotine concentration also showed a linear increase with degree of reported exposure, although this measure was sensitive only to exposure on the day of testing. Carbon monoxide, thiocyanate, and plasma nicotine concentrations were unrelated to exposure.

Data from these studies indicated that cotinine excretion in the urine is a positive indicator of nicotine exposure and potentially of passive smoke exposure. Urine samples are particularly suited to epidemiological investigations, since they can be obtained from all age groups, even infants.

## 3 PHENOL IN URINE AS A MEASURE OF BENZENE EXPOSURE

Benzene is metabolised principally into phenols, which are excreted in the urine as glucuronide and sulphate conjugates. Measurement of phenol in urine has been routinely used earlier for assessing benzene exposure in industry. This is based on the assumption that between 20 and 40 percent of benzene in blood is metabolised to phenol. However, phenol in urine is not a specific indicator of exposure to benzene and the excretion of phenol in urine can be increased by diet, hepatic disturbances, intestinal putrefactive processes, and drugs such as aspirin (Fishbeck *et al.*, 1975). The finding of phenol levels above 20 mg/l suggests exposure to benzene, if other circumstances causing increased phenol excretion have been excluded (Lauwerys, 1979). Phenol levels in urine can be determined by gas chromatography (van Haaften and Sie, 1965), and can thus monitor heavy exposures to benzene. However, sensitive methods are now available for the determination of benzene in blood. Such methods are more reliable than phenol tests for assessing both low level exposure and uptake of benzene, since low levels of benzene can be detected in blood even when phenol levels in urine are regarded as normal (10 mg/l or less) (Braier *et al.*, 1981).

The development of a sensitive technique for the isolation and quantification of *trans*-muconic acid, an open-chain urinary metabolite of benzene, has provided a further method to monitor exposure to benzene (Gad-El Karim *et al.*, 1985).

## 4 *m*-METHYLHIPPURIC ACID IN URINE AS AN INDICATOR OF ABSORBED *m*-XYLENE

*m*-Xylene is the principal component of commercial xylene mixtures widely used as solvents in paints and thinners. Xylene isomers are highly soluble in blood and tissues and undergo metabolism to methylbenzoic acids, which are then conjugated to glycine to yield methylhippuric acids. Thus *m*-xylene yields *m*-methylhippuric acid which is excreted in the urine.

Biological monitoring of occupational exposure to xylene can be carried out by measuring xylene levels in blood and analysis of the metabolites in urine. Measurement of m-methylhippuric acid has been proposed (Ogata *et al.*, 1970; Mikulski *et al.*, 1972) and the method has been tested under experimental conditions by Sedivec and Flek (1976).

Methylhippuric acids in urine can be determined by gas chromatography after alkaline hydrolysis (Engstrom *et al.*, 1976).

Engstrom *et al.* (1978) have evaluated different methods to assess occupational exposure to xylene. They concluded that the determination of methylhippuric acids in urine samples collected at the end of the working day could be used for evaluating the average xylene exposure during the preceding day. The concentration of methylhippuric acid in a morning sample at the end of the work week provides a crude estimate of the xylene exposure during the preceding week.

Senczuk and Orlowski (1978) investigated the absorption of *m*-xylene and the excretion of *m*-methylhippuric acid in the urine in 10 volunteers. They found a relationship between the absorbed dose of m-xylene and the rate of excretion of *m*-methylhippuric acid between the sixth and eighth hour of exposure (correlation coefficient = 0.99). They proposed that exposure to xylene be monitored by determining

(1) the concentration of *m*-methylhippuric acid in urine during the 6th to 8th hours of daily exposure or during the first four hours after the end of exposure (8th to 12th hours); and

(2) the amount of the metabolite in urine collected during the last two hours of exposure.

From these data, it is possible to estimate not only the probable concentration of *m*-xylene during exposure but also the amount absorbed.

## 5 MANDELIC ACID IN URINE AS AN INDEX OF STYRENE EXPOSURE

In humans, styrene is metabolised mainly in the liver, first to styrene oxide, which is biologically active and can bind covalently to cellular macromolecules. Styrene oxide is hydrated to phenylethylglycol and can be conjugated with glutathione. Phenylethylglycol can be conjugated with

glucuronic acid and excreted in the urine or oxidised to mandelic acid (MA), which is partly oxidised further to phenylglyoxylic acid (PGA). A small fraction of styrene can be metabolised to vinyl phenol and phenylethanol. Only small amounts of absorbed styrene are excreted unchanged in exhaled air; most of it is excreted as metabolites into the urine. The two main metabolites, MA and PGA, correspond to about 92 percent of the retained dose of styrene, of which approximately 34 percent is excreted during an 8-hour period of exposure. The ratio of the two metabolites (MA/PGA) is about 3:1 at the end of the exposure, dropping to 4:5 approximately 14 hours after cessation of exposure (Guillemin and Bauer, 1979).

Determination of MA and/or PGA in urine can be used to assess exposure to styrene. Several analytical methods are available, but according to Engstrom (1984) calorimetric determination of MA and PGA in urine is subject to interference and gives a relatively high background level, making the method less suitable for the exact estimation of MA and PGA excretion at low doses of styrene. The relative error decreases towards higher concentrations, as the unspecific interference is relatively constant and not affected by the styrene load. With more specific methods, such as gas and liquid chromatography, the normal values are so low that there is no problem with the variability in normal levels. Exceptions are consumption of some drugs, which may be metabolised to MA, and exposure to ethylbenzene which is excreted in the urine as MA. A greater analytical problem is the instability of samples. In urine, PGA and MA are both prone to change during storage at room temperature as well as at +6° C. A low pH seems to improve the stability.

Droz and Guillemin (1983) described a mathematical model to develop the best strategy for biological monitoring of exposed workers. In their comparison of biological indicators of styrene exposure (MA, PGA, MA + PGA), while taking into account pharmacokinetics, analytical variance, field and laboratory practicability and inter- and intra-individual variance, MA measurement in urine had the highest rating, with respect to measurements both at the end of shifts and the day after exposure.

Engstrom (1984) recommended the determination of MA in urine (whole shift samples) using specific analytical methods for the biological monitoring of exposure to styrene. Such results should be corrected for the creatinine content of the urine, and storage of samples before analysis should be avoided. In assessing results, the type of exposure, the physical work load, and individual factors (such as alcohol intake) should be taken into account.

## 6 DDE IN ADIPOSE TISSUE, MILK FAT, OR CERUMEN AS AN INDEX OF EXPOSURE TO DDT

The extensive, and in some countries continuing, use of large quantities of the persistent insecticide *p,p'*-DDT has led to widespread contamination of

the environment. One result of this is that levels in human milk in some countries are so high that the intake by breast-fed infants may exceed the Acceptable Daily Intake recommended by the World Health Organisation. However, there is no evidence that such an intake has a deleterious effect on the health of infants.

The main metabolite of *p,p'*-DDT in man is *p,p'*-DDE, which is retained in in adipose tissue of the body. The levels of *p,p'*-DDE in adipose tissue, breast milk fat, blood (or fractions thereof), or cerumen can be used as an indicator of exposure to *p,p'*-DDT.

A large number of gas chromatographic methods is available for the analysis of *p,p'*-DDE and related lipid-soluble, persistent organochlorine compounds (Slorach and Vaz, 1983).

Studies of the levels of *p,p'*-DDE and related compounds in breast milk have been carried out since the 1950s and thus there is a considerable body of data to enable the determination of time-trends. However, for a number of reasons the interpretation of the results of studies on the levels of *p,p'*-DDE in adipose tissue or breast milk fat is difficult. First, unless the person concerned is exposed directly to *p,p'*-DDT, as in the case of occupational exposure, it is likely that there will be mixed exposure to both *p,p'*-DDT and *p,p'*-DDE via foodstuffs of animal origin (e.g., fish, meat, and dairy products). In such cases, the level of *p,p'*-DDE in human tissues does not reflect exposure to *p,p'*-DDT alone but also includes exposure to preformed *p,p'*-DDE. In countries where *p,p'*-DDT is still used in agriculture and vector control programmes, the ratio *p,p'*-DDE/*p,p'*-DDT levels in breast milk fat is very much lower than in countries that have stopped using DDT (Slorach and Vaz, 1983; Jensen, 1983). The explanation for this is that in the latter countries exposure is mainly via foodstuffs which contain mainly *p,p'*-DDE and little or no *p,p'*-DDT; whereas, in the former exposure to *p,p'*-DDT may occur both via foodstuffs and via other routes such as inhalation and skin contact.

When *p,p'*-DDE levels in human milk fat were used as an indication of exposure to *p,p'*-DDT, in the same individual those levels can be affected by factors such as parity and length of previous lactation periods and the time post-partum at which the samples are collected.

Levels in adipose tissue or breast milk fat can also be used as an index of exposure to other persistent organochlorine pesticides. For example, exposure to aldrin can be monitored by measuring the levels of its metabolite dieldrin. Likewise, exposure to heptachlor can be assessed by measuring levels of heptachlor epoxide in adipose tissue and breast milk fat.

Readers interested in the use of breast milk for biological monitoring are referred to the excellent review by Jensen (1983).

## 7 CONCLUSIONS

As the above examples show, the determination of metabolites in body fluids and tissues can provide an indication of exposure to certain chemicals. This method is especially useful in cases where metabolism yields a metabolite which is specific and stable. Unfortunately, the interpretation of the results of this type of biological monitoring of exposure to chemicals may be complicated by the facts that there are intra-species and intra-individual differences in the metabolism of some chemicals and that some metabolites are not specific to a single chemical. The development of better analytical methods and advances in our knowledge of the metabolism of environmental chemicals will provide a firmer foundation for this type of biological monitoring in the future.

For further information on the use of metabolites in biological monitoring the reader is referred to Sheldon *et al.* (1986); Carson *et al.* (1986); Linch (1974); Aitio *et al.* (1984); *Patty's Industrial Hygiene and Toxicology* (Cralley, 1985); and a publication from the American Conference of Governmental Industrial Hygienists (ACGIH, 1985).

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