La Medicina del Lavoro Med Lav 2012; *103*, 5: 413-416

CONCLUSION

Biological monitoring as a means to assess exposure and to predict adverse effects of chemical pollutants: an overview of current activities in key Italian laboratories

A. MUTTI
Department of Clinical and Experimental Medicine, University of Parma, Italy

Facilities and technologies are now available to explore the nanomolar range of xenobiotics in biological media. As a consequence of improved analytical capabilities, it is now possible to identify biomarkers useful to assess exposure to environmental contaminants and residual occupational exposure at workplaces where effective prevention measures have been taken. Such increased sensitivity and selectivity of analytical instruments relying on mass spectrometry is particularly suitable to assess exposure to carcinogenic agents, for which even trace amounts are regarded as contributing to overall risk of cancer. This is the reason why biological monitoring (BM) is becoming an important and popular tool to address potential risks to human health associated with air pollution, and contamination of soils, water, and food, thus expanding its scope to involve the general population, even beyond the scope of contaminants polluting working environments, such as bis-Phenol A (2), MTBE (5) or perfluorooctanates (3, 9).

New technologies also pose serious problems, mainly the cost of instrumentation, which can only be afforded in a few specialized centres, and resources needed to hire highly professional and trained personnel. The context of application of biological monitoring is also rapidly changing: more and more risky occupational settings are being delocalized in countries where manpower is less costly, less protected, and health is not considered as an absolute human right. As a whole, the cost per unit of "monitored risk" has increased exponentially over the last ten years. Moreover, exposures close to "background" levels are difficult to study and to interpret from the point of view of the toxicokinetics of individual chemicals. However, at such low levels, toxicokinetics may be less critical than in occupational settings, where biological monitoring was implemented in the past and correct sampling time selected on the basis of toxicokinetics was a pre-requisite to obtain meaningful results. However, this might be an oversimplification, as additional factors, such as polymorphisms of metabolizing enzymes, need to be considered even at low dose levels (17).

This special issue is a collection of papers showing current activities in several Italian centres of Occupational Medicine, where laboratories of Occupational and Environmental Toxicology and Hygiene are still very active. The present overview is aimed at highlighting those articles, trying to grasp the most significant conclusions from a variety of contributions, covering a wide range of contaminants and situations.

Two papers dealing with benzene exposure apparently reach opposite conclusions. Whereas Andreoli et al. (1) emphasize the validity of phenylmercapturate, Campagna et al. (4) conclude that unchanged urinary benzene is the best biomarker of exposure. It ought to be noted that such conclusions have been obtained in subjects exposed to airborne concentrations so low that even a few year ago most samples would have been reported to be below the limit of detection (LOD). Furthermore, as depicted in figure 1, several uses of BM are possible, the main ones being assessment of exposure and prediction of adverse effects, respectively (continuous lines).

Since the parent compound in urine is directly derived from absorbed benzene escaping biotransformation and excreted unchanged, it is not surprising that this biomarker shows the best correlation with personal exposure data (4). What is surprising is that such a correlation could be demonstrated even in the picomolar – low nanomolar range. Probably, kinetic factors or inter-individual variability in biotransformation depicted in figure 1 do not play a confounding role, which is apparent for benzene metabo-

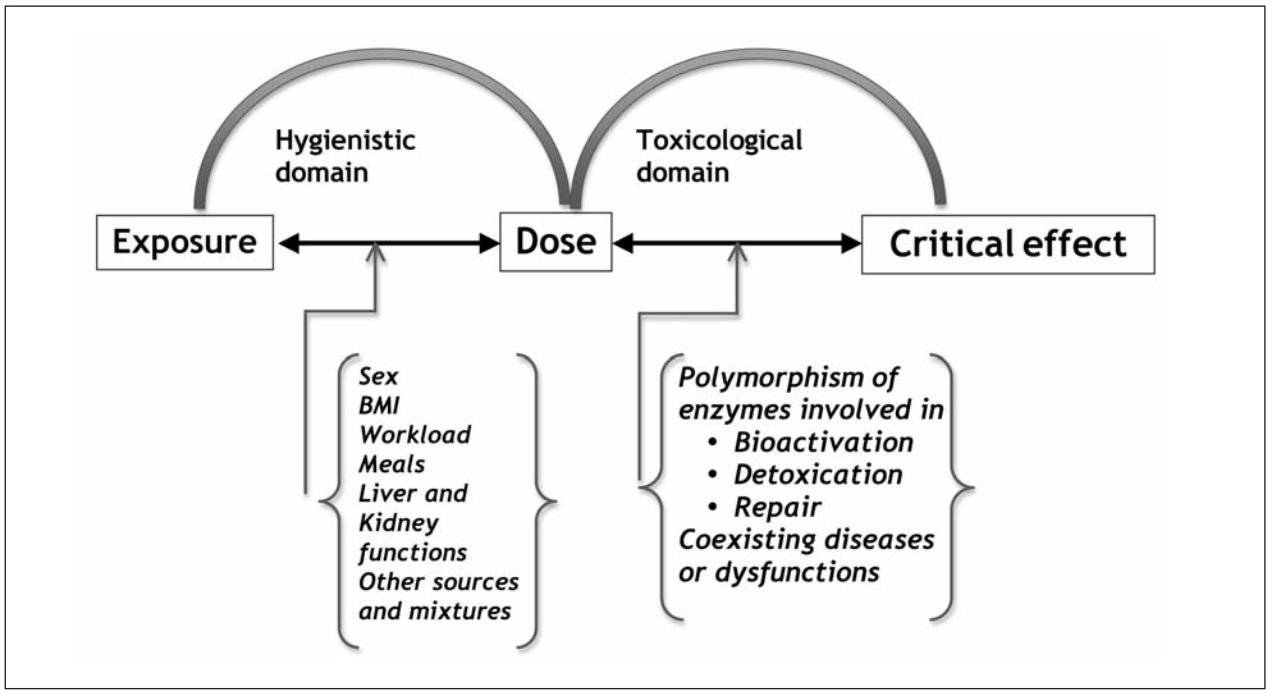

Figure 1 - List of factors affecting the relationship between exposure and dose (hygienistic approach) or the relationship between dose and adverse effects (toxicological approach). The first step is useful for exposure assessment purposes (considering toxicokinetics), whereas the second one should contribute to better understand the mechanistic base of adverse effects and possible threshold levels (considering toxicodynamics)

lites. Andreoli et al. (1) explored the correlation between biomarkers of exposure and oxidative damage to nucleic acids, a potentially adverse effect necessarily associated with biotransformation. Phase I and phase II polymorphic enzymes can introduce inter-individual variability accounting for different oxidative damage to nucleic acids at similar exposure levels. If we are interested in predicting health risks, we should consider the role of biotransformation. Indeed, benzene cannot cause oxidative damage to endogenous macromolecules, unless it is oxidised to hydroquinone, known to give rise to redox cycling and thought to be responsible for the radiomimetic effects of benzene exposure. A purely hygienistic approach can stop at the first step of the correlations depicted in figure 1 (affected by absorption, distribution and excretion), whereas a toxicologic attempt to characterize individual risk needs to rely on subsequent correlations (determined by biotransformation and repair capabilities).

Campo et al. (6) used urinary 1-hydroxy-pyrene (1-OH-P) as a tracer biomarker to assess exposure to complex mixtures of PAH both in occupationally exposed workers and in subjects belonging to the general population (living or not in the vicinity of a coke oven plant). Although 1-OH-P is a metabolite of the non-carcinogenic pyrene, it was correlated with the carcinogenic BaP, occurring at lower concentrations and therefore difficult to detect because of its physic-chemical and metabolic features. Indeed, the target of BaP is represented by the respiratory tract, where BaP is thought to be bioactivated. Despite the interest of these results, caution must be exercised in extrapolation of their data to other contexts. Complex PAH mixtures are known to be highly variable and the "fraction" accounted for by 1-OH-P may change considerably in different settings. Moreover, the use of a systemic biomarker, reflecting not only exposure, but also inter-individual metabolic variability, might not always reflect the target (airways) dose, much more important than the systemic dose as risk determinant.

A similar problem should be considered in the critical evaluation of the interesting paper submitted by De Palma et al. (7), who propose the urinary metabolites 2,4- and 2,6-toluenediamine (TDA) for the biological monitoring of exposure to 2,4-:2,6-toluene diisocyanate (TDI). An accurate study design enabled the authors to pick out information on the kinetics, which was fast enough to conclude that the difference between pre- and post-shift values can be used to characterize daily exposure. It ought to be noted that TDI critical effects are mainly local, affecting the airways (thereby inducing asthma in susceptible individuals).

Whereas one may assume that local effects are associated with exposure intensity, retained dose and haptenization might not be correlated or might be even inversely correlated with systemic dose and biotransformation capability.

The importance of speciation of trace elements is outlined by Lovreglio et al. (10), who were able to characterise urinary arsenic (iAs) species, including As^3, As^5, monomethylarsonic acid and dimethylarsinic acid. Despite a limited sample size, a sophisticated study protocol enabled the authors to conclude that the determination of total arsenic is not very useful to assess exposure to inorganic As. In fact, subjects whose exposure was never detectable through personal air monitoring showed important variations of urinary As after consumption of some food items, particularly crustacean and molluscs, resulting in urinary As exceeding the ACGIH-recommended BEI for occupationally exposed workers. However, such high values were due to organic species of As, mainly the non-toxic arsenobetaine. At a superficial reading, it seems quite obvious that non-exposed people do not excrete toxic species of As. However, the message of the paper is that total As in urine is not a reliable biomarker of exposure, as it can give rise to a high rate of false positives and this should be kept in mind by occupational physicians.

Perchloroethylene is still widely used as a dry cleaning solvent and, as shown by Maccà et al. (11), occupational exposure is still a problem in small shops. Perchloroethylene is poorly metabolized; hence, trichloroacetic acid, accounting for less than 10% of perchloroethylene taken up, is not very useful for biological monitoring purposes, whereas the parent compound can be measured both in blood and urine, blood analysis being recommended by the authors, despite its drawback of being invasive. The reason why an indirect approach was chosen to measure urinary perchloroethylene as chloroform concentrations remains unclear and could account for worse correlations with air concentrations as compared to blood levels. Suitable alternatives are available either to detect urinary concentrations exceeding 0.005 μg/l by solid phase micro-extraction gas chromatography-mass spectrometry analysis (13) or to perform alveolar air analysis as a surrogate of blood sampling, as suggested by Gobba et al. (8).

BM is an elegant approach to assess exposure from all sources when ambient monitoring fails to provide credible measures of exposure. Sabatini et al. (14) summarize their experience on BM of exposure to chemotherapic antineoplastic drugs known to be genotoxic and carcinogenic. Indeed, a contaminated surface may or may not be associated with exposure of the personnel. This paper shows that a problem overlooked for decades has been solved centralizing preparations in a technically efficient unit, making BM a means to rule out rather than to quantify exposure, as should be done for workers potentially exposed to carcinogenic agents.

Finally, Tranfo et al. (15) address an old problem, i.e. styrene exposure in glass fiber reinforced plastics, still representing a potential health risk, due to the relatively poor technology and the need for slow evaporation of styrene from applied resins to obtain commercially suitable products. Data provided by Tranfo et al. (15) confirm earlier results shown by Manini et al. (12) indicating that urinary metabolites of styrene can be found in all urine samples from subjects who are not occupationally exposed to styrene. The source of such widespread exposure remains to be identified and its health significance to be elucidated. The data shown in table 2 by Tranfo et al. (15) are also useful to reflect on the need to rely on the most appropriate measure of central tendency: if mean values were compared, then no difference could be seen between groups. In log-normal distributions, however, arithmetic mean overestimate the central tendency of the sample, which is better defined by either geometric mean or median values, indeed showing that differences between groups existed, as expected. Moreover, actual exposure of the general population resulting from median values was lower by one order of magnitude as compared to what would be apparent relying on arithmetic mean.

Thus, BM is a powerful, albeit delicate, scientific approach to investigate human exposure to environmental pollution. Multidisciplinary skills are required to handle sophisticated analytical techniques and data, difficult to interpret, due to their distribution, which can be affected by a wide range of factors, the main ones being kinetics, physical workload, food intake and, of course, exposure. There are many examples of misuse of BM, which can lead to overemphasize (or underestimate) serious Public Health issues: analytical methods should be validated and applied by skilled technicians, the resulting data should be handled by competent statisticians aware of the complexity of biological systems, in which many factors generate intra- and inter-individual variability. Finally, physicians trained in Occupational Health, Epidemiology, and Toxicology should provide thoughtful risk assessment, incorporating BM in a wider picture integrating ambient monitoring, health surveillance programmes and risk management.

The main challenge today is to make compatible the cost of BM with economic conditions getting worse and worse, but perhaps a better distribution and a rigorous use of resources would be useful to avoid its misuse, which may have serious social consequences and at the same time may undermine the scientific credibility of Industrial and Environmental Hygiene and Toxicology.

Significant developments in the areas of new and existing biomarkers, analytical methodologies, validation studies

and field trials, together with auditing and quality assessment of data, are not covered by this monographic issue dedicated to BM, but can be found in recent reviews (e.g., 16).

Modern techniques have revealed 'backgrounds' in people not knowingly exposed to chemicals and the sources and significance of these need to be determined, particularly in the context of their contribution to background health risks. To address this issue and the complexity of biological systems, genome-wide analyses of susceptibility genes (genomics), gene expression (transcriptomics), protein expression (proteomics), and epigenetic modifications (epigenomics) are being characterized in human populations exposed to benzene to develop biomarkers of exposure, early effect and susceptibility. Comprehensive analysis of these toxicogenomic and epigenomic profiles by bioinformatics in the context of phenotypic endpoints, comprises systems biology, which has the potential to define the mechanisms by which benzene causes leukemia (18).

References

1. Andreoli R, Protano C, Manini P, et al: Association between environmental exposure to benzene and oxidative damage to nucleic acids in children. Med Lav 2012; *103*: 324-337
2. Asimakopoulos AG, Thomaidis NS, Koupparis MA: Recent trends in biomonitoring of bisphenol A, 4-t-octylphenol, and 4-nonylphenol. Toxicol Lett 2012; *210*: 141-154
3. Butenhoff JL, Olsen GW, Pfahles-Hutchens A: The applicability of biomonitoring data for perfluorooctanesulfonate to the environmental public health continuum. Environ Health Perspect 2006; *114*: 1776-1782
4. Campagna M, Satta G, Campo L, et al: Biological monitoring of low-level exposure to benzene. Med Lav 2012; *103*: 338-346
5. Campo L, Cattaneo A, Consonni D, et al: Urinary methyl tert-butyl ether and benzene as biomarkers of exposure to urban traffic. Environ Int 2011; *37:* 404-411
6. Campo L, Vimercati L, Carrus A, et al: Environmental and biological monitoring of PAHs exposure in coke-oven workers at the Taranto plant compared to two groups from the general population of Apulia, Italy. Med Lav 2012; *103*: 347-360
7. De Palma G, Cortesi I, Ghitti R, et al: Biological monitoring as a valid tool to assess occupational exposure to mixtures of 2,4-:2,6-toluene diisocyanate. Med Lav 2012; *103*: 361-371
8. Gobba F, Righi E, Fantuzzi G, et al: Perchloroethylene in alveolar air, blood, and urine as biologic indices of low-level exposure. J Occup Environ Med 2003; *45*: 1152-1157
9. La Rocca C, Alessi E, Bergamasco B, et al: Exposure and effective dose biomarkers for perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) in infertile subjects: preliminary results of the PREVIENI project. Int J Hyg Environ Health 2012; *215:* 206-211
10. Lovreglio P, D'Errico MN, De Pasquale P, et al: Environmental factors affecting the urinary excretion of inorganic arsenic in the general population. Med Lav 2012; *103*: 372-381
11. Maccà I, Carrieri M, Scapellato ML, et al: Biological monitoring of exposure to perchloroethylene in dry cleaning workers. Med Lav 2012; *103*: 382-393
12. Manini P, De Palma G, Andreoli R, et al: Determination of urinary styrene metabolites in the general Italian population by liquid chromatography-tandem mass spectrometry. Int Arch Occup Environ Health 2004; *77*: 433-436
13. Poli D, Manini P, Andreoli R, et al: Determination of dichloromethane, trichloroethylene and perchloroethylene in urine samples by headspace solid phase microextraction gas chromatography-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2005; *820*: 95-102
14. Sabatini L, Barbieri A, Lodi V, Violante FS: Biological monitoring of occupational exposure to antineoplastic drugs in hospital settings. Med Lav 2012; *103*: 394-401
15. Tranfo G, Gherardi M, Paci E, et al: Occupational exposure to styrene in the fibreglass reinforced plastic industry: comparison between two different manufacturing processes. Med Lav 2012; *103*: 402-412
16. Watson WP, Mutti A: Role of biomarkers in monitoring exposures to chemicals: present position, future prospects. Biomarkers 2004; *9*: 211-42
17. Weisel CP: Benzene exposure: an overview of monitoring methods and their findings. Chem Biol Interact 2010; *184*: 58-66
18. Zhang L, McHale CM, Rothman N, et al: Systems biology of human benzene exposure. Chem Biol Interact 2010; *184*: 86-93