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Toxicology Letters 168 (2007) 210–218
Toxicology Letters
www.elsevier.com/locate/toxlet

Exposure assessment at the workplace: Implications of biological variability

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Available online 16 November 2006

Abstract

Biological monitoring (BM) and biomarkers are widely applied in occupational toxicology. BM is mainly aimed at (i) defining the existence of an occupational exposure; (ii) quantifying the level of internal dose; (iii) verifying that exposure limits (BEI®, BAT, BLV) are respected. As compared to ambient monitoring, BM is more expensive and complex. Several biomarkers are available for the same chemical and the meaning of the marker may depend on the sampling time. Therefore, practical issues, including cost and selection of an adequate sampling strategy, should be dealt with when planning a BM program for specific purposes. In addition, several biological and analytical sources of variability may influence biomarker levels, thus making the interpretation of BM data a difficult task. However, we should recognize that the main aim of BM is not to reduce, but to explain biological variance. The decreasing trend in occupational exposure levels highlighted the specificity problems of traditional biomarkers of exposure and prompted the research to the development of new biomarkers, e.g. unchanged volatile compounds in urine, minor metabolites, DNA and protein adducts. Depending on the scope and context (research or routine) different requirements of biomarkers can be envisaged in terms of validation and acceptable variability.

Keywords: Biological monitoring; Biomarkers; Workers; Variability

Abbreviations: BM, biological monitoring; IQC, internal quality control; EQA, external quality assessment; UEN, University Erlangen-Nuremberg; FIOH, Finnish Institute of Occupational Health; ESCODD, European Standards Committee on Oxidative DNA Damage; PHEMAs, phenylhydroxyethyl mercapturic acids; GSTM1, glutathione-*S*-transferase M1-1; 1-HP, 1-hydroxypyrene; PAHs, polycyclic aromatic hydrocarbons; RVs, reference values; LOD, limit of detection; LOQ, limit of quantitation; SCOEL, Scientific Committee for Occupational Exposure Limits; BLV, biological limit value; NOAEL, no-observed-adverse effect-level; OEL, occupational exposure limit; BEI®, biological exposure index; ACGIH, American Conference of Governmental Industrial Hygienists; TLV®, threshold limit value; BAT, Biologischer Arbeitsstoff-Toleranz Wert; DFG, Deutsche Forschungsgemainschaft; MAK, Maximale Arbeitsplatz-Konzentration; CI, confidence interval; LOAEL, lowest-observed-adverse-effect level; BMD, benchmark dose; BMDL, lower limit of a one-sided 95% confidence interval on the BMD; BMR, benchmark response; ED_{10}, median effective dose to a 10% of a population; LED_{10}, lower confidence limit on the dose that produces a 10% risk; Hb, hemoglobin; AAVAL, acrylamide Hb adducts

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0378-4274/$ – see front matter © 2006 Published by Elsevier Ireland Ltd.
doi:10.1016/j.toxlet.2006.09.014

1. Assessment of occupational exposure by biological monitoring

Biological monitoring (BM) has been defined as 'the periodic measurement of xenobiotic(s) or their metabolite(s) in accessible biological media for the comparison with an appropriate reference' (Berlin et al., 1982). BM is mainly aimed at (i) defining the existence of an occupational exposure; (ii) quantifying the dose (internal, effective or cumulated); (iii) verifying that exposure limits are respected. Towards ambient monitoring, which represents the obvious term of comparison, BM should be considered as a complementary rather than an alternative approach (Lauwerys and Hoet, 1993). The characteristics of biological and ambient monitoring are summarized in Table 1. BM takes into consideration all routes of absorption (inhalation, skin, ingestion) in both occupational and leisure activities, accounting for individual differences in absorption rate due to variations in workload or co-exposure to additional components of complex mixtures, and in metabolic capabilities, due to either genetically determined or acquired changes in gene expression and enzyme activity. On the other hand, BM is more complex in terms of standardization and interpretative efforts as compared to ambient monitoring. Since BM rely on the use of biomarkers, a toxicological knowledge is needed for their interpretation and ethical issues should be addressed as generally required in human studies.

Biomarkers – used to model the interaction between a xenobiotic and the individual – are more directly related to the adverse effects which one attempts to prevent than any ambient measurement (Lauwerys and Hoet, 1993). According to the National Research Council, biomarkers can be classified as: biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility. A biomarker of exposure has been defined as 'an exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism' (NRC, 1987), whereas a biomarker of effect is "any measurable biochemical, physiological or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease" (NRC, 1987). Biomarkers of susceptibility are effect-modifying factors, including both genetic (e.g., genetic polymorphisms of drug metabolizing and DNA repair enzymes) and acquired conditions. The use of biomarkers rather than their intrinsic properties may define their classification (Watson and Mutti, 2004). Fig. 1 shows the *continuum* of events between exposure and long-term effects through a multistage and multifactorial process, and the use of biomarkers in risk assessment (Albertini, 2001).

Table 1
Biological monitoring versus ambient monitoring: definition, routes of absorption, confounding factors, cost, standardization, interpretation and significance of results

	Biological monitoring (BM)	Ambient monitoring (AM)
Aimed at quantifying	Dose	External exposure
Reference	BEI®, BAT, BLV	TLV®, MAK, OEL
Absorption	All routes	Inhalation only
Confounding	Metabolic phenotype	Protection devices
Cost	Usually high	Usually low
Standardization	Difficult	Easy
Interpretation	Difficult	Easy
Measurement	Biomarkers	Direct
Ethical issues	Possibly important	None
Variability	High	Usually low

Exposure biomarkers are widely used in occupational toxicology for a more accurate risk assessment. In workers exposed to similar air concentrations of chemical pollutants, various factors can determine the actual absorbed dose, including physical workload, additional skin absorption due to bad working practice or, on the contrary, the use of personal protection devices, and differences in individual uptake and metabolism.

Several biomarkers are often available for the same chemical, e.g. the parent compound itself, a metabolite, or a macromolecular adduct (to DNA or protein), and the meaning of the marker may depend on the sampling time. Therefore, the choice of the biomarker should rely on a number of considerations, but mainly on kinetic parameters (Bernard, 1995) and on the knowledge of the mechanistic basis of adverse effects. In addition, practical issues, including cost, exposure levels, analytical requirements, and selection of an adequate sampling strategy, should be dealt with when planning a BM program for specific purposes.

An ideal biomarker of exposure should be specific for the exposure of interest, detectable in small quantities, measurable by noninvasive techniques, inexpensive, associated with prior exposure and provided of an excellent positive predictive value to a specific health status (Henderson et al., 1989). When dose–effect and dose–response relationships are known, an appropriate biomarker of dose may be sufficient to assess the risk of adverse effects. In order to become useful tools in risk assessment, biomarkers must be relevant, e.g. appropriated to provide information on important questions concerning health risks, and valid for both analytical and epidemiological aspects. In particular, external validity

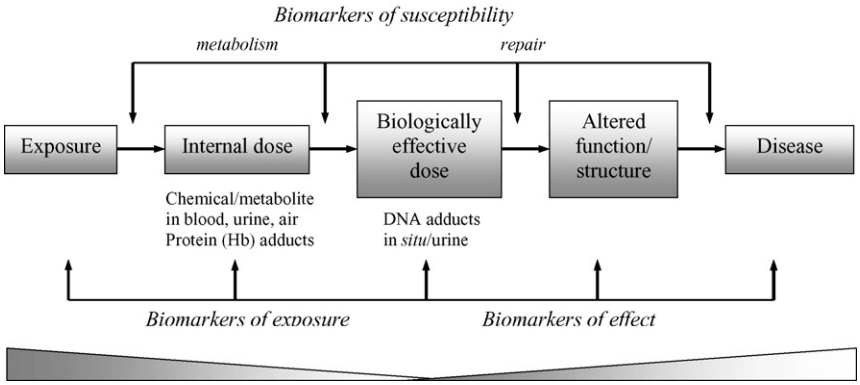

Fig. 1. Continuum of events between exposure and disease, and use of biomarkers in risk assessment. Adapted from NRC (1987); Albertini (2001).

is required to obtain results, which can be generalized to other populations (IPCS, 2001).

2. Biomarkers and variability

Several sources of variability affect BM at several stages, starting from metabolism and sampling, up to analysis and interpretation of results, as shown in Fig. 2. Variability may be classified as analytical and biological in nature. In the past, analytical and biological contributions to variability were comparable, mainly due to the low precision of analytical determinations. Today, analytical variability is generally much lower than the biological one, owing to the improvements in the performance of techniques and to the application of quality assurance in biomarker measurements (Jakubowski and Trzcinka-Ochocka, 2005).

2.1. Analytical variability

Analytical results should be sufficiently accurate and reproducible to avoid misinterpretation of biomonitoring results. To satisfy these requirements, laboratories should use validated methods and apply appropriate internal quality control (IQC). IQC monitors precision on independent replicate analysis of test materials and accuracy using commercially available control samples or reference materials (Aitio and Apostoli, 1995). When not commercially available, control materials can be produced by the laboratory itself and used for IQC. Participation to external quality assessment (EQA) schemes is useful to time monitor laboratory performances and the reliability of results, as well as to warrant the comparability of the results produced by different laboratories in the world, which perform the same analysis. The structure of the external quality control scheme has been described elsewhere (Valkonen and Kallio, 2002; Schaller et al., 2002). In Europe, several institutions organize EQA programs, the most important of them are those run by the University Erlangen-Nuremberg (UEN) on behalf of the German Society for Occupational and Environmental Medicine and by the Finnish Institute of Occupational Health (FIOH), respectively. The UEN quality assurance scheme encompasses the widest range of toxic substances (metals, solvents, organo-chlorine compounds, inorganic and organic compounds) relevant to occupational and environmental medicine, involving about 350 laboratories (Schaller et al., 2002). The FIOH quality assurance program includes a limited number of metabolites and participating laboratories (Valkonen and Kallio, 2002).

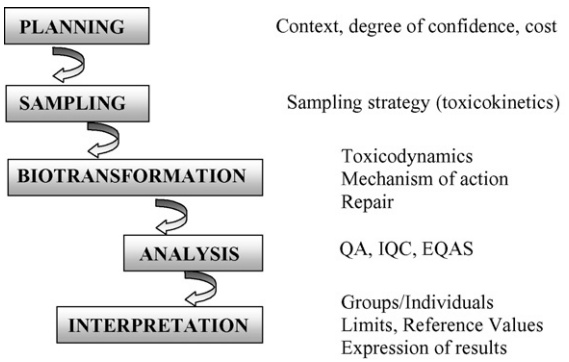

Fig. 2. Sources of variability affecting biological monitoring at several stages, starting from planning and sampling, up to analysis and interpretation of results.

Whereas EQA schemes can be implemented for "traditional" biomarkers of exposure, their application to "novel" biomarkers is hard or even impossible, mainly because of the lack of certified reference materials. In this case, standardization and harmonization of methods used in different laboratories should be pursued as intermediate objective of quality. As an example of standardization, the European Standards Commit-

tee on Oxidative DNA Damage (ESCODD) was set up in 1997 to resolve methodological problems and to reach agreement on the basal level of 8-oxo-2′-deoxyguanosine in biological samples (Riis, 2002). A similar demand of standardization has been claimed for immunochemical methods applied in exposure and effect biomarkers developed for screening purposes. Highly sensitive immunoassays are also extremely sensitive to environmental conditions and to variations in either reagents or analytes, which usually cannot be characterized enough to prepare adequate reference materials (Mutti, 1989). Sharing methods and the exchange of homemade materials within collaborative studies could be useful to the harmonization of measurement procedures in order to achieve consensus values for the analytes.

2.2. Biological variability

If analytical variance can be kept under control by adequate quality assurance programs, inter-individual differences in uptake, biotransformation, mechanism of action, susceptibility to damage, and repair capacity can result in different dose–response relationships for different groups of individuals. The contribution of intra-individual variability in exposure (e.g., day-to-day variation) may also lead to attenuation bias in dose-response relationships, when estimates of workers' exposures relying on single measurements are used to evaluate effects resulting from chronic exposure (Symanski et al., 2001). Increasing the number of repeated individual measurements can reduce the bias in the calculation of slope coefficients (Liljelind et al., 2003). However, biological variance should not be reduced, but explained by kinetics and toxicodynamic factors accounting for inter- and intra-individual differences (Mutti, 2001). In a research context, human variability is the main source of information, as well as the most intriguing challenge.

Differences in physical activity, respiratory rate, and body mass index are known to influence absorption and excretion of chemicals. It is known that the different workload may affect exposure–dose relationships and the interpretation of BM results should consider that a given biomarker could overestimate exposure in the case of heavy tasks and underestimate it in the case of subjects at rest. Similar interferences on absorption and metabolism of chemicals have been described for other factors like sex, fat intake, alcohol consumptions, medication, and in the case of co-exposures to complex mixtures of substances (Viau, 2002). Two approaches involving compartmental toxicokinetic models or physiologically-based toxicokinetic models have been proposed to evaluate the impact of human variability on the practice of exposure biomonitoring using Monte Carlo simulations (Tardif et al., 2002; Pierrehumbert et al., 2002). Thousands of virtual workers corresponding to many profiles of appropriate physiological parameters can be created and used to investigate the effect of changes within a range of exposure conditions. As shown in the case of toluene, these models may be useful to predict the range of the biological exposure parameters (toluene in blood, *o*-cresol) that could occur in a group of workers exposed to the chemical.

Enzymatic induction or inhibition, as well as genetic polymorphisms of enzymes involved in the biotransformation of chemicals may be partly responsible of inter-individual differences in the excretion of metabolites. To this regard, the concentrations of styrene-derived mercapturic acids (phenylhydroxyethyl mercapturic acids, PHEMAs) in the urine of exposed workers were characterized by using novel sensitive and selective analytical methods (Manini et al., 2000). The excretion profile of PHEMAs was strongly influenced by the glutathione-*S*-transferase M1-1 (*GSTM1*) status, with *GSTM1pos* subjects (expressing the *GSTM1-1* enzyme activity) excreting about five-fold higher concentrations of mercapturic acids than *GSTM1null* subjects (lacking the *GSTM1-1* enzyme) (De Palma et al., 2001). In this case, we believe that the modifying role of *GSTM1* polymorphism, which affects about 50% of Caucasian population, limits the practical use of PHEMAs as biomarkers of exposure to styrene. However, other researchers calculated two different biological indices for *GSTM1pos* and *null* subjects, respectively (Haufroid et al., 2001). Accurate modeling would require due attention to additional interfering factors, e.g., body mass, physical workload, sex, etc. Such complex multiple regression models might be useful to address special issues within a research project, but are certainly out of the scope of routine monitoring programs.

Not all biomarkers are characterized by the same degree of variability: biological variability and measurement uncertainty increase as soon as we proceed from exposure to disease, owing to the fact that a major number of biological steps is involved and that biomarker levels decrease moving on from the internal dose to the biological effective dose. The variability of urinary 1-hydroxypyrene (1-HP) as a biomarker of dietary polycyclic aromatic hydrocarbons (PAHs) has been characterized in human volunteers consuming similar amounts of identical food for five days (Viau et al., 2002). Despite the ingestion of identical doses of pyrene,

there was a 50–76% inter-individual variability (as coefficient of variation) in the daily-excreted amount of 1-HP. The inter-individual variation in PAH-DNA adduct levels is even much greater (Godschalk et al., 2003). One study reported a 50-fold inter-individual variation for controls and 100-fold for coke-oven workers in the levels of anti-benzo[a]pyrene diolepoxide-DNA adducts in peripheral mononuclear cells (Rojas et al., 1995). This may be attributed to both biological factors, e.g. differences in the activation or subsequent detoxification of PAH or repair of PAH-DNA adducts, and analytical factors. In fact, the determination of DNA adducts is more difficult and requires a longer time for sample preparation and analysis, as compared to urinary metabolites. It should be noted that, with a few exception, the quantitation of DNA adducts is confined to research exercises by the lack of commercial standards, reference materials and EQA schemes.

3. Interpretation of biomonitoring results

The methods for interpretation of biomonitoring results have become the most important issue today (Jakubowski and Trzcinka-Ochocka, 2005). The interpretation of biomonitoring results is often based on the comparison with an appropriate reference, which could be a reference value or a biological exposure limit. Many analyses are made to ensure that limit values are not exceeded and to take any decision with confidence we need to know: (i) the uncertainty of the result at a given degree of confidence; (ii) how the limit value used as reference is defined.

3.1. Reference values

Reference values (RVs) can be calculated from the upper limit of the frequency distribution of the concentration of the biomarker in the unexposed population. Recommendations for population recruitment (sample size, gender, age, and smoking habit), collection of specimens, and statistical analysis for the production of RVs have been published by the International Federation of Clinical Chemistry (Soldberg, 1987). RVs have been determined mainly for metallic elements, persistent organic pollutants, and pesticides. The production of RVs of organic solvents and their metabolites has been included in the activity of the Italian Society of Reference Values (Minoia and Apostoli, 1999).

It should be noted that RVs should be defined at the local level. In fact, they are influenced by environmental exposure levels in that country, biological conditions (sex, age, fatty mass, diseases), metabolic interferences arising from habits (tobacco and alcohol consumption, diet, use of drugs), genetic polymorphisms, and improvement in analytical procedures. Owing to the application of sensitive and selective analytical methods based on liquid chromatography–mass spectrometry, we have characterized reference values of urinary styrene metabolites, e.g. mandelic acid and phenylglyoxylic acid in an unexposed Italian population, ruling out any role of gender, age, smoking, and alcohol consumption as confounding factors. Nor did genetic polymorphisms act as modifying factors (Manini et al., 2004a) These values were considerably lower (about 20-fold) than those proposed 10 year earlier for the Danish population (Mürer et al., 1994). Such a difference was probably due to the application of more sensitive analytical techniques and methods, greatly lowering both the limit of detection (LOD) and the limit of quantitation (LOQ).

A similar behavior was observed for urinary chromium, when improved techniques (Zeeman effect) have been introduced in laboratories of industrial toxicology to limit interferences on electrothermal atomisation—atomic absorption spectroscopy (Apostoli et al., 1997).

Considering that there is a decreasing trend in occupational exposure levels and an opposite increase in the concentrations of some environmental pollutants, RVs for the general unexposed population are useful to assess a threshold for occupational exposure and to identify subjects with an increased exposure. For example, benzene exposure in the developed countries mainly occurs in urban environments, as benzene is a ubiquitous pollutant arising from motor vehicle emissions. In the case of workers doing their activity in the urban traffic (traffic policemen, bus and taxi drivers), RVs rather than occupational limits are the proper term of comparison. A recent study conducted by our laboratory on a group of taxi drivers showed that the concentration ranges of biomarkers, e.g. *S*-phenylmercapturic acid and *t,t*-muconic acid, observed for both smoking and nonsmoking subjects fell into the reference value intervals proposed for the unexposed population, thus excluding any relevant occupational exposure to benzene (Manini et al., 2006).

3.2. Biological limits for occupational exposure

Two different approaches are possible in data interpretation depending on how biological limits are derived, as clearly stated by the Scientific Committee for Occupational Exposure Limits (SCOEL, 2005) about the origin of Biological Limit Values (BLVs).

When human studies (occupational field studies or experimental laboratories studies on volunteers) are available, linking adverse effects with concentrations

Table 2
Comparison among different biological limits, e.g. BEI® (ACGIH), BAT (DFG), and BLV (SCOEL), in terms of their origin and interpretation

	BEI® (ACGIH)	BAT (DFG)	BLV (SCOEL)
Origin	Exposure-dose (dose–response[a])	Dose–response or exposure–dose	Dose–response (OEL–dose)
Corresponds to	Mean value (NOAEL[a])	Ceiling values	NOAEL, ceiling values
Interpretation	Groups (individuals[a])	Individuals or groups	Individuals and groups
Criterion	TLV®-related (health-based[a])	Health-based or MAK-related	Health-based
Carcinogens	Yes	No (EKA)	No

[a] Only for: Pb-B, inhibitors of cholinesterase activity, methemoglobin.

of the chemical or its metabolites in biological media, the no-observed-adverse effect-level (NOAEL) may be directly used to derive the BLV related to this level. Such BLVs, together with those obtained in the case of biological effect monitoring, are health-based values and are, in principle, to be preferred. Unfortunately, the number of these biomarkers is still limited to few examples, e.g. lead in blood, cadmium in blood and urine, mercury in urine, carboxyhemoglobin, methemoglobin, and inhibitors of cholinesterase activity in erythrocytes. For these biomarkers, interpretation is possible at both the group and the individual level, as shown in Fig. 3. For a group of workers, the cumulated frequency distribution of the biomarker can be constructed (grey area under the curve) and compared with dose-response relationships for one or more effects (curves a, b, and c in Fig. 3). The probability to observe a given biological response for the whole group of workers is obtained by drawing the projection of the 100^{perc} of the cumulated frequency distribution with the corresponding dose–response curves. The intersection gives the probability to observe a certain effect, which is, in the example, 80% for effect c, 35% for effect b, and none for effect a. The same probabilities can be calculated for a single worker, e.g. worker Y in the figure, by positioning on the corresponding point of the cumulated frequency distribution and by using the same method.

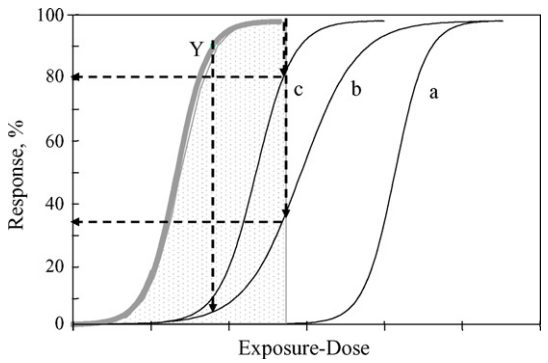

Fig. 3. Possible interpretation of biomonitoring results at the group or at the individual level. The cumulated frequency distribution of the biomarker in a group of workers (grey area under the curve) could be compared with dose-response relationships for one or more effects (curves a–b–c). The intersection between the projection of the 100^{perc} of the cumulated frequency distribution and the corresponding dose-response curve(s) gives the probability to observe a certain effect, that is, 80% for effect c, 35% for effect b, and none for effect a. Using the same method, these probabilities can be calculated for a single worker, e.g. worker y (see also the text).

For most chemicals, only exposure-dose and exposure-effect relationships are known. In this case, it is possible to identify the "mean" level of a biological index in a group of subjects exposed to air concentrations corresponding to the occupational exposure limit (OEL) for a given chemical. Many SCOEL BLVs are obtained from the corresponding OELs. Similarly, the Biological Exposure Indices (BEI®) of the American Conference of Governmental Industrial Hygienists (ACGIH, 2004) are derived from the corresponding Threshold Limit Values (TLV®), with few exceptions (e.g. blood lead, inhibitors of cholinesterase activity, methemoglobin). In this case, interpretation of biomonitoring results is possible at the group level only. Groups must be homogeneous in terms of exposure (e.g., similar job titles and/or similar exposures). Repeated measurements for the same individual can be treated as mean values of collective data. It is possible that some individuals exceed the BEI® value because of occasional confounding by variables other than exposure. This is why intervention aimed at reducing exposure is required only if BEI® values are exceeded for a longer period of time or by a substantial group within the exposed population.

The German Biological Tolerance Values for Occupational Exposures (Biologischer Arbeitsstoff-Toleranz Wert, BAT) of the Deutsche Forschungsgemainschaft (DFG, 2004) are in the middle between BEI® and health-based BLV. BATs are related to the maximum concentrations admissible in the workplace (Maximale Arbeitsplatz-Konzentration, MAK). They are also defined as "the maximum permissible quantity of a chemical substance or its metabolites or the maximum permissible deviation from the norm of a biologi-

cal parameter induced by these substances in humans. BAT values are conceived as *ceiling values* for healthy individuals' and 'are intended to protect employees from impairment of health at work'. As a result, BAT values are expected to be higher than BEI® values. Table 2 summarizes and compares the characteristics of some biological values in terms of their origin and interpretation.

3.3. Impact of variability on interpretation of biomonitoring results

Due to the large inter-individual variability, the correspondence between the ACGIH BEI® and the TLV® has been established on the basis of mean values. In a worker with values corresponding to the BEI®, the probability to have been exposed to concentrations higher (or lower) than the TLV® is exactly 50%. In terms of probability, the association of the biomarker level with a safe exposure level would be ensured if the biological index were extrapolated from the lower limit of the 95th CI of BEI® distribution rather than the mean value, as shown in Fig. 4. In practice, this approach is not feasible, since the variability among CIs reported in various studies is so high that it is impossible to find a consensus among different studies. In addition, as compared to mean values, CIs are strongly affected by sample size.

Traditional approaches for assessing non-carcinogenic risks associated with hazardous compounds are the NOAEL and the lowest-observed-adverse-effect level (LOAEL). In the definition of thresholds, both NOAEL and LOAEL require modifying and uncertainty factors, both are inappropriately related to sample size, and they may be inconsistent from study to study, also because they are constrained to be an experimental or empirical dose (Mutti, 2001). The benchmark dose (BMD) approach (Allen et al., 1994) could be a valid alternative to the NOAEL. The BMD method better uses the biological information available, since the mathematical model fits all the dose–response data in the observable range rather than simply the lowest dose level at which effects are observed. The model is then used to interpolate an estimate of the dose that corresponds to a particular level of response. In the BMD approach, information about the variability within data set and the uncertainty around a BMD are accounted for by the use of the lower confidence limit on the BMD (BMDL). As a result, the BMD is sensitive to the sample size, as a larger study will give narrower confidence limits on the BMD and thus a higher BMDL (Filipsson et al., 2003). Conversely, the NOAEL will be higher in studies with a smaller sample size, the opposite of what is desirable. In the BMD approach, the probability of adverse effects may be derived from the model describing the relationship between the prevalence of abnormalities and the biomarker levels. To this purpose, dichotomous dose–response models can be obtained by identifying a cut-off distinguishing what is "normal" from what is "abnormal". Selection of the response level for deriving the BMD, that is, the benchmark response (BMR), is the more difficult issue and the only factor that affects the magnitude of the BMD (Setzer and Kimmel, 2003). In environmental health, the ED_{10}, that is, the dose level corresponding to a two-fold increase over background occurrence among the reference population, or to a 5% excess of risk is often used as threshold. The BMD (LED_{10}) is defined as the statistical lower bound on a dose corresponding to a specified level of risk (risk of 10% or excess of risk of 5%) on the basis of the logistic regression function describing the dose–response curve, and is obtained from the upper confidence limit (95%) on the dose-response curve.

Although the BMD approach is not widely used in health risk assessment in Europe, it has been applied to several non-cancer end-points. For example, the BMD approach has been applied to assess a dose-response relationship between acrylamide adduct to N-terminal valine in haemoglobin (Hb) and acrylamide-induced neurological damage among workers from a Chinese chemical plant converting acrylonitrile to acrylamide. The application of a logistic regression model on data published by Calleman et al. (1994) showed that 97.5% of subjects with clinical signs of peripheral neuropathy were correctly classified on the basis of acrylamide Hb adducts

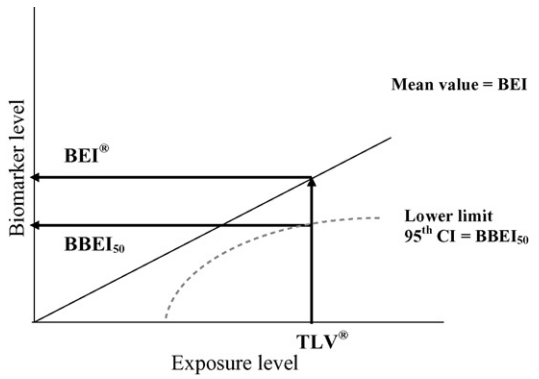

Fig. 4. Regression between airborne concentrations of a chemical and corresponding levels of exposure biomarkers. Contrary to the TLV® – set to protect the vast majority, that is, 95% of workers – the corresponding BEI® would protect only 50% of exposed subjects. In order to assume the same meaning of the TLV®, a benchmark dose 50 should be calculated ($BBEI_{50}$), that is, the value corresponding to the lower limit of the 95th CI of BEI®. In practice, $BBEI_{50}$ derivation is not feasible due to the large variability of the CI as compared to mean values reported in different studies (see also the text).

(AAVAL). On the basis of the parameter of the logistic regression, the calculated BMD corresponds to 0.8 nmol AAVAL/g Hb (IPCS, 2001).

4. Concluding remark

The decreasing trend in occupational exposure levels highlighted the specificity problems of traditional biomarkers of exposure and prompted the research to the development of "novel" biomarkers, e.g. unchanged volatile compounds in urine (Imbriani and Ghittori, 2005), minor metabolites (Manini et al., 2004b), DNA (Koc and Swenberg, 2002) and protein adducts (Törnqvist et al., 2002). Although biomarkers of exposure and effect measured with modern techniques are more sensitive and specific, such biomarkers may require validation, including the characterization of all variability sources (analytical, biological) and the characterization of the *background*. Using very sensitive and selective techniques, we are appreciating the complexity of biomarker research, as we realize that biological and analytical specificity tend to diverge. The ability to determine trace and ultra-trace amounts of parent compounds and their metabolites in biological media may result in the demonstration that such substances are either ubiquitous or shared with endogenous metabolism. As recently noted (Mutti, 2002), very "specific" metabolites are found among "unexposed" people.

Workers (and human beings in general) are exposed to complex mixtures of chemical pollutants and a considerable effort is needed to assess the health risk arising from occupational exposure. To this regard, the production of reference values for the general population is useful to identify individuals with an increased exposure as compared to the background. Depending on the scope and context (research or routine biomonitoring) different requirements of biomarkers can be envisaged in terms of validation and acceptable variability. "Novel" biomarkers can be applied for research purposes by using innovative but not fully validated methods, considering that a relatively wide range of uncertainty is accepted in this context. This is not the case of routine BM or legal litigation, where the application of validated methods is recommended, the analytical uncertainty should be known, and guidelines for data interpretation should be available.

Finally, the analysis of "novel" biomarkers requires the use of complex analytical techniques to achieve the sensitivity and the selectivity needed. On the other hand, the increase in selectivity obtained by multi-dimensional techniques based on mass spectrometry implies also high costs. When we plan BM, we should not forget that cost is a serious limiting factor and that the cost per unit of risk characterized (assessed) tends to increase exponentially as exposure levels become very low or barely appreciable.

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