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Biological Exposure and/or Effect Limits, Facts, Fallacies, and Uncertainties: Practical Aspects

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Summary

In the preceding article general principles in setting biological occupational exposure limits (BOEL) and effect limits (BOEEL) were discussed[1]. Here monitoring in every day occupational health practice is discussed. The specific objectives of biological monitoring (BM) and biological effect monitoring (BEM) determine to a large extent the choice of the parameters to be measured. According to the objective, the assessment may be either simple or sophisticated. The choice of an appropriate reference is essential for a valid evaluation of internal exposure, health risk and state of health. The measurement strategy depends on the working mechanism and the kinetics of the chemical. Protocols for BM and BEM-programmes should be regularly updated.

Different compounds of the same metal may carry widely different health risks. In general it is necessary to correct the excretion of chemicals for dilution of the urine.

Different objectives of monitoring programmes

At least four questions can be asked whenever carrying out BM- and/or BEM-programmes.

1. In groups of workers does any increased internal load (BM) and/or biological response (BEM) exist in comparison to an appropriate reference? In this case the programme may be simple, when a reasonably agent-specific parameter can be measured with a non-sophisticated method. If workers exposed to inorganic lead for at least one month show no increased zinc protoporphyrin (ZPP) in the absence of iron deficiency, then the blood lead (PbB)-level would not be expected to exceed 300 μg/l, and any lead-related health risk in these workers (except in pregnant workers) is unlikely. Only when the easily measured ZPP levels are considerably increased, then the more sophisticated measurement of PbB is needed. When in healthy non-smoking workers exposed to CO the percent carboxy haemoglobin (COHB) does not exceed 4, then probably no health risk exists.
2. When evidence of increased internal exposure and/or response exists, how high is exposure and how strong the response? In that case one has to measure at least the agent-specific parameters of BM and/or BEM, although it should be granted that most BEM-parameters are agent-non-specific[1]. When a close correlation exists between agent-specific BM and agent-non-specific BEM parameters, then a causal relationship in a group, but not necessarily in individuals, can be assumed to exist.
3. Is the BOEL or the BOEEL exceeded? The approach is similar to that described above, but the BOEL or BOEEL are used as a reference. However, compliance with these limits, not necessarily implies that overexposure or adverse health risks do not exist[1]. Moreover, some BOEL and BOEEL are sex dependent, particularly with respect to reproductive risks, which may differ between similarly exposed male and female workers[1].
4. What is the place of BM and BEM in diagnosis and treatment of individual workers with chemical poisoning? In addition to a full occupational and medical history and examination, measurement of maybe all relevant BM and BEM parameters are essential, not only to assess whether the response is work-related or not, but also as guidance for treatment. Moreover, return to work should be accompanied by frequent BM and BEM, in order to prevent repeated poisoning.

Conclusion

The specific objectives of BM and BEM determine to a large extent the choice of the parameters to be measured. According to the objective, the assessment may be either simple or sophisticated.

Reference levels

The choice of an 'appropriate reference' is essential for a valid evaluation of BM and BEM data. One may use either the subject as his own reference, or a group of non-exposed workers as reference. Agent-specific tests by definition have high specificity but not necessarily sensitivity; non-agent specific tests may have high sensitivity but not necessarily specificity.

For BM most parameters are agent-specific (selective), for BEM they usually are non-specific. The reference levels can be made more specific by a proper choice of the parameter. For instance S-phenylmercapturic acid in urine is a more specific indicator for benzene exposure than phenol in urine; measurement of benzene in exhaled air or blood is by definition a specific indicator. An approach more closely related to the carcinogenic risk of benzene may be the formation of phenylguanine in urine as a reaction product of benzene epoxide with DNA[2]. In exposure to carbon disulphide 4-thio-4-thiazolidine carboxylic acid in urine is more specific than the excretion of thioethers. Assessment of diazopositive metabolites in urine in exposure to aromatic amines and of thioethers in urine in exposure to electrophilic mutagenic or carcinogenic chemicals refers to non-specific parameters of exposure; smoking is a confounder. Physical workload, alcohol (enzyme induction), food additives, pesticide residues, drugs or even tobacco may affect internal exposure and/or response. Several biological conditions, such as age (blood flow), sex (sensitivity for Pb), fatty mass, pregnancy, disease (reduced capacity) also may modify internal exposure and/or response. Shiftwork and/or novel work schedules may have an impact on the toxicokinetics and/or -dynamics of workplace chemicals (chronotoxicology)[3].

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Using the subject as his own reference requires repeated testing before exposure starts. One should never rely on one individual measurement. The reference group should be as comparable as possible with the exposed group, except with respect to the exposure of concern. This refers to for instance sex, age, hobbies, physical workload, other chemical, physical or biological exposures at and outside work, state of health, life style (smoking, consumption of alcohol, drugs, medication, nutritional habits) and preferably should live in the same region, and have a similar socio-economic class and/or job classification.

Bernard and Lauwerys[4] presented 'normal values' to be used as reference exposure or response levels in non-exposed workers; however, these levels can only be considered as a guideline, because it is possible that the appropriate reference group deviate from the 'normal value'. Each BM and BEM programme should have its own appropriate reference. Previously, with less specific analytical methods higher phenol, hippuric acid, and metal concentrations were found in urine of non-occupationally exposed subjects than today with very specific gaschromatographic, high performance liquid chromatographic and atomic absorption spectrometric methods.

Another kind of reference is the trend of the BM and BEM data in the course of time, both in the exposed and the reference group. Schulte[5] quite rightly emphasized this for study of biological markers. Markers of exposure precede response markers; early biological effects precede altered structure functions, which again precede clinical disease. Moreover, increasing the amount of data per worker increases the reliability and sensitivity of the evaluation. Graphic presentations of data for individuals and groups over a course of time should be done.

Conclusion

The choice of an appropriate reference is essential for a valid evaluation of internal exposure, health risk and state of health. Repetitive BM and BEM programmes are more valid than single measurements.

Development of protocols

In order to carry out BM and BEM programmes according to the state of the art, one should be aware of several pitfalls. The various issues discussed in the present two papers may emphasize their importance for the evaluation of the data with respect to for instance the measurement strategy, the appropriate sampling specimen, the appropriate parameter to be analysed (including analytical equality assurance), according to the specific objectives of the programmes and the agent of concern. The literature should be consulted, for instance DFG BAT-Werte, Arbeitsmedizinisch-toxikologische Begrundungen[6], documentation of Threshold Limit Values (TLV) and biological exposure index (BEI)[7] and Lauwerys[8]. The occupational health staff that have to carry out BM and BEM programmes, should critically scrutinize the toxicology databases that underpin the BOEL and BOEEL.

There is an urgent need to develop internationally agreed protocols: prescription of how to carry out BM and BEM programmes for periodic assessment of internal overexposure (BM) and of early response according to the state of the art. The protocols should be in a form which permits updating when necessary.

The WHO[9] developed the following criteria (somewhat modified by the present authors) for selection of BM and BEM parameters, particularly for routine programmes: (1) no undue expenditure of time for workers and/or medical and laboratory staff; (2) not inconvenient to workers and not carrying any health risk; (3) present data in the relevant range of occupational exposure and to be used as early predictors of health risks; (4) quantitatively relatable to the agent of concern and adverse health risks; (5) highly sensitive and specific; (6) preferably a steep exposure–response curve; (7) small analytical error and biological variability in comparison with the change in the data to be expected; (8) preferably to be conducted in several stages, advancing from simple to more elaborate procedures, according to the objective of the programmes. It should be realized that almost never will all criteria be fulfilled. Research is needed to increase compliance with these criteria.

Conclusion

There is an urgent need to develop internationally agreed protocols for BM and BEM programmes to be used in occupational health practice, and to develop tests that meet the selection criteria. The protocols should be regularly updated.

Species of the same metal

Different compounds of the same metal may carry widely different health risks. Differences in water or fat solubility, aggregation state, chemical structure and volatility may determine intake, uptake, distribution, accumulation, biotransformation and excretion and also the working mechanisms, critical organs and critical effects.

Different kinetics of organic and inorganic compounds of the same metal can be demonstrated for lead, mercury, arsenic and tin. For arsenic for example, at least three groups of arsenic compounds have to be distinguished: (a) water soluble inorganic compounds: pentoxide, trioxide, Na/K arsenate; (b) non- or low water soluble inorganic compounds: Pb/Ni arsenate, Fe-, Mg-, Pb-arsenate; (c) organic compounds in marine food. The critical organs and effects are for group (a) skin cancer, (b) lung cancer and (c) hardly any health hazard. In recent years it has been shown that in man the water soluble inorganic arsenic compounds are, to a large extent, metabolized into monomethylarsenic acid and subsequently into dimethyl-arsenic acid; the organic arsenic compounds are excreted practically unchanged[10].

For both chromium and nickel, slightly soluble compounds may induce cancer, whereas the soluble salts of hexavalent chromium, but not of nickel, cause bacterial mutations[11].

Methylmercury is very stable and has a long biological half-life compared to inorganic mercury and is predominantly excreted in the faeces, whereas inorganic mercury is mainly excreted in the urine. Other organic mercury compounds are less stable and liberate inorganic mercury.

Conclusion

Different kinetics of compounds of the same metal is the most important source of variability of metal toxicokinetics. Analytical speciation has to be carried out.

Adjustment of urinary excretion

In occupational practice urine sampling is often considered for monitoring without taking into account the inherent difficulties.

Some industrial chemicals or metabolites have a long half-life ($t_{1/2}$); in that case the time and duration of sampling of biological specimens is usually not critical. For other chemicals the duration of sampling may be critical because the compounds and/or their metabolites may be rapidly excreted in urine (or exhaled air). After exposure to trichloroethylene the concentration of the metabolite trichloroethanol in urine increases during exposure and decreases after exposure ($t_{1/2} \approx 10$ h). Therefore, for trichloroethanol not only the time of sampling is critical but also the length of the sampling period, whereas both are not critical for the other metabolite trichloroacetic acid ($t_{1/2} \approx 100$ h). Urine samples are usually collected during the last 4 hours of exposure, just before the next working shift (morning before work: 16 h after exposure), or even after the weekend (i.e. 60–64 hours after exposure).

Since the production and excretion of urine largely depends on the capacity of the kidneys to concentrate and the intake of fluids, the level of most compounds in urine has to be adjusted for the degree of dilution. In practice, various methods have been developed, such as specific gravity, osmolality and creatinine adjustment. The correction for urine dilution by means of the concentration of creatinine is in most cases the best choice. However, the concentration of creatinine in urine also depends on age, sex, muscle mass and food consumption (meat). Satisfactory results were obtained by Sedivic and Flek[12] after experimental exposure to xylenes, when the amount of metabolites excreted within a defined period of time (8 or 24 h) was recalculated according to body weight in kilogrammes. However, the retained dose of methanol correlated very well with the concentration of methanol in urine[13]. Analysis performed in a very dilute or in very concentrated urine samples does not yield reliable data.

Conclusion

It is generally necessary to correct the excretion of chemicals for dilution of the urine. Not only the point of time of sampling is critical, but also the length of the sampling period, especially for chemicals with a short $t_{1/2}$.

Measurement strategies

There does not exist one specific strategy of measurement of BM or BEM parameters. The strategy is determined by the specific toxicokinetics and/or toxicodynamics of the agent and/or its metabolites[1] and by the objectives of the monitoring programmes.

However, to date the working mechanisms and the kinetics for many chemicals are inadequately known to permit using the most appropriate strategy. Some examples may suffice to illustrate the dependence of the measurement strategy on the specific toxicodynamics and -kinetics.

For assessment of health risk in exposure to n-hexane or methyl-n-butylketone, particularly when in combination with methylethylketone, it is more relevant to measure the neurotoxic metabolite 2,5-hexanedione in blood or urine, then the concentration of the solvents in blood or exhaled air. In exposure to toluene two urinary metabolites have to be considered: hippuric acid and o-cresol, both sampled in urine. Hippuric acid appears to be a more accurate parameter than o-cresol; this probably is due to the fact that about 80 per cent of toluene is metabolized into hippuric acid, and only about 0.05 per cent into o-cresol. However, the biotransformation into hippuric acid involves hydroxylation of the side chain, whereas o-cresol results from formation of an epoxide in the aromatic ring; epoxide formation is likely to be more related to toxic risks than side chain oxidation[14]. A drawback for measuring hippuric acid is that the level in urine in the reference group is already up to 1.5 gram/gram creatinine (g/g creat), and highly dependent on consumption of some vegetables, preserved food and beverages containing benzoic acids whereas o-cresol is up to 0.3 mg/g creat in urine of the reference group. Therefore, the individual margin between the reference level and the BOEL of hippuric acid (BEI 2.5 g/g creat) and o-cresol (about 2 mg/g creat) is small or even non-existent. To date, measurement of hippuric acid is usually recommended but it still is not yet decided which of the two metabolites should be preferred in connection with assessment of the health risk. In exposure to trichloroethylene the DFG[6] has chosen as BOEL the concentration of the (free) metabolite trichloroethanol in blood at the end of the workshift, because the effect on the central nervous system appears to be mainly due to free trichloroethanol; trichloroethylene itself appears to exert a narcotic action at very high exposure levels. Trichloroethanol reaches a maximum level in blood almost directly after the end of exposure; peak exposures hardly affect the trichloroethanol level at the end of the workshift. The BOEL of 5 mg trichloroethanol/l blood adopted by the DFG-1989 can be regarded as an individual maximal BOEL because it is based on the percentile distribution[1]. However, the BOEL of 100 mg trichloroacetic acid per litre in urine is not based on the percentile distribution, but on the mean concentration after exposure to trichloroethylene for several weeks. The toxic action of dichloromethane (methylenechloride) is mainly based on the formation of carbon monoxide (CO). However, in exposure to dichloromethane for less than 3 h the percentage COHb has hardly increased; in that case the solvent itself should be measured in blood or alveolar air (narcotic action). Therefore, the duration of exposure determines the parameter to be measured. Moreover, the biotransformation into CO proceeds in the first hours after exposure; consequently the maximum COHb concentration is obtained 0-2 h after exposure and the $t_{1/2}$ seems to be longer (12–16 h) than in workers exposed to CO itself (6–8 h). Sampling of blood (per cent COHb) or alveolar air (CO) should take place not only at the end of the workshift, but preferably also 1 to 2 h thereafter. Peak exposure to dichloromethane hardly affects the per cent COHb in blood or CO in alveolar air, in contrast to exposure to CO itself. Exposure to tetrachloroethylene and most other solvents with a direct action on the central nervous system exerts a narcotic action. The levels in blood or alveolar air during exposure mainly reflect the actual levels in inhaled air. The solvent levels in blood or exhaled the following morning (16 h after the last workshift) are more related to the time weighted average exposure during the last workshift(s); when sampled 64 h after the weekend the levels reflect the cumulative concentration in adipose tissue.

The measurement strategy for chemical parameters of BEM usually are much less dependent on the point of time and duration of sampling than parameters of BM. The ACGIH list[15] of BEI considers the point of sampling blood for the measurement of the cholinesterase activity in red cells 'discretionary'; the DFG-list mentions a point of time of sampling: the end of the workshift or in long term exposure after several workshifts. Measurement of ZPP in erythrocytes after exposure to inorganic lead is independent of the point of time when the workers have been exposed for at least one to two months.

Conclusion

The measurement strategies depend on the working mechanisms and kinetics of the chemical. Therefore, the biological specimens and the time of sampling may be critical. For short term effects (BEM) different parameters have usually to be measured than for long term effects.

Conclusions and recommendations

Conclusions

There exists a geographic difference with respect to the development of BM and BEM programmes and in the methods applied[1].

The validity of the large majority of present OEL is moderate to poor; the same is true for BOEL, derived from these OEL[1].

BOEL derived from the correlation between parameters of BM and BEM are more valid than those derived from parameters of EM and BM[1].

The use of the term 'biomonitoring' creates confusion, because BM and BEM serve different objectives and may lead to different preventive measures[1].

BM and/or BEM programmes differ according to explicitly defined objectives of the programme.

BM and BEM programmes often apply non-appropriate reference data.

For about ninety often widely used industrial chemicals at least tentative BOEL are available[1].

BOE(E)L largely neglect the intra- and interindividual variability in internal exposure and in coping capacity, of groups or individuals at risk[1].

BM of metals often neglects the difference in toxicokinetics and -dynamics between compounds of the same metal. This leads to unwarranted confidence in BOE(E)L.

In analysis of urine samples there is no overall practicable method to adjust for the dilution of urine.

Unethical behaviour of management has a negative impact on the acceptance by workers of BM and BEM programmes[1].

BM and BEM programmes do not lead to infringement on the human rights of exposed workers, if there exists a good confidence relationship between the occupational health staff and the workers[1]

Recommendations

Internationally coordinated research should be carried out in order to develop valid programmes for BM and BEM and to expand the numbers of BOE(E)L. The emphasis should be on filling the gaps in knowledge in toxicokinetics and the factors which affect these[1].

Research should be carried out to improve the sensitivity and the specificity of the parameters of BM and BEM.

In the case of BM analysis of toxic compounds should have preference above analysis of the agent as such[1].

Toxicokinetic models based on data from individual workers should be developed, in order to get information on the variability and the cause of this[1].

Research should be carried out on the intra- and interindividual variability of workers exposed to chemicals; this identifies groups or individuals at extra risk[1].

Development of internationally agreed protocols on how to carry out BM and BEM programmes should have a high priority.

BOE(E)L should also present the 90 per cent confidence intervals[1].

Graphic presentation of BM and BEM data in periodic monitoring should always be made available to the group and individuals examined.

BM of breastmilk of lactating previously or currently exposed mothers should be promoted[1].

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