Genotyping and phenotyping of metabolic enzymes relevant for the interpretation of biomarkers of exposure

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Abstract

In recent years a lot at progress has been made in the field of the identification and the functional significance of genetic polymorphisms for several drug metabolizing enzymes (DME). Powerful technologies are becoming available for the phenotyping of these enzymes. This review attempts to highlight the interest of genotyping and/or phenotyping of DMEs for a more accurate interpretation of biological monitoring in the field of industrial and/or environmental toxicology. It summarizes human field studies already performed in populations exposed to two model compounds, i.e. polycyclic aromatic hydrocarbons (PAHs) or styrene.

Keywords

Biological monitoring, drug metabolizing enzyme, genotype, phenotype.

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Exposure to chemical agents at the workplace or in the environment can be assessed either by measuring the concentration of the agent in the air by stationary or personal sampling (ambient monitoring), or by measuring relevant biological parameters (biological monitoring). Biological monitoring of exposure is based on the measurement of "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" (1). In general, to be of interest to the biological monitoring of occupationally and/or environmentally exposed individuals, a candidate biomarker of exposure must fulfill some conditions among which (a) to specifically assess exposure to the chemical under investigation, (b) to be sufficiently sensitive to detect subjects exposed to low levels of chemicals, (c) to vary quantitatively with the intensity of exposure and/or the risk of development of adverse effects, (d) to yield more information on potential health risk than obtained by ambient monitoring, (e) to be stable enough to allow storage of the sample for a certain period of time, (f) not to entail too much discomfort or any health risk for the subject, (g) to be measured by an analytical method presenting sufficient accuracy, specificity and sensitivity and (h) not to need too time consuming, complex or expensive methods (2). Such ideal biomarkers of exposure do not exist and generally a compromise has to be made.

For the interpretation of biological monitoring data, measured values are to be compared with *reference limit values*, established on the allowance of the uptake of a certain amount of a chemical agent which is considered to be acceptable for the preservation of health of the subject. Such reference limit values are proposed by several national or international organisations such as the American Conference of Governmental Industrial Hygienist (ACGIH) which proposes Biological Exposures Indices (BEI) or the "Deutsche Forschungsgemeinschaft" (DFG) which proposes "Biologischer Arbeitsstoff-Toleranz-Wert" (BAT).

As mentioned in (c), the most useful biomarkers of exposure should be "health-based", i.e. derived from long term follow-up studies of workers allowing the definition of an exposure level without adverse effects in the majority of the workers. Under those conditions limit values may be set on the basis of dose-effect/response relationships and such values are generally called ***"biological action levels"*** (BAL) (figure 1). Examples of biomarkers for which a quantitative relation between internal dose and adverse health effects have been identified are relatively scarce: lead in blood, cadmium in urine or carboxyhaemoglobin.

For most substances however the dose-effect/response relationship is insufficiently assessed and biological limit values are therefore derived
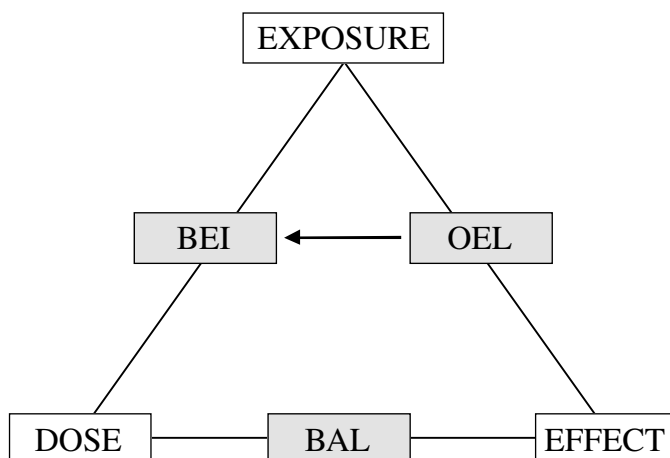
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Fig. 1: Relationship between exposure, internal dose and adverse effects.
While biological action levels (BAL) are derived from dose-effect (response) relationships,
biological exposure indices (BEI) can be estimated for a given occupational exposure
level (OEL) from the relationship between ambient concentration
and the concentration of the appropriate biomarker of internal dose.

from occupational exposure limits (OEL) in air such as threshold limit values (TLVs) proposed by the ACGIH. These limit values are then called *"**biological exposure indexes**"* (BEI) as they represent the concentration of the agent that will occur in the body fluids after an eight hour time weighted average exposure at the OEL (figure 2). Under these conditions, biological monitoring is more an assessment of exposure intensity than of the potential risk to health (2).

So far, BEIs have been developed on the assumption that individuals do not differ significantly in their biotransformation capacities. It is clear, however, that is not the case because wide inter-individual differences exist in the metabolism of xenobiotics. Among the sources of variability, inter-individual differences in uptake (lung or skin), metabolic and nutritional heterogeneity may be cited. As illustrated in figure 2, a fraction of the variability observed in the relationship between the concentration of a biomarker in a body fluid and the ambient concentration of the corresponding toxicant could also be explained by differences in biotransformation capacities. When dealing with these inter-individual variability factors, two situations must be clearly distinguished: (a) the measured biomarker is directly implicated in the toxicity mechanism of the chemical. In this case, only its concentration is of importance for an health assessment without a need to take into account any inter-individual variability factor because, by integrating
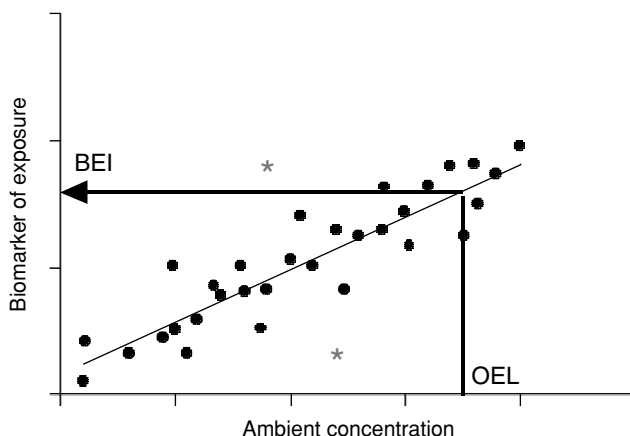
Fig. 2: Relationship between ambient concentration of a xenobiotic
and the concentration of the appropriate biomarkers of exposure.
This figure illustrates (1) the establishment of a biological exposure index (BEI)
corresponding to a given occupational exposure level (OEL) and
(2) the wide inter-individual variation generally encountered when using such approaches.
Two "outliers" representing "high" and "low" responders are represented (*).

such variability factors, this kind of biomarker offers a clear advantage com-
pared to ambient monitoring. The specific carboxylic acids produced *in
vivo* from ethylene glycol derived ethers (e.g. methoxyacetic acid in urine
(MAA) to assess exposure to methoxyethanol,…) or 2,5-hexanedione as
a specific urinary metabolite for n-hexane exposure illustrate this situation.
(b) When the link between the biomarker of exposure and the toxicity
mechanism is not clearly established or even absent, there is a need to
reduce the inter-individual variability observed in figure 2 in order this time
to better assess individual exposure. This is of importance and still remains
a better approach than ambient monitoring especially to assess residual
exposure when individual protective devices are worn (which is a more
and more frequent practice in many countries). To that purpose, integra-
tion of data on individual metabolic capacity could represent a significant
refinement to the interpretation of currently used BEI by integrating, for
example, data on genotype and/or phenotype of metabolic enzyme rele-
vant for the biotransformation pathway of the chemical of interest. This
short overview attempts to highlight the potential interest of the latter
approach and summarizes human field studies already performed in pop-
ulations exposed to polycyclic aromatic hydrocarbons (PAHs) or styrene.

    In order to select adequate genotyping and/or phenotyping methods,
a detailed characterisation of the biotransformation pathways followed by

the chemical of interest and the identification of particular enzyme isoforms implicated in each step of the metabolism are, of course, of primordial importance (3). Most of these preliminary studies are performed *in vitro* using human liver microsomes. The reactions catalyzed by xenobiotic-biotransforming enzymes are generally divided into two groups, called phase I and II. Phase I reactions involve hydrolysis, reduction and oxidation. These reactions expose or introduce a functional group (-OH, -NH$_2$, -SH or –COOH), and usually result in only a small increase in hydrophilicity. Phase II biotransformation reactions include glucuronidation, sulfation, acetylation, methylation, conjugation with glutathione (mercapturic acid synthesis) and conjugation with amino acids (such as glycine, taurine and glutamic acid). The cofactor for these phase II reactions reacts with functional groups that are either present in the xenobiotic or are introduced/ exposed by phase I biotransformation. Most phase II biotransformation reactions result in large increase in xenobiotic hydrophilicity, hence they greatly promote the excretion of foreign chemicals. Among all the enzymes implicated in the biotransformation of xenobiotics, several are of primordial importance for the biotransformation of industrial and/or environmental toxicants (generally smaller molecules than drugs). For phase I reactions, cytochrome P450 (CYP), in particular isoforms CYP1A1 and CYP2E1, have been involved in the metabolic activation of many precarcinogens such as PAHs for CYP1A1 and benzene, dimethylnitrosamine and vinyl chloride for CYP2E1. Another phase I enzyme largely implicated in the biotransformation of industrial and/or environmental toxicants is microsomal epoxide hydrolase (mEH)[1]. This enzyme catalyses the hydrolysis of reactive aliphatic and arene epoxides generated by CYP enzymes to more water soluble dihydrodiol derivatives (detoxification pathway). In certain instances however, chemical products of mEH metabolism (*trans*-dihydrodiols) may be further derivatized to secondary epoxide species which are poor substrates for mEH. These diols-epoxides are often highly reactive and have been involved in processes such as teratogenesis and initiation of cancer. Finally, as phase II enzymes, gluthathione S-transferases (GSTs) are a family of dimeric enzymes which play an important role in the detoxification of numerous industrial and/or environmental toxicants. These enzymes catalyze the conjugation of electrophiles with glutathione thereby inactivating potential cytotoxic and/or genotoxic substances. Other enzymes of importance for the biotransformation of industrial chemical (N-acetyl transferases, UDP-glucuronosyl transferases, sulfotransferases,…) will not be discussed in this minireview.

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[1] mEH is also called EPHX1.

Application of the genotyping methods

For all previously cited enzymes, differences in the gene nucleotide sequence, called genetic polymorphisms, have been described in the general population, including Caucasians. Each gene can be found in a wild and most frequent form, called "wild allele", or in one (for bi-allelic polymorphisms) or more (for multi-allelic polymorphisms) variant forms, called "variant or rare alleles". As each gene is present in double exemplar a particular genotype is defined as a combination of two alleles (hetero- or homozygotes). Such polymorphisms can have an effect on the enzymatic function and ideally, the relationship between a particular genotype and phenotypic catalytic activity of the enzyme should be established at least *in vitro*. For *CYP1A1* (located on chromosome 15), two polymorphic loci have been elaborately studied. The polymorphism of greatest interest is located in the catalytic region of CYP1A1 (exon 7) and leads to the substitution of isoleucine by valine (Ile/Val). The presence of Valine is thought to increase the catalytic activity of the enzyme (4). This mutation is closely linked with another polymorphism which can be detected by a MspI restriction fragment length analysis and is characterised by two alleles: m1 (absence of MspI site) and m2 (presence of MspI site). The m2 allele is believed to be associated with a higher enzyme inductibility (5). For *CYP2E1* (located on chromosome 10), three different polymorphisms detectable with TaqI (A2/A1), DraI (D/C), RsaI and PstI (c1/c2) restriction enzymes (6) as well as a 96 bp-insertion polymorphism (7,8,9) have been described. While reduced CYP2E1 activity in the presence of the rare C allele (*CYPZE1*6*) has been suggested (10, 11), the *in vivo* significance of most of these polymorphisms is, as yet, far from clear. For mEH (located on chromosome 1), two polymorphic sites have been observed in exon 3 (Tyr113/His113) and exon 4 (His139/Arg139). Based on *in vitro* studies, the variant allele of one of these sites (exon 3) correlated with reduced mEH activity whereas the variant in the other site (exon 4) resulted in increased mEH activity (12). Finally, for the cytosolic GSTs which include seven subfamilies (A, K, M, P, S, T and Z), two polymorphic genes have been largely investigated, i.e. *GSTM1* and *GSTT1*, and it has been estimated that about 50 and 15% of Caucasians lack the gene, respectively.

Biological monitoring of exposure to PAHs

As a biomarker of human exposure to PAH, urinary 1-hydroxypyrene (1-OHP) has been largely studied and validated (2) (figure 3). 1-OHP is in fact a highly fluorescent metabolite of pyrene, a component commonly pre-

sent in PAH-containing mixtures and considered as the most abundant PAHs in coal tar. This metabolite is excreted in urine as the corresponding glucuronide. After enzymatic hydrolysis and a purification step, it can be quantified in urine by HPLC with fluorescence detection. This biomarker is very useful because it takes into account all exposure routes, which seems of primordial importance for substances with high degree of per-cutaneous penetration such as PAHs. At least two enzymes (or enzyme families) are of particular interest in the biotransformation of PAHs: CYP1A1 and GSTs, involved in their activation and detoxication.
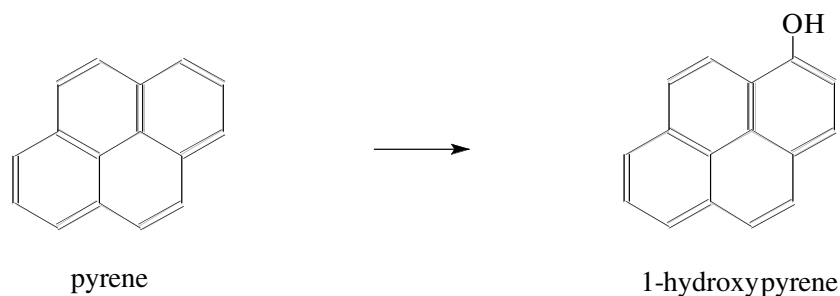

pyrene   1-hydroxypyrene

Fig. 3: Biotransformation of pyrene to 1-hydroxypyrene.

Probably one of the first studies designed to examine the influence of genetic polymorphism on the relationship between external PAH expo-sure and urinary metabolites used as biomarkers of internal exposure was performed by Costa et al. (13). The 46 female non-smokers selected for that study lived and worked most of their lives in highly polluted industrial area of Bohemia (Czech Republic) and spent a significant portion of their working days outdoors. Personal exposure monitors were started at the beginning of the workshift and ran continuously for 24 hours. At the end of this period, urine samples were collected for the measurement of urinary metabolites (data reported as the total of 28 parent PAHs and their hydrox-ylated metabolites including 1-OHP). The ambient PAH concentrations reported were the sum of 6 different PAHs considered as carcinogenic (pyrene measurement not included). Two different genetic polymorphisms were examined in this study: *GSTM1* and *NAT2* (N-acetyltransferase (slow and rapid acetylators)). The latter enzyme metabolizes nitro-PAHs gener-ally found in the air at 10-100 times lower concentrations than PAHs but is not directly involved in PAHs biotransformation. Personal PAH exposure was significantly correlated with urinary PAH metabolites for the whole pop-ulation study (r = 0.36; p = 0.01). In order to test the influence of genetic polymorphisms, the population was divided into high ($\geq$9.4 ng/m^3 air) and

low ($<$ 9.4 ng/m^3 air) PAH exposure groups based on the mean exposure level of the entire cohort. Significantly lower urinary metabolites values were observed with the combination *GSTM1 + /NAT2 rapid* compared with *GSTM1-/NAT2 slow*, especially for the high PAH exposure group (p = 0.03). Thus this study provided evidence that gene-environment interaction influences urinary PAH metabolite levels and suggested "the necessity of including genotype in exposure studies especially when urinary PAH metabolites are used as markers of exposure".

Another field study published in the same year (14) was designed to investigate whether CYP1A1 MspI genotype modulates the relationship between individual occupational exposure to PAHs and urinary 1-OHP concentrations. It was conducted among 80 coke-oven workers in Taiwan. Air measurements were conducted over 3 consecutive days and pre- and post-shift urinary samples were collected (i.e. morning of day 1 and end of day 3, respectively). Multiple linear regression showed significant effects of individual exposure to air PAHs and preshift 1-OHP on post-shift 1-OHP concentrations (p = 0.002 and p $<$ 0.001, respectively). After adjusting for preshift 1-OHP concentrations and air PAHs, subjects with the homozygous variant genotype had a 2-fold higher post-shift 1-OHP levels than the combined wild-type and heterozygous workers (p = 0.04). These authors concluded that "*CYP1A1* MspI variant genotype can modify the metabolism of PAHs in coke-oven workers". It must be stressed however that the observed frequency of the MspI homozygous variant genotypes of CYP1A1 was 15% in this study (Taiwanese population) compared to less than 1% in Caucasians.

Using a different approach, Brescia et al. (15) have analysed the influence of three genetic polymorphisms for *CYP1A1* (MspI and Ile/Val) and *GSTM1* on the relationship between PAH exposure (assessed this time by measuring urinary post-shift excretion of 1-OHP and not by using airborne PAH measurements) and the level of three different biomarkers of effective dose (PAH-DNA adducts, nitro-PAH adducts to Hb and micronuclei frequency). The study sample consisted of 76 coke-oven workers employed at a steel plant (Italy) and 18 non-occupationally PAH-exposed workers recruited from a "ship repairing factory" located in the same town as a control group. In a stratified analysis, individuals were classified in high or low groups ($\geq$66th or $<$66th percentile for the entire population, respectively) for both PAH exposure parameter and biomarkers of effective dose. Significantly (p = 0.03 and p = 0.01) higher percentages of *GSTM1*-subjects compared to *GSTM1 +* subjects and of *CYP1A1* Ile/Val individuals compared to *CYP1A1* Ile/Ile individuals were detected for high levels of PAH-DNA adducts in the high exposure group (namely high levels

of 1-OHP). However, no difference was observed between individuals possessing the MspI site for *CYP1A1* and those with the wild type (MspI-). In a logistic regression modelling, a statistically significant association was observed between increased PAH-DNA adduct levels and the *GSTM1*-genotype (p = 0.03). The authors concluded that "a gene-environment interaction between PAH exposure and two metabolic genotypes involved in activation (CYP1A1) and detoxification (GSTM1) of PAHs, respectively, had been identified". Furthermore, in the case of CYP1A1, they confirmed that the polymorphism of greatest biological interest was the Ile/Val site, which is located in the catalytic region of the enzyme. The influence of CYP1A1 polymorphism detected in this study is, however, difficult to inter-pret because both parameters used to characterize exposure (1-OHP in urine) and biologically effective dose (PAH-DNA adducts) are expected to be equally influenced by this polymorphism.

In a more recent study aiming at (a) evaluating the correlation between external exposure and 1-OHP excretion used as biomarker of exposure and (b) investigating to what extent genetic polymorphism in metabolic enzymes could explain interindividual variation in urinary 1-OHP levels, Alexandrie et al. (16) have measured, at the end of a workweek, airborne PAHs (including pyrene) during a full workday, 1-OHP in pre- and post-shift urinary samples, and eight polymorphisms for *CYP1A1, EPHX, GSTM1, GSTP1* and *GSTT1*. 98 workers of a Swedish aluminium production plant and 55 controls (postmen and city council employees of the same town) were examined. The authors found a weak but significant relationship between end-of-shift 1-OHP excretion (expressed as µmol/mol creatinine) and airborne particulate-associated PAHs individual exposure (r = 0.37; p < 0.001). The inter-individual difference in excretion of 1-OHP was vast (>100-fold) and multivariate regression analysis ultimately showed that the part of the variance that could be explained by differences in bio-transformation genotypes seemed to be of the same order of magnitude as the variance explained by differences in exposure (the total variance explained by the model was 0.28). The only two genotypes retained by this model were *CYP1A1* (Ile/Val) and *GSTM1* (+/−) so that the highest 1-OHP levels were observed in individuals carrying the *CYP1A1* Ile/Val genotype ("variant allele") who were also of the *GSTM1* null genotype. *CYP1A1* MspI genotype was, again, not retained by the model.

All these studies highlight the need to take into account at least two genetic polymorphisms (namely *CYP1A1* Ile/Val and *GSTM1*) for a more accurate interpretation of urinary 1-OHP values when used as a biomarker of exposure in Caucasians. A further step in this approach would be to pro-pose different BEIs based on the genotypic characteristics of the workers.

Biological monitoring of exposure to styrene

So far, biological monitoring of exposure to styrene is routinely achieved by the measurement of mandelic (MA) and phenylglyoxylic (PGA) acids in urine collected at the end of the shift and/or prior to the next shift (i.e. 16 hours after exposure) (figure 4). BEIs have been recommended for this purpose by the ACGIH (1040 and 400 mg/g creatinine for the sum MA + PGA, respectively). Recently, Ghittori et al. (17) proposed the measurement of specific mercapturic acids (M1 and M2, figure 4) in post-shift
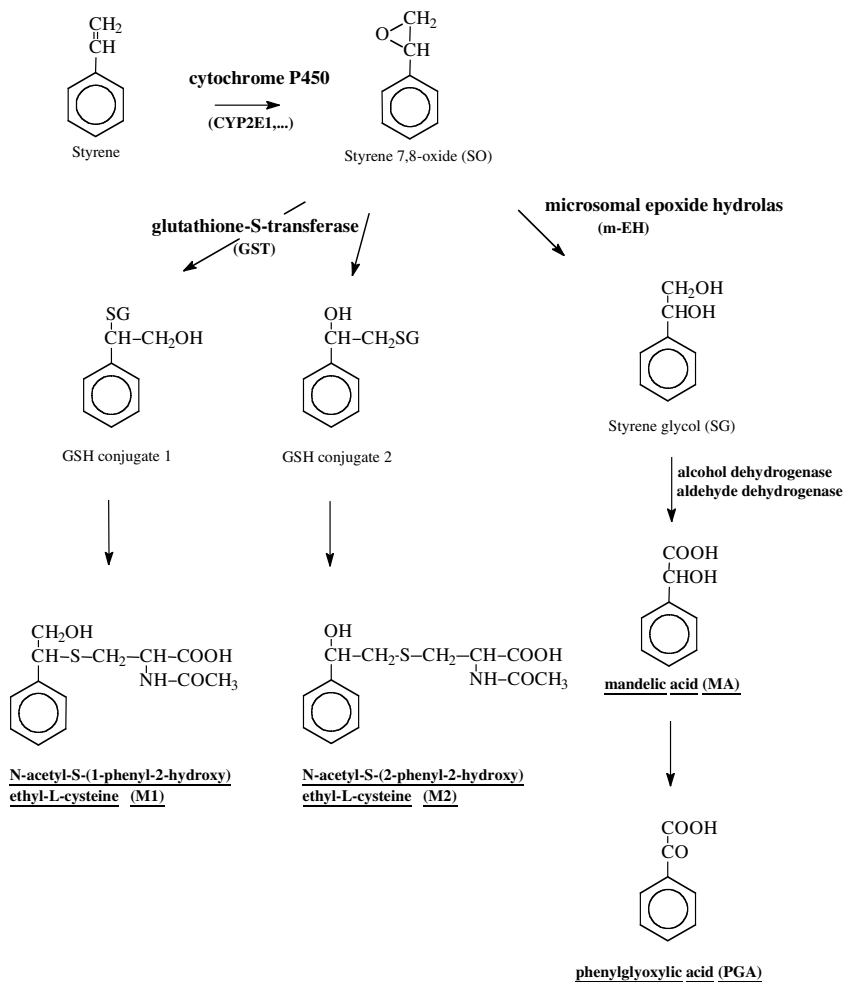
Fig. 4: Biotransformation pathways of styrene.

urine for the biological monitoring of workers exposed to styrene but the authors reported a wide variation in the relationship between air concentrations and urinary metabolites. Several drug metabolizing enzymes are implicated in the biotransformation of styrene in humans (figure 4). The conversion of styrene (S) to styrene 7,8-oxide (SO) is catalysed *in vitro* by CYP2E1 (18, 19) but other isoforms, particularly CYP2B6, could also be involved at higher exposure levels (19, 20) and a contribution of CYP2F1 has also be mentioned (21). In humans, to the best of our knowledge, the exact *in vivo* contribution of CYP2E1 in the formation of SO remains to be determined. The subsequent detoxification of SO involves two distinct metabolic pathways: it can be hydrolysed to styrene glycol (SG) or conjugated with glutathione (GSH). The major pathway in humans consists in hydrolysis of SO by mEH to form SG. This metabolite is then oxidised by alcohol and aldehyde dehydrogenases to MA that can be either excreted as such in urine or further oxidised to PGA, which is also excreted by the kidneys. MA and PGA represent more than 95% of urinary metabolites of styrene. Conjugation represents a minor biotransformation pathway and, following the reaction between GSH and SO (R- and S- enantiomers), each mercapturic acid excreted in urine [N-acetyl-S-(1-phenyl-2-hydroxy) ethyl-L-cysteine (M1) and N-acetyl-S-(2-phenyl-2-hydroxy)ethyl-L-cysteine (M2)] consists of two diastereoisomeric forms: M1-S, M1-R and M2-S, M2-R, respectively (figure 4). The latter metabolites have been measured in low concentrations in urine of workers occupationally exposed to styrene (17).

To the best of our knowledge, the only field study designed to evaluate the influence of genetic polymorphisms on styrene metabolite excretion profile and thus on the interpretation of biological monitoring data has been conducted in our laboratory (22). In this study, we have analysed the influence of seven genetic polymorphisms for *CYP2E1* (c1/c2, D/C and A1/A2), *EPHX* (Tyr113/His113 and His139/Arg139), *GSTM1* and *GSTT1* on the relationship between styrene exposure (assessed by personal ambient measurement during the whole workshift) and the level of two different biomarkers of exposure (sum MA + PGA and sum M1 + M2). The study population consisted of 30 workers of a fiberglass-reinforced plastics factory (Belgium) who did not wear protective devices (mask or gloves) so that this study population provided a relevant model to analyse the relationship between external and internal parameters. Urinary samples were collected at the end of the shift for the determination of MA, PGA, M1 and M2. We found a better correlation between external and internal exposure estimated by urinary MA + PGA ($r = 0.92$; $p < 0.0001$) than when urinary M1 + M2 was used ($r = 0.74$; $p < 0.0001$). Two "metabolic indexes" (derived from the ratio between the sum of urinary metabolites for a
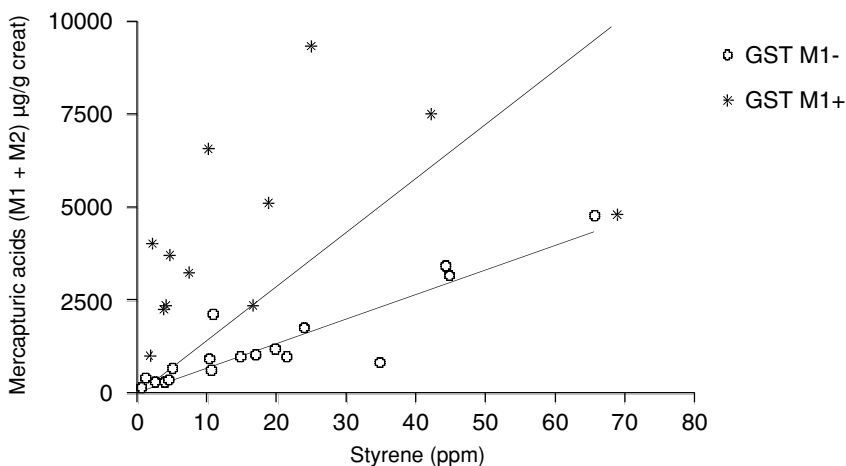
Fig. 5: Relationship between airborne styrene and the sum of mercapturic acids (M1 + M2) in end-of-shift urinary samples taking into account the GSTM1 allelic status (from Haufroid et al. (22)).

specific pathway and ambient styrene concentration) were calculated for each worker and compared for different allelic combinations. Monovariate analyses showed that *GSTM1* polymorphism was clearly the most significant parameter influencing M1 + M2 urinary concentrations. Furthermore, based on *GSTM1* allelic status, two different BEIs for M1 + M2 in post-shift urinary samples corresponding to the recently adopted TLV for styrene (20 ppm) were proposed (*GSTM1*null: 1330 µg/g creatinine, *GSTM1* + : 2878 µg/g creatinine) (figure 5). Multivariate regression analyses revealed that the presence of the rare A1 allele of *CYP2E1* was associated with increased urinary concentrations of metabolites through both pathways (p = 0.007 and p = 0.021 for MA + PGA and M1 + M2 pathways, respectively). The two previously described polymorphisms for *EPHX* gene were also tested but seemed not really relevant for biomarkers interpretation. We concluded that "while *CYP2E1* genotyping, particularly assessment of the A1/A2 allelic status, is useful for a more accurate interpretation of urinary biomarkers concentration, *GSTM1* genotyping is absolutely necessary when considering a biological monitoring program based on urinary mercapturic acids determination". Practically, this knowledge may in the future lead to development of more individualised BEIs calculated on the basis of relevant determinants such as age, BMI but also genetic polymorphisms for DME, for each individual separately (22).

Potential usefulness of phenotyping methods

In association with genotyping methods, the knowledge of the state of induction of a particular drug metabolising enzyme (i.e. its phenotypic status) may be of importance and could have practical applications in the field of the biological monitoring of exposure to chemical agents. Despite the clear advantage of giving a more integrated idea of enzyme activity, a specific limitation of this phenotypic approach in the framework of a biological monitoring programme is, however, that, contrary to data obtained with genotyping methods, phenotype is not constant with time and has to be re-evaluated each time a biological programme is implemented. To illustrate the latter approach, phenotyping of CYP2E1 will be discussed in this review.

Although the most accurate assessment of CYP2E1 activity is based on direct hepatic 2E1 measurement and therefore requires a liver biopsy, alternative approaches have been developed to be used on a large scale with non-invasive methods. Until now, the "gold standard" method requires the use of a probe drug (chlorzoxazone, CZX) which has to be administered to the individual for which a phenotyping test is needed. CZX is a drug formerly used as a myorelaxant and which is extensively metabolised in the liver to 6-hydroxychlorzoxazone (HCZX), a reaction mediated by CYP2E1 (23) (figure 6). The chlorzoxazone test is generally based on the administration of a single oral 500 mg (or 250 mg) dose to fasting individuals. Various CZX pharmacokinetic indexes have been proposed to reflect CYP2E1 activity, as recently reviewed by Streetman et al. (24): plasma CZX $t_{1/2\beta}$, HCZX renal excretion, CZX oral clearance, fractional clearance to HCZX and HCZX area under the concentration curve (AUC). Among these, CZX fractional clearance to HCZX is the most direct measurement of CYP2E1 activity and often serves as a standard method to which other proposed approaches are compared. However this procedure is time consuming, unconvenient (urine collection,…), and requires subject compliance and catheterization for up to 12 hours. The ratio of plasma
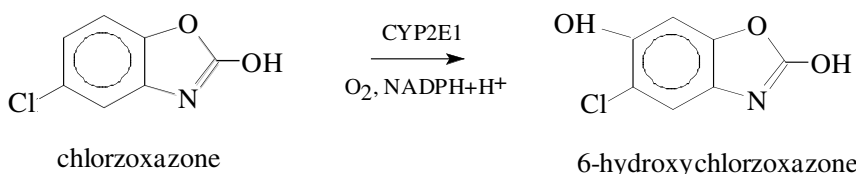

Fig. 6: Biotransformation of chlorzoxazone to 6-hydroxychlorzoxazone.

CZX/HCZX concentrations (chlorzoxazone metabolic ratio, CMR) measured 2 hours after the oral CZX dose has been widely used as an alternative to CZX fractional clearance but the correlation between both methods is quite low (r^2 = 0.16-0.28) suggesting that single-point ratios do not accurately measure CYP2E1 activity (24). Furthermore, recent *in vitro* studies suggest that CYP1A1, CYP1A2 and possibly CYP3A are involved in CZX 6-hydroxylation limiting the specificity of CZX for CYP2E1 phenotyping (24).

CYP2E1 phenotyping on a large scale, such as for the screening of large numbers of samples, for epidemiological studies or even as an adjunct to improve a biological monitoring programme, requires the implementation of more practical methods. Expression of CYP2E1 in the lymphocyte fraction of white blood cells appears to be influenced by the same factors that regulate the expression of the hepatic enzyme, including xenobiotics and physiological states (24). In a recent report (25) involving 51 human subjects (26 alcoholics and 25 controls), pharmacokinetic parameters for chlorzoxazone hydroxylation (CZX clearance rate and CZX AUC) were compared with CYP2E1 protein content and CYP2E1 mRNA content in human peripheral blood lymphocytes (HPBLs). Alcoholics exhibited a 2-fold elevation in lymphocyte CYP2E1 mRNA and protein compared to non-alcoholics. CZX clearances rates were 1.9-fold higher and CZX AUC values 1.8-fold lower in alcoholic individuals compared to non-alcoholics. Furthermore, CZX clearance rates correlated (r = 0.55; p < 0.01) with lymphocyte CYP2E1 mRNA content, and transcript levels further correlated (r = 0.52; p < 0.001) with CYP2E1 protein content in lymphocytes (25). However, determination of CYP2E1 protein content required a large amount of blood. Thus, monitoring for lymphocyte CYP2E1 expression by the measurement of specific mRNA may provide a non-invasive alternative for estimating hepatic activity of this enzyme but practical and easier methods for CYP2E1 mRNA measurement in HPBLs have to be developed for a routine use. We have recently validated a method for CYP2E1 mRNA quantification in HPBLs by real-time reverse transcription PCR (26) and the biological value of this tool is currently investigated.

Conclusion: consequences for every day practice

When considering the inter-individual variability of a biomarker of exposure, two different situations have to be distinguished. In the first case, when the metabolite that is used as a biomarker of exposure is involved in the toxic process (for instance, 2.5-hexanedione), the variability with

which this metabolite is formed measures a difference in susceptibility and this is of course the ideal situation for a biomarker. But these cases are rare and in most situations the metabolite that is used as biomarker of exposure is not directly involved in the toxic process, and it is important to understand the causes of this variability for a proper interpretation. In such cases the knowledge of the genotyping status and/or the level of expression of biotransformation enzymes improve the interpretation of biomonitoring data. In the future, it is likely that different BEIs will be proposed based on genotype and/or phenotype characteristics of a worker. This approach will lead to a new concept of "individualised" interpretation of biological monitoring of chemical exposure taking into account differences (when relevant, i.e. no relationship with toxic process) in biotransformation capacities of the workers.

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