BIOMARKERS, 1998, VOL. 3, NO. 4/5, 239–303

REVIEW

Mercapturic acids as biomarkers of exposure to electrophilic chemicals: applications to environmental and industrial chemicals

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Received 3 July 1997, revised form accepted 9 January 1998

The use of mercapturic acids (N-acetyl-L-cysteine S-conjugates, MAs) in the biological monitoring of human exposure to environmental and industrial chemicals is receiving more and more attention.

Mercapturic acids (MAs) are formed from glutathione (GSH) S-conjugates via the MA-pathway. Although this pathway can lead to different end-products, the formation of MAs is the predominant route in most species, including man. Two GSH S-transferases (GSTs) show genetic polymorphisms in humans and this can have major consequences for individual susceptibility to toxic effects and for MA formation.

In occupational toxicology, adducts to biomacromolecules are also used as biomarkers. DNA adducts are a measure for the effective dose, while protein adducts are related to the dose at critical site. Both type of adducts are normally determined in blood, while MAs are determined in urine. Most MAs are excreted with relatively short half-lifes, allowing a direct evaluation of the occupational circumstances. For many compounds similar (linear) dose-dependency was found for MA excretion, formation of macromolecular adducts, and for various biomarkers of toxic effects. These relations together with fact that MAs relate to the electrophilic character of compounds, allows for the conclusion that MAs are biomarkers of toxicologically relevant internal doses of chemicals or their metabolites.

An overview will be given here of the use of MAs in the assessment of internal human exposure to electrophilic environmental and industrial chemicals. Additionally, the formation of GSH S-conjugates, their catabolism to MAs and several of the frequently used analytical approaches are discussed. When appropriate, the influence of genetic polymorphisms on formation of MAs and on susceptibility to toxicity will be discussed for different chemicals as well.

Keywords: biomarker, glutathione S-conjugate, environmental chemical, industrial chemical, mercapturic acid, urine.

Abbreviations: AC, allyl chloride; α-CH, α-chlorohydrin; ALMA, allyl mercapturic acid; BEI, biological exposure index; BEM, biological effect monitoring; BM, biological monitoring; CCNAT, cysteine S-conjugate N-acetyltransferase; CHPMA, 3-chloro-2-hydroxypropyl mercapturic acid; DCP, E- and Z-1,3-dichloropropene; DCVMA, 1,2- and 2,2-dichlorovinyl mercapturic acid; DHBMA, 3,4-dihydroxybutyl mercapturic acid; DP, cysteinylglycine dipeptidase; CPMA, E- and Z-3-chlor-2-propenyl mercapturic acid; ECH, epichlorohydrin; EM, environmental monitoring; GGT, γ-glutamyltransferase; GSH, glutathione; GST, glutathione S-transferase; HBMA, 2-hydroxy-3-butenyl mercapturic acid (or 1-hydroxymethyl-2-propenyl mercapturic acid); HEMA, 2-bydroxyethyl mercapturic acid; HPMA, 3-hydroxypropyl mercapturic acid; HS, health surveillance; MA(s), mercapturic acid(s) (=N-Acetyl-L-cysteine S-conjugate(s)); MCMA, N-methylcarbamoyl mercapturic acid; PGE, phenyl glycidyl ether; PMA, phenyl mercapturic acid; SIM, selected ion monitoring; TLV, threshold limit value; TWA, time weighted average.

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1354-750X/98 $12.00

Introduction

The first mercapturic acid (MA, N-acetyl-L-cysteine S-conjugate) was already identified in 1879 in urine of dogs treated with bromobenzene (Baumann and Preusse 1879, Jaffe 1879). Since then, numerous MAs have been found in the urine of animals treated with various chemicals (Van Welie *et al.* 1992). Some time ago, the urinary excretion of MAs has been suggested to be a useful tool in biological monitoring (BM) studies (Vermeulen 1989). In recent years many new BM-methods have become available for the assessment of human exposure to toxic chemicals originating from the environment, food or occupation (e.g. Henderson *et al.* 1989, Lowry 1995, Schulte 1995). Meanwhile, MAs have successfully been used as biomarkers of occupational exposure to different chemicals (Van Welie *et al.* 1992).

Mercapturic acids are formed from glutathione S-conjugates via the mercapturic acid pathway (MA-pathway). Glutathione (GSH) conjugation is generally considered to be a detoxification route of potentially toxic electrophilic chemicals or intermediates (Vermeulen 1989, Sipes and Gandolfi 1991). However, in some cases conjugation to GSH and further metabolism may lead to toxicity as well (Koob and Dekant 1991, Baillie and Kassahun 1994, Munday 1994). Various aspects of the MA-pathway, such as enzymes involved in GSH-conjugation and MA-formation, detoxification or toxification by GSH conjugation, and the use of MAs in BM-studies, have been reviewed repeatedly since the early nineties (e.g. Van Welie *et al.* 1992, Monks and Lau 1994, Commandeur *et al.* 1995, Hayes and Pulford 1995). In recent years several new developments were seen in the analysis of MAs, e.g. the use of tandem mass spectrometry which led to very fast and selective methods, as well as immunological approaches which were also applied to this kind of metabolites. Also many new applications of MAs as biomarkers of occupational exposure were reported recently.

The aim of this review is to make an inventory of the use of MAs as biomarkers of human exposure to environmental, industrial and other electrophilic chemicals. Additionally, a brief overview will be given of the functions of GSH, the enzymes involved in GSH conjugation and the biosynthesis of MAs. Finally, relevant aspects of these issues will be discussed for different environmental and industrial chemicals.

Mercapturic acids and biomacromolecular adducts as biomarkers

Most chemicals are not toxic agents *per se*, but require metabolic activation before they express their toxicity (Vermeulen 1995). The presence of activating and inactivating enzyme systems, their specific activity, together with the availability of cofactors, and of course the dose, all determine the tissue concentration of the ultimate toxic intermediate and consequently of the toxic effect (Oesch *et al.* 1990, Vermeulen 1995). On the other hand, the intrinsic chemical reactivity of the intermediate together with that of biomacromolecules involved, determine the nature of the adducts (Vermeulen 1995, La and Swenberg 1996). Many intermediates are electrophilic compounds, while different nucleophilic sites (Lutz 1979) in proteins, DNA, GSH or newly synthesized albumin are available in the cell. When the intermediate is stable enough, other targets e.g. blood albumin, haemoglobin, or lymphocyte DNA may be reached as well. A hypothetical scheme for formation of electrophilic intermediates during hepatic metabolism and their reactions with biomacromolecules is shown in figure 1.

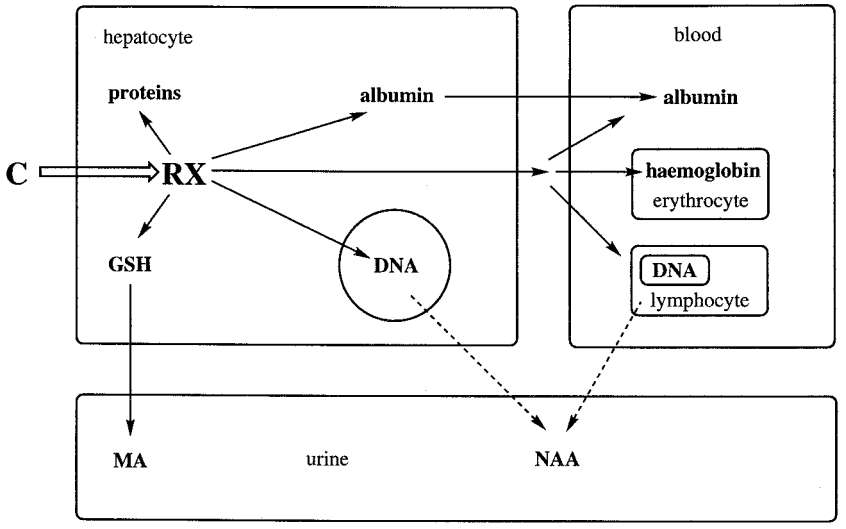

Figure 1. Hypothetical scheme for the formation of adducts of an electrophilic compound or intermediate (RX) to DNA, haemoglobin, albumin and glutathione (GSH) in hepatocytes or blood cells. Adducts to DNA, haemoglobin and albumin are determined in blood cells, while DNA adducts and GSH-adduct derived products, nucleic acid adducts (NAA) and mercapturic acids (MA) are determined in urine. (Modified from Commandeur, personal communication).

In recent years many new biological monitoring methods have become available for the assessment of human exposure (e.g. Henderson *et al.* 1989, Lowry 1995, Schulte 1995). Four types of monitoring are usually distinguished: environmental monitoring (EM), biological monitoring (BM), biological effect monitoring (BEM) and health surveillance (HS) (Henderson *et al.* 1989). These monitoring methods can be placed on a causality chain from exposure of an individual to a xenobiotic to a later developing disease (Henderson *et al.* 1989, Farmer and Sweetman 1995, Lowry 1995). Concomitant with the causality chain a sequence of different biomarkers can be drawn, as outlined in figure 2. Different types of biomarkers for electrophilic compounds are used in occupational toxicology: covalent adducts of xenobiotic compounds or their metabolites with the blood proteins albumin and haemoglobin, with DNA or with GSH (Ehrenberg 1980, Van Welie *et al.* 1992, La and Swenberg 1996, Nestmann *et al.* 1996). Although DNA adducts in the target organs would be the best measure of the target dose of a carcinogenic electrophile, these cannot be used as biomarkers in occupational toxicology because of the inaccessibility of target tissue. As a surrogate, DNA adducts are measured in peripheral lymphocytes and are considered a measure for a cumulative or chronic dose. Protein adducts (albumin adducts in serum or, haemoglobin adducts in erythrocytes) give a measure for a semi-chronic dose. Both type of adducts show that reactive electrophilic compounds were absorbed or formed during metabolism and that these were able to reach, and react with, essential biomacromolecules. GSH adducts are mostly excreted in urine within a few days as their corresponding MAs (Vermeulen 1989, Commandeur *et al.* 1995). Determination of MAs in urine of exposed workers can therefore serve as a marker of recent exposures. The excretion of MAs indicates that electrophilic compounds are absorbed or formed in the body and that they were able to react with the ubiquitous endogenous compound glutathione (GSH). Because glutathione is an es

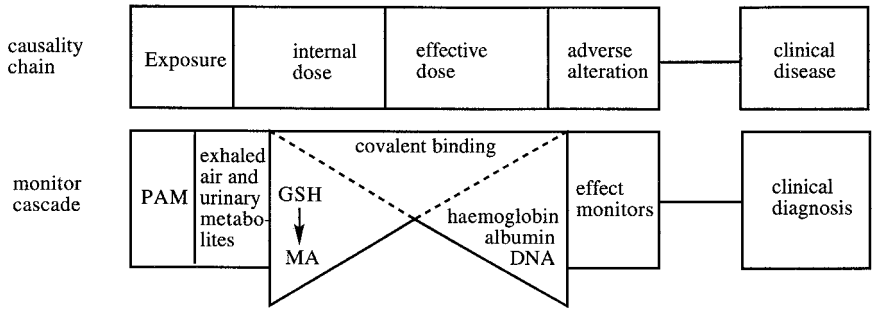

Figure 2. The causality chain from potential exposure to the onset of a disease in different stages, and the monitor cascade representing markers on different levels in the causality chain. PAM = personal air monitoring; GSH = glutathione; MA = mercapturic acid. The causality chain was modified from Henderson *et al.* (1989), Farmer and Sweetman (1995) and Lowry (1995).

compound, involved in protection against electrophiles and radicals, MAs refer to a toxicologically relevant absorbed dose, while other urinary metabolites usually refer to the absorbed dose only.

Adducts to DNA can be stable and detectable for a prolonged period of time (Santella *et al.* 1992), thus allowing the measurement of long-term cumulative exposure. However, many DNA adducts are repaired or removed by depurination as well. Repaired and depurinated nucleotides are usually excreted as nucleic acid adducts in urine (Beach and Gupta 1992, Shuker and Farmer 1992, Hemminki 1995). The repair of DNA adducts can vary substantially among different tissues and cells (Harris 1989, Poirier 1994) and the enzymes involved in this repair may also be subject to large interindividual differences due to polymorphisms, or due to variations caused by diseases (Harris 1989, La and Swenberg 1996). In occupational toxicology, nucleated blood cells are frequently used as a surrogate for target tissues (Henderson *et al.* 1989, Van Welie *et al.* 1992). Often clear relationships are found between DNA adducts in blood cells and such adducts in target tissue, which in turn are related to mutagenic and carcinogenic effects (La and Swenberg 1996). However, different life-spans of various white blood cells and the variations in DNA repair mechanisms can contribute to conflicting results when blood cells are used for dosimetry (Santella *et al.* 1992). Moreover, DNA adducts determined in peripheral blood cells and in target tissues did not correlate significantly in all studies (Phillips *et al.* 1990, Van Schooten *et al.* 1992). This indicates that the use of peripheral blood cells as surrogates for target tissue should be interpreted with caution. Another disadvantage of the use of DNA adducts in exposure assessment is the invasive sampling needed to collect nucleated blood cells. Recently, exfoliated cells were suggested as alternative for blood cells (Talaska *et al.* 1994). Although these cells are collected by non-invasive sampling, the meaning of their determination in occupational toxicology remains to be established. For urinary nucleic acid adducts samples may also be collected by non-invasive means, but the high variability of their excretion and background excretions can limit their application in BM studies (Beach and Gupta 1992, Shuker and Farmer 1992, Hemminki 1995).

Adducts to the blood proteins haemoglobin and albumin are also frequently used in BM studies. Protein adducts are not subject to enzymatic repair mechanisms and their determination is therefore less variable compared to DNA

adducts (Skipper *et al.* 1994). Moreover, proteins are usually present in greater abundance than DNA, consequently resulting in larger amounts of material available for analysis. Reactive electrophiles can react with different nucleophilic sites in proteins, such as thiols in cysteine or amino groups in the N-terminal valine (Lutz 1979, Farmer and Sweetman 1995). A main function of serum albumin is transport of endogenous compounds and metabolites by non-covalent binding. However, reactive products can also covalently be bound to this protein (Van Welie *et al.* 1992, Ong *et al.* 1995, Yeowell-O'Connell *et al.* 1996). Albumin is almost exclusively synthesized in the liver and may therefore directly bind to reactive intermediates formed from xenobiotics within this organ. The blood life-time of haemoglobin (120 days) and albumin (20 days) allows the measurement of an integrated low-level exposure to xenobiotics over a period of 4 months to 3 weeks, respectively, when it is assumed that relatively small adducts to these proteins do not alter their half-lives. This is not always true, however, N-(2-hydroxyethyl)valine, determined in the haemoglobin of rats and mice exposed to high concentrations of ethylene oxide (100 to 300 ppm), was lost in a biphasic manner. In the first phase N-(2-hydroxyethyl)valine was lost from haemoglobin with a shorter half-life than predicted by a normal erythrocyte life-span, while in the second phase N-(2-hydroxyethyl)valine was lost within the life-span of blood cells (Walker *et al.* 1993). In humans exposed to low levels of ethylene oxide, the half-life of this haemoglobin adduct was between 27 and 35 days, indicating that N-(2-hydroxyethyl)valine is removed at the same rate as red blood cells (Van Sittert *et al.* 1993a). Recently, it has been suggested that adducts to histones also can serve as an index of long-term low-level exposures (Skipper *et al.* 1994). Adducts of benzo(*a*)pyrene-7,8-diol-9,10-oxide to histones were identified only recently, and so far, no applications were reported in occupational toxicology. A main disadvantage of protein adducts (as with DNA adducts) is the invasive sampling of blood.

Mercapturic acids constitute an interesting group of metabolites, which are receiving more and more attention in human BM studies. MAs are relatively easily measured and most important, urine may be sampled non-invasively (Henderson *et al.* 1989). Urinary MA excretion is generally less variable compared to the urinary DNA adducts. MA formation always results from GSH conjugation of electrophilic and therefore potentially toxic, compounds and intermediates (Vermeulen 1989). Depending on the parent compound, the amount of a MA metabolite can be relatively high, thus allowing the determination of MA excretion upon very low levels of exposure. Another favourable characteristic of MAs is their generally relatively short elimination half-lifes, allowing the monitoring of recent exposures, as well as a more direct evaluation of occupational hygiene. Therefore MAs, as determinants of a toxicologically relevant internal dose, are frequently applied in chemical industry to monitor human exposure.

Glutathione conjugation and mercapturic acid formation

Homeostasis and functions of glutathione

Glutathione (GSH) is an important endogenous tripeptide (γ-Glu-Cys-Gly) of which relatively high concentrations are found in various organs and tissues (see table 1) (Meister 1983, Ketterer and Mulder 1990, Commandeur *et al.* 1995

Table 1. Organ- and Tissue-distribution of glutathione (GSH) and enzymes involved in formation and disposition of GSH-derived S-conjugates in the rat. Table modified from (12).

Organ/Tissue	[GSH][1]	GSH S-transferase[2] (cytosolic)	GSH S-transferase[2] (microsomal)	γ-Glutamyl-transferase[3]	Dipeptidase[4]	Amino-peptidase M[5]	β-Lyase[6]	N-Acetyl-transferase[7]	Acylase I[8]	Acylase III[9]	3-Mercapto-pyruvic acid S-conjugate reductase[10]
erytrocytes	8·3[11]	–	–	–	–	–	–	–	–	–	–
liver	7·7	1400	126	2	1·3	2·1	0·84	0·46	926	4·4	100
kidney	4·1	336	8·5	560	4·5	10·2	3·05	2·92	8900	40	33
small intestine	2·9	429	60	2–5	0·5	3·1	–	0·04	–	–	17
lung	1·5	79	15	2	12·1	1·2	0·11	–	–	–	6
brain	2·1	190	7·9	1	1·3	1·3	0·21	–	210	0·7	38
spleen	3·4	56	9	2	2	1·1	0·23	–	123	0·6	–
testis	–	3850	129	< 1	–	–	0·4	–	69	0·3	81
adrenal	–	253	52	2	–	–	–	–	–	–	–
heart	1·1	93	7·2	4	–	–	0·47	–	–	–	–
thymus	–	46	4·4	2	–	–	–	–	219	0·5	–
pancreas	1·8	–	–	115	0·8	1·1	0·14	–	730	1·5	–
muscle	0·8	–	–	< 1	–	–	0·44	–	–	–	–
bone marrow	–	–	–	–	–	–	0·03	–	–	–	–
plasma	0·018[11]	–	–	–	–	–	–	–	–	–	–

[1] μmol g^{-1} tissue (35); [2] nmol min^{-1} mg^{-1} protein; substrate: 1-chloro-2,4-dinitrobenzene (36); [3]nmol h^{-1} mg^{-1} protein; substrate: γ-glutamylanilide; in duodenum: , jejunum: 5, ileum: 4 (37); [4] nmol min^{-1} mg^{-1} protein; substrate: glycyldehydrophenylalanine (38); [5] nmol min^{-1} mg^{-1} protein; substrate: S-benzyl-L-cysteine -nitroanilide (38); [6] nmol min^{-1} mg^{-1} protein; substrate: S-(1,2-dichlorovinyl)-L-cysteine (39); [7] nmol min^{-1} mg^{-1} protein; substrate: S-benzyl-L-cysteine (40); [8] nmol $^{-1}$ 10 mg^{-1} tissue; substrate: N-acetylmethionine (41); [9] nmol h^{-1} 10 mg^{-1} tissue; substrate: N-acetyl-L-tryptophan (41); [10] relative activity in 700 g supernatant ompared with that in the liver (10 nmol min^{-1} μl^{-1}); substrate: S-(4-bromophenyl)-3-mercaptopyruvic acid (42), [11] in mmol g^{-1} haemoglobin for humans (43), [12] μmol l^{-1} (44).

Kretzschmar 1996). The concentrations are especially high in the liver (7·7 μmol g^{-1} tissue, (Meister 1983) and erythrocytes (8·3 mmol g^{-1} haemoglobin, (Richie *et al.* 1996) and are not homogeneously distributed within organs (Ketterer and Mulder 1990) and or within cells (Kretzschmar 1996, Smith *et al.* 1996). The mitochondria contain approximately 30 % of the intra-cellular GSH (Smith *et al.* 1996). The major sites of GSH synthesis are liver cells, although some other cells are also able to synthesize this tripeptide (Ketterer and Mulder 1990). GSH is synthesized from glutamate, L-cysteine and glycine in a two-step reaction catalysed by γ-glutamylcysteine synthetase and glutathione synthetase (see figure 3) (Ketterer and Mulder 1990, Meister 1991). This synthesis is feedback inhibited by GSH on the

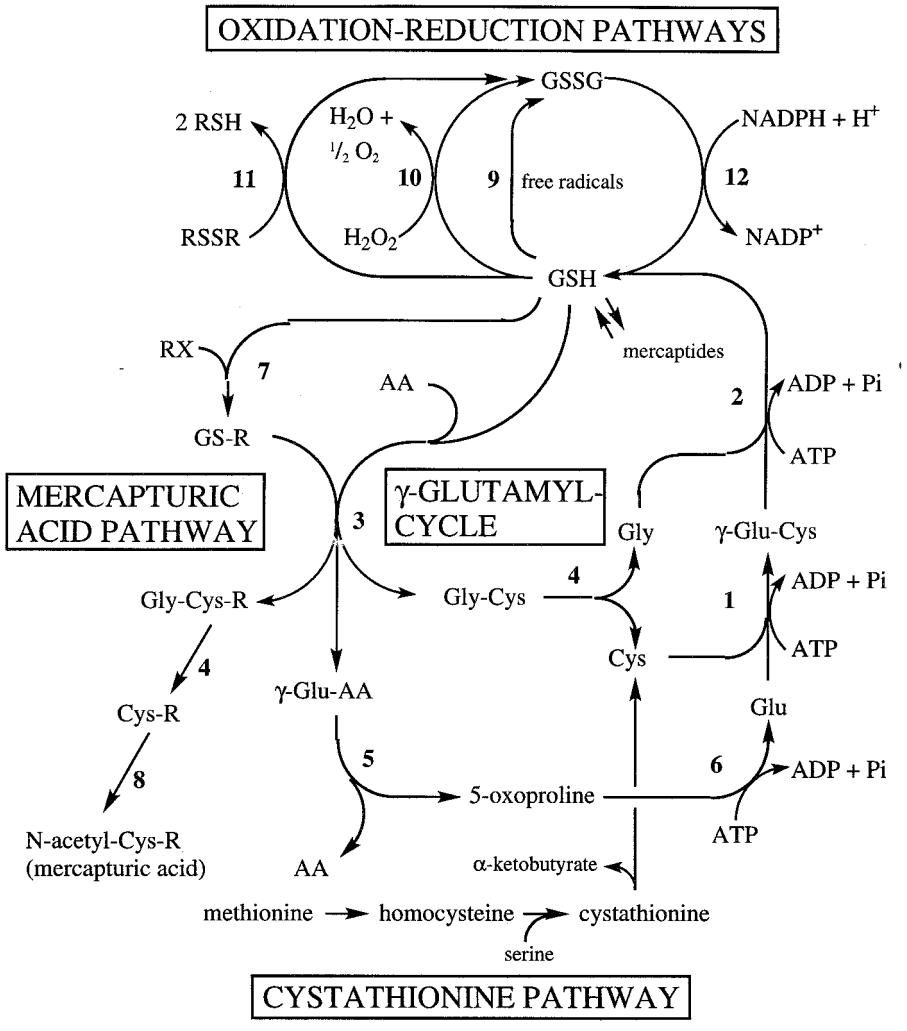

Figure 3. Metabolism of glutathione (GSH). Enzymes involved: (1) γ-glutamylcysteine synthetase, (2) glutathione synthetase, (3) γ-glutamyltranspeptidase, (4) dipeptidases, (5) γ-glutamylcyclotransferase, (6) 5-oxoprolinase, (7) GSH S-transferases, (8) cysteine conjugate N-acetyl transferase, (9) non-enzymatic thiylradical mechanism, (10) GSH-peroxidases, (11) GSH-thioltransferases, (12) GSH-disulphidereductase. AA=amino acid; RX=electrophile. Modified from Meister (1991).

first enzyme. Administration of γ-Glu-Cys can by pass this inhibition and can be used to increase the endogenous GSH concentration (Ketterer and Mulder 1990). A sufficient supply of L-cysteine is essential in GSH biosynthesis. L-Cysteine can be synthesized from methionine by the cystathionine pathway and a sufficient amount of L-cysteine and methionine in the diet is needed to maintain normal GSH levels (Ketterer and Mulder 1990, Taylor *et al.* 1996). GSH degradation is catalysed by γ-glutamyltranspeptidase (by hydrolysis or by transpeptidation) and subsequently dipeptidases (Ketterer and Mulder 1990). When GSH is degraded by transpeptidation an, amino acid is attached to γ-glutamyl and can be transported through the body. After transport glutamate is used again for the synthesis of GSH in the γ-glutamyl cycle (Stryer 1988). GSH also plays an important role in the reduction of hydrogen peroxide (H_2O_2) and organic peroxides (Stryer 1988). During this reduction GSH is oxidized to GSSG, a reaction catalysed by a selenium-dependent glutathione peroxidase. GSSG can be converted back to its reduced form (GSH) by glutathione reductase, at the expense of NADPH (Stryer 1988). The peroxidase activity of GSH is dependent on the thiol-function of cysteine, however free cysteine cannot replace GSH because it is too easily oxidized in the presence of transition element ions, with the formation of free radicals (Ketterer and Mulder 1990). GSH may also play a role in the detoxication of radicals (e.g. superoxide anion radicals, $O_2^{\bullet-}$) under the formation of GSSG via a thiylradical mechanism (Munday 1994). The formation of thiylradicals from GSH, or more favourably from cysteine or other thiols, may also result from molecular oxygen when catalysed by certain transition metals, such as iron and copper. Other metals, such as mercury, lead, arsenic and cadmium, may be transported chelated to GSH as GSH-mercaptides (Ballatori 1994, Dekant *et al.* 1994). The transport may even occur through membranes and the blood-brain barrier. The chelated metals are rapidly exchanged between available sulphydryl groups, e.g. from GSH and proteins by nucleophilic displacement. However, regarding the subject of this review the most important function of GSH is detoxification of electrophilic chemicals or intermediates by conjugation (Ketterer and Mulder 1990, Van Welie *et al.* 1992, Commandeur *et al.* 1995) and this function will be described further.

Glutathione conjugation and glutathione S-transferases

The conjugation of electrophiles to GSH can proceed spontaneously or catalysed by GSH S-transferases (GSTs), of which a microsomal form and a family of cytosolic isoenzyme forms have been identified (Ketterer and Mulder 1990, Anderson *et al.* 1994, Ketterer and Christopoulides 1994, Hayes and Pulford 1995). The catalytic mechanism of cGSTs and of mGST is thought to involve a lowering of the p*Ka* of GSH resulting in increased nucleophilicity of the sulphur (Ketterer and Christopoulides 1994) or alternatively, stabilization of the intermediate (Van Der Aar *et al.* 1996).

Microsomal GST (mGST) is a trimeric enzyme with the liver containing the highest concentrations (Anderson *et al.* 1994). It is also present in most other tissues (see table 1). Common inducers of xenobiotic-metabolizing enzymes do not seem to increase the levels of this enzyme, although small increases in mGST levels were found upon pretreatment with phenobarbital (Aniya *et al.* 1993). The human mGST, purified from liver, is closely related to the rat enzyme with 95% conservation at the amino acid sequence level. mGST shows a broad substrate

specificity and the substrates include many polyhalogenated hydrocarbons, unsaturated hydrocarbons, lipid hydroperoxides, aromatic hydrocarbons and acrolein congeners (Anderson *et al.* 1994). Epoxides are generally poor substrates. The GSH conjugation of the general GST substrate 1-chloro-2,4-dinitrobenzene is also catalysed by microsomal GST (Anderson *et al.* 1994).

At least five subfamilies (alpha (basic), mu (neutral), pi (acidic), sigma and theta) of cytosolic GSTs (cGST) have been identified in rats and humans (Ketterer and Christopoulides 1994, Hayes and Pulford 1995). cGSTs consist of two subunits, formed from the same subunits (homodimers) or from different subunits from the same subfamily (heterodimers). Nowadays the nomenclature proposed by Mannervik *et al.* (1992) is generally used. In this nomenclature a prefix is used to indicate the species (e.g. h for human), while A, P, M, S or T indicate the gene classes alpha, pi, mu, sigma or theta, respectively, while two numbers spaced by a dash indicate the subunits, for example: h GST A 2–2. X-ray crystallography and conformation analysis have revealed that the overall three dimensional structure of the different cGSTs are relatively similar (Ketterer and Christopoulides 1994). All subunits have a binding site for GSH (G-site) and a substrate binding site (H-site) within the active site lying between the two subunits. The cGSTs show broad substrate specificity as well, and a particular reaction can often be carried out by different isoenzymes and with different enzyme kinetics. Toxicologically important substrates of cGSTs include styrene oxide (Pacifici *et al.* 1987, Hiratsuka *et al.* 1989), acrolein (Berhane *et al.* 1994), ethylene oxide (Hallier *et al.* 1993), dichloromethane (methylene chloride) (Reitz *et al.* 1989), dibromoethane (Ploemen *et al.* 1995, Thier *et al.* 1996), 4-hydroxynonenal (Jensson *et al.* 1986, Singhal *et al.* 1994), and (+)-*anti*-benzo(*a*)pyrene-7,8-diol-9,10-oxide (Robertson *et al.* 1986a, Robertson *et al.* 1986b), for a review see Hayes and Pulford (1995). cGSTs are expressed in many tissues, with the highest concentrations found in the testes and liver of rats (see table 1). However, the concentrations of GSTs and their activities may vary considerably within an organ (Ketterer and Mulder 1990). Many enzyme inducers have (small) increasing effects on cGST levels, e.g. benzo(*a*)pyrene, ethanol and phenobarbital (Hayes and Pulford 1995). Almost all GSTs utilize 1-chloro-2,4-dinitrobenzene as a substrate and this compound is generally used as a tool in the characterization of GSTs (Ketterer and Christopoulides 1994, Hayes and Pulford 1995). Recently, it was suggested that 2-chloro-5-nitrobenzonitrile may also serve as a model substrate for GSTs (Van Der Aar *et al.* 1996). For the GST T family, however, 1-chloro-2,4-dinitrobenzene is a weak substrate, but ethacrynic acid (T 2–2 and T 3–3) or 1,2-epoxy-3-(4′-nitrophenoxy)propane (T 1–1) can be used instead as model substrates for this type of GSTs (see table 2) (Hiratsuka *et al.* 1994, Hayes and Pulford 1995, Schrüder *et al.* 1996). Other specific substrates of GST T 1–1 are p-nitrobenzyl chloride (Ketterer and Christopoulides 1994; Schrüder *et al.* 1996), dichloromethane (methylene chloride) (Reitz *et al.* 1989, Mainwaring *et al.* 1996), ethylene oxide (Hallier *et al.* 1993) and methylbromide (Hallier *et al.* 1990, Hallier *et al.* 1993), see table 3. Dichloromethane is an important industrial compound, used as solvent in paint strippers, refrigerant, cleaning agents and in the decaffeination of coffee, and large species differences are found in the toxicity of this compound (Graves *et al.* 1995). The compound is activated by GSH conjugation and causes lung and liver cancer in mice. The relatively low activity of GST T 1–1 together with the activity of DNA repair may explain the lower susceptibility of rats and possibly humans

Table 2. Specific activities of some model substrates for GST T and M in rats and human. Adapted from Hayes and Pulford (1995).

Rat GST isoenzyme	specific activity *1 CDNB	EA	EPNP	Ref.	Human GST isoenzyme	specific activity *1 CDNB	EA	EPNP	Ref.
M 1–1	58	0·08	0·053	(1)	M 1a–1a	190·2	0·47	n.d.	(2, 3)
M 1–2	45	0·34	0·94	(1)	M 1a–1b	161·4	0·05	–	(2)
M 2–2	17	0·62	1·37	(1)	M 1b–1b	172·0	0·12	–	(2)
M 1–3	64	0·54	< 0·1	(1)	M 1b–2	203·1	0·24	–	(2)
M 3–3	45	0·585	–	(4)	M 2–2	276·8	0·29	n.d.	(2, 3)
M 6–6	30·0	0·345	0·1	(4, 5)	M 2–3	171·9	0·48	–	(2)
					M 3–3	15·2	0·31	n.d.	(2, 3)
					M 4–4	1·3	0·04	n.d.	(3)
					M1M2*2	32·6	0·70	< 0·02	(6)
					M2N1*2	46·5	0·40	< 0·02	(6)
T 1–1	< 0·5	–	185	(7)	T 1–1	n.d.	–	4	(7, 8)
T 2–2	< 0·1	0·4	< 0·01	(9)	T 2–2	n.d.	0·29*2	n.d.	(8, 10)
T 2–2′	< 0·1	1·6	< 0·01	(9)					
T 2′–2′	< 0·1	2·5	< 0·01	(9)					
T 3–3	85	26	n.d.	(11)					

*1 CDNB=1-chloro-2,4-dinitrobenzene, EA=ethacrynic acid, EPNP=1,2-epoxy-3-(4′-nitrophenoxy)propane. Specific activities in μmol min^{-1} mg^{-1} protein. – not known, n.d. not detectable. *2 activity of human enzyme expressed in *E. coli*.
References used: 1, Ålin *et al.* 1985; 2, Hussay and Hayes 1993; 3, Ross and Board 1993; 4, Ålin *et al.* 1989; 5, Hayes 1986; 6, Tsuchida *et al.* 1990; 7, Meyer *et al.* 1991; 8, Schrüder *et al.* 1996; 9, Hiratsuka *et al.* 1994; 10, Tan and Board 1996; and 11, Harris *et al.* 1991.

compared with mice, to the genotoxic effects of this compound (Reitz *et al.* 1989, Bogaards *et al.* 1993, Graves *et al.* 1995, Mainwaring *et al.* 1996).

Two cGST subfamilies, T 1–1 and M 1–1, show a genetic polymorphism in humans. Both enzymes are expressed in human blood cells and genotyping of workers can be relatively easily performed using these cells (Bolt 1994, Chen *et al.* 1996a). Approximately 17 % of white Americans lack the GST T 1–1 enzyme activity (Strange 1995), while in Nigerians (39%) and West Indian Britons (32 %) higher percentages were found (Strange 1995). The latter enzyme is expressed in blood cells of humans but not in the blood cells of rats, while in the liver of both species this enzyme is expressed in the GST T 1–1 positive part of the population (Bolt 1994). Specific substrates of GST T 1–1 are given in table 3. Recently, the GST T 1–1 negative genotype was associated with myeloid leukemia (Chen *et al.* 1996b). The second GST showing genetic polymorphism is the GST M 1–1 isoenzyme. This enzyme is lacking in 50–60 % of the Caucasian population (Bolt 1994, Raunio *et al.* 1995), while in North American blacks (28%) and Nigerians (22 %) lower percentages are found (Strange 1995). GST M 1–1 is important in the detoxification of epoxides like *anti* benzo(*a*)pyrene-7,8-diol-9,10-oxide (Robertson *et al.* 1986b). Genetic polymorphisms of xenobiotic-enzymes, such as GSTs, have been associated with a higher susceptibility of certain subgroups for specific types of cancer (Nebert *et al.* 1996). For example, both the GST M 1–1 negative- and the GST T 1–1 positive genotypes were associated with a higher risk of bladder cancer (Brockmöller *et al.* 1996). The GST M 1–1 negative genotype has also been related with a higher risk of lung (Raunio *et al.* 1995), skin (Strange 1995) and gastric cancer (Katoh *et al.* 1996). However, a causal relation with lung cancer was not confirmed in all studies (Brockmöller *et al.* 1993, Strange 1995).

Catabolism of glutathione S-conjugates: the mercapturic acid pathway

In the catabolism of GSH S-conjugates to their corresponding MAs different metabolic steps are recognized (Sipes and Gandolfi 1991), see figure 4. This process also referred to as the MA-pathway (Vermeulen 1989). The first step in this pathway is the removal of the γ-glutamyl group of the GSH-moiety by γ-glutamyltranspeptidase (also known as γ-glutamyltransferase, GGT) forming cysteinylglycine S-conjugates (Commandeur *et al.* 1995). In the second step, the latter S-conjugates are degraded to the corresponding cysteine S-conjugates by cysteinylglycine dipeptidase (DP) and to a minor extent by aminopeptidase-M (APM). All three enzymes involved (GGT, DP and APM) are ectoproteins found on many apical membranes of epithelial cells, showing their active sites to the extra cellular space. Very high activities of GGT are found in the kidney and pancreas in rats (see table 1). DP and APM have their highest activities in the kidney and in the lung. In the liver these enzymes are localized in the bile canaliculus where they contribute to the γ-glutamyl cycle (Stryer 1988). The final step in the formation of MAs is the N-acetylation of cysteine S-conjugates to mercapturic acids (MAs,

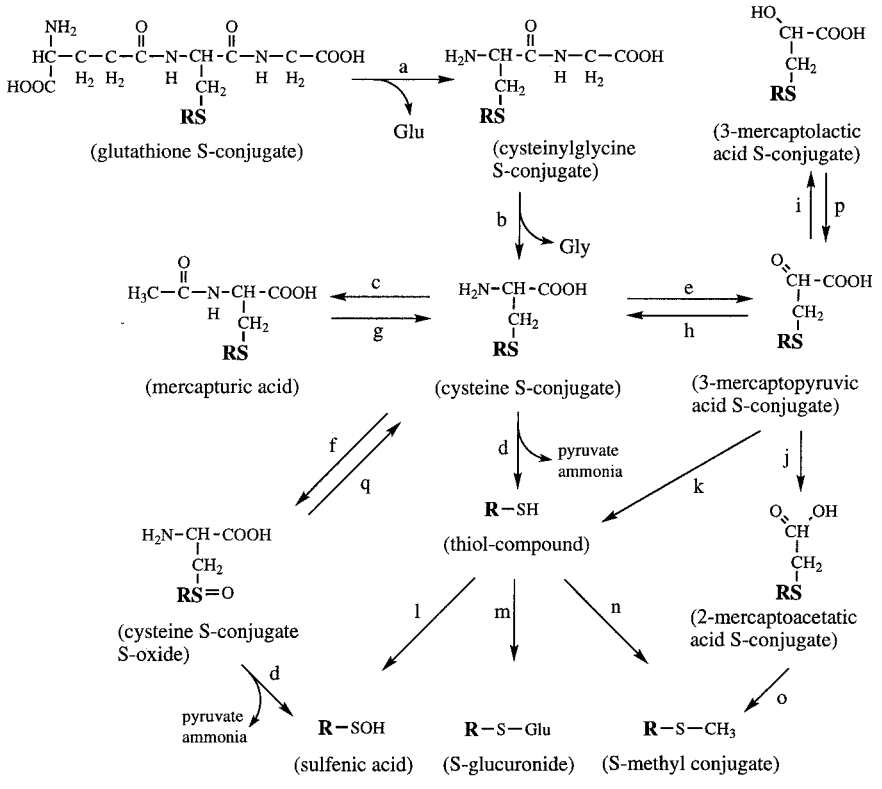

Figure 4. Possible routes of catabolism of GSH S-conjugates. Enzymes involved: (a) γ-glutamyltranspeptidase, (b) cysteineglycine dipeptidase and aminopeptidase M, (c) cysteine conjugate N-acetyltransferase, (d) cysteine conjugate β-lyase, (e) cysteine conjugate transaminase and L-amino acid oxidase, (f) cysteine confugate S-oxidase, (g) N-deacetylase, (h) transaminases, (i) 3-mercaptopyruvic acid S-conjugate reductase, (j) decarboxylase, (k) enzyme unknown, (l) S-oxygenase, (m) uridine diphosphate-glucuronyl transferase, (n) S-methyl transferase, (o) decarboxylase, (p) 3-mercaptolactic acid S-conjugate oxidase and (q) sulphoxide reductase. Adapted from Commandeur *et al.* (1995).

Table 3. Specific activities of rat and human GSTs for various industrial chemicals. If the specific activity is known, it is given between parentheses in μmol min^{-1} mg^{-1} protein, unless otherwise stated. Many bifunctional chemicals are activated by GSH conjugation, as indicated.

Chemical	activation/ inactivation	rat GST isoenzyme	Ref.	human GST isoenzyme	Ref.
± anti benzo(*a*)pryene- -7,8-diol-9,10-oxide	inactivation	M 1–1 (0·68) P 1–1 (5·5) M 2–2 and A 3–3 low activity	(1)	M 1–1 (0·57) P 1–1 (0·83) (M 1–1 equal active with + and – isomer, P 1–1 for + only 2·9) Note – isomer inhibits P 1–1	(2)
3,4-epoxybutene (butadiene monoepoxide)	inactivation	T 1–1 low/no activity	(3)	M 1–1*1 T 1–1 low/no activity	(4) (5)
1,2,3,4-diepoxybutane (butadiene diepoxide)	activation	T 1–1 *2	(5,3)	T 1–1 *1*2 M 1–1 low/no activity	(6) (7)
ethylene oxide (epoxyethane)	inactivation			T 1–1 *1	(8)
1,2-epoxypropane (propylene oxide)	inactivation	T 1–1 *2 (weak)	(3)	T 1–1 *2 (weak)	(5)
methylchloride	inactivation			T 1–1 *1 erytrocytes (1·14)	(8) (9)
methylbromide	inactivation			T 1–1 *1 erythrocytes (1·14) Note: high spontaneous conjugation	(8) (9)
methyl iodide	inactivation			T 1–1 *1 erythrocytes (0·26)	(8) (9)
dichloromethane (methylenechloride)	activation	T 1–1 *2 rat liver *3 (0·007) rat lung *3 (0·001) mice liver *3 (0·026) mice lung *3 (0·007)	(10) (12) (12) (12) (12)	T 2–2 no activity *1 T 1–1 *1 human lung *3 (0·0002–0·0004) human liver *3 (0·003) purified from liver (1·6) purified from erythrocytes (1·4) other GSTs no activity	(11) (8) (13,14) (12) (15) (16) (17)
large species differences in DNA damage of GSH-activated metabolites					
dibromomethane	activation	T 1–1 *2	(10) (5)	T 1–1 *2 erythrocytes (11·4)	(5) (16)
chlorobromomethane	activation	T 1–1 *2	(10)		
1,2-dibromoethane (ethylene dibromide)	activation	T 1–1 *2 (high activation) A 2–2 A 1–1, A 1–2 also act. M 1–1, M 1–2 or 2–2 also activation *4	(5) (10) (20) (21)	T 1–1 *3 erythrocytes cytosols (0·002) A 2–2 A 1–1 *2 P 1–1 no substrate	(5) (19) (20) (22)
1,2-dichloroethane	activation, weak			A 1–1 *2 P 1–1 no substrate	(22)
chloroethane	inactivation	rat, mice liver cytosol unspecified (0·02, 0·11) also spontaneous conjugation			(23)
Z-1,3-dichloropropene	inactivation			M 1a–1a (0·12) M 1a–1b (0·17) M 1b–1b (0·16) A 1, A 2 and P 1 also active	(24)

Table 3. (*cont.*)

Chemical	activation/ inactivation	rat GST isoenzyme	Ref.	human GST isoenzyme	Ref.
E-1,3-dichloropropene	inactivation			M 1a–1a (0·070) M 1a–1b (0·050) M 1b–1b (0·054) A 1, A 2, and P 1 also active	(24)
1,3-dichloroacetone	activation	T 1–1 *2	(3,5)	T 1–1 *2	(5)
epibromohydrin	activation	T 1–1 *2 (weak act.)	(3,5)	T 1–1 *2 (weak act.)	(5)
4-bromo-1,2-epoxy-butane	activation	T 1–1 *2	(3)		
1,4-dibromobutane	activation	T 1–1 *2 (low act.)	(3)		
1,4-dibromo-2, 3-epoxy-butane	activation	T 1–1 *2	(3)		
1,4-dibromo-2, 3-dihydroxybutane	activation	T 1–1 *2	(3)		
1-bromopropane-2,3-diol		T 1–1 *2 no substrate	(3)		
1,2-dibromo-3-chloropropane	activation	T 1–1 *2 (weak act.) M 1–1 (0·017) M 2–2 (0·002) T 2–2 (0·009) other enzymes lower act.	(3) (25)	M 1a–1a (0·005) M 3–3 (0·002) A 1 and A 2 enzymes higher activity activity	(25)
2,3-dibromopropion-aldehyde	activation	T 1–1 *2 (no substrate)			
1,3-dibromopropanol	activation	T 1–1 *2	(3)		
chloroethene (vinyl chloride)	?	conjugation identified, no specific activities known			(26)
styrene-7,8-oxide	inactivation	M 1–1 (0·8 mM^{-1} s^{-1}) M 1–2 (0·7 mM^{-1} s^{-1}) M 2–2 (0·5 mM^{-1} s^{-1}) A 1–1 and P 1–1 low activity (activity as kcat/Km)	(27)	M 1 biphasic dependent on GSH: [GSH] < mM: Vmax 1·3 μmol mg min Km 0·32 mM [GSH] > 1 mM: Vmax 2·3 μmol mg min Km 1·24 mM M (unspecified, 2·6) A (unspecified 0·02) P 1–1 (0·14)	(13) (28)
2-propenal (acrolein)	inactivation			M 1–1 (7·1) P 1–1 (26·3) A 1–1 (0·86)	(29)
4-hydroxynonenal	inactivation	A 4 (170)	(30)	A 4 (177) M 1–1 (3·2) A others except A 4 (0·58) P 1 (0·54)	(31)

*1 substrate specificity deduced from GSH conjugation upon incubation with human blood cells, no specific activity was determined. *2 substrate specificity deduced from the effect of GSH conjugation on mutagenicity in *Salmonella Typhimurium* expressing GSTs from rat or human. *3 activity of GST determined in cytosolic fraction. *4 activity of GST deduced from binding to DNA in the presence of GST isoenzymes.

References used: 1, Robertson *et al.* 1986a; 2, Robertson *et al.* 1986b; 3, Thier *et al.* 1995; 4, Uusküla *et al.* 1995; 5, Thier *et al.* 1996; 6, Norppa *et al.* 1995; 7, Landi *et al.* 1996; 8, Hallier *et al.* 1993; 9, Hallier *et al.* 1990; 10, Guengerich *et al.* 1995; 11, Tan and Board 1996; 12, Reitz *et al.* 1989; 13, Pacifici *et al.* 1987; 14, Hiratsuka *et al.* 1994; 15, Juronen *et al.* 1996; 16, Schrüder *et al.* 1996; 17, Bogaards *et al.* 1993; 18, Graves *et al.* 1995; 19, Ploemen *et al.* 1995; 20, Cmarij *et al.* 1990; 21, Inskeep and Guengerich 1984; 22, Simula *et al.* 1993; 23, Fedtke *et al.* 1994; 24, Vos *et al.* 1991; 25, Søderlund *et al.* 1995; 26, Chasseaud 1979; 27, Hiratsuka *et al.* 1989; 28, Warholm *et al.* 1987; 29, Berhane *et al.* 1994; 30, Jensson *et al.* 1986; and 31, Singhal *et al.* 1994.

N-acetyl-L-cysteine S-conjugates). This reaction is carried out by cysteine S-conjugate N-acetyltransferase (CCNAT). This enzyme is different from the well-known genetically polymorphic N-acetyltransferase (NAT2) involved in the metabolism of drugs such as isoniazid (Sipes and Gandolfi 1991, Commandeur *et al.* 1995). CCNAT is located on the endoplasmatic reticulum. The highest specific activity is found in the kidney (see table 1), especially in the proximal tubular region. Although the liver contains a lower specific activity of CCNAT, the liver contributes considerably to the N-acetylation of cysteine S-conjugates due to its relatively high weight (Inoue *et al.* 1984). MAs can be deacetylated again by N-deacetylases, mainly acylase I and III, two enzymes with high activities in the kidney and liver (see table 1) (Commandeur *et al.* 1995).

Other enzymes may also be involved in the metabolism of GSH S-conjugates leading to different end-products, such as mercaptolactic acids (3-mercapto-2-hydroxypropionic acid), mercapturic S-oxides and mercaptoacetic acids. The different routes reviewed by Commandeur *et al.* (1995) are outlined in figure 4. Apart from metabolism to MAs, cysteine S-conjugates can follow various metabolic routes, such as transamination or oxidative deamination to 3-mercaptopyruvic acid S-conjugates (α-keto acids or 3-mercapto-2-oxopropionic acids), oxidation to cysteine S-conjugate S-oxides and β-elimination to thiol-compounds. The 3-mercaptopyruvic acid S-conjugates are generally excreted in extremely low concentrations because rapid further metabolism occurs. An exception is the mercaptopyruvic acid from benzylisothiocyanate, a natural compound present in foodstuff such as garden cress (Görler *et al.* 1982). The mercaptopyruvic acid of benzylisothiocyanate is stabilized by internal cyclization to 4-hydroxy-4-carboxy-3-benzylthiazolidin-2-thione, thus preventing further metabolism. Benzylisothiocynate administered to guinea pigs resulted in a urinary excretion of approximately 23 % of the dose as this particular metabolite (Görler *et al.* 1982). In other cases 3-mercaptopyruvic acid S-conjugates may undergo reduction to the corresponding 3-mercaptolactic acid S-conjugates, decarboxylation to 2-mercaptoacetic acid S-conjugates and S-methyl conjugates, or β-elimination (enzyme not specified) to the corresponding thiol-compounds (Commandeur *et al.* 1995). β-Elimination of cysteine S-conjugates and cysteine conjugate S-oxides, catalyzed by β-lyase results in the formation of thiol-compounds and sulphenic acids. Many of these thiols and sulphenic acids are toxicologically relevant as will be discussed later. Guinea pigs and rabbits are poor excretors of MAs and therefore in these species GSH conjugation often leads to the excretion in urine of the other end products mentioned. For instance, administration of S-pentylcysteine to these species led predominantly to the urinary excretion of 3-(pentylthio)-pyruvic acid, 3-(pentylthio)-lactic acid and pentyl-MA sulphoxide (James and Needham 1973). In rats and hamsters, however, the excretion of pentyl-MA and other MA metabolites predominated, and was explained by a higher acetylation capacity in these species for cysteine S-conjugates. When rats were treated with S-pentyl-3-mercaptopyruvic acid, the major urinary metabolite was still the corresponding MA, indicating that the reverse transamination and subsequent N-acetylation (see figure 4) is also an important metabolic route for this compound in the rat. Sulphoxides of cysteine S-conjugates and related metabolites were also found in the urine of animals and humans exposed to different compounds. For instance, small amounts of mercapturic acid sulphoxides were identified in the urine of rats treated with allyl

halides (Kaye *et al.* 1972) and hexachloro-1,3-butadiene (Birner *et al.* 1995). Human volunteers treated with the mucolytic drug S-carboxymethylcysteine excreted S-carboxymethylcysteine, methylcysteine, carboxymethyl-MA and thiodiglycolic acid (thiodiacetic acid) and their respective sulphoxides in urine (Woolfson *et al.* 1987, Staffeldt *et al.* 1991). Thiodiglycolic acid is formed from S-carboxymethylcysteine by deamination and decarboxylation. In contrast to what these examples might suggest, humans are generally excellent excretors of MAs (Van Welie *et al.* 1992). However, in most studies the other products are often not looked for and therefore still should be considered as potentially useful biomarkers.

The mercapturic acid pathway is a multi-organ process

The MA-pathway is generally believed to be a multi-organ process (see figure 5) (Hinchman and Ballatori 1990, Hinchman and Ballatori 1994, Commandeur *et al.* 1995). GSH conjugation mainly takes place in the liver and under normal circumstances the GSH S-conjugates are excreted into the biliary canalicula. GSH S-conjugates formed in other organs (e.g. in the lung or in erythrocytes) are transported via the systemic circulation to the liver and other organs. In the bile, GSH S-conjugates are degraded to the corresponding cysteine S-conjugates by GGT and dipeptidases. The cysteine S-conjugates may be reabsorbed by hepatocytes and acetylated within these cells. Both cysteine S-conjugates and MAs are excreted in the blood via the sinusoidal membrane while MAs may be excreted

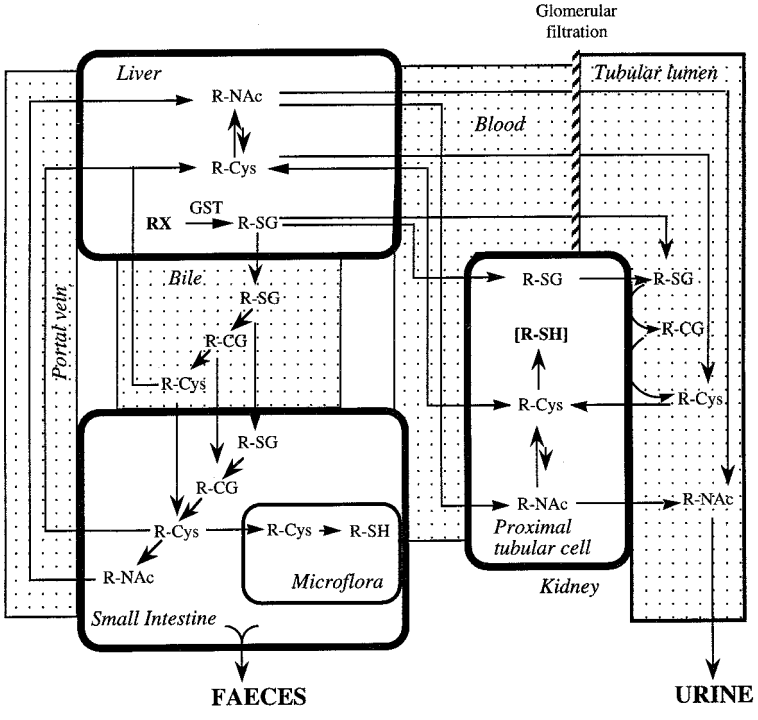

Figure 5. Schematic representation of the interorgan transport of GSH S-conjugates (R-SG) and their metabolites (e.g. glycine-cysteine S-conjugates (R-CG), cysteine S-conjugates (R-Cys) and N-acetyl-cysteine S-conjugates (R-NAc = mercapturic acids), leading to hepato-biliary cycling, hepato-intestinal cycling or hepato-renal cycling. R-SH = thiol compound derived from R-Cys. Adapted from Commandeur *et al.* (1995).

again in the bile. This cycle is called the hepato-biliary cycling (Hinchman and Ballatori 1994). Both GSH S-conjugates and cysteine S-conjugates may also be excreted via the biliary tree into the intestinal lumen, where high concentrations of GGT and dipeptidases are present. Free GSH, GSH S-conjugates and cysteine S-conjugates all can be absorbed by the epithelial cells of the intestinal microvilli and be delivered again to the liver by the portal vein (entero-hepatic cycling). The S-conjugates which are not re-absorbed from the intestinal epithelial cells are removed via the faeces. Cysteine S-conjugates can also be acetylated in the intestinal epithelial cells or can be metabolized by β-lyases present in the gut flora.

The S-conjugates and MAs that are eventually excreted in the blood by hepatocytes (after hepato-biliary, entero-hepatic or hepato-renal cycling) are further distributed by the systemic circulation to the kidneys and other organs. MAs in the blood are strongly bound to plasma albumin (Inoue *et al.* 1987) and are therefore not eliminated by glomerular filtration. However, MAs are absorbed actively by renal proximal tubular cells from the blood. Glomerular filtration is only responsible for 25 % of the renal GSH clearance, while the rest is removed by active transport across the basolateral membrane (Häberle *et al.* 1979). GSH S-conjugates and cysteine S-conjugates are also absorbed from the blood across the basolateral membrane into the tubular cells and GSH S-conjugates, cysteine S-conjugates, and MAs can be excreted in the primary urine in the lumen of the proximal tubules. In this lumen GGT and dipeptidases are active in the hydrolysis of GSH S-conjugates. Cysteine S-conjugates may be absorbed from the primary urine into proximal tubules cells via the apical membrane. In the proximal tubular cells cysteine S-conjugates may be subject to acetylation, cleavage by β-lyase or they are transported to the blood and subsequently distributed to the liver for acetylation.

The different enzymes involved in the MA-pathway have their highest specific activities in different organs. Their active sites are located either intra- or extracellularly and therefore the different metabolites of this pathway have to be transported across different membranes. Various active or facilitated transport systems (like the GS-X pump, GSH carriers, system A, -L, ASC or -T and the organic anion transporter) have been identified and found to be involved in the transport of GSH-conjugates and their metabolites. For a recent review on this field see Commandeur *et al.* (1995).

The exact route that GSH S-conjugates follow in the body during the MA-pathway is strongly dependent on the activity of GGT, dipeptidases, and CCNAT in various organs and large species differences have been found in the activities of the enzymes involved. In rats and mice relatively high activities of GGT and the dipeptidases are found in the kidney, while CCNAT has its highest activity in the liver and kidney. In these species GSH S-conjugates are transported from the liver via the bile, intestine and systemic circulation to the kidney, where hydrolysis to cysteine S-conjugates takes place. The cysteine S-conjugates are transported back to the liver for acetylation and again transported to the kidney for urinary excretion. This is called hepato-renal cycling. In other species, like guinea pig and rabbit the relative activities of GGT and dipeptidases in the liver are higher compared to that in the kidney (see table 4) and therefore the contribution of the hepato-biliary cycling to the MA-pathway is much higher in these species (Hinchman and Ballatori 1994). Attempts have been made to estimate the kidney-liver ratios of GGT in humans, and these estimates indicated that the relative distribution of this enzyme in humans resembles that of guinea pigs (Shaw *et al.*

Table 4. Species-differences in the activities of enzymes involved in formation and disposition of GSH-derived S-conjugates, modified from Commandeur *et al.* (12)

	Liver					Gallbladder		Kidney				
	GSH S-transferase									β-Lyase[5]		
Species	(micr.)[1]	(cyt.)[1]	(cyt.)[2]	γ=Glutamyl-transfrase[3]	Dipeptidase[4]	γ-Glutamyl-transferase[3]	Dipeptidase[4]	γ-Glutamyl-transferase[3]	Dipeptidase[4]	Vmax[6]	Km (mM)	Vmax/Km
rat	1·39	0·350	7	3·8	3·3	n.d.[7]	n.d.[7]	3325	65	4·00 (3·64)	0·68 (1·26)	5·88 (2·88)
mouse	0·16	0·109	26	1·5	5·4	2·4	9·4	619	31	1·15 (1·66)	5·69 (4·45)	0·20 (0·37)
rabbit	0·14	0·042	–	4·8	0·7	8·4	2·1	482	48	–	–	–
guinea pig	0·03	0·038	–	19·8	5·5	5·8	14·3	294	155	–	–	–
hamster	0·25	0·011	1·3	–	–	–	–	–	–	–	–	–
pig	–	–	–	6·5	8·3	3·8	3·6	123	78	–	–	–
monkey	1·39	0·074	–	9·7	3·7	10·9	4·1	454	60	–	–	–
human	1·17	0·031	3	27	–	–	–	570	–	0·49 (0·64)	2·53 (2·67)	0·21 (0·24)

[1]nmol min^{-1} mg^{-1} protein; substrate: hexachlorobutadiene (116); [2] nmol min^{-1} mg^{-1} protein; substrate: dichloromethane (61); [3] nmol min^{-1} mg^{-1} protein; substrate: γ-glutamyl-p-nitroanilide (113, 117); [3] nmol min^{-1} mg^{-1} protein; substrate: alanine-p-nitroanilide (113); [5] cytosolic β-lyase; substrate: S-(1,2,2-trichlorovinyl)-L-cysteine, in parentheses from females (118); [6] nmol min^{-1} mg^{-1} protein; [7] n.d., not determined (rats do not have a gallbladder).

1978, Hinchman and Ballatori 1990). This would mean that hepato-biliary cycling contributes to the MA-pathway in humans. However, the mRNA encoding for dipeptidase was only transcribed in the kidney of humans (Satoh *et al.* 1994). Therefore, it is not yet clear which route is followed in humans during the MA-pathway. As mentioned earlier, the MA-pathway leads predominantly to other end-metabolites in guinea pigs (James and Needham 1973, Görler *et al.* 1982), whereas in humans and rats the formation of MAs is much more prominent (Van Welie *et al.* 1992, Commandeur *et al.* 1995). This kind of species variation is dominated by differences in acetylation/deacetylation and other reactions involved in the metabolism of GSH S-conjugates, and rat seems to be a good model for humans to investigate the urinary excretion of MAs resulting from GSH conjugation of electrophilic compounds.

Glutathione conjugation leading to toxicity

Although GSH-conjugation of electrophilic, and therefore potentially toxic, chemicals or intermediates is generally regarded as a detoxification pathway, some GSH S-conjugates can lead to toxicity as well. Different mechanisms have been identified by which GSH conjugation can lead to increased toxicity. These include formation of reactive intermediates from GSH S-conjugates such as episulphonium ions, β-elimination of the C-S bond in cysteine S-conjugates catalysed by β-lyase, transport and accumulation of redox-active GSH S-conjugates of hydroquinones and aminophenols, transport of reversible GSH S-conjugates and metals, and depletion of cellular GSH levels. The mechanisms involved in the bioactivation by GSH-conjugation will be discussed briefly. For more extensive discussions on this subject see references Koob and Dekant (1991), Baillie and Kassahun (1994), Monks and Lau (1994).

Dihalogenated alkanes such as 1,2-dibromoethane and 1,2-dichloroethane, can be activated by GSH conjugation (Guengerich 1994). 1,2-Dibromoethane is a former pesticide and anti-knock agent, and 1,2-dichloroethane is still used as precursor of vinyl chloride. Both compounds react with GSH to give a half-mustard (S-(2-haloethyl)-glutathione) from which a reactive episulphonium ion can be formed. The episulphonium ion reacts with bio-macromolecules, including DNA, leading to mixed GSH-DNA adducts. In DNA, the S-[2-(N^7-guanyl)ethyl]-glutathione (GE-SG) is preferentially formed and the corresponding MA (S-[2-(N^7-guanyl)ethyl]-MA) is excreted in urine. As can be expected, the formation of S-[2-(N^7-guanyl)ethyl]-MA is much lower with the chlorine compound compared with the bromine analogue (Guengerich 1994). Many other dihalogenated alkanes are activated by GSH conjugation (see table 3). GSH conjugation enhanced mutagenicity in the *Salmonella typhimurium* assay of dibromomethane (Thier *et al.* 1995), dichloromethane (Thier *et al.* 1996), 1,2-dibromoethane (Van Bladeren *et al.* 1980, Thier *et al.* 1996) but not of 1,4-dibromobutane (Thier *et al.* 1995). These compounds, having one or two carbon atoms, readily form reactive sulphur intermediates (sulphide ions, episulphonium ions or sulphonium ions), after GSH conjugation. Bifunctional compounds with other chain lengths (e.g. n=3) do not readily form such reactive sulphur intermediates and the 5-membered ring resulting from 1,4-dihalobutanes is rather stable to nucleophilic attack resulting in a lower mutagenic activity compared to the compounds with one or two carbon atoms (Inskeep and Guengerich 1984, Buijs 1985). Mixed GSH-DNA adducts are

formed by the same mechanism from 1,2-dibromo-3-chloropropane (Humphereys *et al.* 1991) and 1,2,3-trichloropropane (La *et al.* 1996). GSH conjugation of 1,2-dibromoethane (Ploemen *et al.* 1995), and many other dihalogenated compounds, is in humans generally catalysed by the genetically polymorphic GST T 1–1 (see table 3). GST A 2–2 also has high activity for 1,2-dibromoethane (Cmarij *et al.* 1990), while GST M and GST P enzyme families show low and no activity, respectively (Inskeep and Guengerich 1984).

β-Elimination of cysteine S-conjugates is catalysed by the enzyme cysteine conjugate β-lyase (also C-S lyase). This enzyme is present in many intestinal bacteria, and different forms are also found in the liver and kidney (Cooper 1994, Commandeur *et al.* 1995). In the kidney a mitochondrial and a cytosolic form of β-lyase has been found. The cytosolic form is identical to glutamine transaminase K. Different forms are also found in the liver and one of these forms is identical to kynureninase. β-Lyase enzymes are dependent on pyridoxal 5′-phosphate, and when a Schiff' base is formed with the co-factor pryidoxal 5′-phosphate, α-proton abstraction followed by the β-elimination occurs. Many L-Cysteine S-conjugates from halogenated alkenes are recognized as substrates by β-lyase. The corresponding D-cysteine S-conjugates and MAs (N-acetyl-L-cysteine S-conjugates) are not substrates for β-lyase (Commandeur *et al.* 1995). The β-elimination results in a free thiol, pyruvate and ammonia. Many thiol-compounds are toxic, because they may be auto-oxidized with subsequent formation of thiyl radicals and reactive oxygen species (Ballatori 1994, Dekant *et al.* 1994, Munday 1994). The auto-oxidation can be catalysed by metal ions. Secondary reactions may lead to formation of disulphides, for instance with cysteine residues in proteins. Chemically stable thiols, e.g. from 6-mercaptopurine, may be oxidized enzymatically to highly reactive sulphenic acids (R-SOH) (Van Den Broek *et al.* 1990). β-Elimination of cysteine S-conjugates from haloalkenes results in thiol-compounds which may rearrange to highly reactive intermediates such as thionoacyl halides (R–C=S), thiiranes (R–CSC–R, CSC in three membered ring) and thioketenes (R–C=C=S) Koob and Dekant 1991, Commandeur *et al.* 1996). The first two types are formed from 2-chloro-1,1,2-trifluoroethyl-cysteine (CTFEC, from 1,1-dichloro-2,2-difluoroethene) and 1,1,2,2-tetrafluoroethyl-cysteine (TFEC, from tetrafluoroethene) (Boogaard *et al.* 1989, Commandeur *et al.* 1996). The latter type of intermediate is formed from 1,2-dichlorovinyl-cysteine (from 1,1,2-trichloroethene) (Dekant *et al.* 1988b, Koob and Dekant 1991), 1,2,2-trichlorovinyl-cysteine (TCVC, from tetrachloroethene, also known as perchloroethylene) (Dekant *et al.* 1988b) and 1,2,3,4,4-pentachlorobutadienyl-cysteine (PCBC, from hexachlorobutadiene) (Birner *et al.* 1995, Dekant 1996). For unidentified cysteine S-conjugates from 1,1-dichloroethene (vinylidene chloride) (Cavelier *et al.* 1996), β-lyase mediated mechanisms as well as oxidative mechanisms were suggested to cause renal toxicity (Dekant 1996).

Hydroquinones and 4-aminophenols can produce oxidative stress by a redox-cycling mechanism and multiple addition of GSH to these compounds has been shown (Monks and Lau 1994). However, GSH conjugation does not eliminate the redox activity. The effect of GSH conjugation is that the hydroquinone and aminophenol S-conjugates are efficiently transported to the kidney and accumulated in this organ. The high GGT and dipeptidase activity in the kidney seems to facilitate this accumulation.

Many electrophilic compounds can be transported as conjugates to GSH

(Baillie and Kassahun 1994, Monks and Lau 1994). Additional to accumulation in the kidney, reversible binding to GSH can deliver the reactive compounds to tissues distant from the site of conjugation, including the brain. GSH conjugation of isothiocyanates, isocyanates, and α, β-unsaturated aldehydes are reversible, and their GSH-conjugates are in equilibrium with their parent compounds. Isothiocyanates (R–N=C=S) from natural products (e.g. allyl isothiocyanate from cruciferous vegetables and mustard (Jiao *et al.* 1994) and benzylisothiocyanate from garden cress and other vegetables (Görler *et al.* 1982), and from synthetic origin readily react with thiols, forming dithiocarbamidic esters (Baillie and Kassahun 1994, Monks and Lau 1994). The corresponding MAs are generally prominent urinary metabolites. However, the equilibrium depends on pH (dithiocarbamidic esters are base labile), and the relative concentrations of the parent chemical, GSH, and the conjugate in a particular tissue. Therefore, GSH can transport the isothiocyanate from the original site of formation to other tissues. Isocyanates (R–N=C=O) are generally more reactive compared with the corresponding isothiocyanates. Because of their reactivity with peptides, proteins, and nucleic acids they are generally highly toxic (Baillie and Kassahun 1994). Isocyanates are widely used in the manufacture of pesticides, polyurethanes, paints, and are also formed from drugs such as fotemustine (Brakenhoff *et al.* 1994), so it is an interesting group of chemicals from an occupational point of view. Like isothiocyanates, thiol conjugates from isocyanates are reversible and the equilibrium is also dependent on physico-chemical circumstances such as the pH and the relative concentrations. A notorious isocyanate is methylisocyanate, which will be discussed in some depth later in this review.

α, β-Unsaturated aldehydes react with GSH, by Michael addition on the β-position, and the reaction is theoretically reversible (Baillie and Kassahun 1994, Monks and Lau 1994). However, S-conjugates from α, β-unsaturated aldehydes are quite stable and the reverse reaction (*retro*-Michael cleavage) may require further metabolism of the S-glutathionyl moiety before the original aldehyde is liberated again. For example, oxidation of the GSH-conjugate of acrolein to its mercapturic acid sulphoxide favours β-elimination of acrolein from the molecule (Hashmi *et al.* 1992, Ramu *et al.* 1996).

The last mechanism by which GSH conjugation may lead to toxicity concerns GSH-depletion. However, at the current low exposure levels significant GSH-depletion happens only rarely, while at higher doses in animal studies, GSH-depletion may occur. GSH plays an important role in the detoxification of radicals and peroxides and depletion of GSH leaves the cell less well protected against these reactive species, (section III.1 Homeostasis and functions of glutathione) (Meister 1991, Munday 1994).

Analysis of urinary mercapturic acid excretion

Mercapturic acids consists of a N-acetylated cysteine moiety and an S-bound group originating from the conjugated electrophile. The N-acetylated cysteine moiety is relatively hydrophillic, whereas the S-bound group is usually lipophilic in nature. MAs are non-volatile compounds and the analytical matrix, urine, contains numerous salts and organic compounds. Many selective methods that have been developed for the determination of MAs are based on HPLC or GC separations and therefore salts (HPLC and GC) and water (GC) have to be removed from

urine. As a non-selective method to determine urinary thioether concentration, the often thioether assay is used. In this assay, the thiol content in samples is determined spectrophotometrically after alkaline hydrolysis and reaction with Ellman reagent (5,5′-dithiobis(2-nitrobenzoic acid)) (Ellman 1959, Seutter-Berlage *et al.* 1977). Major disadvantages of this method are that no information is obtained about the structure of the thioethers determined, and that relatively high background values occur making this method less suitable for the determination of low exposure levels. However, it can be successfully applied in occupational settings when high exposure levels to mixtures of compounds occur. For instance, significant differences between exposed and unexposed workers were found in tyre manufacturing and rubber synthesis workers, when groups of workers were divided according to smoking behaviour (Vianio *et al.* 1978).

Many chromatographic methods have been used to selectively detect individual MAs in urine (Van Welie *et al.* 1992). These methods consist of three steps: (1) sample pretreatment (extraction) and derivatization, (2) chromatographic separation and (3) detection by (selective) detectors. An overview of the different methods used to analyse MAs is given in table 5.

For the preparation of urine samples for HPLC and GC analysis, extraction of acidified urine by ethylacetate, diethylether, or by solid phase extraction (SPE; RP18), with simultaneous salting out or the use of counter-ions as needed, is used to remove salts and water and for partial sample clean-up. These extraction procedures are only successful when the xenobiotic restgroup is sufficiently lipophilic. For MAs with small or relatively hydrophillic xenobiotic S-bound groups, e.g. 3,4-dihydroxybutyl-MA, lyophilization combined with repeated salt precipitation by methanol/acetone, seems to be more appropriate (Bechtold *et al.* 1994).

In principle, MAs can be analysed by HPLC without further derivatization when electrochemical detection is used. When fluorescence detection is applied, derivatization (using *o*-phthaldialdehyde for example) is often used. Before MAs can be derivatized by *o*-phthaldialdehyde, the MAs must be deacetylated enzymatically, by acylase (Sakai *et al.* 1995) or chemically by acid hydrolysis (Stommel *et al.* 1989, Wu *et al.* 1993). MAs may be unstable in strong acidic environments used in acid hydrolysis; 2-carbamoylethyl-MA, a metabolite of acrylamide, was converted to carboxyethyl-cysteine during acid hydrolysis (Wu *et al.* 1993). An alternative way to prepare MAs for fluorescence detection is alkaline hydrolysis followed by monobromobimane derivatization (Dehnen 1990). This method is in fact a more specific application of the thioether assay. By this method, labile MAs, e.g. formed from diethylmaleate, are converted to N-acetyl-L-cysteine and the total urinary N-acetyl-L-cysteine can be determined by HPLC. More stable MAs, e.g. formed from benzene, may undergo β-elimination by this method and monobromobimane derivatives of the resulting thiols can be determined by HPLC (Dehnen 1990).

High pressure liquid chromatography (HPLC) is frequently used in bioanalytical work, especially for thermolabile or nonvolatile analytes which can not be determined by GC. HPLC separation of urinary MAs is mostly performed on reverse-phase (RP18) columns, using gradient or isocratic elution systems. Simple UV detection (λ=210–256 nm) is frequently applied, resulting in method detection limits ranging from 1 to 14 mg l^{-1} (Norström *et al.* 1986, Truchon *et al.* 1990). When more sophisticated detection systems are used, e.g. fluorescence detection after *o*-phthalaldehyde derivatization (Gérin and Tardif 1986, Stommel

Table 5. Comparison of different analytical methods used for the determination of mercapturic acids in urine samples.

Method	Selectivity	Cheapness	Det. limit. (mg L^{-1})	Mercapturic acid determined	Parent compound	Ref.
Thioether assay	– – – –	+ + + +	high back-ground	general thioethers	general exposure to electrophiles	(1, 2)
HPLC-UC	+	+	2	benzyl-MA	toluene	(3, 4)
			1	*o*-methylbenzyl-MA	*o*-xylene	(3, 4)
			14	1-phenyl-2-hydroxyethyl-MA (+ isomer)	styrene	(5)
				3-hydroxypropyl-MA	allylamine allylhalides	(6)
			1·5	phenyl-MA	benzene	(7)
HPLC-Fluorescence	+	+	0·5	phenyl-MA	benzene	(8)
			2·5	propionamide-MA (as carboxyethyl-MA)	acrylamide	(9)
			3·0	hydroxyethyl-MA	ethylene oxide and other	(10)
	+	+	0·004 (?)	alkaline hydrolysis followed by mono-bromobiman derivatization	total thiophenol	(11)
HPLC electro-chemical detection	+	+	0·5	isovalerylurea-2-MA	α-bromo-iso-valerylurea	(12)
GC-FID	++	+	6	3-hydroxypropyl-MA	acrolein	(13)
			6	2-carboxyethyl-MA	acrolein/ethyl- or butylacrylate	(13,14)
			1	2-cyanoethyl-MA	acrylonitrile	(15)
GC-NPD	++	+	0·07 (Z) 0·04 (E)	Z- and E-3-chloro-propenyl-2-MA	Z- and E-1, 3-dichloropropene	(16)
			0·2	N-methylcarbamoyl-MA (after hydrolysis to ethyl-N-methyl-carbamate)	N,N-demethyl formamide and N-methylformamide methylisocyanate	(17,18)
GC-FPD	++	+	0·1	Z- and E-3-chloro-propenyl-2-MA	Z- and E-DCP	(19)
			0·06 0·02	Z- and E-3-chloropro-penyl-2-MA	Z- and E-DCP	(16)
GC-MS (PIEL, SIM)	+++	– –	1	N-methylcarbamoyl-MA	N,N-dimethylfor-mamide N-methylformamide methylisocyanate	(20)
			0·01	benzyl-MA	toluene	(21)
			0·5	benzyl-MA	toluene	(4)
			0·3	*o*-methylbenzyl-MA	*o*-xylene	(4)
			0·1	3,4-dihydroxy-butyl-MA	1,3-butadiene	(22)
			0·01	phenyl-MA	benzene	(8)
			0·001			(23)
GC-MS (NICI, SIM)	+++	– –	0·006	trichlorovinyl-MA	tetrachloroethene	(24)
GC-MS (NICI, SIM)	+++	– –	13 10–9	1,2- and 2,2-dichloro-vinyl-MA	1,1,2-trichloro-ethene	(25)
GC-MS/MS (PICI)	++++	– – – –	0·0005	phenyl-MA	benzene	(26)
GC-MS/MS (NICI)	++++	– – – –	0·0001	trichlorovinyl-MA	tetrachloroethene	(24)
LC-MS (thermospray, SIM)	++++	– – – –	11	N-methylcarbamoyl-MA	N,N-dimethylfor-mamide N-methylformamide	(27)

Table 5. (*cont.*)

Method	Selectivity	Cheapness	Det. limit. (mg L^{-1})	Mercapturic acid determined	Parent compound	Ref.
LC-MS/ MS (ionspray, SIM)	++++	----	not mentioned	2-chloroethylcarbamoyl-MA	2-chloroethylisocyanate intermediate from BCNCI	(28)
	NOTE: in general, detection limits down to 0·0005 mg L^{-1} are possible					(29)
DIP-MS/ MS (PIEI)	++++	----	0·05	3-hydroxypropyl-MA	acrolein	(26)
ELISA	+++	++	0·001	1,2-dihydro-1-hydroxy-2-naphthyl-MA (and isomers)	naphthalene	(30)
ELISA	+++	++	0·0025	4-ethene-6-isopropylamino-1,3,5-triazin-2-MA	atrazine	(31)

Abbreviations used: HPLC or LC, high pressure liquid chromatography; UV, ultraviolet absorption detection; FLU, fluorescence detection; MA, mercapturic acid moiety; GC, gas chromatography; FID, flame ionisation detector; NPD, nitrogen-phosphor detector; FPD, flame photometric detector (sulphur selective); MS, mass spectrometric detector; PI, positive ion; EI, electron impact ionization; SIM, selected ion monitoring; NI, negative ion; CI, chemical ionization; MS/MS, tandem mass spectrometry; DIP, direct insertion probe; ELISA, enzyme linked immunosorbent analysis.

References used: 1, Seutter-Berlage *et al.* 1977; 2, Vianio *et al.* 1978; 3, Tanaka *et al.* 1990; 4, Norström *et al.* 1986; 5, Truchon *et al.* 1990; 6, Sanduja *et al.* 1989; 7, Jongeneelen *et al.* 1987; 8, Stommel *et al.* 1989; 9, Wu *et al.* 1993; 10, Gérin and Tardif 1986; 11, Dehnen 1990; 12, Te Koppele *et al.* 1988; 13, Linhart *et al.* 1996; 14, Linhart *et al.* 1994b; 15, Jakubowski *et al.* 1987; 16, (Onkenhout *et al.* 1986; 17, Sakai *et al.* 1995; 18, Mràz 1988; 19, Van Welie *et al.* 1989; 20, Casal-Lareo *et al.* 1995; 21, Takahashi *et al.* 1994; 22, Bechtold *et al.* 1994; 23, Boogaard and Van Sittert 1995; 24, Bartels 1994; 25, Bernauer *et al.* 1996; 26, Stanek *et al.* 1993; 27, Slatter *et al.* 1991; 28, Davis *et al.* 1993; 29, Gelpi 1995; 30, Marco *et al.* 1993; and 31, Lucas *et al.* 1993.

et al. 1989) or electrochemical detection using a bromine generation cell (Te Koppele *et al.* 1988), slightly lower detection limits ranging from 0·5 to 3 mg l^{-1} may be reached. In a bromine generation cell, electrochemically generated bromine reacts with thioethers in the eluate. The detection limits reached by HPLC are mostly too high to be used for human exposure assessment when exposure occurs at low levels. Additionally, no structural information is obtained by HPLC based methods. However, HPLC analysis is a convenient analytical tool in the quantitative determination of MAs excreted by animals treated with higher doses of various compounds. Examples of MAs which have been determined by HPLC in urine of laboratory animals are: benzyl-MA (from toluene) (Norström *et al.* 1986, Tanaka *et al.* 1990), *o*-methyl-benzyl-MA (from *o*-xylene) (Norström *et al.* 1986, Tanaka *et al.* 1990), isovalerylurea-2-MA (from α-bromoisovalerylurea) (Te Koppele *et al.* 1988), 1-phenyl-2-hydroxyethyl-MA (and *regio* isomer, from styrene) (Truchon *et al.* 1990), 2-hydroxyethyl-MA (from ethylene oxide) (Gérin and Tardif 1986), 3-hydroxypropyl-MA (from allyl amine or from allyl halides) (Sanduja *et al.* 1989) and phenyl-MA (from benzene) (Jongeneelen *et al.* 1987, Stommel *et al.* 1989).

HPLC combined with mass spectrometry (LC-MS or LC-MS/MS) is receiving increasing attention in bioanalytical work. Mass spectrometers are usually coupled to HPLC-systems by thermospray or ionspray interfaces (Arpino 1990). An ionspray LC-MS/MS method was recently applied to the identification and quantitative determination of 2-chloroethylcarbamoyl-MA in urine and the corresponding GSH S-conjugate in bile from rats treated with th

N,N′-bis(2-chloroethyl)-N-nitrosurea (BCNU) (Davis *et al.* 1993). Unfortunately, the limit of detection was not mentioned. N-Methylcarbamoyl-MA (MCMA), a metabolite of methylisocyanate and of caracemide, was identified in the urine of rats treated with methylisocyanate or caracemide, respectively, by thermospray LC-MS (Slatter *et al.* 1991, Slatter *et al.* 1993). However, only a relative high limit of detection (11 mg l^{-1} urine) was reported for MCMA in rat urine (Slatter *et al.* 1991). In general extremely low limits of detection (down to 0·5 μg l^{-1}) are possible by using LC-MS, especially when tandem mass spectrometry (MS/MS) and selected ion monitoring (SIM) is used (Gelpi 1995).

Gas chromatography (GC) is also used for the determination of MAs in urine. When MAs are analysed by GC-methods, they also have to be isolated from urine as described above. Additionally the volatility needs to be improved by derivatization (Dinoff *et al.* 1992). The most common derivatization used for this purpose is methylation by diazomethane or methanolic hydrochloride, but trimethylsilylation, acetylation, n-butylation, and pentafluorobenzylation have also been used. Some alternative destructive procedures for the isolation and derivatization of urinary MAs have been developed: alkaline permethylation, Raney-Nickel catalysis and derivatization with acetic anhydride/methanesulphonic acid. These methods are again variants of the thioether assay. During alkaline permethylation, a base catalysed β-elimination of the N-acetylcysteine moiety occurs, and the xenobiotic S-bound group is liberated attached to the sulphur atom of the MA. The thiols are methylated by the methyliodide present and the resulting methylthioethers are subsequently extracted with n-pentane. This procedure has been applied to MA-metabolites (Zheng and Hanzlik 1992) and protein S-adducts (Slaughter *et al.* 1993) of bromobenzene and for GSH S-conjugates of paracetamol (Bessems *et al.* 1996). The alkaline permethylation procedure can in principle also be used to investigate GSH and cysteine S-adducts. In another destructive method, cysteine S-adducts are cleaved using Raney-Nickel catalysis, liberating the xenobiotic S-bound group. When the S-bound group contains a hydroxyl, it can be derivatized further to a pentafluorobenzoate ester prior to GC-ECD or GC-MS analysis. Raney-Nickel catalysis was applied to GSH S-conjugates and MAs synthesized from 4-hydroxynonenal (Uchida and Stadtman 1992) and to haemoglobin and albumin adducts of styrene oxide (Rappaport *et al.* 1993). In the third destructive method, β-elimination of cysteine-thioethers and concomitant acetylation of the resulting thiols is performed by addition of acetic anhydride and methanesulphonic acid to dry samples. Therefore, extraction-evaporation, drying or lyophilization must precede this type of derivatization. After destruction of the excess anhydride and subsequent extraction of the derivatives, GC and GC-MS analysis can proceed. When trifluoroacetic anhydride is used instead of acetic anhydride, derivatives with high responses to GC-ECD analysis are formed. This method has been applied to GSH S-conjugates, cysteine S-conjugates and MAs of naphthalene and other aromatic compounds (Bakke 1982) and to haemoglobin-cysteine adducts of benzene oxide (Yeowell-O'Connell *et al.* 1996). Unfortunately the detection limits were not given and the methods occasionally resulted in relatively high amounts of side products (Bakke 1982). A very elegant alternative method to prepare MAs in urine for GC analysis concerns a phase-transfer procedure (Dinoff *et al.* 1992). In this procedure the extraction of benzyl-MA from urine to a dichloromethane phase is facilitated using tetrabutylammonium to form an ion pair. The benzyl-MA extracted was derivatized to its

pentafluorobenzyl ester by the addition of pentafluorobenzylbromide to the organic layer. The resulting derivatives usually are readily detectible with GC-ECD or negative ion chemical ionization measurement mass spectrometry (NICI-MS) (Poole and Schuette 1986).

Different detectors are used in the GC determination of MAs: flame ionization (FID), electron-capture (ECD), nitrogen-phosphor (NPD), nitrogen selective flame thermoionic (FTD) and sulphur selective flame photometric detector (FPD). The detection limits which are reached (0·02–0·2 mg l^{-1} are 2 to 25 times more sensitive compared to the corresponding limits reached by HPLC-based methods, and application of these methods in human exposure measurements is often possible. Examples of MAs which have been determined by GC are: 3-hydroxypropyl-MA (from acrolein and others) (Linhart *et al.* 1996), 2-carboxyethyl-MA from acrolein (Linhart *et al.* 1996) and acrylate esters (Linhart *et al.* 1994b), N-methylcarbamoyl-MA from N,N-dimethylformamide (Mràz 1988, Sakai *et al.* 1995) and Z- and E-3-chloro-2-propenyl-MA from Z- and E-1,3-dichloropropene (Van Welie *et al.* 1989). The detectors used and the respective detection limits are given in table 5.

The application of GC-MS based methods is becoming increasingly popular in human biological monitoring. Its flexibility allows it to be used for the identification of metabolites in the scan mode, and for the quantitative determination of low concentrations when characteristic ions are monitored in the selected ion mode (SIM). Electron impact ionization (EI) is mostly used to generate ions, but chemical ionization (CI) using methane or ammonia as reagent gases is also often applied in the determination of MAs (Vermeulen *et al.* 1983). Reasonably good sensitively, ranging from 0·001 to 0·5 mg l^{-1} is attained for MAs in urine when splitless injection is used for the GC. With high volume injection techniques, like solvent purge injection systems, even lower detection limits should be possible (Hinshaw 1989). Examples of MAs determined by GC-MS are: benzyl-MA from toluene (Norström *et al.* 1986, Takahashi *et al.* 1994), 3,4-dihydroxybutyl-MA from 1,3-butadiene (Bechtold *et al.* 1994), trichlorovinyl-MA from tetrachloroethene (Bartels 1994) and phenyl-MA from benzene (Stommel *et al.* 1989, Boogaard and Van Sittert 1995).

Tandem mass spectrometry (MS/MS) techniques have also been used for the structural elucidation and quantitative determination of MA metabolites (Deterding *et al.* 1989, Stanek *et al.* 1991, Jones *et al.* 1993). Recently, 3-hydroxypropyl-MA was determined without derivatization down to 0·05 mg l^{-1} in ethylacetate extracts of human urine (Stanek *et al.* 1993). The method was selective enough to omit a GC separation. Instead a direct insertion probe was used to introduce the sample into the MS/MS. In this method the first MS was used to focus on a fragment of 3-hydroxypropyl-MA (HPMA) (at *m/z* 162 (M-59, PIEI conditions)). In the second MS, daughter ions of *m/z* 162, at *m/z* 144, 99 and 58, were used to identify and quantify the MA. For other MAs, e.g. phenyl-MA (PMA), alone MS/MS, was not selective enough, and a GC separation had to precede the MS/MS. Extremely good sensitivity down to 0·0005 or 0·0001 mg l^{-1}, is possible using MS/MS based methods (Stanek *et al.* 1993, Bartels 1994). For HPMA and PMA, these methods were sensitive enough to determine concentrations in the urine of apparently non-exposed humans (Stanek *et al.* 1993). However, one disadvantage of MS/MS techniques is the very expensive equipment needed which may not be available to most laboratories.

Recent developments in other bioanalytical techniques, like capillary electrophoresis (Smith *et al.* 1993, Huang and Kok 1995) and on-line LC-GC coupling (Chappell *et al.* 1992), have not yet been used for the determination of MAs in urine. With capillary electrophoresis an extremely high separation efficiency is reached and this may be beneficial for such a complex matrix. The recent development of enzyme-linked immunosorbent assays (ELISA) for the specific detection of the MAs of naphthalene (Marco *et al.* 1993) and atrazine (Lucas *et al.* 1993) is also interesting. The sensitivity is excellent, 0·001 and 0·0025 mg l^{-1} urine, respectively. In general, enzyme-linked immunosorbent assay (ELISA) based methods are inexpensive, but it is usually time consuming to raise the antibodies (1–2 months). Antibodies have also been produced to 2-hydroxyethyl-MA (HEMA) and to PMA, and these antibodies were suggested as being useful in an immunoenrichment procedure (Wright *et al.* 1995).

In the preceding sections it was stated that the MA-pathway might lead as well to the urinary excretion of other end-products instead of MAs, like cysteine S-conjugates, mercaptopyruvic acids, mercaptolactic acids, mercaptoacetic acids and sulphoxides of cysteine S-conjugates and of MAs (see figure 4). However, in most cases MAs are quantitatively the most important metabolites in rats (Commandeur *et al.* 1995). All mentioned alternative end-products of the MA-pathway are more polar and more hydrophillic than MAs, therefore extraction of these compounds from urine into an organic phase is more difficult. Ion exchange methods in sample clean-up or in analytical HPLC separation, however, were successful. Examples are the clean-up of urine for the determination of S-[2-carboxy-1-(1H-imidazole)ethyl]-3-thiolactic acid (the mercaptolactic acid metabolite of histidine, Kinuta *et al.* 1994) and the determination of the mucolytic drug carboxymethylcysteine in blood (Iwase *et al.* 1995). Alternatively, after deproteinization by means of precipitation or filtering and subsequent derivatization, urine samples have also been used directly for reverse phase HPLC separation. This was done for carboxymethylcysteine, and its metabolites carboxymethyl-MA, methylcysteine, thiodiglycolic acid and the corresponding sulphoxides (Woolfson *et al.* 1987, Staffeldt *et al.* 1991) and for the metabolites of hexachlorobutadiene, 1,2,3,4,4-pentachlorobutadiene-MA and corresponding sulphoxide (Birner *et al.* 1995). Mainly UV, electrochemical or fluorescence detection was applied to detect the analytes. Derivatization generally improves the lipophilicity, thus facilitating extraction prior to separation (Koike and Koike 1984). Mercaptopyruvic acids, mercaptolactic acids, and mercaptoacetic acids can be determined by GC-based methods after derivatization (e.g. methylation) of the carboxylic acid groups (James and Needham 1973, Görler *et al.* 1982). Extraction, drying or an LC-separation usually precedes this analysis. For cysteine S-conjugates a quantitative acetylation procedure, forming MAs, has been described for aqueous solutions of cysteine S-conjugates (Dekant *et al.* 1988a, Commandeur *et al.* 1989). Sulphoxides are generally thermolabile and can therefore not be determined by GC. HPLC-based methods are more appropriate for their isolation and analysis. After isolation, mass spectrometric techniques can then be used for their identification. Thus the MA-sulphoxide of hexachlorobutadiene was identified by thermospray mass spectrometry (Birner *et al.* 1995). Although the previously mentioned alternative end-products of the MA-pathway have been detected in the urine of animals, little is known about excretion of these products in human urine. Therefore, no applications of determination of those alternative products are currently made in occupational exposure assessments.

Mercapturic acids as biomarkers of exposure to industrial chemicals

Many toxicologically relevant compounds are electrophilic and can react with nucleophilic centers in biomacromolecules such as DNA, proteins and GSH, forming covalent adducts. These adducts, together with MAs (Van Welie *et al.* 1992), as metabolites of GSH S-conjugates, can all be used in the assessment of internal exposure to the respective toxic parent compounds (Nestmann *et al.* 1996). The urinary excretion of MAs is a very useful tool in biological monitoring (BM) studies (Vermeulen 1989) and their use in the assessment of internal human exposure to toxic chemicals has become more or less routine (Van Welie *et al.* 1992). The main advantages of their application in human exposure measurement are the non-invasive sampling of urine, and their relatively short half-lifes which allow repeated sampling and the establishment of direct relationships between internal exposure and specific job-types (Henderson *et al.* 1989, Van Welie *et al.* 1992). Depending on the parent compound, the amount of the internal dose excreted as MA can be reasonably high, which enables the determination of low exposure levels.

In the following sections an inventory is made of applications of MAs as biomarkers of human exposure to industrial compounds. The specificity for the anticipated parent compounds of the MAs used, sources of background, relationships with adverse effects and, where appropriate, genetic polymorphisms influencing the dose-excretion relationships will be discussed.

Ethylene oxide and related compounds

Ethylene oxide is a reactive epoxide widely used in industry and as sterilant in hospitals (IARC 1994b). Ethylene oxide is also an environmental air pollutant, and a proven carcinogen in animals and humans (IARC 1994b). Reproductive toxicity in females was also reported (Rowland *et al.* 1996). In haemodialysis patients, severe allergic reactions have been reported, resulting from sterilization of dialysis membranes with ethylene oxide (Grammer *et al.* 1984, Rumpf *et al.* 1985). Ethylene oxide is the principal metabolite of ethene (Filser and Bolt 1983), which is also an important industrial chemical, an air pollutant and is detected in the smoke of cigarettes (IARC 1994b, Löfroth *et al.* 1989, Bostrom *et al.* 1994).

In the urine of rats treated with ethylene oxide, 2-hydroxyethyl-MA (HEMA) accounted for 30 % of the ethylene oxide dose. Remarkable species differences were found in the HEMA excretion in various rodent species (Tardif *et al.* 1987). In mice HEMA accounted for 8 % of the ethylene oxide dose, while the cysteine analogue (2-hydroxyethyl-cysteine) and the oxidized form of HEMA (carboxymethyl-MA) were also detected. In rabbits none of these metabolites were identified.

Recently, HEMA was determined in the urine of ethylene oxide-exposed hospital workers (disinfectors) (Popp *et al.* 1994). HEMA was extracted from acidified urine by ethyl acetate, deacetylated to 2-hydroxyethyl-cysteine, separated with HPLC and subsequently detected by electrochemical or fluorescence detection as a *o*-phthaldialdehyde derivative. Unfortunately, the detection limits were not mentioned by these authors. The disinfectors excreted 558 ± 642 μg HEMA in 24 h, whereas non-exposed controls excreted 33 ± 57 μg HEMA in 24 h. Although urinary HEMA excretion was suitable to discriminate between ethylene oxide exposed and non-exposed individuals, no correlation with individual peak

exposure levels was found. It was suggested that interindividual differences in conjugative metabolism of ethylene oxide might have obscured such a relationship. Incubation of human blood samples with radiolabeled ethylene oxide resulted in low and high GSH-conjugator subgroups (Föst *et al.* 1991). An eight-fold higher binding to the low molecular weight fraction (including GSH), isolated from the erythrocytes in the blood samples, was identified for the high conjugators compared to the low conjugators. Recently, the polymorphic GST T 1–1 was shown to be involved in the GSH conjugation of ethylene oxide (Hallier *et al.* 1993) (table 3). Approximately one quarter of the population tested (n =36) showed a markedly slower ethylene oxide metabolism upon incubation with blood samples. In these so-called 'non-conjugators' more DNA damage could be detected in the lymphocytes upon incubation with ethylene oxide.

For many industrial chemicals, other than ethene and ethylene oxide, the urinary excretion of HEMA (or 2-hydroxyethyl-cysteine or the corresponding MA) has been shown in animals. Such chemicals are 2-bromoethanol, 1,2-dibromoethane, 1,2-di(methanesulphonate)ethane, vinylchloride, acrylonitrile and

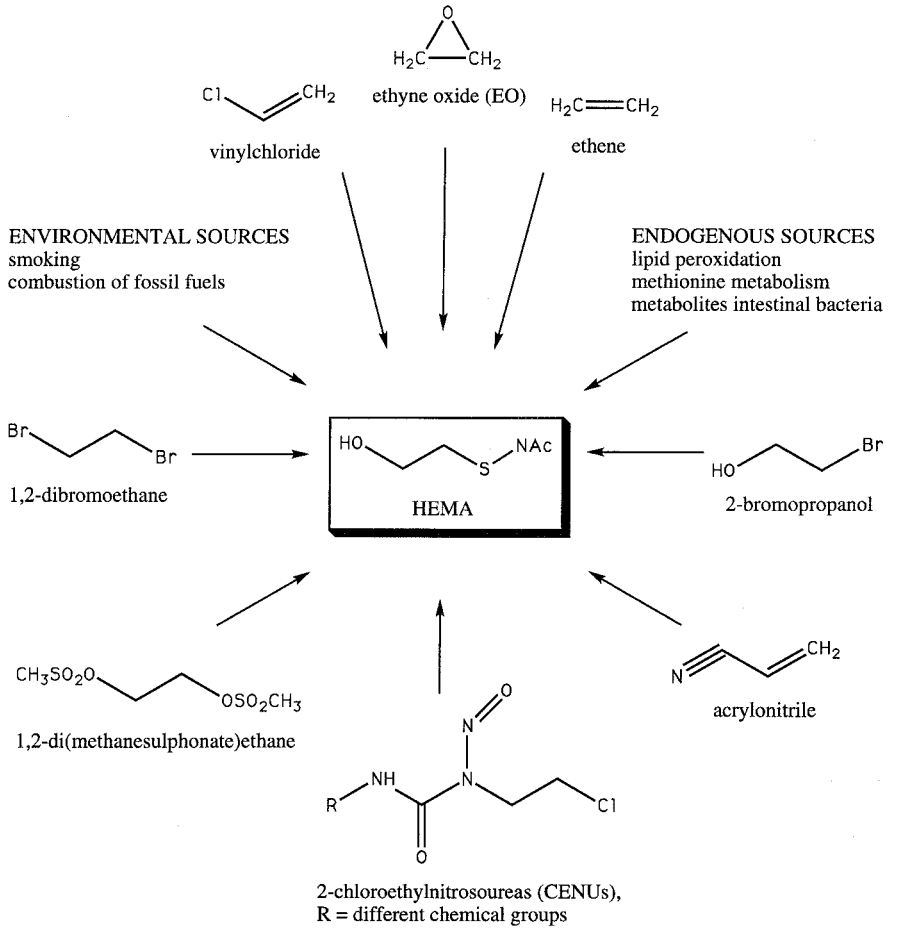

Figure 6. Different compounds and processes probably leading to urinary excretion of 2-hydroxyethyl mercapturic acid (HEMA). S-NAc=N-acetyl-cysteine.

chloroethylnitroso ureas (CENUs) (Vermeulen *et al.* 1989). The compounds and processes leading to HEMA are summarized in figure 6. It has been suggested that urinary HEMA excretion can be a tool in the biological monitoring of human exposure to these compounds. HEMA is therefore regarded as a relatively unselective biomarker of exposure. When using HEMA as biomarker of human exposure to ethylene oxide or other compounds, one might expect large interindividual differences due to genetic polymorphisms (Föst *et al.* 1991, Hallier *et al.* 1993), environmental factors and due to variable background excretions (Fu *et al.* 1979, Törnqvist *et al.* 1986, Van Sittert *et al.* 1993a).

Monitoring of long term low level exposures to ethylene oxide is generally performed by the determination of N-terminal 2-hydroxyethylvaline in haemoglobin, because it may be more selective for ethylene oxide than HEMA (Törnqvist *et al.* 1986, Van Sittert *et al.* 1993a). These adducts were detected both in smokers and in non-smokers (Törnqvist *et al.* 1986, Van Sittert *et al.* 1993a). The N-terminal 2-hydroxyethylvaline adducts in non-smokers may also arise from passive smoking, combustion of fossil fuels, or from endogenous processes such as lipid peroxidation, metabolism of methionine or from metabolism in intestinal bacteria (Fu *et al.* 1979, Törnqvist *et al.* 1986). It is suggested that polymorphism of GST T 1–1 would lead to a different response of haemoglobin adducts in humans (Föst *et al.* 1995). DNA adducts of ethylene oxide, mainly N^7-hydroxyethyl-guanine, are also well known. Differences in the distribution of ethylene oxide originating from endogenous or exogenous sources were also suggested to lead to different amounts of DNA- and haemoglobin adducts formed (Bolt 1996). However, solid proof to explain this paradox is not available so far.

Acrolein and related compounds

α,β-Unsaturated aldehydes, also referred to as acrolein congeners, are generally highly reactive compounds that may react with endogenous macromolecules via Michael-type additions (Eder *et al.* 1982a). Many α,β-unsaturated aldehydes are positive in the *Salmonella typhimurium* mutagenicity assay, and various DNA adducts have been shown to be formed (Eder *et al.* 1982a, Eder and Hoffman 1993). An important representative of this group of reactive compounds is acrolein (propenal) (Eder *et al.* 1982a).

Acrolein is an industrial compound, used in the synthesis of acrylic acid (propenoic acid), ethylacrylate, butylacrylate, methionine and several homo- or copolymers (IARC 1985). Acrolein is also a product of incomplete combustion (IARC 1985) and is detectable in tobacco smoke (Löfroth *et al.* 1989), vehicle exhaust, and in the smoke of burning organic materials (Scheepers and Bos 1992). For some other chemicals the formation of acrolein as a metabolic intermediate is proposed, i.e. allyl halides (Kaye *et al.* 1972), allyl amine (Sanduja *et al.* 1989), allyl alcohol (Kaye *et al.* 1972, Sanduja *et al.* 1989) and the chemotherapeutic agents iphosphamide and cyclophosphamide (Giles 1979). The oxidation of allyl alcohol by liver alcohol dehydrogenase (ALDH) to acrolein has been proposed to lead to extensive periportal necrosis of the liver (Reid 1972). Acrolein is also formed, together with other aldehydes and 4-hydroxyalkenals during lipid peroxidation (Poli *et al.* 1985).

Upon administration of radiolabelled acrolein to rats, exhalation of CO_2 and urinary excretion were recently found to be important routes of elimination (Parent

et al. 1996). No gender differences were identified in distribution of the radiolabelled acrolein. Two MA metabolites were identified already earlier in urine of rats treated with acrolein (Sanduja *et al.* 1989, Linhart *et al.* 1996): 3-hydroxypropyl-MA (HPMA) and carboxyethyl-MA. Both MAs are believed to be formed after reduction of oxidation of a GSH-acrolein adduct, 2-aldehydoethyl-GSH, by liver alcohol dehydrogenase (ALDH) and aldehyde dehydrogenase (ADH) (Mitchell and Petersen 1989) (see figure 7). GSH conjugation of acrolein is catalysed by the polymorphic GST M 1–1, but the specific activity of GST P 1–1 is higher, and therefore genetic polymorphism of GST M 1–1 is expected to have only a minor influence on the formation of HPMA upon exposure to acrolein (see table 3) (Berhane *et al.* 1994). Carboxyethyl-MA and the corresponding sulphoxide were also identified in the urine of rats treated with ethyl- or butylacrylate (Linhart *et al.* 1994a, b).

In patients treated with cyclophosphamide, the urinary excretion of HPMA was confirmed also (Honjo *et al.* 1988). An important side-effect of cyclophosphamide, which limits its therapeutic use, is bladder cancer. It has been suggested that sulphoxidation of 2-aldehydoethyl-MA followed by alkaline β-elimination would release acrolein in the bladder, leading to toxicity (Hashmi *et al.* 1992, Ramu *et al.* 1995) (see figure 7). This concept was recently confirmed upon incubation with human lung adenoma A549 cells. An analogous MA (3-aldehydropropyl-MA), which can not release an acrolein congener, did not lead to this toxicity (Ramu *et al.* 1996).

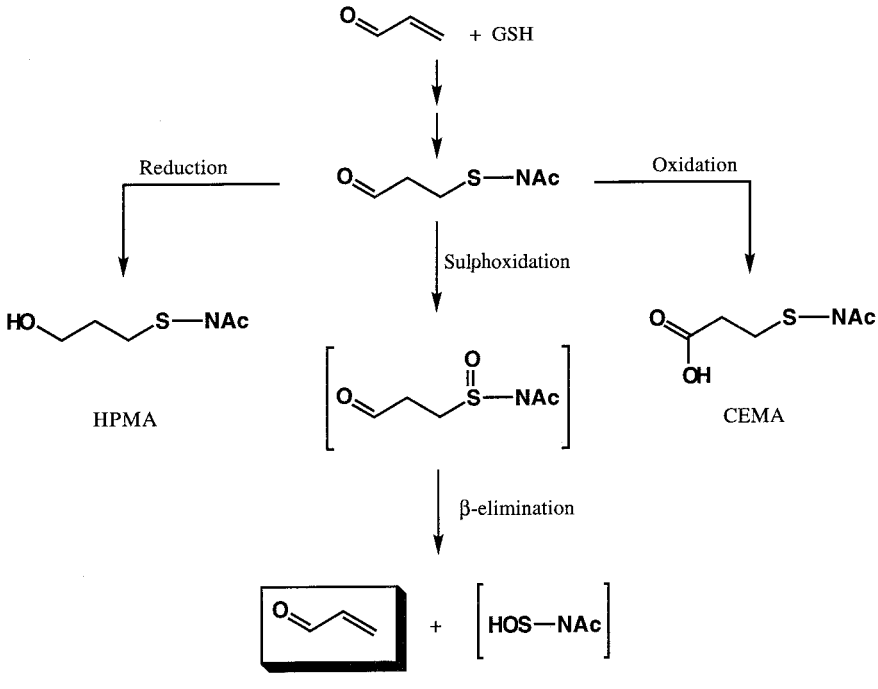

Figure 7. Formation of mercapturic acid metabolites from acrolein and the liberation of acrolein from aldehydoethyl mercapturic acid-sulphoxide by base catalysed β-elimination. HPMA=3-hydroxypropyl mercapturic acid; CEMA=carboxyethyl mercapturic acid; S-NAc=N-acetyl-cysteinyl. See Hashmi *et al.* (1992), Ramu *et al.* (1995) and Linhart *et al.* (1996).

Acrolein has been proposed to be an intermediate metabolite of various industrial compounds, and therefore urinary HPMA can probably be used as a more general biomarker of chemical exposure. In urine of rats treated with acrolein, or different precursors of acrolein, like allyl amine, allyl alcohol and allyl chloride, HPMA was found (Sanduja *et al.* 1989). Other compounds (i.e. 3-bromopropenal, 3-chloropropenal and bromopropane) led to urinary HPMA excretion without formation of acrolein as intermediate (Jones and Walsh 1979).

Using HPLC-UV and GC-FID for the determination of HPMA in urine, relatively high detection limits, i.e. 14 and 6 mg l^{-1}, were reached (see table 5) (Linhart *et al.* 1996). However, with tandem mass spectrometry (MS/MS) HPMA could be detected down to 0·05 mg l^{-1}, thus allowing the determination of background excretion in smokers (0·8–2·3 mg g^{-1} creatinine) and non-smokers (0·006–0·6 mg g^{-1} creatinine) (Stanek *et al.* 1993). Background levels of HPMA measured in human urine can originate from tobacco smoke, environmental or endogenous sources (Poli *et al.* 1985, Linhart *et al.* 1996). This indicates that when HPMA is used as biomarker of the various compounds mentioned, one should realize possible contributions to the urinary HPMA excretion from life-style factors, e.g. smoking. Various compounds and processes leading to HPMA excretion are shown in figure 8.

Acrylamide, acrylonitrile and methacrylonitrile

Acrylamide is an α,β-unsaturated carbonyl compound, utilized in the production of polyacrylamides. These are used as flocculants in the purification of drinking water, in the production of certain textiles and in gels for chromatography. Acrylamide is a genotoxic agent (Dearfield *et al.* 1995), potent cumulative neurotoxin (Calleman *et al.* 1994), germ-line mutagen (Dearfield *et al.* 1995), an animal carcinogen, and possibly also a human carcinogen (IARC 1994a). The main application of acrylonitrile is in the production of acrylic fibres. Acrylonitrile has been found to be carcinogenic in rats, however human carcinogenicity is still uncertain at this moment (Kedderis *et al.* 1995). A structural analogue of acrylonitrile, methacrylonitrile, is also used in the production of plastics. However, minimal information on the toxicity of methacrylonitrile is available (Burka *et al.* 1994).

Acrylamide

Acrylamide reacts readily with GSH and a high percentage of the dose (50 %) is excreted as 2-carbamoylethyl-MA by rats (Miller *et al.* 1982, Wu *et al.* 1993). Other MAs identified in animals treated with acrylamide arise from glycidamide, the epoxidation product of acrylamide, i.e. 1- and 2-carbamoyl-2-hydroxyethyl-MA (Sumner 1992). 2-Carbamoylethyl-MA was determined by fluorescence HPLC after hydrolysis to 2-carboxyethyl-cysteine and *o*-phthaldialdehyde derivatization with a detection limit of 0·23 mg l^{-1} (Wu *et al.* 1993, Calleman *et al.* 1994). Workers involved in the production and polymerization of acrylamide excreted up to 75 mg l^{-1} urine as 2-carbamoylethyl-MA in urine (Wu *et al.* 1993). For acrylamide it was difficult to investigate dose-excretion relationships since dermal exposure plays an important role (Wu *et al.* 1993). The main toxic effect of acrylamide, peripheral polyneuropathy, develops slowly and a biomarker of integrated long term exposure seems therefore to be appropriate. N^7-(2-Carbamoyl-2-hydroxyethyl)guanine, a

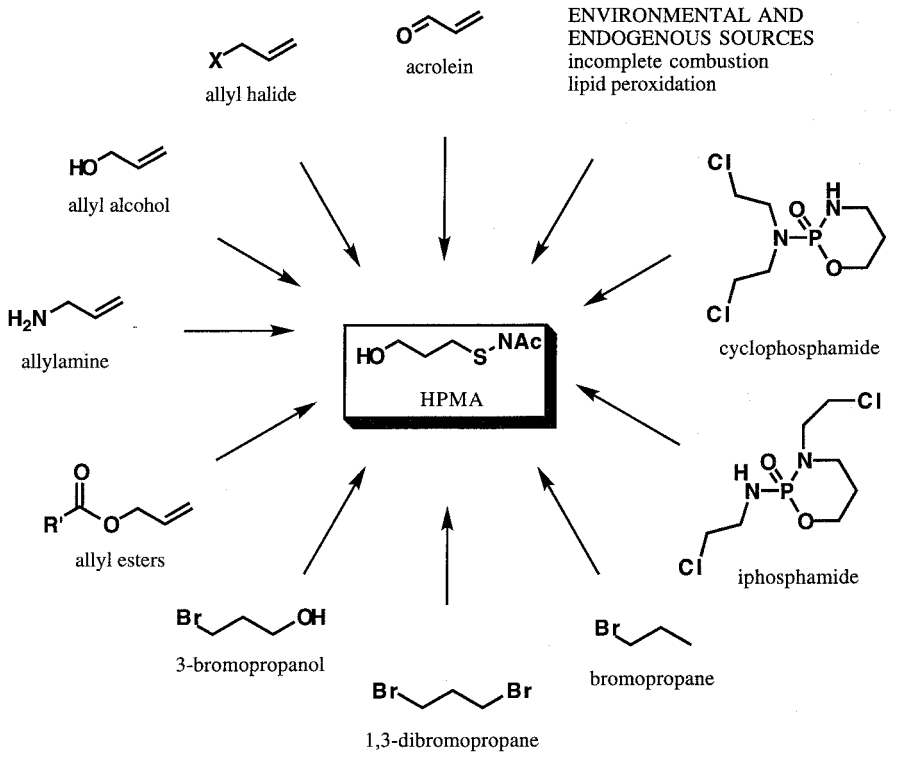

Figure 8. Various compounds and processes leading to urinary excretion of 3-hydroxypropyl mercapturic acid (HPMA). S-NAc=N-acetyl-cysteinyl.

DNA adduct originating from glycidamide, was identified in various organs of mice and rats (Segerback *et al.* 1995). The DNA adduct has not been determined in blood cells of humans exposed to acrylamide, so far. For acrylamide an N-terminal valine adduct (2-carbamoylethyl-valine, determined as carboxyethylvaline after hydrolysis) was used as indicator of long-term combined dermal and ambient exposures (levels not specified) to acrylamide in a group of workers involved in the production of polyacrylamide (Calleman *et al.* 1994). The levels of 2-carbamoylethyl-valine in blood correlated with the determined neurotoxicity index. The urinary excretion of 2-carbamoylethyl-MA also correlated significantly with the neurotoxicity index. Unfortunately, relationships between exposure and mercapturic acid excretion were not given. A possible confounder in the biological monitoring of acrylamide exposure is acrylonitrile exposure, which can also lead to carboxyethyl-MA and to carboxyethyl-valine in the hydrolysis procedures applied (Bergmark *et al.* 1993). This is important because acrylamide is frequently synthesized by catalytic hydration of acrylonitrile. This was neglected in the correlation with the neurotoxicity index given. N-terminal valine adducts in haemoglobin originating from acrylamide and acrylonitrile can now be determined separately using the modified Edman degradation procedure, thus avoiding this confounding factor when investigating exposure to acrylamide (Bergmark *et al.* 1993, Osterman-Golkar *et al.* 1994).

Acrylonitrile

In the metabolism of acrylonitrile two important routes leading to MA metabolites have been described. Direct conjugation of acrylonitrile to GSH leads to the urinary excretion of 2-cyanoethyl-MA and the corresponding 2-cyanoethylthioacetic acid in rats and mice (Fennel *et al.* 1991, Kedderis *et al.* 1993b). In the second metabolic route, epoxidation leads to 2-cyanoethene oxide, a reaction mainly catalysed by the CYP 2E1 isoenzyme (Kedderis *et al.* 1993a). 2-Cyanoethene oxide is believed to be the genotoxic species. Upon incubation of 2-cyanoethene oxide with deoxynucleic acid-monophosphates, adducts to the nucleic acid moiety as well as phosphodiesters were found (Yates *et al.* 1994). The latter adducts were suggested to cause the observed DNA-strand breaks. A detoxification pathway for 2-cyanoethene oxide is GSH conjugation, leading to three MA metabolites in rats and mice. In both species 2-hydroxyethyl-MA (HEMA), carboxymethyl-MA and 1-cyano-2-hydroxyethyl-MA were identified in urine as metabolites of acrylonitrile (Fennel *et al.* 1991, Kedderis *et al.* 1993b). Additionally, thiodiglycolic acid, formed from carboxymethyl-MA, was also identified in the urine of those animals. In humans, a second detoxification pathway for 2-cyanoethene oxide is catalysed by microsomal epoxide hydrolase. The corresponding enzyme in microsomal fractions from rats or mice was not active towards 2-cyanoethene oxide (Kedderis and Batra 1993). The GSH conjugation of acrylonitrile and its metabolite 2-cyanoethene oxide can occur spontaneously at physiological pH, while enzyme mediated GSH conjugation can accelerate this process about four fold (Kedderis *et al.* 1995). Unfortunately, the isoenzymes involved have not been identified.

In human volunteers exposed to acrylonitrile concentration between 5 or 10 mg m^{-3} air for 8 h (former TLV: 10 mg m^{-3} TWA), 22 % of the absorbed dose of acrylonitrile was excreted as 2-cyanoethyl-MA (Jakubowski *et al.* 1987); HEMA excretion was not determined. No applications of the MA-metabolites of acrylonitrile DNA adducts have been described in occupational settings so far. However, an N-terminal valine adduct of acrylonitrile was recently detected in haemoglobin from smokers, but not from non-smokers. It was suggested that the method is sufficiently sensitive to determine occupational exposures to acrylonitrile (Osterman-Golkar *et al.* 1994).

Methacrylonitrile

The metabolism of methacrylonitrile follows qualitatively the same routes as acrylonitrile in rats, although at different levels (Burka *et al.* 1994). Rats excreted approximately 66 % of the acrylonitrile dose in urine, while 22 % of the methacrylonitrile dose was excreted in urine by these animals. For methacrylonitrile, exhalation of small volatile metabolites, including CO_2, is much more important, compared to acrylonitrile. Direct GSH conjugation is the main metabolic route for acrylonitrile, while for methacrylonitrile, epoxidation prior to GSH conjugation is more important. No MA-metabolites of methacrylonitrile have been determined in humans so far.

N,N-Dimethylformamide, N-methylformamide and methylisocyanate

N,N-Dimethylformamide is a highly polar industrial solvent, used in the production of various polymers, coatings, printing inks and adhesives (IARC

1989). Occupational exposure of N,N-dimethylformamide may occur via inhalation or dermal absorption, and can lead to irritating and toxic effects at different sites. N,N-Dimethylformamide is a suspected carcinogen (IARC 1989). A metabolite of N,N-dimethylformamide, N-methylformamide is also used as an industrial solvent (Cross *et al.* 1990), and has been tested as an antitumour agent. However, serious noxious effects to the liver and other organs diminished its therapeutic application (McGuire *et al.* 1990). Both N,N-dimethylformamide and N-methylformamide can form methylisocyanate as a reactive metabolite (Mutlib *et al.* 1990, Slatter *et al.* 1991). Methylisocyanate is used in the chemical industry, particularly in the manufacture of carbamate pesticides and polyurethane foams. Methylisocyanate became notorious after the Bhopal (India) incident (Bucher 1987). In addition to the direct effects of methylisocyanate on the lungs, skin and mucous membranes, effects on the cardiovascular, neuromuscular and immunological systems, and to the foetus were also evident (Bucher 1987, Anderson 1989). A summarizing scheme for MA formation from N,N-dimethylformamide, N-methylformamide and methylisocyanate is given in figure 9.

N,N-Dimethylformamide is found to be partly excreted unchanged in urine (Casal-Lareo and Perbellini 1995). However, most N,N-dimethylformamide is rapidly metabolized. Oxidation of one methyl group of N,N-dimethylformamide results in the formation of N-hydroxymethyl-N-methylformamide. Other

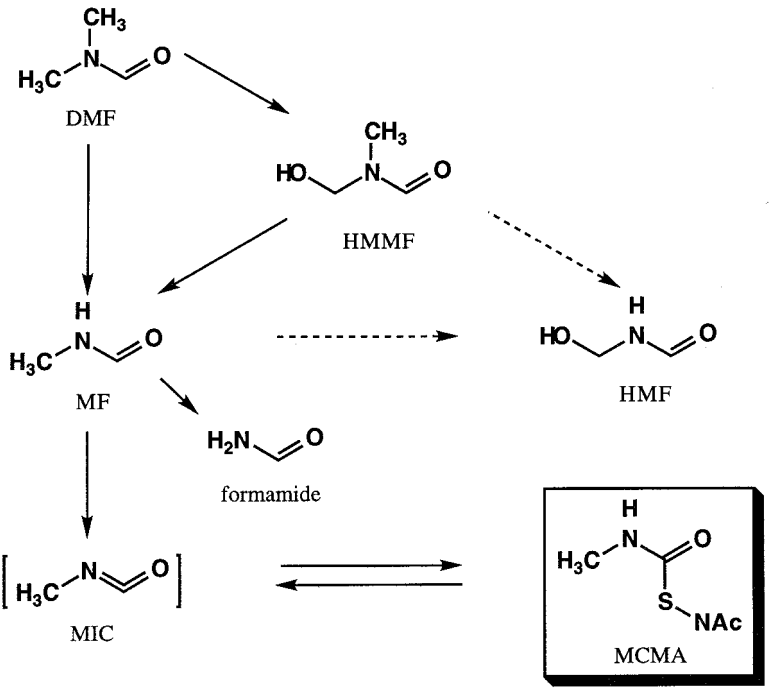

Figure 9. Schematic representation of the metabolic routes of methylformamides and methylisocyanate, leading to a 'reversible' glutathione conjugate. Abbreviations used are: DMF = N,N-dimethylformamide; HMMF = N-hydroxymethyl-N-methylformamide; HMF = hydroxymethylformamide; MF = N-methylformamide; MIC = methylisocyanate; MCG = N-methylcarbamoyl-glutathione and MCMA = N-methylcarbamoyl mercapturic acid; NAc = N-acetyl-cysteinyl for MCMA. See Cross *et al.* (1990), Pearson *et al.* (1990), Pearson *et al.* (1991), Casal-Lareo and Perbellini (1995) and Sakai *et al.* (1995).

metabolites of N,N-dimethylformamide are hydroxymethylformamide, N-methylformamide and formamide. The formation of methylisocyanate from N-methylformamide is supposed to lead to N-methylcarbamoyl-glutathione and the corresponding MA (N-methylcarbamoyl-MA, MCMA). Unchanged N,N-dimethylformamide excreted in urine and the urinary metabolites N-methylformamide and formamide correlated significantly with exposure to N,N-dimethylformamide in end-shift samples (Casal-Lareo and Perbellini 1995). However, MCMA concentrations in urine did not correlate with N,N-dimethylformamide exposure when such end-shift samples were used (Casal-Lareo and Perbellini 1995). MCMA was determined in urine by GC-NPD (Mràz 1988, Sakai *et al.* 1995), GC-MS (Casal-Lareo *et al.* 1995) and by LC-MS (Slatter *et al.* 1991). In another study, however, a clear relationship between MCMA excretion and exposure to N,N-dimethylformamide was found when next-morning urine samples were used, suggesting a relatively slow excretion rate for this MA (Sakai *et al.* 1995). For MCMA a relatively long elimination half-life of 23 h was found in humans (Mràz and Nohova 1992). However MCMA excretion was fast, upon administration of the corresponding cysteine S-conjugate (Mràz and Nohova 1992). This discrepancy was explained by a rate-limiting reversible protein binding of the intermediate methylisocyanate before GSH conjugation (Mràz and Nohova 1992). Again, both N-methylformamide and MCMA concentrations in end-shift urine samples correlated with N,N-dimethylformamide exposure. For both compounds a biological exposure index (BEI) was proposed; 83 mg g^{-1} creatinine for MCMA and 63 mg g^{-1} creatinine for N-methylformamide. The latter value is only slightly higher compared to the value proposed by the ACGIH (40 mg g^{-1} creatinine) ACGIH 1990). Although N-methylformamide is excreted in urine and its determination is recommended as a biomarker of exposure to N,N-dimethylformamide, it is in fact an artefact when GC analysis is used. N-Hydroxymethyl-N-methylformamide is also excreted in urine, but it decomposes rapidly and completely to N-methylformamide during GC analysis and the N-methylformamide determined therefore corresponds to the sum of N-hydroxymethyl-N-methylformamide and N-methylformamide present in urine (Casal-Lareo and Perbellini 1995). N-Hydroxymethyl-N-methylformamide and N-methylformamide can be determined separately by a more recently developed HPLC-based method (Santoni *et al.* 1992). During the working-week relatively stable MCMA excretion levels were reached, showing that MCMA can be used as a marker for cumulative exposure to N,N-dimethylformamide during several days, while urinary N-methylformamide can be used for daily exposures (Sakai *et al.* 1995).

The use of MCMA as a biomarker for human exposure to N-methylformamide or methylisocyanate was not reported until recently. Rats treated with methylisocyanate excreted 25 % of the dose as MCMA in urine (Schulte 1995). In addition to the direct effects of methylisocyanate, systemic effects are also known (Bucher 1987, Anderson 1989). These systemic effects can only occur when methylisocyanate is transported from the initial site of exposure to distant organs where methylisocyanate is released again (Slatter *et al.* 1991). The GSH S-conjugate of methylisocyanate, MCG, is suggested to act as a transport vehicle for methylisocyanate. This hypothesis is supported by the transfer of the N-methylcarbamoyl-group from MCG to cysteine, (and *vice versa*) and to bovine serum albumin (Pearson *et al.* 1990, Pearson *et al.* 1991). That the transformed

intermediate is the isocyanate was derived from the identification of such an intermediate in the case of a more stable isocyanate, i.e. N-(1-methyl-3,3-diphenylpropyl)isocyanate (Mutlib *et al.* 1990) and by the identification of a GSH-conjugate of diethyl (1-ethyl)phosphonate-isocyanate, a reactive isocyanate-metabolite of fotemustine (Brakenhoff *et al.* 1994). From the corresponding GSH S-conjugates the parent isocyanates were liberated under basic conditions.

1,3-Butadiene

1,3-Butadiene is used in the production of polybutadiene- and styrene-butadiene rubbers (Fajen *et al.* 1993). Air concentrations of 1,3-butadiene ranging between 0·044 μg m^{-3} and 800 μg m^{-3} have been determined in different industrial settings (Fajen *et al.* 1993). Additionally, 1,3-butadiene is found in the smoke of cigarettes (main- and side-stream smoke) and at low levels (< 1 μg m^{-3}) in outdoor air (Löfroth *et al.* 1989, Sorsa *et al.* 1995). The main toxic effect is genotoxicity and mice are more susceptible to this effect than rats and humans (Adler *et al.* 1995, Omenn 1996).

Various metabolites of 1,3-butadiene have been identified in urine of different laboratory animals and in humans, see figure 10. Interesting quantitative species differences have been found (Henderson *et al.* 1993). Epoxidation of 1,3-butadiene to 3,4-epoxybutene is the initial activating step. This oxidation is catalysed by CYP 2E1 and, at high concentrations, by CYP 2A6 (Csanády *et al.* 1992). 3,4-Epoxybutene can undergo a second oxidation to 1,2,3,4-diepoxybutane, which is also catalysed by CYP 2E1 and, at high concentrations, by CYP 3A4 (Seaton *et al.* 1995).

3,4-Epoxybutene can be inactivated by epoxide hydrolases to 3,4-dihydroxybutene (IV, figure 10) or by GSH conjugation, which leads to the urinary excretion of MAs. Although 1,2,3,4-diepoxybutane was shown to react with GSH *in vitro*, the corresponding MA metabolites have not yet been identified in urine of rats and humans exposed to 1,3-butadiene (Boogaard *et al.* 1996). The relatively low amounts of 1,2,3,4-diepoxybutane and its GSH S-conjugates formed and analytical problems may have prevented their detection. Only mice were shown to excrete traces of 1-hydroxymethyl-3,4-dihydroxypropyl-MA (**VI**) which is derived from a GSH S-conjugate of 1,2,3,4-diepoxybutane (Nauhaus *et al.* 1996). 3,4-Epoxybutene is a substrate for the genetically polymorphic GST M 1–1 isoenzyme and not for GST T 1–1 (table 3) (Uusküla *et al.* 1995, Thier *et al.* 1996). In contrast, 1,2,3,4-diepoxybutane is a substrate for GST T 1–1 and not for GST M 1–1 (Norppa *et al.* 1995, Landi *et al.* 1996). Both substrate specificities result in higher amounts of DNA damage upon incubation with isolated human lymphocytes in GST T 1–1 or GST M 1–1 negative persons (Norppa *et al.* 1995, Uusküla *et al.* 1995).

Three MA metabolites, i.e. 2-hydroxy-3-butenyl-MA (**I**, HBMA), is *regio*isomer (1-hydroxymethyl-2-propenyl-MA (**II**), and 3,4-dihydroxybutyl-MA (**III**, DHBMA) were found to be excreted by rats inhalation-exposed to 1,3-butadiene (Bechtold *et al.* 1994). The first two MAs result from GSH conjugation of 3,4-epoxybutene, while the latter is supposed to be formed from 3,4-epoxybutene after epoxide hydrolysis, oxidation by alcohol dehydrogenase to an α,β-unsaturated aldehyde intermediate and a final reduction step (see figure 10). In addition to the metabolites mentioned above for 1,3-butadiene in rats, minor

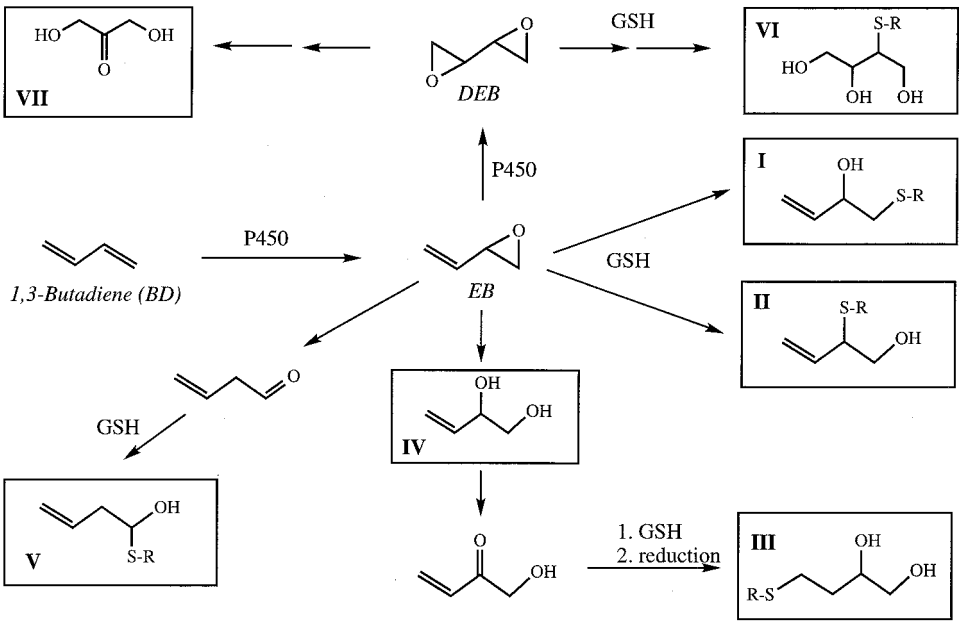

Figure 10. Schematic representation of the metabolic routes of 1,3-butadiene (*BD*). *EB*, 3,4-epoxybutene; *DEB*, 1,2,3,4-diepoxybutane. Compounds indicated in boxes represent identified urinary metabolites of 1,3-butadiene: **I**, 2-hydroxy-3-butenyl-MA (HBMA); **II**, 1-hydroxymethyl-2-propenyl-MA; **III**, 3,4-dihydroxybutyl-MA (DHBMA); **IV**, 3,4-dihydroxybutene; **V**, 1-hydroxy-3-butenyl-MA; **VI**, 1-hydroxymethyl-3,4-dihydroxypropyl-MA; **VII**, 1,3-dihydroxypropanone.

urinary metabolites were identified recently as 3-butene-1,2-diol (**IV**) and 1,3-dihydroxypropanone (**VII**), while in mice other minor urinary metabolites were 3,4-dihydroxybutene, 1-hydroxymethyl-2-propenyl-cysteine (**II**, R=Cys), and 1-hydroxy-3-butenyl-MA (**V**). 3-Hydroxypropyl mercapturic acid (HPMA) and 2-carboxyethyl mercapturic acid (CEMA) have also been analysed previously, but it is not clear whether these originate from acrolein or from further metabolism of DHBMA (Nauhaus *et al.* 1996).

For the metabolism of 1,3-butadiene remarkable species differences have been found. The MAs (HBMA and DHBMA) were identified and quantified by SIM GC-MS in urine samples, after drying and trimethylsilylation with detection limits of about 0·1 mg l^{-1} (Bechtold *et al.* 1994). Rats were found to excrete equal amounts of DHBMA and HBMA, whereas mice excreted predominantly HBMA (DHBMA/HBMA ca. 1/3). Humans, in contrast, almost exclusively excreted DHBMA (Sabourin *et al.* 1992, Bechtold *et al.* 1994). In urine of non-smoking workers in a 1,3-butadiene extraction plant, with potential occupational exposure levels of 3–4 ppm, only DHBMA was detected (3·2 ± 1·6 mg l^{-1}. In urine samples collected from a non-smoking control population, DHBMA excretion was significantly lower (0·320 ± 0·070 mg l^{-1}). The background excretion may have originated from butadiene in cigarette smoke or from traffic emissions (Löfroth *et al.* 1989, Sorsa *et al.* 1995). The marked species differences may be explained by higher epoxide hydrolase activities and the lower GST activities towards 3,4-epoxybutene in human liver when compared to rat liver (Be[illegible] *et al.* 1994).

Another species difference is the higher blood concentration of 1,3-butadiene, 3,4-epoxybutene, and 1,2,3,4-diepoxybutane in mice compared to rats or monkeys (Dahl *et al.* 1991, Henderson *et al.* 1993, Himmelstein *et al.* 1994). Different explanations are suggested to explain this species difference, e.g. a higher respiratory absorption for 1,3-butadiene in mice (Dahl *et al.* 1991, Henderson *et al.* 1993), physiological differences in ventilation rate, lung-air partition coefficients, relative organ volumes and differences in hepatic blood flow (Kohn and Melnick 1993). The higher rate of formation of epoxides from 1,3-butadiene in mice resulted in the well-known higher susceptibility of mice to develop 1,3-butadiene related lung carcinogenicity (Csanády *et al.* 1992, Himmelstein *et al.* 1994).

As biomarkers of long-term exposure, haemoglobin or DNA adducts of 1,3-butadiene may also be useful. A haemoglobin adduct of 3,4-epoxybutene, N-terminal N-(2-hydroxybutenyl)-valine, was determined in the haemoglobin of 1,3-butadiene exposed workers (Sorsa *et al.* 1995). However, no clear relationship was found between exposure levels and amounts of haemoglobin adducts. A DNA adduct of 3,4-epoxybutene, N^6-2-hydroxybutenyl-deoxyadenosine, was detected in a dose-related manner in lung and liver tissue from rats and mice exposed to 1,3-butadiene (Sorsa *et al.* 1995). Recently, DNA adducts of 3,4-epoxybutene (N^7-(1-hydroxy-3-buten-2-yl)guanine) and 1,2,3,4-diepoxybutane (N^7-(2,3,4-trihydroxybutyl)guanine) were identified in liver tissue from mice exposed to 1,3-butadiene (Bolt and Jelitto 1996). DNA adducts in blood have, so far, not been used in human exposure assessment (Legator *et al.* 1993). Apparently, the urinary excretion of DHBMA is a valuable biomarker of human exposure to 1,3-butadiene (Bechtold *et al.* 1994). Interestingly, significant correlations were found between urinary DHBMA concentrations and the *hprt* (hypoxanthine-guanine phosphoribosyl transferase locus) mutant frequency (Legator *et al.* 1993) and the reduced DNA repair capacity (challenge assay) (Au *et al.* 1995) in blood cells isolated from 1,3-butadiene exposed workers, showing that in this case DHBMA excretion in urine can be used to calculate the target dose of 1,3-butadiene. Combined exposure to styrene and 1,3-butadiene is likely in the rubber industry, which may lead to a toxico-kinetic interaction. Both compounds are oxidized *in vivo* by CYP 2E1 and accumulation of styrene may result in a competitive inhibition of oxidation of 1,3-butadiene, as was found in rats (Laib *et al.* 1992).

Styrene

Styrene is used as organic solvent and in the production of plastics, resins and synthetic rubber. Styrene has toxic effects on the central and peripheral nervous systems (Campagna *et al.* 1996, Tsai and Chen 1996, Welp *et al.* 1996). Genotoxic effects were also described as well and these effects were supposed to arise from the metabolite styrene-7,8-oxide (Brenner *et al.* 1991, Hallier *et al.* 1994, Uusküla *et al.* 1995, Vodicka *et al.* 1995). Oxidation of styrene to styrene-7,8-oxide is mainly catalysed by CYP 2B6 (Nakajima *et al.* 1993), while GSH conjugation of styrene-7,8-oxide is mainly catalysed by GST M isoenzymes (see table 3) (Pacifici *et al.* 1987, Hiratsuka *et al.* 1989, Uusküla *et al.* 1995). Rats treated with styrene excreted reasonable fractions of the dose as 1-phenyl-2-hydroxyethyl-MA (12 %) and 2-phenyl-2-hydroxyethyl-MA (4 %) in urine (Truchon *et al.* 1990). In humans, conversion of styrene to mercapturic acids appears to be much lower (below 1 %). Recently, however, both mercapturic acids could be detected in urine of

occupationally exposed workers using an HPLC method, consisting of enzymic deacetylation to the corresponding cysteine conjugates followed by derivatization with *o*-phtalaldehyde and mercaptoethanol (Maestri *et al.* 1997). The mercapturic acids could not be detected in unexposed subjects.

Two major urinary metabolites of styrene, mandelic acid and phenylglyoxylic acid, have been recommended as biomarkers and BEIs were proposed for the latter two metabolites, i.e. 800 mg g^{-1} creatinine and 240 mg g^{-1} creatinine in end-shift urine samples, respectively, with a TLV-TWA of 213 mg m^{-3} (ACGIH 1990). DNA-, haemoglobin- and albumin adducts of styrene oxide have also been identified in humans upon occupational exposure to styrene (Brenner *et al.* 1991, Christakopoulos *et al.* 1993, Phillips and Farmer 1994, Vodicka *et al.* 1995). Interestingly, linear relationships were found between a single *i.p.* dose of styrene to rats with adducts of styrene-7,8-oxide to cysteine in haemoglobin and albumin and with the urinary excretion of phenylglyoxylic acid, mandelic acid, 1-phenyl-2-hydroxyethyl-MA and the 2,2-isomer (see figure 11) (Truchon *et al.* 1990, Rappaport *et al.* 1993). Due to the different levels of formation for the various compounds, the data in figure 11 is presented double-logarithmically. The relationships show that, for single doses of styrene to rats, the urinary MA excretion can be used to assess doses at other internal sites, i.e. blood cells.

Benzene

Benzene is a constituent of many petrochemical products, such as gasoline and petroleum (Bechtold and Henderson 1993, Ghittori *et al.* 1995), and is formed during combustion (Snyder *et al.* 1993). It is also a ubiquitous air pollutant (Gold *et al.* 1993, Bostrom *et al.* 1994) and a component of smoke (Löfroth *et al.* 1989). Benzene is an animal carcinogen and a human leukemogen (Snyder *et al.* 1993).

Many methods were applied in the past to monitor internal doses of benzene. Benzene concentrations in exhaled air, blood or urine, the urinary excretion rates of benzene metabolites phenol, 1,2- and 1,4-hydroquinone (catechol), and the ring-opened metabolite muconic acid all showed significant relationships to airborne

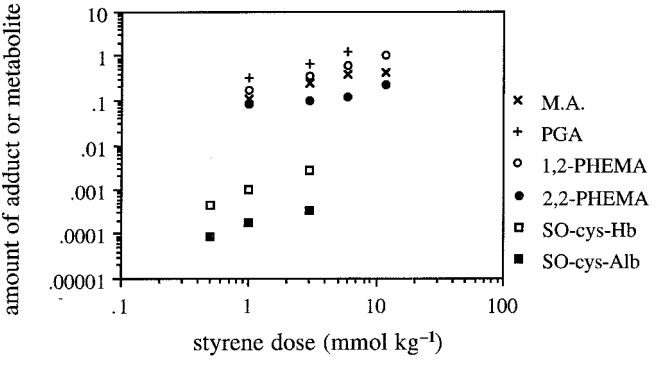

Figure 11. Relationships between a single *i.p.* dose of styrene, the urinary excretion of phenylglyoxylic acid (PGA), mandelic acid (M.A.), 1-phenyl-2-hydroxyethyl mercapturic acid (1,2-PHEMA) and 2-phenyl-2-hydroxyethyl mercapturic acid (2,2-PHEMA), and the formation of cysteine adducts of styrene-7,8-oxide to haemoglobin (SO-cys-Hb) and to albumin (SO-cys-Alb) in rats. The data are expressed in mmol kg^{-1} (SO-cys-Hb) and μmol kg^{-1} (SO-cys-Alb).

benzene (Pekari *et al.* 1992, Boogaard and Van Sittert 1995, Ghittori *et al.* 1995, Ong *et al.* 1995). Biological exposure indices (BEIs) have been proposed: exhalation of 0·08 ppm benzene in next-morning expired air, or urinary excretion of phenol (50 mg l^{-1}) in end-shift samples (ACGIH 1990). These BEIs are based on an 8 h exposure to the TLV-TWA of 10 ppm (32 mg m^{-3}) for airborne benzene (ACGIH 1990). Additionally, phenyl-MA (PMA) was used as a biomarker of human exposure to benzene (Jongeneelen *et al.* 1987, Stommel *et al.* 1989, Boogaard and Van Sittert 1995, Ghittori *et al.* 1995, Ong *et al.* 1995). Although only 0·1 % of the inhaled dose of benzene is excreted as PMA by humans, sensitive GC-MS techniques offer sufficient sensitivity for the quantitative determination of urinary PMA excretion upon low occupational exposure and of background PMA excretion (Boogaard and Van Sittert 1995, Ong *et al.* 1995). The limits of detection for PMA by different methods are given in table 5. PMA is excreted with a half-life of 9·1 ± 3·1 h (n=27), with the highest concentrations generally at the end of the work-shifts (Boogaard and Van Sittert 1995). In some cases a second PMA excretion phase was found with a half-life of 45 ± 4 h (n=5) (Van Sittert *et al.* 1993b). Correlation coefficients between airborne benzene and urinary PMA, range between 0·70 to 0·96 (Jongeneelen *et al.* 1987, Stommel *et al.* 1989, Van Sittert *et al.* 1993b, Boogaard and Van Sittert 1995, Ghittori *et al.* 1995, Ong *et al.* 1995). Exposure at the level of the TLV would lead to urinary PMA excretion rates between 185 and 383 μg g^{-1} creatinine (Boogaard and Van Sittert 1995, Ghittori *et al.* 1995).

Cumulative exposures to benzene are determined by measuring S-phenylcysteine adducts to serum albumin (Bechtold and Henderson 1993). Haemoglobin adducts (S-phenylcysteine- and N-terminal phenylvaline adducts) have not been detected in blood collected from benzene exposed workers so far (Bechtold and Henderson 1993, Bader *et al.* 1994). Although benzene can cause DNA damage in human lymphocytes (Tompa *et al.* 1994), no attempts have been made to determine DNA adducts of benzene in these blood cells. However, in laboratory animals, adducts of the benzene metabolites benzene-oxide, 1,2-benzoquinone, and 1,4-benzoquinone to cysteine in albumin and haemoglobin, and to DNA in lymphocytes and in bone marrow have been identified. Moreover, in rats, linear relationships were found between a single dose of benzene and cysteine adducts
in haemoglobin, bone marrow proteins, and urinary excretion of PMA (Stommel *et al.* 1989, Bechtold and Henderson 1993, McDonald *et al.* 1994). Due to the different levels of formation for the various compounds, the data in figure 12 is presented double-logarithmically. The relationship between the dose of benzene and cysteine adducts in albumin was less clear. These relationships show that, with single doses of benzene to rats, the urinary PMA excretion can be used to calculate the dose at a critical site, i.e. bone marrow.

Z- and E-1,2-dichloropropene

Extensive research on occupational exposure to the soil fumigant (nematocide) 1,3-dichloropropene (DCP) was performed in the Dutch flower bulb industry, because of the known carcinogenicity in animals and the large amounts used for soil fumigation (Yang 1986). DCP consists of a Z- and E-DCP isomer. DCP is injected into the soil, and occupational exposure may occur during loading of

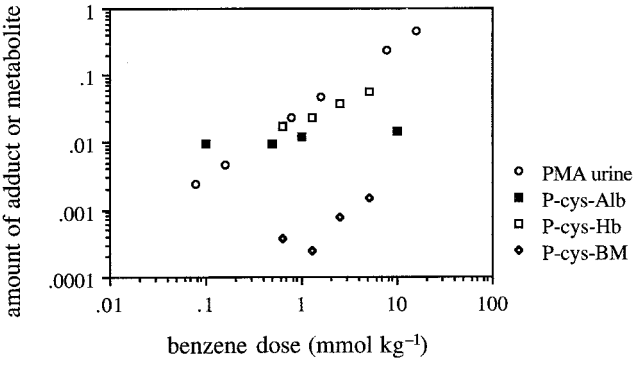

Figure 12. Relationships between a single dose (*i.p.* or *p.o.*) of benzene, the urinary excretion of phenyl mercapturic acid (PMA in mmol kg^{-1}) in urine and the formation of adducts to cysteine residues in haemoglobin (P-cys-Hb in μmol g^{-1} globin), albumin (P-cys-Alb in μmol g^{-1} albumin) or bone marrow proteins (P-cys-BM in μmol g^{-1} protein) of benzene-7,8-oxide. Data taken from Stommel *et al.* (1989) and Bechtold and Henderson (1993), McDonald *et al.* (1994).

injection devices and during injecting procedures due to evaporation or spilling of the liquid. Dermal absorption is generally considered to be caused by the liquid, however, recently it has been shown that DCP vapours also may play a role (Kezic *et al.* 1996). Liver- and renal-function tests indicated subclinical adverse effects in applicators, by the end of the spraying season (Brouwer *et al.* 1991). Additionally, erythrocyte GST activity, and blood GSH concentrations were significantly depressed. However, the clinical significance of these observations was debated (Van Sittert *et al.* 1991). Moreover, in workers involved in industrial production of DCP, no clinical significant effects on kidney and liver were found (Boogaard *et al.* 1993).

Rats exposed to a mixture of Z- and E-DCP excrete relatively high percentages of the absorbed doses as the corresponding Z- and E-3-chloro-2-propenyl-MA (Z-CPMA and E-CPMA, 55 % and 45 %) (Onkenhout *et al.* 1986) and the bioavailability by inhalation of the vapour is reasonably high in those animals (50–66 %) (Stott and Kastl 1986). Z- and E-CPMA were determined in urine by GC-NPD, GC-FPD, GC-(CI)MS and by GC-(EI)MS, see table 5 (Osterloh *et al.* 1984, Onkenhout *et al.* 1986). The correlation coefficients for the relationship between ambient human exposure to Z- and E-DCP and the urinary excretion of Z- and E-CPMA were found between 0·83 and 0·93 (Osterloh *et al.* 1984, Van Welie *et al.* 1991a, Van Welie *et al.* 1991b). A biological exposure index (BEI), based on a TLV-TWA value of 5 mg m^{-3}, was proposed as total cumulative excretion of 14·4 or 3·2 mg of Z- and E-CPMA, respectively (Van Welie *et al.* 1991a). Urinary MA excretion showed first-order linear kinetics and the half-lifes of elimination for Z- and E-CPMA were 5·0 ± 1·2 h and 4·7 ± 1·3 h, respectively (Van Welie *et al.* 1991a). The percentages of the internal dose of DCP excreted as Z- or E-CPMA were slightly lower compared to rats and differed substantially between the two isomers (45 % and 14 %, respectively). This observation was explained by the different enzyme kinetics of Z- and E-DCP towards GST A, P, and M isoenzymes (see table 3) (Vos *et al.* 1991). The specific activities of these GSTs for Z-DCP is higher compared to the specific activities for E-DCP. For E-DCP the specific activities of GST M, A, and P were compara

Z-DCP the specific activity of the polymorphic GST M 1 is higher compared to the specific activities of GST A and GST P. This did not lead to differences in the kinetic behaviour of Z-CPMA in humans exposed to mixtures of DCP-isomers (Vos *et al.* 1991). Apparently, GST A and GST P isoenzymes contributed substantially to the GSh conjugation of Z-DCP in this case. This example indicates that the total metabolic capacity should be considered, rather than substrate specificities of polymorphic enzymes, when attempting to predict the metabolic fate of xenobiotics.

1,1,2-Trichloroethene

1,1,2-Trichloroethene (trichloroethylene) is mainly used to degrease metals and occupational exposure may occur via inhalation of its vapour (IARC 1995). Low levels of 1,1,2-trichloroethene have also been found in water supplies, groundwater, the atmosphere, and indoor domestic air (Hughes *et al.* 1994, IARC 1995). The carcinogenicity of 1,1,2-trichloroethene in humans is subject of considerable debates (e.g. Clewell *et al.* 1995, Fahrig *et al.* 1995, Henschler *et al.* 1995, IARC 1995, Goeptar *et al.* 1995, Weiss 1996) and it is classified differently by various agencies (e.g. IARC (1995) ECETOC (1994) and ACGIH (Clewell *et al.* 1995).

Glutathione (GSH) conjugation of 1,1,2-trichoroethene and the subsequent metabolism via the MA-pathway leads to urinary excretion of 1,2-dichlorovinyl-MA (1,2-DCVMA) and its *regio*isomer 2,2-dichlorovinyl-MA (2,2-DCVMA) in rats and humans, although the excreted percentages of the dose are very low (< 0·1 % (Dekant *et al.* 1986), 0·0026 % 1,2-DCVMA and 0·0013 % 2,2-DCVMA (Commandeur and Vermeulen 1990) in rats). Traces of both 1,2-DCVMA and 2,2-DCVMA were detected in humans exposed to high (Birner *et al.* 1993) and low (Bernauer *et al.* 1996) airborne concentrations of 1,1,2-trichloroethene. The two isomers were detected in a GC-(NICI)-MS based method with detection limit of 13 ng l^{-1} urine (Bernauer *et al.* 1996). Humans excreted equal amounts of both isomers while rats excreted more of the 2,2-DCVMA isomer. Surprisingly, a biphasic urinary excretion with peak concentrations at 10 and 34 h after exposure was seen in humans.

Both the cysteine S-conjugates and mercapturic acids formed from 1,1,2-trichloroethene were mutagenic in the Ames test (Commandeur *et al.* 1991) and showed nephrotoxicity in rats (Vamvakas *et al.* 1992, Ilinskaja and Vamvakas 1996), which may be explained by formation of a reactive chlorothioketene intermediate upon β-lyase mediated β-elimination of 1,2- and 2,2-dichlorovinyl-cysteine (Dekant *et al.* 1988b, Zhang *et al.* 1995). Cysteine S-conjugates of other halogenated compounds, e.g. tetrachloroethene (Dekant *et al.* 1988b) and hexachlorobutadiene (Birner *et al.* 1995, Dekant 1996), are also substrates for β-lyase. For tetrachloroethene, the corresponding MA (1,1,2-trichlorovinyl-MA) was detected in the urine of workers (n=4) in a dry-cleaning facility (Birner *et al.* 1996), while the corresponding MA from hexachlorobutadiene has not been used in human exposure monitoring so far.

The metabolites trichloroethanol and trichloroacetic acid in blood or urine have been determined, as alternative biomarkers of 1,1,2-trichloroethene exposure (IARC 1995). For both metabolites BEI values were proposed for urine; trichloroacetic acid concentrations of 100 mg l^{-1} in end-shift samples at the end of a workweek, and the sum trichloroacetic acid and trichloroethanol concentrations

of 300 mg l^{-1} in end-shift samples at the end of a workweek, with a TLV-TWA of 269 mg m^{-3} (ACGIH 1990). Binding to the blood proteins haemoglobin and albumin was identified in rats and mice, but these adducts have not been used in human exposure measurements yet (Stevens *et al.* 1992).

Allyl chloride, epichlorohydrin and phenyl glycidyl ether

Allyl chloride, epichlorohydrin and phenyl glycidyl ether are three successive products in a so called 'production chain' in the petrochemical industry (Waddams 1978). In this chain allyl chloride (AC, 3-chloro-1-propene) is used as an intermediate in the synthesis of epichlorohydrin (ECH, 1,2-epoxy-3-chloropropane) (IARC 1985). ECH in turn is used in the synthesis of glycidyl ethers, agricultural chemicals, and drugs (IARC 1976). ECH and glycidyl ethers are also used in the formulation of epoxy resins (Gardiner *et al.* 1992, Jolamki *et al.* 1994). In these resins, glycidyl ethers are used as reactive diluents, phenyl glycidyl ether (PGE, 1,2-epoxy-3-phenoxypropane) being one of the most important.

In rodents treated with high doses of AC, toxic effects on the liver, kidneys and peripheral nerves have been reported (Torkelson *et al.* 1959, Boqin *et al.* 1982). Peripheral neurotoxicity and liver damage were also found in humans exposed to extremely high concentrations of AC (Häusler and Lenich 1968, Fengsheng and Sou-lin 1985). However, when humans were exposed to low concentrations of AC, such effects were not observed (Boogaard *et al.* 1993). Although AC was shown to be mutagenic to various *Salmonella Typhimurium* strains (McCoy *et al.* 1978, Eder *et al.* 1982b), and to show carcinogenic properties in mice (Theiss *et al.* 1979, Van Duuren *et al.* 1979), there are no indications that AC is a human carcinogen (IARC 1985). A relationship between human exposure of ECH and the incidence of cancer and heart disease has been suggested (Enterline *et al.* 1990). However, the validity of this relationship is still under debate (e.g. Tsai *et al.* 1990, Olsen *et al.* 1994, Tsai *et al.* 1996, Giri 1997). For exposed workers the development of occupational epoxy dermatitis and skin sensitization to epoxy resins, ECH, and glycidyl ethers is of most concern (Van Joost *et al.* 1990, Jolamki *et al.* 1994). Additionally PGE is classified as a possible human carcinogen (IARC 1989).

Allyl chloride

In rats treated with AC, two MA-metabolites were detected in urine. Allyl-MA (ALMA) was suggested to be a minor urinary metabolite, being excreted for 1·7 % of the AC dose (Kaye *et al.* 1972), while 3-hydroxypropyl-MA (HPMA) was reported to be excreted as 22 % of the AC dose (Sanduja *et al.* 1989). HPMA was believed to be formed after GSH conjugation of the intermediate metabolite acrolein, see Section IV.2, Acrolein and related compounds. In contrast, in a more recent study, ALMA was found to be the major urinary metabolite (30 % of the AC dose), ALMA excretion being linear with the AC dose. HPMA was a minor metabolite (< 3 %) and no clear dose-excretion relationship could be found for HPMA (De Rooij *et al.* 1996b). Differences in the strain of rats and in the route of administration may explain the observed differences. In addition to HPMA and ALMA, two other urinary metabolites, namely 3-chloro-2-hydroxypropyl-MA (CHPMA) and α-chlorohydrin (α-CH), were detected in the urine of rats upon administration of AC (De Rooij *et al.* 1996b). Although the excreted percentages were low (0·2 and 0·1 %, respectively) this suggests the formation of

epichlorohydrin (ECH) as a metabolite of AC *in vivo*. The urinary excretion of ALMA was relatively stable upon pretreatment with three enzyme inducers, i.e. pyrazole, β-naphthoflavone and phenobarbital, while the urinary excretion of CHPMA (phenobarbital) and α-CH (phenobarbital and pyrazole) was increased 2·4 to 15·4-fold (De Rooij *et al.* 1996b). Therefore, ALMA seems to be the most promising metabolite to be used as biomarker of human exposure to AC.

During three annual maintenance shut-down periods in a production facility for AC, ALMA was recently used as a biomarker of AC exposure (De Rooij *et al.* 1997b). In some of the cases, where no respiratory protection equipment was used, the inhalatory air concentrations of AC ranged between < 0·1 (not detectable) to 17 mg m^{-3}. ALMA was easily detected in urine samples collected before and after the work-shifts. For each worker, time courses for urinary ALMA excretion were constructed (figure 13) and clear relationships were found between ALMA excretion and potential airborne exposure to AC. However, in some cases relatively high

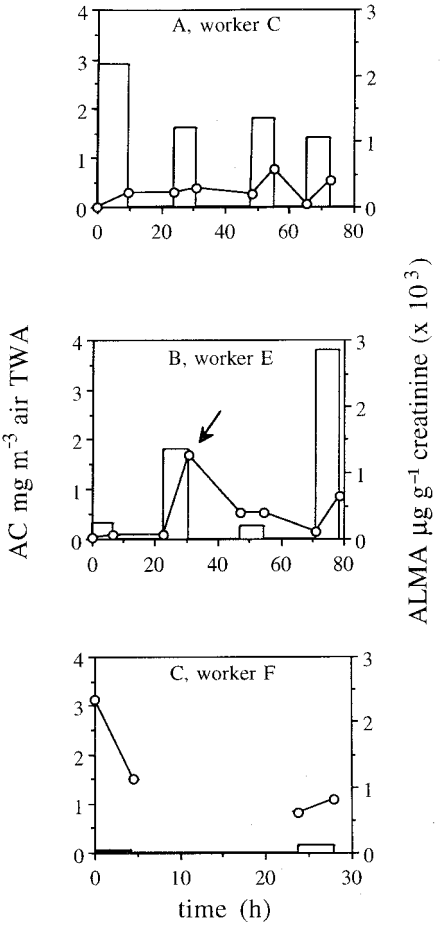

Figure 13. Time courses for urinary ALMA excretion (circles) in relation to airborne allyl chloride (AC) (blocks) in three different workers involved in a maintenance shut-down period in a production unit for AC. A; typical example of a clear relationship between the work-shifts and the urinary excretion of ALMA. B; during the second day a dermal exposure to AC occurred (indicated by arrow). C; Relative high urinary concentrations of ALMA possibly resulting from garlic consumption. See De Rooij *et al.* (1997b).

ALMA concentrations were related to low AC exposure levels. In some of these cases the relatively high ALMA excretion resulted from dermal exposure, while in other cases garlic consumption was suggested as source of ALMA. In garlic some compounds, e.g. γ-glutamyl-S-allyl-cysteine or diallyl sulphide are present (Block 1992, Mütsch-Eckner *et al.* 1992), which may be metabolized to ALMA *in vivo*. A volunteer study recently confirmed that garlic consumption can lead to urinary ALMA concentrations (up to 2·2 mg l^{-1}), which may interfere with ALMA determinations in occupationally exposed humans (up to 3·5 mg l^{-1}) (De Rooij *et al.* 1996a). Upon garlic consumption the urinary elimination half-life of ALMA was found to be 6 h (n=5) (De Rooij *et al.* 1996a). Despite this possible confounder, the increase of ALMA excretion over work-shifts correlated significantly (r^2=0·82, p=0·0001) with inhalatory air concentrations of AC determined (De Rooij *et al.* 1997b). Based on this relationship and on the TLV-value of AC (3 mg m^{-3}) (Ministry of Social Affairs 1995) a BEI was proposed as 0·35 mg g^{-1} creatinine (De Rooij *et al.* 1997b).

Alternative biomarkers of exposure to AC have not yet been reported. AC was shown to be able to conjugate with DNA isolated from salmon sperm (e.g. Eder *et al.* 1987). The value of this observation for human exposure monitoring remains to be proven, however.

Epichlorohydrin

For ECH, high percentages of the dose (31–36 %) were found to be excreted as 3-chloro-2-hydroxypropyl-MA (CHPMA) by rats (Gingell *et al.* 1985, De Rooij *et al.* 1996c). 2,3-Dihydroxypropyl-MA and α-chlorohydrin (α-CH) were minor urinary metabolites of ECH in rats. Urinary excretion products of ECH were investigated recently in workers in a production unit for glycidyl ethers, exposed to low ambient concentrations of ECH, (De Rooij *et al.* 1997a). 2,3-Dihydroxypropyl-MA could not be identified, while α-CH was only identified in traces in the urine of these workers. In contrast, CHPMA was easily identified upon respiratory and dermal exposure to ECH. The concentrations of ECH determined in air correlated with the increase in CHPMA excretion over the work-shifts (r^2=0·94, n=7), and with CHPMA concentrations in end-of-shift samples (r^2=0·95, n=7). Although these concentrations of ECH were below the current TLV-value of 4 mg m^{-3} (Ministry of Social Affairs 1995), a BEI of 14 mg CHPMA g^{-1} creatinine was tentatively proposed by extrapolation of the relationship found, an increase of CHPMA excretion over a work-shift. CHPMA was determined by SIM GC-MS of its methyl ester, or tetramethylsilyl derivative of the methyl ester, with method limits of detection of 0·025 and 0·5 mg l^{-1}, respectively (De Rooij *et al.* 1996c, De Rooij *et al.* 1997a). By comparing successive urine samples, the urinary elimination half-life of CHPMA was calculated, to be 2·5 h (n=8). These observations show that the urinary metabolic profile of ECH is comparable for these three metabolites in rats and humans, and that CHPMA is a selective biomarker for daily exposures to ECH. Other halogenated compounds were also shown to lead to CHPMA excretion in animals, i.e. allyl chloride (De Rooij *et al.* 1996b), 1,2-dibromo-3-chloropropane (Gingell *et al.* 1987), 1,2,3-trichloropropane (Mahmood *et al.* 1991). For these compounds, the use of CHPMA as a biomarker of human exposure still needs to be evaluated, however.

As an alterative biomarker of ECH, an N-terminal valine adduct in haemoglobin (N-(2,3-dihydroxypropyl)valine) might be used (Landin *et al.* 1996).

This adduct was recently detected in blood of ECH treated rats, and not in unexposed humans. ECH is also reactive towards deoxynucleosides and to DNA *in vitro* (Singh *et al.* 1996). However, practical applications of these adducts as biomarkers of human exposure to ECH have not been described so far.

Phenyl glycidyl ether

As yet, the urinary metabolite profile of phenyl glycidyl ether (PGE) has been investigated in rats only, see figure 14 (James *et al.* 1978, De Rooij 1997, De Rooij *et al.* 1997c). GSH conjugation leads to 2-hydroxy-3-phenoxypropyl-MA, which was excreted in urine in considerable amounts of the dose (5 to 27 %) and the dose excretion relationship was linear (r^2=0·998), (De Rooij 1997, De Rooij *et al.* 1997c). 3-Phenyloxylactic acid was another major metabolite, accounting for 15 to 94 % of the dose (James *et al.* 1978). The observed differences in excretion levels of both metabolites may be due to variations in applied doses of PGE, resulting in relatively higher MA excretion at lower dose levels. Recently, a new urinary metabolite was identified as N-acetyl-O-(phenyl)-serine (De Rooij 1997, De Rooij *et al.* 1997c). This metabolite was suggested as being formed from the corresponding lactic acid metabolite by subsequent oxidation, transamination and N-acetylation. Similar metabolites, i.e. N-acetyl-O-(butyl)-serine and 2-hydroxy-3-[2-methylphenoxy]propyl-MA, were also identified in urine of butyl glycidyl ether and *o*-cresyl glycidyl ether treated rats (Eadsforth *et al.* 1985, De Rooij 1997, De Rooij *et al.* 1997c). For *o*-cresyl glycidyl ether, which is a structural analog of PGE, the metabolic profile was comparable to that of PGE. Rats treated with *o*-cresyl glycidyl ether (*o*-CGE) excreted 3-[2-methylphenoxy]lactic acid and 2-hydroxy-3-[2-methylphenoxy]propyl-MA, which are structurally related to the respective metabolites of PGE (De Rooij 1997, De Rooij *et al.* 1997c).

MA metabolites of PGE and *o*-PGE have not been used in human biomonitoring experiments, as yet. Recently, however, adducts to N-terminal valine of haemoglobin were described in mice treated with a series of glycidyl ethers, including PGE (Perez *et al.* 1997). Additionally, different adducts of PGE to deoxynucleosides were identified *in vitro* (e.g. Hemminki and Vainio 1980, Van den Eeckhout *et al.* 1990, Lemiere *et al.* 1993). Adducts to deoxynucleoside have been identified in the same way for the diglycidyl ether of bisphenol A (Vanhoutte *et al.* 1995) and for allyl glycidyl ether (Plna *et al.* 1996). From the latter compound, the reactive intermediate glycidaldehyde was found to react with DNA, in mice treated with diglycidyl ether of bisphenol A (Steiner *et al.* 1992), and other glycidyl ethers. However, none of these adducts are being used for human biomonitoring purposes.

Summary and concluding remarks

An overview is given of the use of MAs in the assessment of internal human exposure to electrophilic industrial chemicals. Additionally, the formation of GSH S-conjugates, their catabolism to MAs, and several of the frequently used analytical approaches are discussed.

MAs are formed via the MA-pathway after GSH conjugation of electrophilic compounds or electrophilic intermediates (Vermeulen 1989, Van Welie *et al.* 1992). Although GSH-conjugation is generally a detoxification pathway, GSH S-conjugation can sometimes lead to toxicity as well (Vermeulen 1989, Sipes and Gandolfi 1991). The conjugation of electrophiles to GSH can proceed

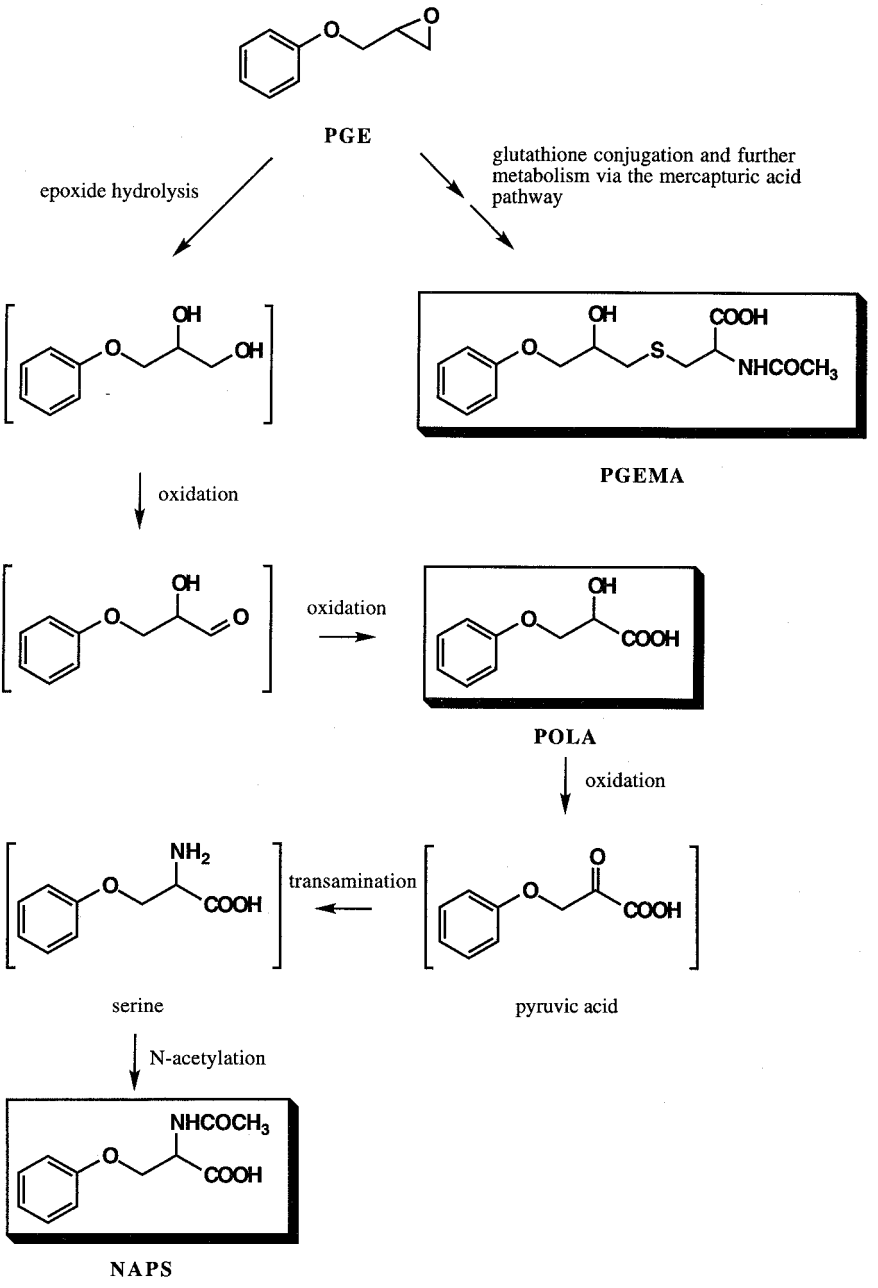

Figure 14. Metabolic scheme for phenyl glycidyl ether (PGE) in rats. Hypothetical intermediates are indicated between brackets and identified urinary metabolites in boxes. PGE is metabolized via glutathione conjugation and the mercapturic acid pathway, to the corresponding mercapturic acid (PGEMA). Metabolism via epoxide hydrolysis and further oxidation leads to 3-phenyloxy-lactic acid (POLA). POLA may be further oxidized to pyruvic acid and after transamination and N-acetylation to N-acetyl-O-(phenyl)-serine (NAPS). See Eadsforth *et al.* (1985) and De Rooij *et al.* (1997c).

spontaneously, or be catalysed by GSH S-transferases (GST), of which one microsomal form and a family of cytosolic isoenzymes have been identified (Mannervik *et al.* 1992, Hayes and Pulford 1995). Two GST enzymes show genetic polymorphisms in humans, i.e. GST T 1–1 and GST M 1–1 (Strange 1995). Both enzymes are expressed in human blood cells and genotyping of workers can be relatively easily performed using these cells (Bolt 1994, Chen *et al.* 1996a). GST T 1–1 plays an important role in the detoxification of certain industrial compounds such as methylbromide, methylchloride, and ethylene oxide (Hallier *et al.* 1993), while GST M 1–1 is involved in the GSH conjugation of epoxides such as *anti* benzo(*a*)pyrene-7,8-diol-9,10-oxide (Robertson *et al.* 1986b) and styrene-7,8-oxide (Pacifici *et al.* 1987). Enzyme polymorphisms can have major consequences for the balance between toxication and detoxification, and for formation of metabolites. When human blood cells are incubated with ethylene oxide for example, clear differences in DNA damage were found, related to GST T 1–1 polymorphism (Föst *et al.* 1991, Hallier *et al.* 1993). In contrast when other GST isoenzymes or spontaneous GSH conjugation play a role, only minor influences of GST T 1–1 and GST M 1–1 polymorphisms are expected. This was noted for Z-DCP and acrolein, both substrates for GST M 1–1 (Vos *et al.* 1991, Berhane *et al.* 1994).

In the metabolism of glutathione S-conjugates to the corresponding MAs, the MA-pathway, different metabolic steps are recognized (Ketterer and Mulder 1990, Commandeur *et al.* 1995): removal of the γ-glutamyl- and glycinyl residues from the GSH S-conjugate by γ-glutamyltranspeptidase and dipeptidases, followed by acetylation to mercapturic acids (N-acetyl-L-cysteine S-conjugates, MAs) by cysteine S-conjugate N-acetyltransferase. The extent and routes GSH S-conjugates follow in the body during the MA-pathway are dependent on the relative activities of the enzymes involved in the different organs resulting in hepato-renal cycling, entero-hepatic cycling or hepato-biliary cycling (Hinchman and Ballatori 1994, Commandeur *et al.* 1995). Other enzymes may also be involved in the catabolism of GSH S-conjugates and this can lead to different end-products: mercaptolactic acids, mercapturic S-oxides, and mercaptoacetic acids (Commandeur *et al.* 1995). In rats, the formation of MAs is a predominant metabolic route in most cases, while the relative importance of MA formation in humans remains to be elucidated (Van Welie *et al.* 1992).

In recent years many new applications of MAs were made in human exposure assessment. Two MAs, 2-hydroxyethyl-MA (HEMA) and 3-hydroxypropyl-MA (HPMA), can be used as more or less unselective biomarkers of exposure, because many compounds can lead to their excretion (Poli *et al.* 1985, Sanduja *et al.* 1989, Vermeulen *et al.* 1989). Examples of more selectively excreted MAs are: 2-carbamoylethyl-MA (from acrylamide) (Wu *et al.* 1993), cyanoethyl-MA (from acrylonitrile) (Jakubowski *et al.* 1987), N-methylcarbamoyl-MA (MCMA, from N,N-dimethylformamide, N-methylformamide or methylisocyanate) (Sakai *et al.* 1995, Schulte 1995), 3,4-dihydroxybutyl-MA (DHBMA, from 1,3-butadiene) (Sabourin *et al.* 1992, Bechtold *et al.* 1994), phenyl-MA (PMA, from benzene) (Jongeneelen *et al.* 1987, Stommel *et al.* 1989, Boogaard and Van Sittert 1995, Ghittori *et al.* 1995, Ong *et al.* 1995), Z- and E-3-chloro-2-propyl-MA (CPMA, from Z- and E-1,3-dichloropropene) (Osterloh *et al.* 1984, Van Welie *et al.* 1991a, Van Welie *et al.* 1991b), 1,2,2-trichlorovinyl-MA (from tetrachloroethene) (Birner *et al.* 1996). In most of these cases good relationships are found between ambient exposure levels and the urinary MA excretion. For some chemicals, e.g. 2

carbamoylethyl-MA from acrylamide, these relationships were difficult to investigate due to contributions of dermal exposure (Wu *et al.* 1993). This shows that MA excretion relates to total internal doses integrated over all possible routes of exposure. For some other compounds the urinary MA excretion was less marked in humans. For instance, exposure to 1,1,2-trichloroethene led to extremely low levels of 1,2- and 2,2-dichlorovinyl-MA. Although the excreted levels were low, extremely sensitive analytical approaches were successful in the identification of these metabolites despite low level occupational exposures. In humans occupationally exposed to styrene, 1-phenyl-2-hydroxyethyl-MA (or its *regio* isomer) could only be found in a small group of the exposed individuals (Hallier *et al.* 1995). This can possibly be explained by genetic polymorphisms of GSTs and other enzymes.

Background excretion is sometimes seen, e.g. for the MAs derived from benzene (Jongeneelen *et al.* 1987, Stommel *et al.* 1989, Boogaard and Van Sittert 1995, Ghittori *et al.* 1995, Ong *et al.* 1995), ethylene oxide (Popp *et al.* 1994) and butadiene (Bechtold *et al.* 1994). Despite some confounding background excretion, however, clear relationships were found between occupational exposure and urinary MA excretion.

Excretion kinetics are usually important to determine the most appropriate time of sampling in BM studies. Most MAs, like other urinary metabolites, are excreted with relatively short half-lifes, ranging from 1·5 to 9 h (Van Welie *et al.* 1992), and in most cases excretion levels at the end of work-shifts are successfully related to exposure levels. Ideally, urine samples are taken at the beginning as well as at the end of the work-shifts, to show the increase in biomarker excretion during that particular work-shift. Sometimes relatively long excretion half-lifes are found, e.g. 23 h for MCMA (from N,N-dimethylformamide) (Mraz and Nohova 1992). In this case, next-morning or even end-of-week urine samples are more appropriate. Although linear excretion kinetics are mostly found for MA excretion, some exceptions have been reported. For PMA (from benzene) a second slow excretion phase was identified after the initial linear excretion phase in some of the workers monitored (Boogaard and Van Sittert 1995). Another exception is 1,2- and 2,2-dichlorovinyl-MA (from 1,1,2-trichloroethene). For these MAs a bi-phasic excretion pattern was found (Bernauer *et al.* 1996). Hepatic-intestinal cycling was suggested as a reason for this phenomenon. However, this explanation is probably not satisfactory because many other MAs may also be subject to cycling during the MA-pathway.

Adducts to biomacromolecules are also often used as biomarkers in occupational toxicology. Determination of DNA adducts, for instance in peripheral lymphocytes, gives a measure of cumulative or chronic exposure, whereas protein adducts (albumin adducts in serum or, haemoglobin adducts in erythrocytes) are considered as a measure of semi-chronic exposure. Both type of adducts at least show that reactive compounds were absorbed or formed during metabolism and that these were able to reach and react with essential biomacromolecules (Skipper *et al.* 1994, La and Swenberg 1996, Nestmann *et al.* 1996). MAs, as degradation products of GSH conjugates, relate to the electrophilic character of parent compounds or metabolites, and are therefore usually an indicator of toxicologically relevant absorbed doses of such chemicals (Van Welie *et al.* 1992). The amount of compound covalently bound to albumin is sometimes found to be higher when compared to the amount covalently bound to haemoglobin (Van Welie *et al.* 1992). This is seen in the case of 1,1,2-trichloroethene (Stevens *et al.* 1992). However, in animals treated with

styrene (Rappaport *et al.* 1993) or benzene (Bechtold and Henderson 1993) adducts to haemoglobin were more abundant than albumin adducts when normalized to blood volume, albumin and haemoglobin content (Prosser 1973, Altman and Dittmer 1974), see also figures 11 and 12. After single doses to animals, linear relationships were found for ethylene oxide with urinary HEMA excretion, haemoglobin adduct formation, and the formation of DNA adducts in the liver (Van Welie *et al.* 1992). Moreover, since the same (linear) dose-dependency was found for MA excretion and adduct formation, with single doses to animals of ethylene oxide (Van Welie *et al.* 1992), 1,2-dibromoethane (Van Welie *et al.* 1992), styrene (see figure 11) (Truchon *et al.* 1990, Rappaport *et al.* 1993) and benzene (see figure 12) (Stommel *et al.* 1989, Bechtold and Henderson 1993, McDonald *et al.* 1994) it is apparently possible to use urinary MA excretion to calculate target doses as well, provided that these relationships have been previously determined. In other examples clear relationships were found between MA excretion and markers of toxic effects. Thus good correlations were found between 2-carbamoylethyl-MA (from acrylamide) and a neurotoxicity index, and between DHBMA excretion, the *hprt* (hypoxanthine-guanine phosphoribosyl transferase locus) mutant frequency (Legator *et al.* 1993) and the reduced DNA repair capacity in blood cells isolated from 1,3-butadiene exposed workers (challenge assay) (Au *et al.* 1995). These examples indicate that urinary MA excretion can indeed be related to the toxic internal dose.

In conclusion: Urinary mercapturic acid (MA) excretion has in recent years successfully been used in the assessment of internal doses in humans exposed to environmental and industrial chemicals. As degradation products of GSH S-conjugates, MAs are related to the electrophilic character of the parent compound or reactive metabolites and are therefore biomarkers of a potentially toxicologically relevant absorbed dose. Generally, short urinary excretion half-lifes are found for MAs, allowing a direct evaluation of occupational circumstances. While other frequently used biomarkers, like haemoglobin-, albumin- or DNA adducts are determined in blood cells, MAs can be determined in urine which is obtained by non-invasive sampling. This is important, especially when repeated sampling strategies are used in order to evaluate specific occupational circumstances. We conclude that urinary MA excretion is a useful tool in the assessment of a toxicologically relevant absorbed dose in humans exposed to environmental or industrial chemicals.

Acknowledgement

We like to express our gratitude to Mrs. F. Dolman for her valuable help in preparing this manuscript.

References

ACGIH (American Conference of Governmental Industrial Hygienists) 1990, 1990–1991 Threshold limit values for chemical substances and physical agents and biological exposure indices. ACGIH, No. 0205.

ADLER, I-D., COCHRANE, J., OSTERMAN-GOLKAR, S., SKOPEK, T. T., SORSA, M. and VOGEL, E. 1995, 1,3-Butadiene working group report. *Mutation Research*, **330**, 101–114.

ÅLIN, P., JENSSON, H., GUTHENBERG, C., DANIELSON, U. H., TAHIR, M. K. and MANNERVIK, B. 1985, Purification of major basic glutathione transferase isoenzymes from rat liver by use of affinity chromatography and fast protein liquid chromatofocussing. *Analytical Biochemistry*, **146**, 313–320.

ÅLIN P., JENSSON, H., CEDERLUND, E., JÖRNVALL, H. and MANNERVIK, B. 1989, Cytosolic glutathione transferases from rat liver. *Biochemical Journal*, **261**, 531–539.

ALTMAN, P. L. and DITTMER, D. S. (Eds) 1974, *Biology data book*, **3**, (Federation of American Societies for Experimental Biology, Bethesda), pp. 1851, 1984, 1969.

ANDERSON, N. 1989, *Lancet*, **June 3** 1259.

ANDERSON, C., MOSIALOU, E., WEINANDER, R. and MORGENSTERN, R. 1994, Enzymology of microsomal glutathione S-transferase. In *Conjugation dependent carcinogenicity and toxicity of foreign compounds*, M. W. Anders and W. Dekant, eds. *Advanced Pharmacology*, **27**, (Academic Press, San Diego, USA), pp. 19–36.

ANIYA, Y., SHIMOJI, M. and NAITO, A. 1993, Increase in liver microsomal glutathione S-transferase activity by phenobarbital treatment of rats. *Biochemical Pharmacology*, **46**, 1741–1747.

ARPINO, P. 1990, Coupling techniques in LC-MS and SFC-MS. *Fresenius Journal of Analytical Chemistry*, **337**, 667–685.

AU, W. W., BECHTOLD, W. E., WHORTON, JR E. B. and LEGATOR, M. S. 1995, Chromosome aberrations and response to γ-ray challenge in lymphocytes of workers exposed to 1,3-butadiene. *Mutation Research*, **334**, 125–130.

BADER, M., LEHNERT, G. and ANGERER, J. 1994, GC-MS determination of N-phenyl, a possible biomarker for benzene exposure in human haemoglobin by the "N-alkyl Edman method". *International Archives of Occupational Health*, **65**, 411–414.

BAILLIE, T. A. and KASSAHUN, K. 1994, Reversibility in glutathione-conjugate formation. In *Conjugation dependent carcinogenicity and toxicity of foreign compounds*, M. W. Anders and W. Dekant, eds. *Advanced Pharmacol* **27**, (Academic Press, San Diego) pp. 163–182.

BAKKE, J. E. 1982, Conversion of the prosthetic moieties of glutathione pathway conjugates to the corresponding S-acetates. *Biomedical Mass Spectrometry*, **9**, 74–77.

BALLATORI, N. 1994, Glutathione mercaptides as transport forms of metals. In *Conjugation dependent carcinogenicity and toxicity of foreign compounds*. M. W. Anders and W. Dekant, ed. *Advanced Pharmacology*, **27**, (Academic Press, San Diego) pp. 271–300.

BARTELS, M. J. 1994, Quantitation of the tetrachloroethylene metabolite N-acetyl-S-(trichlorovinyl)cysteine in rat urine via negative ion chemical ionization gas chromatography/tandem mass spectrometry. *Biological Mass Spectrometry*, **23**, 689–694.

BAUMANN, E. and PREUSSE, C. 1879, Ueber Bromphenylmercaturäre. *Chemische Berichte*, (Berichte der Deutschen Chemischen Gesellschaft), **12**, 806–812.

BEACH, A. C. and GUPTA, R. C. 1992, Human biomonitoring and the ^{32}P-postlabeling assay. *Carcinogenesis*, **13**, 1053–1074.

BECHTOLD, W. E. and HENDERSON, R. F. 1993, Biomarkers of human exposure to benzene. *Journal of Toxicology and Environmental Health*, **40**, 377–386.

BECHTOLD, W. E., STRUNK, M. R., CHANG, I. Y., WARD JR, J. B. and HENDERSON, R. F. 1994, Species differences in urinary butadiene metabolites: comparisons of metabolite ratios between mice, rats, and humans. *Toxicology and Applied Pharmacology*, **127**, 44–49.

BERGMARK, E., CALLEMAN, C. J., HE, F. and COSTA, L. G. 1993, Determination of haemoglobin adduct in humans occupationally exposed to acrylamide. *Toxicology and Applied Pharmacology*, **120**, 45–54.

BERHANE, K., WIDERSTEN, M., ENGSTRÖM, A., KOZARICH, J. W. and MANNERVIK, B. 1994, Detoxication of base propenals and other α,β-unsaturated aldehyde products of radical reactions and lipid peroxidation by human glutathione transferases. *Proceedings of the National Academy of Sciences*, **91**, 1480–1484.

BERNAUER, U., BIRNER, G., DEKANT, W. and HENSCHLER, D. 1996, Biotransformation of trichloroethene: dose-dependent excretion of 2,2,2-trichloro-metabolites and mercapturic acids in rats and humans after inhalation. *Archives of Toxicology*, **70**, 338–346.

BESSEMS, J. G. M., TE KOPPELE, J. M., VAN DIJK, P. A., VAN STEE, L. L. P., COMMANDEUR, J. N. M. and VERMEULEN, N. P. E. 1996, Rat liver microsomal cytochrome P450-dependent oxidation of 3,5-disubstituted analogues of paracetamol. *Xenobiotica*, **26**, 647–666.

BIRNER, G., VAMVAKAS, S., DEKANT, W. and HENSCHLER, D. 1993, Nephrotoxic and genotoxic N-acetyl-S-dichlorovinyl-L-cysteine is a urinary metabolite after occupational 1,1,2-trichloroethene exposure in humans: Implications for the risk of trichloroethene exposure. *Environmental Health Perspectives*, **99**, 281–284.

BIRNER, G., WERNER, M., OTT, M. M. and DEKANT, W. 1995, Sex differences in hexachlorobutadiene biotransformation and nephrotoxicity. *Toxicology and Applied Pharmacology*, **132**, 203–212.

BIRNER, G., RUTKOWSKA, A. and DEKANT, W. 1996, N-acetyl-S-(1,2,2-trichlorovinyl)-L-cysteine and 2,2,2-trichloroethanol: Two novel metabolites of tetrachlorethene in humans after occupational exposure. *Drug Metabolism and Disposition*, **24**, 41–47.

BLOCK, E. 1992, Die Organoschwefelchemie der Gattung Allium und ihre Bedeutung fur die organische Chemie des Schwefels. *Angewandte Chemie*, **104**, 1158–1203.

BOGAARDS, J. J. P., VAN OMMEN, B. and VAN BLADEREN, P. J. 1993, Interindividual differences in the *in vitro* conjugation of methylene chloride with glutathione by cytosolic glutathione S-transferase in 22 human liver samples. *Biochemical Pharmacology*, **45**, 2166–2169.

BOLT, H. M. 1994, Genetic and individual differences in the process of biotransformation and their relevance for occupational medicine. *La Medicina del Lavoro*, **1**, 37–48.

Bolt, H. M. 1996, Quantification of endogenous carcinogens, the ethylene oxide paradox. *Biochemical Pharmacology*, **52**, 1–5.

Bolt, H. M. and Jelitto, B. 1996, Biological formation of the 1,3-butadiene DNA adducts 7-N-(2-hydroxy-3-buten-1-yl)guanine, 7-N-(1-hydroxy-3-buten-2-yl)guanine and 7-N-(2,3,4-trihydroxy-butyl) guanine. *Toxicology*, **113**, 328–330.

Boogaard, P. J., Commandeur, J. N. M., Vermeulen, N. P. E., Mulder, G. J. and Nagelkerke, J. F. 1989, Toxicity of the cysteine S-conjugates and mercapturic acids of four structurally related difluoroethylenes in isolated proximal tubular cells from rat kidney. *Biochemical Pharmacology*, **38**, 3731–3741.

Boogaard, P. J., Rocchi, P. S. J., Van Sittert, N. J. 1993, Effects of exposure to low concentration of chlorinated hydrocarbons on the kidney and liver of industrial workers. *British Journal of Industrial Medicine*, **50**, 331–339.

Boogaard, P. J., Sumner, S. C-J. and Bond, J. A. 1996, Glutathione conjugation of 1,2:3,4-Diepoxybutane in human liver and rat and mouse liver and lung *in vitro*. *Toxicology and Applied Pharmacology*, **136**, 307–316.

Boogaard, P. J. and Van Sittert, N. J. 1995, Biological monitoring of exposure to benzene: a comparison between S-phenylmercapturic acid, trans, trans-muconic acid, and phenol. *Occupational and Environmental Medicine*, **52**, 611–620.

Boqin, L., Shuwel, D., Airu, Y., Yinlin, X., Taibao, G. and Tao, C. 1982, Studies on the toxicity of allyl chloride. *Ecotoxicology and Environmental Safety*, **6**, 19–27.

Boström, C-E., Almén, J., Steen, B. and Westerholm, R. 1994, Human exposure to urban air pollution. *Environmental. Health Perspectives*, **102**, suppl. 4, 39–47.

Brakenhoff, J. P. G., Commandeur, J. N. M., De Kamter, F. J. J., Van Baar, B. L. M., Luijten, W. C. L. M. and Vermeulen, N. P. E. 1994, Chemical and glutathione conjugation-related degradation of fotemustine: Formation and characterization of a GSH-conjugate of diethyl (1-ethyl phosphonate-isocyanate, a reactive metabolite of fotemustine. *Chemical Research in Toxicology*, **7**, 380–389.

Brenner, D. D., Jeffrey, A. M., Latriano, L., Wazneh, L., Warburton, D., Toor, M., Pero, R. W., Andrews, L. R., Walles, S. and Perera, F. P. 1991, Biomarkers in styrene-exposed boatbuilders. *Mutation Research*, **261**, 225–236.

Brockmöller, J., Kerb, R., Drakoulis, N., Nitz, M. and Roots, I. 1993, Genotype and phenotype of glutathione S-transferase class μ isoenzymes θ and ψ in lung cancer patients and controls. *Cancer Research*, **53**, 1004–1011.

Brockmöller, J., Cascorbi, I., Kerb, R. and Roots, I. 1996, Combined analysis of inherited polymorphisms in arylamine N-acetyltransferase 2, glutathione S-transferase M1 and T1, microsomal epoxide hydrolase, and cytochrome P450 enzymes as modulators of bladder cancer risk. *Cancer Research*, **56**, 3915–3925.

Brouwer, E. J., Elevo, C. I. A., Verplanke, A. J. W., Van Welie, R. T. H. and De Wolff, F. A. 1991, Biological effect monitoring of occupational exposure to 1,3-dichloropropene: effects on liver and renal function and on glutathione conjugation. *British Journal of Industrial Medicine*, **48**, 167–172.

Bucher, J. R. 1987, Methyl isocyanate: A review of health effects research since Bhopal. *Fundamental and Applied Toxicology*, **9**, 367–379.

Burka, L. T., Sanchez, I. M., Ahmed, A. E. and Ghanayem, B. I. 1994, Comparative metabolism and disposition of acrylonitrile and methacrylonitrile in rats. *Archives of Toxicology*, **68**, 611–618.

Buijs, W. 1985, The relation between structure, biotransformation and mutagenic activity of α, ω-dihalogenoalkenes. Academic Thesis, pp. 53–76.

Calleman, C. J., Wu, Y., He, F., Tian, G., Bergmark, E., Zhang, S., Deng, H., Wang, Y., Crofton, K. M., Fennell, T. and Costa, L. G. 1994, Relationships between biomarkers of exposure and neurological effects in a group of workers exposed to acrylamide. *Toxicology and Applied Pharmacology*, **126**, 361–371.

Campagna, D., Gobba, F., Mergler, D., Moreau, T., Galassi, C., Cavalleri, A. and Huel, G. 1996, Color vision loss among styrene-exposed workers neurotoxicological threshold assessment. *Neurotoxicology*, **17**, 367–374.

Casal-Lareo, A. and Perbellini, L. 1995, Biological monitoring of workers exposed to N,N-dimethylformamide; II. Dimethylformamide and its metabolites in urine of exposed workers. *International Archives of Occupational and Environmental Health*, **67**, 47–52.

Casal-Lareo, A., Perico, A., Bavazzano, P., Soave, D. and Perbellini, L. 1995, Biological monitoring of workers exposed to N,N-dimethylformamide. I. Methods of analysis. *International Archives of Occupational Health*, **67**, 41–46.

Cavelier, L., Bonnet, P., Morel, G. and De Ceaurriz, J. 1996, Role of cysteine conjugation in vinylidene chloride-induced nephrotoxicity and hepatotoxicity in fasted rats. *Journal of Applied Toxicology*, **16**, 109–113.

Chappell, C. G., Creaser, C. S. and Shepherd, M. J. 1992, Modified on-column interface for coupled high-performance liquid chromatography-gas chromatography and its application to the determination of levamisole in milk. *Journal of Chromatography*, **626**, 223–230.

CHASSEAUD, L. F. 1979, The role of glutathione and glutathione S-transferases in the metabolism of chemical carcinogens and other electrophilic agents. *Advanced Cancer Research*, **29**, 175–274.

CHEN, C-L., LIU, Q. and RELLING, M. V. 1996a, Simultaneous characterization of glutathione S-transferase M1 and T1 polymorphisms by polymerase chain reaction in American whites and blacks. *Pharmocogenetics*, **6**, 187–191.

CHEN, H., SANDLER, D. P., TAYLOR, J. A., SHORE, D. L., LIU, E., BLOOMFIELD, C. D. and BELL, D. A. 1996b, Increased risk for myelodysplastic syndromes in individuals with glutathione transferase theta 1 (GSTT1) gene defect. *Lancet*, **347**, 295–297.

CLEWELL, H. J., GENTRY, P. R., GEARHART, J. M., ALLEN, B. C. and ANDERSON, M. E. 1995, Considering pharmacokinetic and mechanistic information in cancer risk assessment for environmental contaminants: Examples with vinyl chloride and trichloroethylene. *Chemosphere*, **31**, 2561–2578.

CMARIJ, J. L., INSKEEP, P. B., MEREDITH, M. J., MEYER, D. J., KETTERER, B. and GUENGERICH F. P. 1990, Selectivity of rat and human glutathione S-transferases in activation of ethylene dibromide by glutathione conjugation and DNA binding and induction of unscheduled DNA synthesis in human hepatocytes. *Cancer Research*, **50**, 2747–2752.

COMMANDEUR, J. N. M., DE KANTER, F. J. J. and VERMEULEN, N. P. E. 1989, Bioactivation of the cysteine S-conjugate and mercapturic acid of tetrafluoroethylene to thioacylating reactive intermediates in the rat. *Molecular Pharmacology*, **36**, 654–664.

COMMANDEUR, J. N. M., BOOGAARD, P. J., MULDER, G. J. and VERMEULEN, N. P. E. 1991, Mutagenicity and cytotoxicity of two regioisomeric mercapturic acids and cysteine S-conjugates of trichloroethylene. *Archives of Toxicology*, **65**, 373–380.

COMMANDEUR, J. N. M., STIJNTJES, G. J. and VERMEULEN, N. P. E. 1995, Enzymes and transport systems involved in the formation and disposition of glutathione S-conjugates. *Pharmacological Reviews*, **47**, 271–330.

COMMANDEUR, J. N. M., KING, L. J., KOYMANS, L. and VERMEULEN, N. P. E. 1996, Bioactivation of S-(2,2-dihalo-1,1-difluoreoethyl)-L-cysteines and S-(trihalovinyl)-L-cysteines by cysteine S-conjugate β-lyase: Indications for formation of both thionoacylating species and thiiranes as reactive intermediates. *Chemical Research in Toxicology*, **9**, 1092–1102.

COMMANDEUR, J. N. M. and VERMEULEN, N. P. E. 1990, Identification of N-acetyl (2,2-dichlorovinyl)- and N-acetyl(1,2-dichlorovinyl)-L-cysteine as two regioisomeric mercapturic acids of trichloroethylene in the rat. *Chemical Research in Toxicology*, 3, 212–218.

COOPER, A. J. L. 1994, Enzymology of cysteine S-conjugate β-lyases. In *Conjugation dependent carcinogenicity and toxicity of foreign compounds*, M. W. Anders and W. Dekant eds. *Advanced Pharmacology*, **27**, (Academic Press, San Diego), pp. 71–114.

CHRISTAKOPOULOS, A., BERGMARK, E., ZORCEC, V., NORPPA, H., MÄKI-PAAKKANEN, J. and OSTERMAN-GOLGAR, S. 1993, Monitoring occupational exposure to styrene from haemoglobin adducts and metabolites in blood. *Scandinavian Journal of Work, Environment & Health*, **19**, 255–263.

CROSS, H., DAYAL, R., HYLAND, R. and GESCHER, A. 1990, N-alkylformamides are metabolized to N-alkylcarbamoylating species by hepatic microsomes from rodents and humans. *Chemical Research in Toxicology*, **3**, 357–362.

CSANÁDY, G. A., GUENGERICH, F. P. and BOND, J. A. 1992, Comparison of the biotransformation of 1,3-butadiene and its metabolite, butadiene mono-epoxide, by hepatic and pulmonary tissues from humans, rats and mice. *Carcinogenesis*, **13**, 1143–1153.

DAHL, A. R., SUN, J. D., BIRNBAUM, L. S., BOND, J. A., GRIFFITH, W. C., MADERLY, J. L., MUGGENBURG, B. A., SABOURIN, P. J. and HENDERSON, R. F. 1991, Toxicokinetics of inhaled 1,3-butadiene in monkeys: comparison to toxicokinetics in rats and mice. *Toxicology and Applied Pharmacology*, **110**, 9–19.

DAVIS, M. R., KASSAHUN, K., JOCHHEIM, C. M., BRANDT, K. M. and BAILLIE, T. A. 1993, Glutathione and N-acetylcysteine conjugates of 2-chloroethyl isocyanate. Identification as metabolites of N,N′-bis (2-chloroethyl)-N-nitrosourea in the rat and inhibitory properties toward glutathione reductase in vitro. *Chemical Research in Toxicology*, **6**, 376–383.

DEARFIELD, K. L., DOUGLAS, G. R., EHLING, U. H., MOORE, M. M., SEGA, G. A. and BRUSICK, D. J. 1995, Acrylamide: A review of its genotoxicity and an assessment of heritable genetic risk. *Mutation Research*, **330**, 71–99.

DEHNEN, W. 1990, A study on urinary thioethers by detecting N-acetylcysteine and thiophenol after alkaline hydrolysis. *Zeitblatt der Hygiene*, **189**, 441–451.

DEKANT, W. 1996, Biotransformation and renal processing of nephrotoxic agents. *Archives of Toxicology*, **18**, suppl. 163–172.

DEKANT, W., METZIER, M. and HENSCHLER, D. 1986, Identification of S-1,2-dichlorovinyl-N-acetyl-cysteine as a urinary metabolite of trichloroethylene: A possible explanation for its nephrocarcinogenicity in male rats. *Biochemical Pharmacology*, **35**, 2455–2458.

DEKANT, W., SCHRENK, D., VAMVAKAS, S. and HENSCHLER, D. 1988a, Metabolism of hexachloro-1,3-butadiene in mice: in vivo and in vitro evidence for activation by glutathione conjugation. *Xenobiotica*, **18**, 803–816.

DEKANT, W., BERTHOLD, K., VAMVAKAS, S., HENCHLER, D. and ANDERS, M. W. 1988b, Thioacylating intermediates as metabolites of S-(1,2-dichlorovinyl)-L-cysteine and S-(1,2,2-trichlorovinyl)-L-cysteine formed by cysteine conjugate β-lyase. *Chemical Research in Toxicology*, **1**, 175–178.

DEKANT, W., VAMVAKAS, S. and ANDERS, M. W. 1994, Glutathione mercaptides as transport forms of metals. In *Conjugation dependent carcinogenicity and toxicity of foreign compounds*, M. W. Anders and W. Dekant eds. *Advanced Pharmacology*, **27**, (Academic Press, San Diego), pp. 115–162.

DEPIERRE, J. W. AND MORGENSTERN, R. 1983, Comparison of the distribution of microsomal and cytosolic glutathione S-transferase activities in different organs of the rat. *Biochemical Pharmacology*, **12**, 465–474.

DE ROOIJ, B. M. 1997, Mercapturic acids in biological monitoring; Applications to industrial chemicals, PhD thesis, Vrije Universiteit, Amsterdam, The Netherlands, pp. 189–206.

DE ROOIJ, B. M., BOOGAARD, P. J., RIJKSEN, D. A., COMMANDEUR, J. N. M. and VERMEULEN, N. P. E. 1996a, Urinary excretion of N-acetyl-S-allyl-L-cysteine upon garlic consumption by human volunteers. *Archives of Toxicology*, **70**, 635–639 (1996).

DE ROOIJ, B. M., COMMANDEUR, J. N. M., GROOT, E. J., BOOGAARD, P. J. and VERMEULEN, N. P. E. 1996b, Biotransformation of allyl chloride in the rat: Influence of inducers on the urinary metabolic profile. *Drug Metabolism and Disposition*, **24**, 765–772.

DE ROOIJ, B. M., COMMANDEUR, J. N. M., RAMCHARAN, J. R., SCHUILENBURG, H. C. P., VAN BAAR, B. L. M. and VERMEULEN, N. P. E. 1996c, Identification and quantitative determination of 3-chloro-2-hydroxypropylmercapturic acid and α-chlorohydrin in urine of rats treated with epichlorohydrin. *Journal of Chromatography B*, **685**, 241–250.

DE ROOIJ, B. M., BOOGAARD, P. J., COMMANDEUR, J. N. M. and VERMEULEN, N. P. E. 1997a, 3-Chloro-2-hydroxypropylmercapturic acid and α-chlorohydrin as biomarkers of occupational exposure to epichlorohydrin. *Environmental Toxicology and Pharmacology*, **3**, 175–185.

DE ROOIJ, B. M., BOOGAARD, P. J., COMMANDEUR, J. N. M., VAN SITTERT, N. J. and VERMEULEN, N. P. E. 1997b, Allylmercapturic acid as urinary biomarker of human exposure to allyl chloride. *Occupational and Environmental Medicine*, **54**, 653–661.

DE ROOIJ, B. M., COMMANDEUR, J. N. M., HOMMES, J. W., AALBERS, T., GROOT, E. J. and VERMEULEN, N. P. E. 1997c, Urinary Metabolite Profile of Phenyl- and *ortho*-Cresyl Glycidyl Ether in Rats: Identification of a Novel Pathway leading to N-acetyl-serine-O-conjugates. *Chemical Research in Toxicology* (submitted).

DETERDING, L. J., SRINIVAS, P., MAHMOOD, N. A., BURKA, L. T. and TOMER, K. B. 1989, Fast atom bombardment and tandem mass spectrometry for structure determiantion of cysteine, N-acetylcysteine, and glutathione adducts of xenobiotics. *Analytical Biochemistry*, **183**, 94–107.

DINOFF, T. M., WINTER, C. K., JONES, A. D. and NEW, R. 1992, An evaluation of sample preparation techniques for the GC-MS analysis of urinary mercapturic acid conjugates. *Journal of Applied Toxicology*, **16**, 147–151.

EADSFORTH, C. V., HUTSON, D. H., LOGAN, C. J. and MORRISON, B. J. 1985, The metabolism of n-butyl glycidyl ether in the rat and rabbit. *Xenobiotica*, **15**, 579–589.

ECETOC 1994, Trichloroethylene: Assessment of human carcinogenic hazard. European centre for ecotoxicology and toxicology of chemicals, *Technical Report*, **60**, (ECETOC, Brussels), pp. 1–77.

EDER, E., HENSCHLER, D. and NEUDECKER, T. 1982a, Mutagenic properties of allylic and α,β-unsaturated compounds: consideration of alkylating mechanism. *Xenobiotica*, **12**, 831–848.

EDER, E., NEUDECKER, T. and HENSCHLER, D. 1982b, Correlating of alkylating and mutagenic activities of allyl and allylic compounds: Standard alkylation test vs. kinetic investigation. *Chemico-Biological Interactions*, **38**, 303–315.

EDER, E., LUTZ, D. and JÖRNS, M. 1987, Allylic compounds bind directly to DNA: Investigation of the binding mechanisms *in vitro*. *Chemico-Biological Interactions*, **61**, 97–108.

EDER, E. and HOFFMAN, C. 1993, Identification and characterization of deoxyguanosine adducts of mutagenic β-alkyl-substituted acrolein congeners. *Chemical Research in Toxicology*, **6**, 486–494.

EHRENBERG, L., MOUSTACCHI, E., OSTERMAN-GOLKAR, S. and EKMAN, G. 1983, Dosimetry of genotoxic agents and dose-response relationships of their effects. *Mutation Research*, **123**, 121–182.

ELLMAN, G. L. 1959, Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics*, **82**, 70–77.

ENDO, Y. 1978, N-Acyl-L-aromatic amino acid deacylase in animal tissues. *Biochimica et Biophysica Acta*, **523**, 207–214.

ENTERLINE, P. E., HENDERSON, V. and MARSH, G. 1999), Mortality of workers potentially exposed to epichlorohydrin. *British Journal of Industrial Medicine*, **47**, 269–276.

FAHRIG, R., MADLE, S. and BAUMANN, H. 1995, Genetic toxicology of trichloroethylene, *Mutation Research*, **340**, 1–36.

FAJEN, J. M., LUNSFORD, R. A. and ROBERTS, D. R. 1993, Industrial exposure to 1,3-butadiene in monomer, polymer and end-user industries. In *Butadiene and styrene: assessment of health hazards* M. Sorsa, K. Peltonen, H. Vianio and K. Hemminki eds. *IARC Scientific Publications*. **127**, pp. 3–13.

FARMER, P. B. and SWEETMAN, G. M. A. 1995, Mass spectrometric detection of carcinogen adduxts. *Journal of Mass Spectrometry*, **30**, 1369–1379.

FEDTKE, N., CERTA, H., EBERT, R. and WIEGAND, H-J. 1994, Species differences in the biotransformation of ethyl chloride; GSH-dependent metabolism. *Archives of Toxicology*, **68**, 217–223.

FENGSHENG, H. and SOU-LIN, Z. 1985, Effects of allyl chloride on occupationally exposed subjects. *Scandinavian Journal of Work, Environment & Health*, **11** (suppl. 4), 43–45.

FENNEL, T. R., KEDDERIS, G. L. and SUMNER, S. C. J. 1991, Urinary metabolites of [1,2,3-^{13}C acrylonitrile in rats and mice detected by ^{13}C nuclear magnetic resonance spectroscopy. *Chemical Research in Toxicology*, **4**, 678–687.

FILSER, J. G. and BOLT, H. M. 1983, Exhalation of ethylene oxide by rats on exposure to ethylene. *Mutation Research*, **120**, 57–60.

FÖST, U., HALLIER, E., OTTENWÄLDER, H., BOLT, H. M. and PETER, H. 1991, Distribution of ethylene oxide in human blood and its implications for biomonitoring. *Human and Experimental Toxicology*, **10**, 25–31.

FÖST, U., TÖRNQVIST, M., LEUTBECHER, M., GRANATH, F., HALLIER, E. and EHRENBERG, L. 1995, Effects of variation in detoxication rate on dose monitoring through adducts. *Human and Experimental Toxicology*, **14**, 201–203.

FU, P. C., ZIC, V. and OZIMY, K. 1979, Studies of ethylene-forming system in rat liver extract. *Biochmica et Biophysica Acta*, **585**, 427–434.

GARDINER, T. H., WAECHTER, J. M., WIEDOW, M. A. and SOLOMON, W. T. 1992, Glycidyloxy compounds used in epoxy resin systems: A toxicology review. *Regulatory Toxicology and Pharmacology*, **15**, S1–S77.

GELPI, E. 1995, Biomedical and biochemical applications of liquid chromatography-mass spectrometry. *Journal of Chromatography*, **703**, 59–80.

GÉRIN, M. and TARDIF, R. 1986, Urinary N-acetyl-S-2-hydroxyethyl-L-cysteine in rats as biological indicator of ethylene exide exposure. *Fundamental and Applied Toxicology*, **7**, 419–423.

GHITTORI, S., MAESTRI, L., FIORENTINO, M. L. and IMBRIANI, M. 1995, Evaluation of occupational exposure to benzene by urinalysis. *International Occupational and Environmental Health*, **67**, 195–200.

GILES, P. M. 1979, The biosynthesis of 3-hydroxypropyl mercapturic acid from cyclophosphamide. *Xenobiotica*, **9**, 745–762.

GINGELL, R., MITSCHKE, H. R., DZIDIC, I., BEATTY, P. W., SAWIN, V. L. and PAGE, A. C. 1985, Disposition and metabolism of [2-^{14}C] epichlorohydrin after oral administration to rats. *Drug Metabolism and Disposition*, **13**, 333–341.

GINGELL, R., BEATTY, P. W., MITSCHKE, H. R., MUELLER, R. L., SAWIN, V. L. and PAGE, A. C. 1987, Evidence that epichlorohydrin is not a toxic metabolite of 1,2-dibromo-3-chloropropane. *Xenobiotica*, **17**, 229–240.

GIRI, A. K. 1997, Genetic toxicology of epichlorohydrin: A review. *Mutation Research*, **386**, 25–38.

GOEPTAR, A. R., COMMANDEUR, J. N. M., VAN OMMEN, B., VAN BLADEREN, P. J. and VERMEULEN, N. P. E. 1995, Metabolism and kinetics of trichloroethylene in relation to toxicity and carcinogenicity. Relevance of the mercapturic acid pathway. *Chemical Research in Toxicology*, **8**, 3–21.

GOLD, K. W., NAUGLE, D. F. and BERRY, M. A. 1993, Indoor concentrations of environmental carcinogens. In *Environmental carcinogens methods of analysis and exposure measurement* B. Seifert, H. J. Van Der Wiel, B. Bodet and I. K. O'Neil eds, *IARC Scientific Publications*, **109**, (IARC, Lyon, pp. 41–71.

GOLDBARG, J. A., FRIEDMAN, O. M., PINEDA, E. P., SMITH, E. E., CHATTERJI, R., STEIN, E. H. and RUTENBERG, A. M. 1960, The colorimetric determination of γ-glutamyltranspeptidase with a synthetic substrate. *Archives of Biochemistry and Biophysics*, **91**, 61–70.

GÖRLER, K., KRUMBIEGEL, G., MENNICKE, W. H. and SIEHL, H-U. 1982, The mechanism of benzyl isothiocyanate and its cysteine conjugate in guinea-pigs and rabbits. *Xenobiotica*, **12**, 535–542.

GRAMMER, L. C., ROBERTS, M., NICHOLLS, A. J., PLATTS, M. M. and PATTERSON, R. 1984, IgE against ethylene oxide-altered human serum albumin in patients who have had acute dialysis reactions. *Journal of Allergy and Clinical Immunology*, **74**, 544–546.

GRAVES, R. J., COUTTS, C. and GREEN, T. 1995, Methylene chloride-induced liver damage: An interspecies comparison. *Carcinogenesis*, **16**, 1919–1926.

GREEN, T., ODUM, J., NASH, J. A. and FOSTER, J. R. 1990, Perchloroethylene-induced rat kidney tumors: An investigation of the mechanisms involved and their relevance to humans. *Toxicology and Applied Pharmacology*, **103**, 77–89.

GUENGERICH, F. P. 1994, Metabolism and genotoxicity of dihaloalkanes. In *Conjugation dependent carcinogenicity and toxicity of foreign compounds*, M. W. Anders and W. Dekant eds. *Advanced Pharmacology*, **27**, (Academic Press, San Diego), pp. 211–236.

GUENGERICH, F. P., THIER, R., PERSMARK, M. TAYLOR, J. B., PEMBLE, S. E. and KETTERER, B. 1995, Conjugation of carcinogens by θ class glutathione S-transferases: Mechanisms and relevance to variations in human risk. *Pharmacogenetics*, **5**, S103–S107.

HÄBERLE, D., WAHLLÄNDER, A. and SIES, H. 1979, Assessment of the kidney

of plasma glutathione concentrations and redox state in anesthetized rats. *FEBS Letters*, **108**, 335–340.

HALLIER, E., DEUTSCHMANN, S., REICHEL, C., BOLT, H. M. and PETER, H. 1990, A comparative investigation of the metabolism of methyl bromide and iodide in human erythrocytes. *International Archives of Occupational and Environmental Health*, **62**, 211–225.

HALLIER, E., LANGHOF, T., DANNAPPEL, D., LEUTBECHER, M., SCHRÖDER, K., GEORGENS, H. W., MÜLLER, A. and BOLT, H. M. 1993, Polymorphism of glutathione conjugation of methylbromide, ethylene oxide and dichloromethane in human blood: influence on the induction of sister chromatid exchanges (SCE) in lymphocytes. *Archives of Toxicology*, **67**, 173–178.

HALLIER, E., GOERGENS, H. W., HALLIER, K. and BOLT, H. M. 1994, Intervention study on the influence of reduction of occupational exposure to styrene on sister chromatid exchanges in lymphocytes. *International Archives of Occupational and Environmental Health*, **66**, 167–172.

HALLIER, E., GOERGENS, H. W., KARELS, H. and GOLKA, K. 1995, A note on individual differences in the excretion of optical enantiomers of styrene metabolites and of styrene-derived mercapturic acids in humans. *Archives of Toxicology*, **69**, 300–305.

HARRIS, C. C. 1989, Interindividual variation among humans in carcinogen metabolism, DNA adduct formation and DNA repair. *Carcinogenesis*, **10**, 1563–1566.

HARRIS, J. M., MEYER, D. J., COLES, B. and KETTERER, B. 1991, A novel glutathione transferase (13–13) isolated from the matrix of rat liver mitochondria having structural similarity to class theta enzymes. *Biochemical Journal*, **278**, 137–141.

HASHMI, M., VAMVAKAS, S. and ANDERS, M. W. 1992, Bioactivation mechanism of S-(3-oxopropyl)-N-acetyl-L-cysteine, the mercapturic acid of acrolein. *Chemical Research in Toxicology*, **5**, 360–365.

HÄUSLER, M. and LENICH, R. 1968, Zur wirkung von allylchlorid bei chronischer gewerblicher exposition. *Archives of Toxicology*, **23**, 209–214.

HAYES, J. D. 1986, Purification and physical characterization of glutathione S-transferase K. *Biochemical Journal*, **233**, 789–798.

HAYES, J. D. and PULFORD, D. J. 1995, The glutathione S-transferase supergene family: Regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Critical Reviews in Biochemistry and Molecular Biology*, **30**, 445–600.

HEMMINKI, K. 1995, DNA adducts in biomonitoring. *Journal of Occupational and Environmental Medicine*, **37**, 44–51.

HEMMINKI, K. and VAINIO, H. 1980. Alkylation of nucleic acid bases by epoxides and glycidyl ethers. *Developments in Toxicological and Environmental Sciences*, **8**, 241–244.

HENDERSON, R. F., BECHTOLD, W. E., BOND, J. A. and SUN, J. D. 1989, The use of biological markers in toxicology. *Critical Reviews in Toxicology*, **20**, 65–82.

HENDERSON, R. F., BECHTOLD, W. E., SABOURIN, P. J., MAPLES, K. R. and DAHL, A. R. 1993, Species differences in the metabolism of 1,3-butadiene *in vivo*. In *Butadiene and styrene: assessment of health hazards*, M. Sorsa, K. Peltonen, H. Vianio and K. Hemminki eds, *IARC Scientific Publications*, **127**, pp. 57–64.

HENSCHLER, D., VAMVAKAS, S., LAMMERT, M., DEKANT, W., KRAUS, B., THOMAS, B. and ULM, K. 1995, Increase incidence of renal cell tumors in a cohort of cardboard workers exposed to trichloroethylene. *Archives of Toxicology*, **69**, 291–299.

HIMMELSTEIN, M. W., TURNER, M. J., ASGHARIAN, B. and BOND, J. A. 1994, Comparison of blood concentrations of 1,3-butadiene and butadiene epoxides in mice and rats exposed to 1,3-butadiene by inhalation. *Carcinogenesis*, **15**, 1479–1486.

HINCHMAN, C. A. and BALLATORI, N. 1990, Glutathione-degrading capacities of liver and kidney in different species. *Biochemical Pharmacology*, **40**, 1131–1135.

HINCHMAN, C. A. and BALLATORI, N. 1994, Glutathione conjugation and conversion to mercapturic acids can occur as an intrahepatic process. *Journal of Toxicological and Environmental Health*, **41**, 387–409.

HINSHAW, J. V. 1989, Large-volume injection in capillary GC: Problems and solutions. *LC-GC International*, **2**, 24–29.

HIRATSUKA, A., YOKOI, A., IWATA, H., WATABE, T., SATOH, K., HATAYAMA, I. and SATO, K. 1989, Glutathione conjugation of styrene 7,8-oxide enantiomers by major glutathione transferase isoenzymes isolated from rat livers. *Biochemical Pharmacology*, **38**, 4405–4413.

HIRATSUKA, A., OKADA, T., NISHIYAMA, T., FUJIKAWA, M., OGURA, K., OKUDA, H., WATABE, T. and WATABE, T. 1994, Novel theta class glutathione S-transferases Yrs-Yrs' and Yrs'–Yrs' in rat liver cytosol: Their potent activity towards 5-sulphoxymethylchrysene, a reactive metabolite of the carcinogen 5-hydroxymethylchrysene. *Biochemical and Biophysical Research Communications*, **202**, 278–284.

HIROTA, T., NISHIKAWA, Y., TANAKA, M., IGARASHI, T. and KITAGAWA, H. 1986, Characterization of dehydropeptidase-Iin the rat lung. *European Journal of Biochemistry*, **160**, 521–525.

HONJO, I., SUOU, T. and HIRAYAMA, C. 1988, Hepatotoxicity of cyclophosphamide in man: pharmacokinetic analysis. *Research Communications in Chemical Pathology and Pharmacology*, **61**, 149–165.

HUANG, X. and KOK, W. TH. 1995, Determination of thiols by capillary electrophoresis with electrochemical detection using a palladium field-decoupler and chemically modified electrodes. *Journal of Chromatography*, **716**, 347–353.

HUGHES, K., MEEK, M. E. and WINDLE, W. 1994, Trichloroethylene: Evaluation of risks to health from environmental exposure in Canada. *Environmental Carcinogens and Ecotoxicology Reviews*, **C12**, 527–543.

HUMPHEREYS, W. G., KIM D-H. and GUENGERICH, F. P. 1991, Isolation and characterization of N7-guanyl adducts derived from 1,2-dibromo-3-chloropropane. *Chemical Research in Toxicology*, **4**, 445–453.

HUSSAY, A. J. and HAYES, J. D. 1993, Human mu-class glutathione S-transferases present in liver, skeletal muscle and testicular tissue. *Biochimica et Biophyica Acta*, **1203**, 131–141.

IARC, 1976, Epichlorohydrin. *IARC monograph Evaluation of Carcinogenic Risks of chemicals in Humans*, **11**, 131–139.

IARC, 1985, Allyl compounds, aldehydes, epoxides and peroxides. *IARC monograph Evaluation of Carcinogenic Risks of chemicals in Humans*, **36**, pp. 39–54, 133–162 and 189–226.

IARC, 1989, Some organic solvents, resin monomers and related compounds. *IARC monograph Evaluation of Carcinogenic Risks of chemicals in Humans*, **47**, dimethylformamide, pp. 171–198 and 237–261.

IARC, 1994a, Some industrial chemicals; Acrylamide. *IARC monograph Evaluation of Carcinogenic Risks of chemicals in Humans*, Volume **60**, pp. 389–433.

IARC, 1994b, Some industrial chemicals; Ethylene oxide. *IARC monograph Evaluation of Carcinogenic Risks of chemicals in Humans*, Volume **60**, pp. 73–159.

IARC, 1995, Dry cleaning, some chlorinated solvents and other industrial chemicals; trichloroethylene. *IARC monograph Evaluation of Carcinogenic Risks of chemicals in Humans*, Volume **63**, pp. 75–158.

ILINSKAJA, O. and VAMVAKAS, S. 1996, Alterations of the renal function in the isolated perfused rat kidney system after in vivo and in vitro application of S-(1,2-dichlorovinyl)-L-cysteine and S-(2,2-dichlorovinyl)-L-cysteine. *Archives of Toxicology*, **70**, 224–229.

INOUE, M., OKAJIMA, K. and MORINO, Y. 1984, Hepato-renal cooperation in biotransformation, membrane transport, and elimination of cysteine S-conjugates of xenobiotics. *Journal of Biochemistry (Tokyo)*, **95**, 247–254.

INOUE, M., OKAJIMA, K., NAGASE, S. and MORINO, Y. 1987. Inter-organ metabolism and transport of a cysteine S-conjugate of xenobiotics in normal and mutant analbuminetic rats. *Biochemical Pharmacology*, **36**, 2145–2150.

INSKEEP, P. B. and GUENGERICH, F. P. 1984, Glutathione-mediated binding of dibromoalkanes to DNA: specificity of rat glutathione S-transferase and dibromoalkane structure. *Carcinogenesis*, **5**, 805–808.

IWASE, H., OZAWA, S., IKUTA, M. and ONO, I. 1995, Determination of amino acids in human plasma by liquid chromatography with postcolumn ninhydrin derivatization using a hydroxyapatite cartridge for precolumn deproteination. *Journal of Chromatography B*, **663**, 15–24.

JAFFE, M. 1879, Ueber die nach Einführung von Brombenzol und Chlorbenzol im Organismus entstehenden schwefelhaltigen Säuren. *Chemische Berichte*, (Berichte der Deutschen Chemischen Gesellschaft), **12**, 1092–1098.

JAKUBOWSKI, M., LINHART, I., PIELAS, G. and KOPECKY, J. 1987, 2-Cyanoethyl mercapturic acid (CEMA) in the urine as a possible indicator of exposure to acrylonitrile. *British Journal of Industrial Medicine*, **44**, 834–840.

JAMES, S. P. and NEEDHAM, D. 1973, Some metabolites of S-pentyl-L-cysteine in the rabbit and other species. *Xenobiotica*, **4**, 207–218.

JAMES, S. P., PHEASANT, A. E. and SOLHEIM, E. 1978, Metabolites of 1,2-epoxy-3-phenoxy- and 1,2-epoxy-3-(p-nitrophenyxy)propane. *Xenobiotica*, **8**, 219–228.

JENSSON, H., GUTHENBERG, C., ÅLIN, P. and MANNERVIK, B. 1986, Rat glutathione transferase 8-8, an enzyme efficiently detoxifying 4-hydroxyalk-2-enals. *FEBS Letters*, **203**, 207–209.

JIAO, D., HO, C-T., FOILES, P. and CHUNG, F-L. 1994, Identification and quantification of the N-acetylcysteine conjugate of allyl isothiocyanate in human urine after ingestion of mustard. *Cancer Epidemiological Biomarkers & Prevention*, **3**, 487–492.

JOLANKI, R., KANERVA, L., ESTLANDER, T. and TARVIANEN, K. 1994, Epoxy dermatitis. *Occupational Medicine – State of the Art Reviews*, **9**, 97–110.

JONES, A. R. and WALSH, D. A. 1979, The oxidative metabolism of 1-bromopropane in the rat. *Xenobiotica*, **9**, 763–772.

JONES, T. W., QIN, C., SCHAEFFER, V. H. and STEVENS, J. L. 1988, Immunohistochemical localization of glutamine transanimase K, a rat kidney cysteine conjugate β-lyase, and the relationship to the segment specificity of cysteine conjugate nephrotoxicity. *Molecular Pharmacology*, **34**, 621–627.

JONES A. D., WINTER, C. K., BUONARATI, M. H. and SEGALL, H. J. 1993, Analysis of mercapturic acid conjugates of xenobiotic compounds using negative ionization and tandem mass spectrometry. *Biological Mass Spectrometry*, **22**, 68–76.

JONGENEELEN, F. J., DRIVEN, H. A. A. M., LEIJDEKKERS, C-M., HENDERSON, [illegible]

and Halm, K. 1987, S-Phenyl-N-acetylcysteine in urine of rats and workers after exposure to benzene. *Journal of Analytical Toxicology*, **11**, 100–104.

Juronen, E., Tasa, G., Uusküla, M., Pooga, M. and Mikelsaar, A-V. 1996, Purification, characterization and tissue distribution of human class theta glutathione S-transferase T1–1. *Biochemical Molecular and Biological Interactions*, **39**, 21–29.

Katoh, T., Nagata, N., Kuroda, Y., Itoh, H., Kawahara, A., Kuroki, N., Ookuma, R. and Bell, D. A. 1996, Glutathione S-transferase M1 (GSTM1) and T1 (GSTT1) genetic polymorphism and susceptibility to gastric and colorectal adenocarcinoma. *Carcinogenesis*, **17**, 1855–1859.

Kaye, C. M., Clapp, J. J. and Young, L. 1972, The metabolic formation of mercapturic acids from allyl halides. *Xenobiotica*, **2**, 129–139.

Kedderis, G. L. and Batra, R. 1993, Species differences in the hydrolysis of 2-cyanoethylene oxide, the epoxide metabolite of acrylonitrile. *Carcinogenesis*, **14**, 685–689.

Kedderis, G. L., Batra, R. and Koop, D. R. 1993a, Epoxidation of acrylonitrile by rat and human cytochromes P450. *Chemical Research in Toxicology*, **6**, 866–871.

Kedderis, G. L., Sumner, S. C., Held, S. D., Batra, R., Turner, M. J. Jr, Roberts, A. E. and Fennell, T. P. 1993b, Dose-dependent urinary excretion of acrylonitrile metabolites in rats and mice. *Toxicology and Applied Pharmacology*, **120**, 288–297.

Kedderis, G. L., Batra, R. and Turner Jr, M. J. 1995, Conjugation of acrylonitrile and 2-cyanoethylene oxide with hepatic glutathione. *Toxicology and Applied Pharmacology*, **135**, 9–17.

Ketterer, B. and Christopoulides, L. G. 1994, Enzymology of cytosolic glutathione S-transferase. In *Conjugation dependent carcinogenicity and toxicity of foreign compounds* M. W. Anders and W. Dekant eds. *Advanced Pharmacology*, **27**, (Academic Press, San Diego), pp. 37–70.

Ketterer, B. and Mulder, G. J. 1990, Glutathione conjugation. In *Conjugation reactions in drug metabolism: An integrated approach* G. J. Mulder ed, (Taylor & Francis, London), pp. 307–364.

Kezic, S., Monster, A. C., Verplanke, A. J. W. and De Wolff, F. A. 1996, Dermal absorption of cis-1,3-dichloropropene vapour: Human experimental exposure. *Human and Experimental Toxicology*, **15**, 396–399.

Kinuta, M., Ubuka, T., Yao, W-B., Zhao, Y-Q. and Shimizu, H. 1994, Isolation of S-[2-carboxy-1-(1H-imidazol-4) ethyl]-3-thiolactic acid, a new metabolite of histidine, from normal human urine and its formation from S-[2-carboxy-1-(1H-imidazol-4-yl)ethyl] cysteine. *Biochemical Journal*, **297**, 475–478.

Kohn, M. C. and Melnick, R. L. 1993, Species differences in the production and clearance of 1,3-butadiene metabolites: a mechanistic model indicates predominantly physiological, not biochemical, control. *Carcinogenesis*, **14**, 619–628.

Koike, K. and Koike M. 1984, Fluorescent analysis of α-keto acids in serum and urine by high-performance liquid chromatography. *Analytical Biochemistry*, **141**, 481–487.

Koob, M. and Dekant, W. 1991, Bioactivation of xenobiotics by formation of toxic glutathione conjugates. *Chemico-Biological Interactions*, **77**, 107–136.

Kretzschmar, M. 1996, Regulation of hepatic glutathione metabolism and its role in hepatotoxicity. *Experimental and Toxicologic Pathology*, **48**, 439–446.

La, D. K., Schoonhoven, R., Ito, N. and Swenberg, J. A. 1996, The effects of exposure route on DNA adduct formation and cellular proliferation by 1,2,3-trichloropropane. *Toxicology and Applied Pharmacology*, **140**, 108–114.

La, D. K. and Swenberg, J. A. 1996, DNA adducts: Biological markers of exposure and potential applications to risk assessment. *Mutation Research*, **365**, 129–146.

Laib, R. J., Tucholski, M., Filser, J. G. and Csanády, G. A. 1992, Pharmacokinetic interaction between 1,3-butadiene and styrene in Sprague-Dawley rats. *Archives of Toxicology*, **66**, 310–314.

Landi, S., Ponzanelli, I., Hirvonen, A., Norppa, H. and Barale, R. 1996, Repeated analysis of sister chromatid exchange induction by diepoxybutane in cultured human lymphocytes: Effect of glutathione S-transferase T1 and M1 genotype. *Mutation Research*, **351**, 79–85.

Landin, H. H., Osterman-Golkar, S., Zorcec, V. and Törnqvist, M. 1996, Biomonitoring of epichlorohydrin by hemoglobin adducts. *Analytical Biochemistry*, **240**, 1–6.

Legator, M. S., Au, W. W., Ammenheuser, M. and Ward Jr, J. B. 1993, Elevated somatic cell mutant frequencies and altered DNA repair responses in nonsmoking workers exposed to 1,3-butadiene. In M. Sorsa, K. Peltonen, H. Vainio and K. Hemminki eds, *IARC Scientific Publications*, **127**, (IARC, Lyon), pp. 253–263.

Lemiere, F., Esmans, E. L., Van Dongen, W., Van den Eeckhout, E. and Van Onckelen, H. 1993, Evaluation of liquid chromatography-thermospray mass spectrometry in the determination of some phenyl glycidyl ether-2′-deoxynucleoside adducts. *Journal of Chromatography*, **647**, 221–218.

Linhart, I., Frantík, E., Vodickova, L., Vosmanská, M., Smejkal, J. and Mitera, J. 1996, Biotransformation of acrolein in rat: Excretion of mercapturic acids after inhalation and intraperitoneal injection. *Toxicology and Applied Pharmacology*, **136**, 155–160.

Linhart, I., Hrabal, R., Smejkal, J. and Mitera, J. 1994a, Metabolic pathways of 1-butyl [3-^{13}C] acrylate. Identification of urinary metabolites in rat using nuclear magnetic resonance and mass spectroscopy. *Chemical Research in Toxicology*, **7**, 1–8.

LINHART, I., VOSMANSKÁ, M. and SMEJKAL, J. 1994b, Biotransformation of acrylates. Excretion of mercapturic acids and changes in urinary carboxylic acid profile in rat dosed with ethyl- and 1-butyl acrylate. *Xenobiotica*, **24**, 1043–1052.

LÖFROTH, G., BURTON, R. M., FOREHAND, L., HAMMOND, S. K., SEILA, R. L., ZWEIDINGER, R. B. and LEWTAS, J. 1989, Characterization of environmental tobacco smoke. *Environmental Science and Technology*, **23**, 610–614.

LOWRY, L. K. 1995, Role of biomarkers of exposure in the assessment of health risks. *Toxicology Letters*, **77**, 31–38.

LUCAS, A. D., JONES, A. D., GOODROW, M. H., SAIZ, S. G., BLEWETT, C., SEIBER, J. N. and HAMMOCK, B. D. 1993, Determination of atrazine metabolites in human urine: Development of a biomarker of exposure. *Chemical Research in Toxicology*, **6**, 107–116.

LUTZ, W. K. 1979, In vivo covalent binding of organic chemicals to DNA as a quantitative indicator in the process of chemical carcinogenesis. *Mutation Research*, **65**, 289–356.

MAESTRI, L., IMBRIANI, M., GHITTORI, S., CAPODAGLIO, E., GOBBA, F. and CAVALERRI, A., 1997, Excretion of N-acetyl-S-(1-phenyl-2-hydroxyethyl)-cysteine and N-acetyl-S-(2-phenyl-2-hydroxyethyl)-cysteine in workers exposed to styrene. *Science of the Total Environment*, **199**, 13–22.

MAHMOOD, N. A., OVERSTEER, D. and BURKA, L. T. 1991, Comparative disposition and metabolism of 1,2,3-trichloropropane in rats and mice. *Drug Metabolism and Disposition*, **19**, 411–418.

MAINWARING, G. W., WILLIAMS, J. R., FOSTER, J. R., TUGWOOD, J. and GREEN, T. 1996, The distribution of theta-class glutathione S-transferases in liver and lung of mouse, rat and human. *Biochemical Journal*, **318**, 297–303.

MANNERVIK, B., AWASTHI, Y. C., BOARD, P. G., HAYES, J. D., DI ILIO, C., KETTERER, B., LISTOWSKY, I., MORGENSTERN, R., MURAMATSU, M., PEARSON, W. R., PICKETT, C. B., SATO, K., WIDERSTEN, M. and WOLF, C. R. 1992, Nomenclature for human glutathione transferases. *Biochemical Journal*, **282**, 305–306.

MARCO, M-P., NASIRI, M., KURTH, M. J. and HAMMOCK, B. D. 1993, Enzyme-linked immuno-sorbent assay for the specific detection of the mercapturic acid metabolites of naphthalene. *Chemical Research in Toxicology*, **6**, 284–293.

MCCOY, E. C., BURROWS, L. and ROSENKRANZ, H. S. 1978, Genetic activity of allyl chloride. *Mutation Research*, **57**, 11–15.

MCDONALD, T. A., YEOWELL-O'CONNELL, K. and RAPPAPORT, S. M. 1994, Comparison of protein adducts of benzene oxide and benzoquinone in blood and bone marrow of rats and mice exposed to [$^{14}C/^{13}C_6$] benzene. *Cancer Research*, **54**, 4907–4914.

MCGUIRE, W. P., BLESSING, J. A., HATCH, K. D. and BERMAN, M. L. 1990, Phase II study of N-methylformamide in patients with advanced squamous cancer of the cervix. *Investigational New Drugs*, **8**, 195–197.

MEISTER, A. 1983, Metabolism and transport of glutathione and other γ-glutamyl compounds. In *Functions of glutathione: Biochemical, physiological, toxicological and clinical aspects*, A. Larsson, S. Orrenius, A. Holmgren and B. Mannervik eds, (Raven Press, New York), pp. 1–22.

MEISTER, A. 1991, Glutathione deficiency produced by inhibition of its synthesis, and its reversal: Applications in research and therapy. *Pharmacology and Therapeutics*, **51**, 155–194.

MEYER, D. J., COLES, B., PEMBLE, S. E., GILMORE, K. S., FRASER, G. M. and KETTERER, B. 1991, Theta, a new class of glutathione transferases purified from rat and man. *Biochemical Journal*, **274**, 409–414.

MILLER, M. J., CARTER, D. E. and SIPES, I. G. 1982, Pharmacokinetics of acrylamide in Fischer-334 rats. *Toxicology and Applied Pharmacology*, **63**, 36–44.

MINISTRY OF SOCIAL AFFAIRS, 1995, *De Nationale MAC-lijst 1995* (in Dutch) (Sdu, The Hague).

MITCHELL, D. Y. and PETERSEN, D. R. 1989, Metabolism of the glutathione-acrolein adducts, S-(2-aldehydo-ethyl) glutathione, by rat liver alcohol and aldehyde dehydrogenase. *Journal of Pharmacology and Experimental Therapeutics*, **251**, 193–198.

MONKS, T. J. and LAU, S. S. 1994, Glutathione conjugation as a mechanism for the transport of reactive metabolites. In *Conjugation dependent carcinogenicity and toxicity of foreign compounds*, M. W. Anders and W. Dekant eds, *Advanced Pharmacology*, **27**, (Academic Press, San Diego), pp. 183–210.

MRÀZ, J. 1988, Gas chromatographic method for the determination of N-acetyl-S-(N-methylcarbamoyl) cysteine, a metabolite of N,N-dimethylformamide and N-methylformamide, in human urine. *Journal of Chromatography B*, **431**, 361–368.

MRÀZ, J. and NOHOVA, H. and 92, Absorption, metabolism and elimination of N,N-dimethylformamide in humans. *International Archives of Occupational and Environmental Health*, **64**, 85–92.

MUNDAY, R. 1994, Bioactivation of thiols by one-electron oxidation. In *Conjugation dependent carcinogenicity and toxicity of foreign compounds*, M. W. Anders and W. Dekant eds, *Advanced Pharmacology*, **27**, (Academic Press, San Diego), pp. 237–270.

MUTLIB, A. E., TALAAT, R. E., SLATTER, J. G. and ABBOTT, F. S. 1990, Formation and reversibility of S-linked conjugates of N-(1-methyl-3,3-diphenylpropyl)isocyanate, an in vivo metabolite of N-(1-methyl-3,3-diphenylpropyl)formamide, in rats. *Drug Metabolism and Disposition*, **18**, 1038–1045.

Mütsch-Eckner, M., Meier, B., Wright, A. D. and Sticher, O. 1992, γ-Glutamyl peptides from allium sativum bulbs. *Phytochemistry*, **31**, 2389–2391.

Nakajima, T., Elovaara, E., Gonzales, F. J., Gelboin, H. V., Vainio, H. and Aoyama, T. 1993, Characterization of human P-450 isoenzymes responsible for styrene metabolism. In *Butadiene and styrene: assessment of health hazards*, M. Sorsa, K. Peltonen, H. Vianio and K. Hemminki eds, *IARC Scientific Publications*, **127**, pp. 101–108.

Nauhaus, S. K., Fennell, T. R., Asgharian, B., Bond, J. A. and Sumner, S. C. J. 1996, Characterization of urinary metabolites from Sprague-Dawley rats and B6C3F1 mice exposed to [1,2,3,4-^{13}C]butadiene. *Chemical Research in Toxicology*, **9**, 764–773.

Nebert, D. W., McKinnon R. A. and Puga, A. 1996, Human drug-metabolizing enzyme polymorphisms: Effects on risk of toxicity and cancer. *DNA and Cell Biology*, **15**, 273–280.

Nestmann, E. R., Bryant, D. W. and Carr, C. J. 1996, Toxicological significance of DNA adducts: Summary of discussions with an expert panel. *Regulatory Toxicology and Pharmacology*, **24**, 9–18.

Norström, Å., Andersson, B. and Levin, J-O. 1986, A procedure for the analysis of S-benzyl-N-acetylcysteine and S-(o-methylbenzyl)-N-acetylcysteine in human urine. *Xenobiotica*, **16**, 525–529.

Norström, Å., Löf, A., Aringer, L., Samuelsson, R., Andersson, B., Levin, J-O. and Näslund, P. 1992, Determination of N-acetyl-S-(2-phenyl-2-hydroxyethyl) cysteine in human urine after experimental exposure to styrene. *Chemosphere*, **24**, 1553–1561.

Norppa, H., Hirvonen, A., Järventaus, H., Uusküla, M., Tasa, G., Ojajärvi, A. and Sorsa, M. 1995, Role of GSTT1 and GSTM1 genotypes in determining individual sensitivity to sister chromatid exchange induction by diepoxybutane in cultured human lymphocytes. *Carcinogenesis*, **16**, 1261–1264.

Oesch, F., Doehmer, J., Friedberg, T., Glatt, H. R., Oesch-Bartlomowicz, B., Platt, K. L., Steinberg, P., Utesch, D. and Thomas, H. 1990, Toxicological implications of enzyme control of reactive metabolites. *Human and Experimental Toxicology*, **9**, 171–177.

Oesch, F. and Wolf, C. R. 1989, Properties of the microsomal and cytosolic glutathione transferases involved in hexachloro-1,3-butadiene conjugation. *Biochemical Pharmacology*, **38**, 353–359.

Olsen, G. W., Lacy, S. E., Chamberlin, S. R., Albert, D. L., Arceneaux, T. G., Bullard, L. F., Stafford, B. A. and Boswell, J. M. 1994, Retrospective cohort mortality study of workers with potential exposure to epichlorohydrin and allyl chloride. *American Journal of Industrial Medicine*, **25**, 205–218.

Omenn, G. S. 1996, Risk assessment for butadiene: introductory and summary comments. *Toxicology*, **113**, 5–11.

Ong, C. N., Kok, P. W., Lee, B. L., Shi, C. Y., Ong, H. Y., Chia, K. S., Lee, C. S. and Luo, X. W. 1995, Evaluation of biomarkers for occupational exposure of benzene. *Occupational and Environmental Medicine*, **52**, 528–533.

Onkenhout, W., Mulder, P. P. J., Boogaard, P. J., Buys, W. and Vermeulen, N. P. E. 1986, Identification and quantitative determination of mercapturic acids formed from Z- and E-1,3-dichloropropene by the rat, using gas chromatography with three different detection techniques. *Archives of Toxicology*, **59**, 235–241.

Osterman-Golkar, S. M., MacNeela, J. P., Turner, M. J., Walker, V. E., Swenberg, J. A., Sumner, J. A., Youtsey, N. and Fennell, T. R. 1994). Monitoring exposure to acrylonitrile using adducts with N-terminal valine in hemoglobin. *Carcinogenesis*, **15**, 2701–2707.

Osterloh, J. D., Popendorf, W., Cohen, B-S. and Pond, S. M. 1984, Urinary excretion of the N-acetyl cysteine conjugate of cis-1,3-dichloropropene by exposed individuals. *Archives of Environmental Health*, **39**, 271–275.

Pacifici, G. M., Warholm, M., Guthenberg, C., Mannervik, B. and Rane, A. 1987, Detoxication of styrene oxide by human liver glutathione transferase. *Human Toxicology*, **6**, 483–489.

Parent, R. A., Caravello, H. E. and Sharp, D. E. 1996, Metabolism and distribution of [2,3-^{14}C]acrolein in Sprague-Dawley rats. *Journal of Applied Toxicology*, **16**, 449–457.

Pearson, P. G., Slatter, J. G., Rashed, M. S., Han, D-H., Grillo, M. P. and Baillie, T. A. 1990, S-(N-methylcarbamoyl)glutathione: A reactive S-linked metabolite of methyl isocyanate. *Biochemical and Biophysical Research Communications*, **166**, 245–250.

Pearson, P. G., Slatter, J. G., Rashed, M. S., Han, D-H. and Baillie, T. A. 1991, Carbamoylation of peptides and proteins in vitro by S-(N-methylcarbamoyl)glutathione and S-(N-methylcarbamoyl)cysteine, two electrophilic S-linked conjugates of methyl isocyanate. *Chemical Research in Toxicology*, **4**, 436–444.

Pekari, K., Vainiotalo, S.,Heikkilä, P., Palotie, A., Luotamo, M. and Riihimäki, V. and 92, Biological monitoring of occupational exposure to low levels of benzene. *Scandinavian Journal of Environmental Health*, **18**, 317–322.

Perez, H. L., Plna, K. and Osterman-Golgar, S. 1997, Dosimetry of glycidyl ethers in mice by quantification of haemoglobin adducts. *Chemico-Biological Interactions*, **103**, 1–16.

Phillips, D. H. and Farmer, P. B. 1994, Evidence for DNA and protein binding by styrene and styrene oxide. *Critical Reviews in Toxicology*, **24**, S35–S46.

Phillips, D. H., Schoket, B., Hewer, A., Bailey, E., Kostic, S. and Vin[illegible]

cigarette smoking on the level of DNA adducts in human bronchial epithelium and white blood cells. *International Journal of Cancer*, **46**, 569–575.

PLOEMEN, J. H. T. M., WORMHOUDT, L. W., VAN OMMEN, B., COMMANDEUR, J. N. M., VERMEULEN, N. P. E. and VAN BLADEREN, P. J. 1995, Polymorphism in the glutathione conjugation activity of human erythrocytes towards ethylene dibromide and 1,2-epoxy-3-(p-nitrophenoxy)-propane. *Biochimica et Biophysica Acta*, **1243**, 469–476.

POIRIER, M. C. 1994, Human exposure monitoring, dosimetry, and cancer risk assessment: The use of antisera specific for carcinogen-DNA adducts and carcinogen modified DNA. *Drug Metabolism Reviews*, **26**, 87–109.

POLI, G., DIANZANI, M. U., CHEESEMAN, K. H., SLATER, T. F., LANG, J. and ESTERBAUER, H. 1985, Separation and characterization of the aldehydic products of lipid peroxidation stimulated by carbon tetrachloride or ADP-iron in isolated rat hepatocytes and rat liver microsomal suspensions. *Biochemical Journal*, **227**, 629–638.

POOLE, C. F. and SCHUETTE, S. A. eds 1986, *Contemporary practice of chromatography* (Elsevier, Amsterdam), pp. 485–510.

POPP, W., VAHRENHOLZ, C., PRZYGODA, H., BRAUKSIEPE, A., GOCH, S., MÜLLER, G., SCHELL, C. and NORPOTH, K. 1994, DNA-protein cross-links and sister chromatid exchange frequencies in lymphocytes and hydroxyethyl mercapturic acid in urine of ethylene oxide-exposed hospital workers. *International Archives of Occupational and Environmental Health*, **66**, 325–332.

PROSSER, C. L. ed 1973, *Comparative animal physiology* (Saunders, Philadelphia), pp. 824, 839.

RAMU, K., FRAISER, L. H., MAMIYA, B., AHMED, T. and KEHRER, J. P. 1995, Acrolein mercapturates: Synthesis, characterization, and assessment of their role in the bladder toxicity of cycloposphamide. *Chemical Research in Toxicology*, **8**, 515–524.

RAMU, K., PERRY, C. S., AHMED, T., PAKENHAM, G. and KEHRER, J. P. 1996, Studies on the basis for the toxicity of acrolein mercapturates. *Toxicology and Applied Pharmacology*, **140**, 487–498.

RAPPAPORT, S. M., TING, D., JIN, Z., YEOWELL-O'CONNELL, K., WAIDYANATHA, S. and MCDONALD, T. 1993, Application of Raney nickel to measure adducts of styrene oxide with hemoglobin and albumin. *Chemical Research in Toxicology*, **6**, 238–244.

RAUNIO, H., HUSGAFVEL-PURSIAINEN, K., ANTTILA, S., HIETANEN, E., HIRVONEN, A. and PELKONEN, P. 1995, Diagnosis of polymorphisms in carcinogen-activating and inactivating enzymes and cancer susceptibility. *Gene*, **159**, 113–121.

REID, W. D. 1972, Mechanism of allyl alcohol-induced hepatic necrosis. *Experientia*, **28**, 1058–1061.

REITZ, R. H., MENDRALA, A. L. and GUENGERICH, F. P. 1989, In vitro metabolism of methylene chloride in human and animal tissues: Use of physiologically based pharmocokinetic models. *Toxicology and Applied Pharmacology*, **97**, 230–246.

RICHIE JR J. P., ABRAHAM, P. and LEUTZINGER, Y. 1996, Long-term stability of blood glutathione and cysteine in humans. *Clinical Chemistry*, **42**, 1100–1105.

PLNA, K., SEGERSBACK, D. and SCHWEDA, E. K. H. 1996, DNA adduct formation by allyl glycidyl ether. *Carcinogenesis*, **17**, 1465–1471.

ROBERTSON, I. G. C., JENSSON, H., MANNERVIK, B. and JERNSTRÖM, B. 1986a, Glutathione transferases in rat lung: The presence of transferase 7-7, high efficient in the conjugation of glutathione with the carcinogenic (+)-7β, 8α-dihydroxy-9α,10α-oxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene. *Carcinogenesis*, **7**, 295–299.

ROBERTSON, I. G. C., GUTHENBERG, C., MANNERVIK, B. and JERNSTRÖM, B. 1986b, Differences in stereoselectivity and catalytic efficiency of three human glutathione transferases in the conjugation of glutathione with 7β, 8α-dihydroxy-9α,10α-oxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene. *Cancer Research*, **46**, 2220–2224.

ROSS, V. L. and BOARD, P. G. 1993, Molecular cloning and heterologous expression of an alternatively spliced human Mu class glutathione S-transferase transcript. *Biochemical Journal*, **294**, 373–380.

ROWLAND, A. S., BAIRD, D. D., SHORE, D. L., DARDEN, B. and WILCOX, A. J. 1996, Ethylene oxide exposure may increase the risk of spontaneous abortion, preterm birth, and postterm birth. *Epidemiology*, **7**, 363–368.

RUMPF, K. W., SEUBERT, A., VALENTIN, R., IPPEN, H., SEUBERT, S., LOWITZ, H. D., RIPPE, H. and SCHELER, F. 1985, Association of ethylene-oxide-induced IgE antibodies with symptoms in dialysis patients. *Lancet*, **2**, 1385–1387.

SABOURIN, P. J., BURKA, L. T., BECHTOLD, W. E., DAHL, A. R., HOOVER, M. D., CHANG, I. Y. and HENDERSON, R. F. 1992, Species differences in urinary butadiene metabolites; identification of 1,2-dihydroxy-4-(N-acetylcysteinyl)butane, a novel metabolite of butadiene. *Carcinogenesis*, **13**, 1633–1638.

SAKAI, T., KAGEYAMA, H., ARAKI, T., YOSIDA, T., KURIBAYASHI, T. and MASUYAMA, Y. 1995, Biological monitoring of workers exposed to N,N-dimethylformamide by determination of the urinary metabolites, N-methylformamide and N-acetyl-S-(N-methylcarbamoyl) cysteine. *International Archives of Occupational and Environmental Health*, **67**, 125–129.

SANDUJA, R., ANSARI, G. A. S. and BOOR, P. J. 1989, 3-Hydroxypropyl mercapturic acid: a biologic marker of exposure to allylic and related compounds. *Journal of Applied Toxicology*, **9**, 235–238.

Santella, R. M., Grinberg-Funes, R. A., Lan Young, T., Dickey, C., Nath Singh, V., Wen Wang, L. and Perera, F. P. 1992, Cigarette smoking related polycyclic aromatic hydrocarbon-DNA adducts in peripheral mononuclear cells. *Carcinogenesis*, **13**, 2041–2045.

Santoni, G., Bavazzano, P., Perico, A., Colzi, A., Benassi, S., Medica, A., La Morgia, R. and Giuliano, G. 1992, High performance liquid chromatographic determination of N-methylformamide and N-methyl-N-(hydroxymethyl)-formamide in human urine. *Journal of Chromatography B*, **581**, 287–292.

Satoh, S., Ohtsuka, K., Keida, Y., Kusunoki, C., Konta. Y., Niwa, M. and Kohsaka, M. 1994, Gene structural analysis and expression of human renal dipeptidase. *Biotechnology Progress*, **10**, 134–140.

Scheepers, P. T. J. and Bos, R. P. 1992, Combustion of diesel fuel from a toxicological perspective. *International Archives of Occupational and Environmental Health*, **64**, 149–161.

Schrüder, K. R., Hallier, E., Meyer, D. J., Wiebel, F. A., Müller, A. M. F. and Bolt, H. M. 1996, Purification and characterization of a new glutathione S-transferase class θ, from human erythrocytes. *Archives of Toxicology*, **70**, 559–566.

Schulte, P. A. 1995, Opportunities for the development and use of biomarkers. *Toxicology Letters*, **77**, 25–29.

Seaton, M. J., Follansbee, M. H. and Bond, J. A. 1995, Oxidation of 1,2-epoxy-3-butene to 1,2:3,4-diepoxybutane by cDNA-expressed human cytochromes P450 2E1 and 3A4 and human, mouse and rat liver microsomes. *Carcinogenesis*, **16**, 2287–2293.

Segerback, D., Calleman, C. J., Schroeder, J. L., Costa, L. G. and Faustman, E. M. 1995, Formation of N-7-(2-carbamoyl-2-hydroxyethyl)guanine in DNA of the mouse and the rat following intraperitoneal administration of [^{14}C]acrylamide. *Carcinogenesis*, **16**, 1161–1165.

Seutter-Berlage, F., Van Dorp, H. L., Kosse, H. G. J. and Henderson, P. Th. 1977, Urinary mercapturic acid excretion as a biological parameter of exposure of alkylating agents. *International Archives of Occupational and Environmental Health*, **39**, 45–51.

Shaw, L. M., London, J. W. and Petersen, L. E. 1978, Isolation of γ-glutamyltransferase from human liver and comparison with the enzyme from human kidney. *Clinical Chemistry*, **24**, 905–915.

Simula, T. P., Glancey, M. J. and Wolf, C. R. 1993, Human glutathione S-transferase-expressing Salmonella typhimurium tester strains to study the activation/detoxication of mutagenic compounds: Studies with halogenated compounds, aromatic amines and aflatoxin B_1. *Carcinogenesis*, **14**, 1371–1376.

Singh, U. S., Decker-Samuelian, K. and Solomon, J. J. 1996, Reaction of epichlorohydrin with 2′-deoxynucleosides: characterization of adducts. *Chemico-Biological Interactions*, **99**, 109–128.

Singhal, S. S., Zimniak, P., Sharma, R., Srivastava, S. K., Awasthi, S., Awasthi, Y. C. 1994, A novel glutathione S-transferase isoenzyme similar to GST 8-8 of rat and mGSTA4-4 (GST 5·7) of mouse is selectivity expressed in human tissues. *Biochimica et Biophyica Acta*, **1204**, 279–286.

Sipes, I. G. and Gandolfi, A. J. 1991, Biotransformation of toxicants. In *Toxicology, the basic science of poisons*, M. O. Amdur, J. Doull and C. D. Klaassen eds (Pergamon Press, New York), pp. 88–126.

Shuker, D. E. and Farmer, P. B. 1992, Relevance of DNA adducts as markers of carcinogen exposure. *Chemical Research in Toxicology*, **5**, 450–460.

Skipper, P. L., Peng, X., Sochoo, C. K. and Tannenbaum, S. R. 1994, Protein adducts as biomarkers of human carcinogen exposure. *Drug Metabolism Review*, **26**, 111–124.

Slatter, J. G., Rashed, M. S., Pearson, P. G., Han, D-H. and Baillie, T. A. 1991, Biotransformation of methyl isocyanate in the rat. Evidence for glutathione conjugation as a major pathway of metabolism and implications for isocyanate-mediated toxicities. *Chemical Research in Toxicology*, **4**, 157–161.

Slatter, J. G., Davis, M. R., Han, D-H., Pearson, P. G. and Baillie, T. A. 1993, Studies on the metabolic fate of caracemide, an experimental antitumor agent, in the rat. Evidence for the release of methyl isocyanate in vivo. *Chemical Research in Toxicology*, **6**, 335–340.

Slaughter, D. E., Zheng, J., Harriman, S. and Hanzlik, R. P. 1993, Identification of covalent adducts to protein sulphur nucleophiles by alkaline permethylation. *Analytical Biochemistry*, **208**, 288–295.

Smith, R. D., Wahl, J. H., Light-Wahl, J. and Winger, B. E. 1993, New developments in micro scale separations and mass spectrometry for biomonitoring: capillary electrophoresis and electrospray ionization mass spectrometry. *Journal of Toxicological and Environmental Health*, **40**, 147–158.

Smith, C. V., Jones, D. P., Guenthner, T. M., Lash, L. H. and Lauterburg, B. H. 1996, Compartmentation of glutathione: Implications for the study of toxicity and disease. *Toxicology and Applied Pharmacology*, **140**, 1–12.

Snyder, R., Witz, G. and Goldstein, B. D. 1993, The toxicology of benzene. *Environmental Health Perspectives*, **100**, 293–306.

Søderlund, E. J., Meyer, D. J., Ketterer, B., Nelson, S. D., Dybing, E. and Holme, J. A. 1995, Metabolism of 1,2-dibromo-3-chloropropane by glutathione S-transferases. *Chemico-Biological Interactions*, **97**, 257–272.

SORSA, M., PELTONEN, K., ANDERSON, D., DEMOPOULOS, N. A., NEUMANN, H-G. and OSTERMAN-GOLKAR, S. 1996, Assessment of environmental and occupational exposures to butadiene as a model for risk estimation of petrochemical emissions. *Mutagenesis*, **11**, 9–17.

STANEK, W., HAYEK, E. W. H., KRENMAYR, P. and SCHMID, E. R. 1991, Tandem mass spectrometric studies of mercapturic acid derivatives: fragmentation, structure elucidation of fragment ions and development of an analytical method. *Fresenius Journal of Analytical Chemistry*, **340**, 201–206.

STANEK, W., KRENMAYR, P., SCHERER, G. and SCHMID, E. R. 1993, Quantitative determination of N-acetyl(-L-)cysteine derivatives in human urine by tandem mass spectrometry. *Biological Mass Spectrometry*, **22**, 133–142.

STAFFELDT, B., BROCKMÖLLER, J. and ROOTS, I. 1991, Determination of S-carboxymethyl-L-cysteine and some of its metabolites in urine and serum by high performance liquid chromatography using fluorescent pre-column labelling. *Journal of Chromatography*, **571**, 133–147.

STEINER, S., HONGER, G. and SAGELSDORFF, P. 1992, Molecular dosimetry of DNA adducts in C3H mice treated with bisphenol A diglycidyl ether. *Carcinogenesis*, **13**, 969–972.

STEVENS, D. K., EYRE, R. J. and BULL, R. J. 1992, Adduction of hemoglobin and albumin in vivo by metabolites of trichloroethylene, trichloroacetate, and dichloroacetate in rats and mice. *Fundamental and Applied Toxicology*, **19**, 336–342.

STOMMEL, P., MÜLLER, G., STÜCKER, W., VERKOYEN, C., SCHÖBEL, S. and NORPOTH, K. 1989, Determination of S-phenyl mercapturic acid in the urine: an improvement in the biological monitoring of benzene exposure. *Carcinogenesis*, **10**, 279–282.

STOTT, W. T. and KASTL, P. E. 1986, Inhalation pharmacokinetics of technical grade 1,3-dichloropropene in rats. *Toxicology and Applied Pharmacology*, **85**, 332–341.

STRANGE, R. C. 1995, Glutathione S-transferases and cancer susceptibility. In *ISSX workshop on Glutathione S-transferases*, Amsterdam, (Taylor & Francis, London), pp. 239–247.

STRYER, L. 1988, *Biochemistry* (Freeman, New York), pp. 436, 592.

SUMNER, S., MACNEELA, J. and FENNELL, T. 1992, Characterization and quantitation of urinary metabolites of [1,2,3-^{13}C]-acrylamide in rats and mice using ^{13}C nuclear magnetic resonance spectroscopy. *Chemical Research in Toxicology*, **5**, 81–89.

TAKAHASHI, S., KAGAWA, M., SHIWAKU, K. and MATSUBARA, K. 1994, Determination of S-benzyl-N-acetyl-L-cysteine by gas chromatography/mass spectrometry as a new marker of toluene exposure. *Journal of Applied Toxicology*, **18**, 78–80.

TALASKA, G., CUDNIK J., JAEGER, M., ROTHMAN, N., HAYES, R., BHATNAGAR, V. J. and KAYSHUP, S. J. 1996, Development and application of non-invasive biomarkers for carcinogen-DNA adduct analysis in occupationally exposed populations. *Toxicology*, **111**, 207–212.

TAN, K-L. and BOARD, P. G. 1996, Purification and characterization of a recombinant human theta-class glutathione transferase (GSTT2-2). *Biochemical Journal* **315**, 727–732.

TANAKA, M., SHIMIZU, M. and TSUJIMOTO, Y. 1990, High-performance liquid chromatographic determination of urinary mercapturic acids in rat. *Journal of Chromatography*, **526**, 289–292.

TARDIF, R., GOYAL, R., BRODEUR, J. and GÉRIN, M. 1987, Species differences in the urinary disposition of some metabolites of ethylene oxide. *Fundamental and Applied Toxicology*, **9**, 448–453.

TAYLOR, C. G., NAGY, L. E. and BRAY, T. M. 1996, Nutritional and hormonal regulation of glutathione homeostasis. In *Current Topics in Cellular Regulation*, E. R. Stadtman and P. B. Chock eds, **34**, pp. 189–208.

TE KOPPELE, J. M., VAN DER MARK, E. J. and MULDER, G. J. 1988, Liquid chromatographic determination of diastereomeric glutathione conjugates and further derivatives of α-bromoisovalerylurea in rat bile and urine by electrochemically generated bromine. *Journal of Chromatography*, **427**, 67–77.

THEISS, J. C., SHIMKIN, M. B. and POIRIER, L. A. 1979, Induction of pulmonary adenomas in strain A mice by substituted organohalides. *Cancer Research*, **39**, 391–395.

THIER, R., MÜLLER, M., TAYLOR, J. B., PEMBLE, S. E., KETTERER, B. and GUENGERICH, F. P. 1995, Enhancement of bacterial mutagenicity of bifunctional alkylating agents by expression of mammalian glutathione S-transferase. *Chemical Research in Toxicology*, **8**, 465–472.

THIER, R., PEMBLE, S. E., KRAMER, H., TAYLOR, J. B., GUENGERICH, F. P. and KETTERER, B. 1996, Human glutathione S-transferase T1-1 enhances mutagenicity of 1,2-dibromoethane, dibromomethane and 1,2,3,4-diepoxybutane in *Salmonella typhimurium*. *Carcinogenesis*, **17**, 163–166.

TOMISAWA, H., OKAMOTO, A., HATTORI, K., OZAWA, N., UDA, F. and TATEISHI, M. 1990, Purification and characterization of 3-mercaptopyruvic acid S-conjugate reductases. *Biochemical Pharmacology*, **40**, 2047–2057.

TOMPA, A., MAJOR, J and JAKAB, M. G. 1994, Monitoring of benzene-exposed workers for genotoxic effects of benzene: improved-working-condition-related decrease in the frequencies of chromosomal aberrations in peripheral blood lymphocytes. *Mutation Research*, **304**, 159–165.

TORKELSON, T. R., WOLF, M. A., OYEN, F. and ROWE, V. K. 1959, Vapor toxicity of allyl chloride as determined on laboratory animals. *American Industrial Hygiene Associati*

Törnqvist, M., Osterman-Gölkar, S., Kautiainen, A., Jensen, S., Farmer, P. B. and Ehrenberg, L. 1986, Tissue doses of ethylene oxide in cigarette smokers determined from adduct levels in hemoglobin. *Carcinogenesis*, **7**, 1519–1521.

Truchon, G., Brodeur, J. and Gérin, M. 1990, Simultaneous determination of urinary mandelic, phenylglyoxylic, and mercapturic acids of styrene by high-performance liquid chromatography. *Journal of Analytical Toxicology*, **14**, 227–230.

Tsai, S-Y. and Chen, J-D. 1996, Neurobehavioral effects of occupatioal exposure to low-level styrene. *Neurotoxicology and Teratology*, **18**, 463–469.

Tsai, S. P., Cowles, S. R., Tackett, D. L., Barclay, M. T. and Ross, C. E. 1990, Morbidity prevalence study of workers with potential exposure to epichlorohydrin. *British Journal of Industrial Medicine*, **47**, 392–399.

Tsai, S. P., Gilstrap, E. L. and Ross, C. E. 1996, Mortality study of employees with potential exposure to epichlorohydrin: A 10 year update. *Occupational and Environmental Medicine*, **53**, 299–304.

Tsuchida, S., Maki, T. and Sato, K. 1990, Purification and characterization of glutathione transferases with an activity towards nitroglycerin from human aorta and heart: Multiplicity of the human mu forms. *Journal of Biological Chemistry*, **265**, 7150–7157.

Uchida, K. and Stadtman, E. R. 1992, Selective cleavage of thioether linkage in proteins modified with 4-hydroxynonenal. *Proceedings of the National Academy of Sciences*, **89**, 5611–5615.

Uusküla, M., Järventaus, H., Hirvonen, A., Sorsa, M. and Norppa, H. 1995, Influence of GSTM1 genotype on sister chromatid exchange induction by styrene-7,8-oxide and 1,2-epoxy-3-butene in cultured human lymphocytes. *Carcinogenesis*, **16**, 947–950.

Vamvakas, S., Bittner, D., Dekant, W. and Anders, M. W. 1992, Events that precede and that follow S-(1,2-dichlorovinyl)-L-cysteine-induced release of mitochondrial Ca^{2+} and their association with cytotoxicity to renal cells. *Biochemical Pharmacology*, **44**, 1131–1138.

Van Bladeren, P. J., Breimer, D. D., Rotteveel-Smijs, G. M. T., De Jong, R. A. W., Buijs, W., Van Der Gen, A. and Mohn, G. R. 1980, The role of glutathione conjugation in the mutagenicity of 1,2-dibromoethane. *Biochemical Pharmacology*, **29**, 2975–2982.

Van den Eeckhout, E., De Bruyn, A., Pepermans, H., Esmans, E. L., Vryens, I., Claereboudt, J., Claeys, M. and Sinsheimer, J. E. 1990, Adduct formation identification between phenyl glycidyl ether and 2′-deoxyadenosine and thymidine by chromatography, mass spectrometry and nuclear magnetic resonance spectroscopy. *Journal of Chromatography*, **504**, 113–128.

Van Der Aar, E. M., Buikema, D., Commandeur, J. N. M., Te Koppele, J. M., Van Ommen, B., Van Bladeren, P. J. and Vermeulen, N. P. E. 1996, Enzyme kinetics and substrate selectivities of rat glutathione S-transferase isoenzymes towards a series of new 2-substituted 1-chloro-4-nitrobenzenes. *Xenobiotica*, **26**, 143–155.

Van Den Broek, L. A. G. M., Delbrissine, L. P. C. and Ottenheijm, H. C. J. 1990, Biochemistry and metabolic pathways of sulfenic acids and their derivatives. In *The chemistry of sulfenic acids and their derivatives*, S. Patai ed (John Wiley and Sons, New York), pp. 701–721.

Van Duuren, B. L., Goldschmidt, B. M., Leowengart, G., Smith, A. C., Melchionne, S., Seidman, I. and Roth, D. 1979, Carcinogenicity of halogenated olefinic and aliphatic hydrocarbons in mice. *Journal of the National Cancer Institute*, **63**, 1433–1439.

Vanhoutte, K., Joos, P., Lemiere, F., Van Dongen, W., Esmans, E. L., Claeys, M. and Van den Eeckhout, E. 1995, Thermospray liquid chromatography-mass spectrometry of the DNA adducts formed between 2′-deoxynucleosides and bisphenol A diglycidyl ether. *Journal of Mass Spectrometry*, **30**, 1453–1461.

Van Joost Th., Roesyanto, I. D. and Satyawan, I. 1990, Occupational sensitization to epichlorohydrin and bisphenol-A during the manufacture of epoxy-resin. *Contact Dermatitis*, **22**, 125–126.

Van Sittert, N. J., Veenstra, G. E., Dumas, E. P. and Tordoir, W. F. 1991, Biological effect monitoring of occupational exposure to 1,3-dichloropropene: Effects on liver and renal function and on glutathione conjugation. *British Journal of Industrial Medicine* (letter), **48**, 646–647.

Van Sittert, N. J., Beulink, G. D. J., Van Vliet, E. and Van der Waal, H. 1993a, Monitoring occupational exposure to ethylene oxide by the determination of hemoglobin adducts. *Environmental Health Perspectives*, **99**, 217–220.

Van Sittert, N. J., Boogaard, P. J. and Beulink, G. D. J. 1993b, Application of the urinary S-phenyl mercapturic acid test as a biomarker for low levels of exposure to benzene in industry. *British Journal of Industrial Medicine*, **50**, 460–469.

Van Schooten, F. J., Hillebrand, M. J. X., Van Leeuwen, F. E., Van Zandwijk, N., Jansen, H. M., Den Enselse, L. and Krick, E. 1992, Polycyclic aromatic hydrocarbon-DNA adducts in white blood cells from lung cancer patients: no correlation with adduct levels in lung. *Carcinogenesis*, **13**, 987–993.

Van Welie, R. T. H., Van Duyn, P. and Vermeulen, N. P. E. 1989, Determination of two mercapturic acid metabolites of 1,3-dichloropropene in human urine with gas chromatography and sulphur-selective detection. *Journal of Chromatography*, **496**, 463–471.

Van Welie, R. T. H., Van Duyn, P., Brouwer, D. H., Van Hemmen, J. J., Brouwer, E. J. and Vermeulen, N. P. E. 1991a, Inhalation exposure to 1,3-dichloropropene in the Dutch flower-bulb culture. Part II. Biological monitoring by measurement of urinary excretion of two mercapturic acid metabolites. *Archives of Environmental Contamination and Toxicology*, **20**, 6–12.

Van Welie, R. T. H., Van Duyn, P. and Vermeulen, N. P. E. 1991b, Environmental and biological monitoring of non-occupational exposure to 1,3-dichloropropene. *International Archives of Occupational and Environmental Health*, **63**, 169–173.

Van Welie, R. T. H., Van Dijck, R. G. J. M., Vermeulen, N. P. E. and Van Sittert, N. J. 1992, Mercapturic acids, protein adducts and DNA adducts as biomarkers of electrophilic chemicals. *Critical Reviews in Toxicology*, **22**, 271–306.

Vermeulen, N. P. E. 1989, Analysis of mercapturic acids as a tool in biotransformation, biomonitoring and toxicological studies. *Trends in Pharmacological Sciences*, **10**, 177–181.

Vermeulen, N. P. E. 1995, Role of metabolism in chemical toxicology. In *Cytochrome P450: Metabolic and toxicological aspects*, C. Ioannides ed. (CRC Press, Boca Raton), pp. 49–73.

Vermeulen, N. P. E., Onkenhout, W. and Van Bladeren, P. J. 1983, The analysis of mercapturic acids in biological fluids; applications of gas-liquid chromatography and (EI- and CI-) mass spectrometry. In *Chromatography and Mass Spectrometry in Biomedical Sciences*, A. Frigerio ed. (Elsevier Scientific Publishing Co., Amsterdam), **2**, pp. 39–52.

Vermeulen, N. P. E., De Jong, J., Van Bergen, E. J. C. and Van Welie, R. T. H. 1989, N-acetyl-S-(2-hydroxyethyl)-L-cysteine as a potential tool in biological monitoring studies? *Archives of Toxicology*, **63**, 173–184.

Vianio, H., Savolianen, H. and Kilpikari, I. 1978, Urinary thioether of employees of a chemical plant. *British Journal of Industrial Medicine*, **35**, 232–234.

Vodicka, P., Bastlova, T., Vodickova, L., Peterkova, K., Lambert, B and Hemminki, K. 1995, Biomarkers of styrene exposure in lamination workers: Levels of O^6-guanine DNA adducts, DNA strand breaks and mutant frequencies in the hypoxanthine guanine phosphoribosyltransferase gene in T-lymphocytes. *Carcinogenesis*, **16**, 1473–1481.

Vos, R. M. E., Van Welie, R. T. H., Peters, W. H. M., Evelo, C. T. A., Boogaards, J. J. P., Vermeulen, N. P. E. and Van Bladeren, P. J. 1991, Genetic deficiency of human class mu glutathione S-transferase isoenzymes in relation to the urinary excretion of the mercapturic acids of Z- and E-1,3-dichloropropene. *Archives of Toxicology*, **65**, 95–99.

Waddams, A. L. 1978, *Chemicals from petroleum* (J. Murray Ltd., London), pp. 150–192.

Walker, V. E., Fennell, T. R., Upton, P. B., MacNeela, J. P. and Swenberg, J. A. 1993, Molecular dosimetry of DNA and hemoglobin adducts in mice and rats exposed to ethylene oxide. *Environmental Health Perspectives*, **99**, 11–17.

Warholm, M., Guthenberg, C. and Mannervik, B. 1987, Molecular and catalytic properties of glutathione transferase μ from human liver: An enzyme efficiently conjugating epoxides. *Biochemistry*, **22**, 3610–3617.

Weiss, N. S. 1996, Cancer in relation to occupational exposure to trichloroethylene. *Occupational and Environmental Medicine*, **53**, 1–5.

Welp, E., Kogevinas, M., Andersen, A., Bellander, T., *et al.* 1996, Exposure to styrene and mortality from nervous system diseases and mental disorders. *American Journal of Epidemiology*, **144**, 623–633.

Woolfson, A. D., Millership, J. S. and Karim, El. IA. 1987, Determination of the sulphoxide metabolites of S-carboxymethyl-L-cysteine by high-performance liquid chromatography with electrochemical detection. *Analyst*, **112**, 1421–1425.

Wright, A. S., Aston, J. P., Van Sittert, N. J. and Watson, W. P. 1995, Molecular approaches to assess cancer risks. In *Toxicology of industrial compounds*, H. Thomas, R. Hess and F. Waechter (Taylor & Francis, London), pp. 185–200.

Wu, Y-Q., Yu, A-R., Tang, X-Y., Zhang, J. and Cui, T. 1993, Determination of acrylamide metabolite, mercapturic acid by high performance liquid chromatography. *Biomedical and Environmental Sciences*, **6**, 273–280.

Yang, S. H. 1986, 1,3-dichloropropene. *Residue Reviews*, **97**, 19–35.

Yates, J. M., Fennell, T. R., Turner, M. J. Jr, Recio, L. and Sumner, S. C. 1994, Characterization of phosphodiester adducts produced by the reaction of cyanoethylene oxide with nucleotides. *Carcinogenesis*, **15**, 277–283.

Yeowell-O'Connell, K., McDonald, T. and Rappaport, S. M. 1996, Analysis of hemoglobin adducts of benzene oxide by gas chromatography-mass spectrometry. *Analytical Biochemistry*, **237**, 49–55.

Zhang, T-I., Wang, L., Hasjmi, M., Anders, M. W., Thorpe, C. and Ridge, D. P. 1995, Fourier-transform ion cyclotron resonance mass spectrometric evidence for the formation of α-chloroenethiolates and thioketenes from chloroalkene-derived cytotoxic 4-thio-alkanoates. *Chemical Research in Toxicology*, **8**, 907–910.

Zheng, J. and Hanzlik, R. P. 1992, Dihydroxylated mercapturic acid metabolites of bromobenzene. *Chemical Research in Toxicology*, **5**, 561–567.