

Biotransformation of organic solvents¹

A review

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TOFTGÅRD R, GUSTAFSSON J-Å. Biotransformation of organic solvents: A review. *Scand j work environ health* 6 (1980) 1—18. This review is intended to provide a summary of the present state of knowledge and the need for research with regard to the biotransformation of commonly used solvents.

Key terms: biological monitoring, biotransformation, chemical carcinogenesis, cytochrome P-450, epoxide, epoxide hydrolase, glucuronide, glutathione-S-transferase, metabolic activation, sulfate, organic solvents.

In connection with exposure to solvents and other xenobiotics, it is essential to know how the substance is absorbed via the lungs, gastrointestinal tract and skin, how it is subsequently distributed to various parts of the body, metabolized (biotransformed), and finally eliminated. Knowledge about all of these steps is important for the understanding of the mechanisms of toxic effects and dose-response relationships and for the establishment of well-founded hygienic standards.

The present review is intended to give a summary of the present state of knowledge and need for research with regard to the biotransformation of commonly used solvents.

Background

Biotransformation is a process in which a chemical substance is transformed into another compound (metabolite) in the body. The metabolite is usually more polar (water soluble) than the original compound, and it can therefore be eliminated more easily from the body.

The liver is the organ with the largest metabolic capacity, but also other organs and organ systems, such as the lungs, kidneys, gastrointestinal tract, etc, are active in biotransformation that may be important with respect to special toxic effects.

Biotransformation consists of one or several chemical reactions catalyzed by enzymes localized in the soluble (cytoplasmic), mitochondrial or microsomal (endoplasmic) fraction of the cell. The reactions may be classified into the following four main types: oxidation, reduction, hydrolysis, and conjugation. Oxidative reactions usually occur in the microsomal fraction and are, in general, catalyzed by cytochrome P-450-dependent enzymes, so-called monooxygenases. The cytochrome

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P-450-dependent reactions may, eg, be aliphatic hydroxylation; aromatic hydroxylation; epoxidation; deamination; N-, O-, S-dealkylation; N-oxidation; N-hydroxylation; sulfoxidation; and desulfonation. Reductive reactions occur in both the microsomal and other cell fractions, whereas the hydrolytic reactions mainly take place in the soluble fraction.

The most important conjugation reactions are conjugation with glucuronic acid, sulfuric acid, glutathione and glycine; methylation; acetylation; and formation of thiocyanates. These reactions need adenosine 5'-triphosphate (ATP) as a source of energy, as well as coenzymes and specific transferases. In these reactions endogenous substrates are conjugated to xenobiotics (or their metabolites) that contain suitable functional groups (hydroxyl, amino, carboxyl or epoxide groups). This type of reaction requires the participation of both soluble enzymes and enzymes localized in the microsomal fraction of the cell.

The enzyme cytochrome P-450 contains an iron atom which is bound in a heme molecule. The heme molecule is very important during the cytochrome P-450-dependent catalysis of oxidative reactions. Heme binds molecular oxygen (O_2) that is taken up from the blood, and then one of the two oxygen atoms is bound to the substrate that is metabolized. The other oxygen atom is eliminated in the form of water. The name cytochrome P-450 stems from the fact that under certain conditions the enzyme may be detected and measured spectrophotometrically due to its capacity to absorb light at 450 nm. P stands for pigment.

Cytochrome P-450 metabolizes both endogenous and exogenous compounds. Examples of endogenous compounds that are metabolized are fatty acids, sex hormones, adrenal cortical hormones, bile acids, and prostaglandins. Cytochrome P-450 is present in many organs of the body, eg, the gonads (testes and ovaries) and the adrenal cortex, where cytochrome P-450 participates in the formation of sex hormones, glucocorticoids, and mineralocorticoids. Since the same enzyme can metabolize both endogenous and exogenous compounds, it is quite possible that the administration of large quantities of exogenous compounds can affect the biotrans-

formation of the endogenous compounds.

Cytochrome P-450 can be measured either directly by spectrophotometric estimation of the amount of enzyme or indirectly by the measurement of the catalytic activity of the enzyme. In the latter case, one measures the rate whereby the enzyme transforms a substrate into a metabolite. The analytical technique varies depending upon the type of compound under investigation. Examples of common methods are gas-liquid chromatography, thin-layer chromatography, mass spectrometry, and spectrophotometry. With SDS(sodium dodecyl sulfate)-polyacrylamide gel electrophoresis, it is possible to separate several types of cytochrome P-450 with different molecular weights.

Significance of biotransformation

Biotransformation of xenobiotics usually leads to the formation of more water-soluble compounds that are more easily excreted via urine and bile than the compound itself. Earlier, this process was generally thought to lead to a detoxification of the compound in question. Nowadays, however, we know that biotransformation, especially via cytochrome P-450, often leads to the formation of reactive metabolites that are more toxic than the original compounds. This phenomenon, which is called metabolic activation, is important with respect to the occurrence of several different toxic effects and also occurs in connection with solvents. Examples are the hepatotoxic effect of carbon disulfide (26, 61) and the neurotoxic effect of n-hexane and methyl-n-butyl ketone (24, 31, 90).

The appearance of a specific toxic effect in a special organ may depend upon different metabolic routes in various parts of the body. For example p-xylene gives a significant decrease in the concentration of cytochrome P-450 in the lung (toxic effect) but does not affect the concentration of cytochrome P-450 in the liver. The reason is thought to be that a reactive metabolite, p-tolualdehyde, is rapidly detoxified in the liver via aldehyde dehydrogenase, while the absence of this enzyme in the lung leads to toxic effects from p-tolualdehyde in this organ (78).

Induction of metabolizing enzymes

Biotransformation of a solvent may be influenced by several factors such as age, sex, dose, and simultaneous exposure to other exogenous compounds. Several chemicals can increase the concentration of different metabolizing enzymes (enzyme induction). This increase is the most obvious for enzymes of the cytochrome P-450 type. The different forms of cytochrome P-450 vary among other things with regard to their substrate specificity (43). Certain types of cytochrome P-450 are induced by phenobarbital (49), others by 3-methylcholanthrene (49), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (80) and β -naphthoflavone (49). Induction can also occur following exposure to cigarette smoke (4), which leads to an increase in the levels of the enzymes that activate benzo(a)pyrene (a polycyclic aromatic hydrocarbon) to carcinogenic metabolites. This induction takes place both in the lungs and in other organs. Also solvents can induce cytochrome P-450 in the liver after inhalation, as shown for xylene and toluene (Toftgård et al, unpublished results).

The occurrence and levels of different forms of cytochrome P-450 may, in this way, decide whether a metabolic activation or detoxification takes place and may thus greatly influence the toxic effects of a solvent. An example of this phenomenon is the fact that the hepatotoxic effect of trichloroethylene, perchloroethylene, and carbon disulfide is significantly increased if the levels of cytochrome P-450 are increased, eg, following induction with phenobarbital (61, 70). The neurotoxic effect of methyl-n-butyl ketone is enhanced by simultaneous exposure to methyl ethyl ketone (7), which has been reported to induce cytochrome P-450 (102). Another factor of great importance for the occurrence of a toxic effect is the level of those enzymes that can deactivate a reactive metabolite, eg, epoxide hydrase and glutathione-S-transferases.

In the following discussion a summary of the biotransformation of the most commonly occurring types of solvents is given. The intention is not to give a complete review but to outline the general patterns exemplified by solvents that occur in industry.

BIOTRANSFORMATION

Aromatic hydrocarbons

Aromatic hydrocarbons, including benzene, toluene, xylene and styrene, have been relatively thoroughly investigated with regard to biotransformation, especially benzene and styrene. Nevertheless, no definitive knowledge exists about which metabolite is responsible for the toxic effects of benzene.

Benzene C_6H_6

The metabolism of benzene has been described in two recently published reviews (88, 93). In contrast to other compounds in the aromatic hydrocarbon group, benzene does not have any substituents on the ring, and therefore it has a slightly different metabolism when compared to, eg, toluene and styrene. The special toxic effect of benzene on blood-forming organs has created great interest in the biotransformation of benzene.

In vivo metabolism of benzene. The *in vivo* metabolism of benzene has been studied mainly in rabbits, rats and mice, but its metabolism in man seems to follow principally the same pattern (88). Fig 1 gives a summary of the demonstrated metabolic routes, as well as estimations of the part of a given dose that is transformed to a certain metabolite. The most-detailed studies have been performed with rabbits and ^{14}C -labeled benzene (76, 77). Following oral administration of 0.34–0.5 ml/kg (3.8–5.6 mmol/kg), a recovery of 84–89 % of the administered dose was obtained. Forty-three percent was expired unchanged and 1.5 % as $^{14}CO_2$. In the urine, 23.5 % was excreted as phenol (conjugated), 4.8 % as hydroquinone, 2.2 % as catechol, 0.3 % as hydroxyhydroquinone, 1.3 % as transtransmuconic acid, and 0.5 % as L-phenylmercapturic acid. No free phenol could be detected in urine. Feces and other tissues contained 5–10 % of the dose, and in another study the same authors have shown that about 1 % of the dose is excreted in the bile (3). Phenol is excreted mainly as a sulfuric or glucuronic acid conjugate with species variations with regard to the relative amounts of the two conjugates (93). Man eliminates benzene

mainly as a sulfate conjugate, but glucuronic acid conjugates occur at high concentrations of phenol (400 mg/l or 4.2 mmol/l) in the urine. Unmetabolized benzene is excreted in the urine only to a very small extent (0.1–0.2 %) (96).

In one experiment 15 persons were exposed to 500 ppm (1,500 mg/m³) of benzene for 5 h and an uptake of 46 % was reported (98). Of the quantities taken up, 26 % was eliminated in the form of unmetabolized benzene via the lungs, and in the urine 61 % was excreted as phenol, 6.3 % as catechol and 2.4 % as hydroquinone. The rate of metabolism has been shown to depend both on the dose and on possible simultaneous exposure to compounds that either induce or inhibit the biotransformation of benzene. Toluene for instance inhibits the metabolism of benzene in both the rat and the mouse (53, 107), while pretreatment of the rat with phenobarbital seems to have very little effect *in vivo* although a significant increase in the metabolism of benzene *in vitro* has been shown (44). Pretreatment with benzene increased the rate of metabolism of benzene *in vivo* in the rat. The excretion of hydroquinone in the urine was increased six times, and at the same time the toxic effect of benzene was potentiated (101).

In vitro metabolism of benzene. The major part of the biotransformation of benzene seems to occur in the liver, and it

has been shown that liver microsomes metabolize benzene. Studies using this cell fraction from the liver have also shown that benzene is a substrate for cytochrome P-450 and that it is transformed to benzene epoxide via this enzyme system. The epoxide, which is very reactive and short-lived (half-time 2 min) can then spontaneously be transformed to phenol, conjugated with glutathione, or hydrolyzed via the enzyme epoxide hydrazide to a dihydrodiol (54) (see fig 1).

The induction of cytochrome P-450 with phenobarbital or 3-methylcholanthrene resulted in both cases in an increase in the amount of phenol (44, 95). Also pretreatment with benzene itself has been shown to increase the metabolism of benzene (42). This increase was not accompanied by any detectable increase in the concentration of cytochrome P-450. One explanation may be that benzene induces a form of cytochrome P-450 that occurs in small amounts, and therefore no increase in the total concentration of the enzyme system is registered. However, an increase in the level of cytochrome P-450 by 65 % has been shown in the rat after exposure to 450 ppm (1,350 mg/m³) of benzene, 5 h/d for 10 d (74).

The toxic effect of benzene is thought to be due to the formation of a reactive metabolite (93). Experiments using radioactively labeled benzene have shown that a metabolite of benzene is covalently

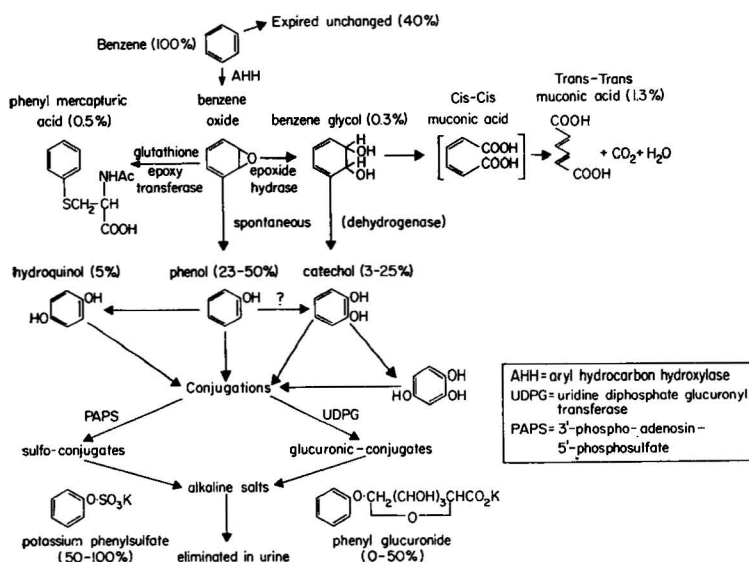


Fig 1. Pathways of benzene metabolism and elimination. The figure has been reproduced from Rusch et al (88) with approval from the authors and the publishing company.

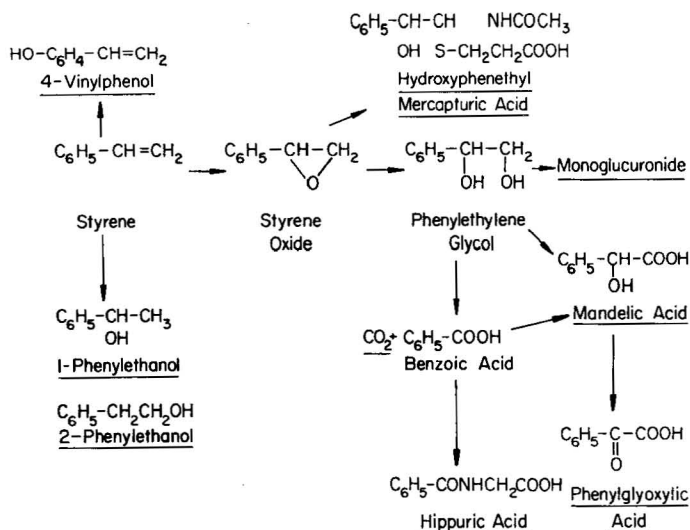


Fig 2. Pathways of the biotransformation of styrene. Underlined metabolites are those excreted in urine (or in expired air in the case of carbon dioxide). The figure has been reproduced from Leibman (57) with approval from the author and the publishing company.

bound to bone marrow (94) and DNA (deoxyribonucleic acid) in liver (60). This reactive metabolite has, by many authors, been proposed to be benzene epoxide. In a recently published study, however, it was shown that benzene epoxide added to rat liver microsomes gave only an insignificant covalent binding to microsomal protein, whereas phenol caused a covalent binding in the same quantity as with benzene (103). The authors suggested that a hitherto unknown metabolite of phenol is responsible for the covalent binding. Phenol is possibly metabolized further via cytochrome P-450, and this may result in the formation of a diol epoxide analogous to the metabolism of benzo(a)pyrene.

Styrene C_8H_8

The metabolic route for styrene in mammals is summarized in fig 2. In man the main metabolites excreted in the urine are mandelic acid and phenylglyoxylic acid (57). In rodents, the major part of the dose is excreted in urine as mandelic acid, phenylglyoxylic acid or hippuric acid. Unchanged styrene is also eliminated via exhalation.

The most important metabolic route proceeds via formation of an epoxide, a reaction catalyzed by cytochrome P-450 in the liver microsomal fraction. The epoxide is then transformed to styrene glycol by the enzyme epoxide hydrazine and is then further metabolized to, among others,

mandelic acid. Ring hydroxylation occurs to a smaller extent, as does the formation of phenyl ethanol (7, 34).

The induction of liver microsomal enzymes with phenobarbital has been shown to increase the amount of hippuric acid, mandelic acid, phenylglyoxylic acid and glucuronic acid conjugates in the rat, while simultaneous exposure to toluene causes a decrease in the formation of these metabolites (53).

The formation of styrene oxide may be classified as a metabolic activation since the acute toxicity of this metabolite is about four times greater than that of styrene (75). Styrene oxide has also been shown to be mutagenic in the Ames test (166).

Toluene C_7H_8

Man absorbs about 53 % of an inhaled dose of toluene. About 18 % of the absorbed dose is excreted unchanged via expired air, while only small amounts (0.06 %) are excreted in the urine (99). Toluene is metabolized to a greater extent than benzene, and about 80 % of the administered dose is metabolized to benzoic acid, which is then conjugated with glycine and secreted as hippuric acid in the urine (71). Also small amounts of benzoic acid and glucuronic acid conjugates are excreted in the urine (30). Less than 2 % of the toluene metabolites are excreted via the bile (3).

Hence, it is mainly the methyl group in

toluene that, via a cytochrome P-450-dependent hydroxylation, is metabolized under the formation of benzoic acid [this compound has been detected in the urine of rats exposed to toluene] (7)], which is rapidly oxidized to benzoic acid, probably by the enzymes alcohol dehydrogenase and aldehyde dehydrogenase. Ring hydroxylation also occurs but only to a limited extent. After the administration of an oral dose of 100 mg/kg (1.1 mmol/kg) to rats, 0.04–0.11 % was recovered as o-cresol and 0.4–1.0 % as p-cresol.

Treatment of rats with phenobarbital, which induces the cytochrome P-450-system, resulted in a shorter biological half-time for toluene in blood and also in a decreased sensitivity to the effects of toluene on the central nervous system (51). This result may be explained by a significant increase in the hydroxylation of toluene to benzoic acid via cytochrome P-450 (7, 30).

Xylene C_8H_{10}

Technical xylene consists of four isomers, o-, m- and p-xylene and ethyl benzene. When volunteers were exposed to 200 or 400 mg/m³ during 8 h, 64 % of the o-, m- and p-xylene was taken up via the lungs (91). Only 5 % of the absorbed dose was excreted unchanged in the expired air, and the excretion of xylene in the urine was negligible. The main metabolites

(> 95 %) are isomers of methylbenzoic acid, formed by oxidation of one of the methyl groups. These acids are excreted in urine conjugated with glycine (methylhippuric acid). Ring hydroxylated metabolites (xlenols) excreted in the urine correspond to 0.86 % (o-xylene), 1.98 % (m-xylene) and 0.05 % (p-xylene) of the administered dose. In animal experiments using higher doses, also other metabolites have been shown, such as free o-methylbenzoic acid, sulfate conjugates, and glucuronic acid conjugates (14, 15). A summary of the metabolic routes of xylene is given in fig 3.

Recently, it has been shown that, in the rat, p-xylene causes a selective decrease in the level of cytochrome P-450 in the lungs and that this toxic effect may be ascribed to the aldehyde which is an intermediate step in the formation of methylbenzoic acid (78). In the liver, this aldehyde is rapidly oxidized further to the corresponding acid via aldehyde dehydrogenase. This enzyme, however, hardly occurs in the lungs, and therefore the reactive aldehyde may react with various cell components. The authors also suggested that aldehydes may be toxic intermediates in the metabolism of toluene, styrene, etc. Several aldehydes have the capacity to inactivate microsomal cytochrome P-450 (48).

Ethyl benzene is mainly metabolized by hydroxylation of the side-chain with subsequent conjugation to glucuronic acid (30).

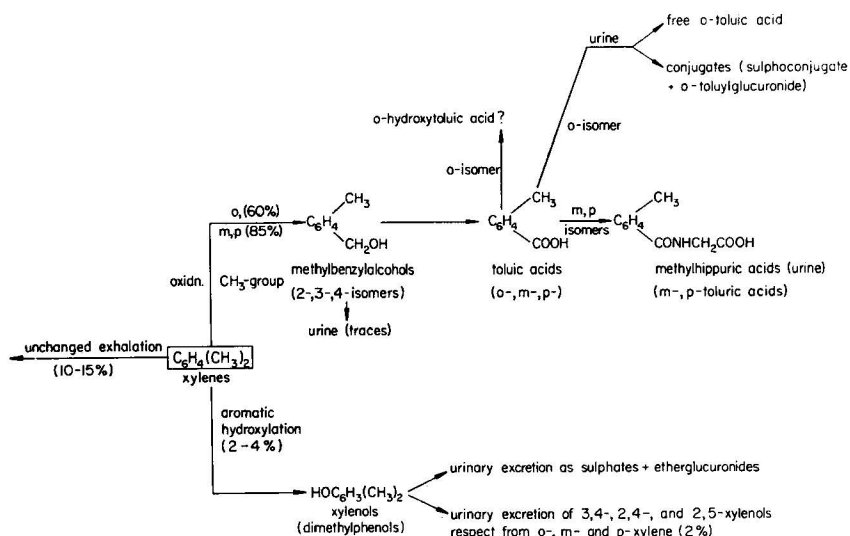


Fig 3. Metabolic scheme for isomeric xylenes. The figure has been reproduced from De Bruin (30) with approval from the author and the publishing company.

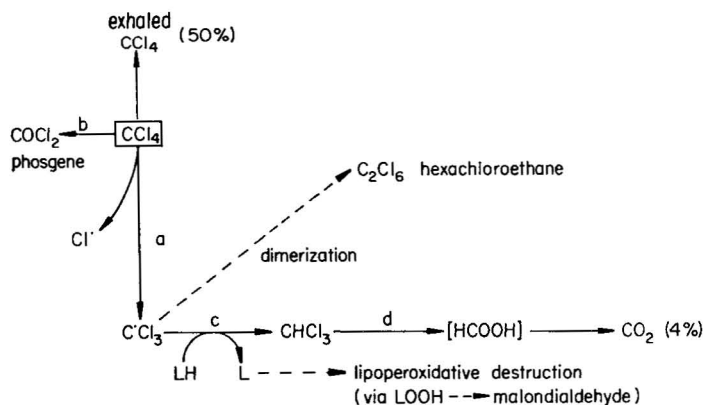


Fig 4. Metabolic pathway of tetrachloromethane. [a: homolytic cleavage; b: heterolytic cleavage; a & c: reductive dechlorination; d: hydrolytic dechlorination; L: target (unsaturated lipid) for free radical attack] The figure has been reproduced from DeBruin (28) with approval from the author and the publishing company.

Halogenated hydrocarbons

Chlorinated hydrocarbons such as methylchloroform, methylene chloride, and trichloroethylene are extensively used in industry, and most of the solvents belonging to this group are more or less hepatotoxic. Following the demonstration that vinyl chloride may cause cancer, much interest has been directed towards this group of compounds, especially towards trichloroethylene and tetrachloroethylene, which have chemical structures similar to vinyl chloride.

Metabolic activation via the liver microsomal cytochrome P-450 system is a prerequisite for the toxic effects of both saturated and unsaturated chlorinated hydrocarbons (31, 92). In the case of 1,2-dichloroethane, however, the activation to a mutagenic metabolite probably occurs via a glutathione-S-transferase present in the cytoplasm of the cell (82). The metabolite is thought to be S-chloroethyl-glutathione.

Chlorinated alkanes

Metabolic studies of chlorinated alkanes have mainly dealt with compounds with one or two carbon atoms, especially chlorinated methanes. With increasing substitution with chlorine, these compounds are more and more destabilized, and for this reason the formation of radicals is favored (11). Dechlorination may occur either reductively or oxidatively, in both cases via the enzyme cytochrome P-450 (105). Regardless of the reaction mechanism, it is the carbon atom that is

first attacked, and then the chlorine atom is eliminated. An optimal configuration for dehalogenation seems to be the occurrence of a dihalomethyl group. Carbon tetrachloride, for instance, is activated reductively, and chloroform oxidatively (92). If a reductive reaction takes place, there is also the possibility of the formation of unsaturated compounds from saturated, halogenated compounds with two carbon atoms, eg, 1,1,2-trichloroethane (105). The formation of reactive metabolites and the toxic effects are increased for carbon tetrachloride and chloroform following the induction of cytochrome P-450 with phenobarbital (65, 79), and the increase shows the significance of this enzyme system also for this type of compounds.

A scheme of the probable metabolic route for carbon tetrachloride is shown in fig 4. Experiments with monkeys showed that about 50 % of the absorbed dose was expired unchanged, while the major part of the residual dose was excreted in urine and feces. A small part was expired as carbon dioxide (4 %) or incorporated into urea and excreted in the urine (9, 63, 64). Only a small part of the absorbed dose was metabolized. Carbon tetrachloride is thought to be cleaved homolytically, and the formed trichloromethyl radical reacts with unsaturated fatty acids under the formation of chloroform and a fatty acid radical which may then form peroxides (83). The trichloromethyl radical may also bind to macromolecules in the cell or may react with reduced glutathione under the formation of chloroform and oxidized glutathione, which may then be reduced back to glutathione by glutathione reductase (33). It has been shown in vitro that glu-

tathione significantly inhibits the binding of carbon tetrachloride to macromolecules (92).

Contrary to carbon tetrachloride, chloroform seems to be dechlorinated oxidatively by oxidation to trichloromethanol by cytochrome P-450. Trichloromethanol is then transformed to phosgene by spontaneous dehydrochlorination (79). Phosgene can then react with water and form carbon dioxide or be covalently bound to cellular macromolecules. In an investigation with mice it was shown that 80 % of an administered dose of chloroform was excreted as carbon dioxide (16). Preliminary studies also show that at least three non-volatile metabolites are excreted in the bile of rats following the administration of chloroform and that the amount of these metabolites may be correlated to the decrease in the liver concentration of glutathione caused by chloroform (33).

In the rat, up to 76 % of the inhaled dose of methylene chloride is transformed to carbon monoxide and carbon dioxide via a mechanism as yet unknown (17, 86). Furthermore, methylene chloride is metabolized to formaldehyde and inorganic chloride, probably via a soluble glutathione-S-transferase (5). It has been suggested that an intermediate metabolite, S-chloromethylglutathione, should be responsible for the covalent binding of ^{14}C -methylene chloride that has been observed after the administration of the solvent to rats (85).

Methylchloroform, 1,1,1-trichloroethane, is dechlorinated only to a very small extent (105) and is excreted mainly unchanged via the lungs. However, trichloroethanol and, to a smaller extent, trichloroacetic acid may be recovered in the urine of both rodents and man (52). The occurrence of a toxic metabolite is indicated by the fact that the induction of cytochrome P-450 in the rat with phenobarbital increases the hepatotoxic effect of methylchloroform (18).

Chlorinated alkenes

Unlike chlorinated alkanes, chlorinated ethylenes are stabilized as a result of increasing substitution with chlorine (11),

and an increasing proportion is metabolized in the series tetra-, tri- and cis-1,2-dichloroethylene (12). The first step in the biotransformation is the formation of an epoxide which is a reactive intermediate, especially for epoxides with unsymmetrical chlorine substitution. The unsymmetrical epoxides formed from vinyl chloride, vinylidene chloride, and trichloroethylene have also been shown to be mutagenic in contrast to symmetrical epoxides formed from trans-1,2- and cis-1,2-dichloroethylene and tetrachloroethylene (11).

The chlorinated alkenes of greatest industrial importance are trichloroethylene and tetrachloroethylene, and therefore the following presentation is limited to these compounds. During exposure during rest about 55 % of the trichloroethylene is absorbed in man (1) and is expired unchanged only to a very small extent (72). The main metabolites excreted in urine are trichloroacetic acid and trichloroethanol. The latter metabolite is also excreted as a glucuronic acid conjugate. The possible metabolic routes for trichloroethylene are illustrated in fig 5. Trichloroethylene may possibly be conjugated directly to glutathione via glutathione-S-transferase as indicated by the low recovery obtained for trichloroethylene and its metabolites in expired air and urine and by the changes in the level of glutathione in the liver of rats exposed to trichloroethylene (84). The most important metabolic route is the cytochrome P-450-catalyzed formation of an epoxide that is rearranged by the migration of a chlorine atom under the formation of trichloroacetic aldehyde which is rapidly hydrated to chloral hydrate. The occurrence of an epoxide has been shown by spectral studies of microsomal preparations (104), and chloral hydrate has been detected in plasma in man following exposure to trichloroethylene (21). The transformation of chloral hydrate to trichloroacetic acid is catalyzed by alcohol dehydrogenase and an NADH-dependent³ dehydrogenase different from aldehyde dehydrogenase, respectively (59). Trichloroethanol is thereafter conjugated to glucuronic acid. The excretion of trichloro-metabolites in the urine of trichloroethylene-exposed rats is increased after

³ NADH = reduced form of nicotinamide adenine dinucleotide.

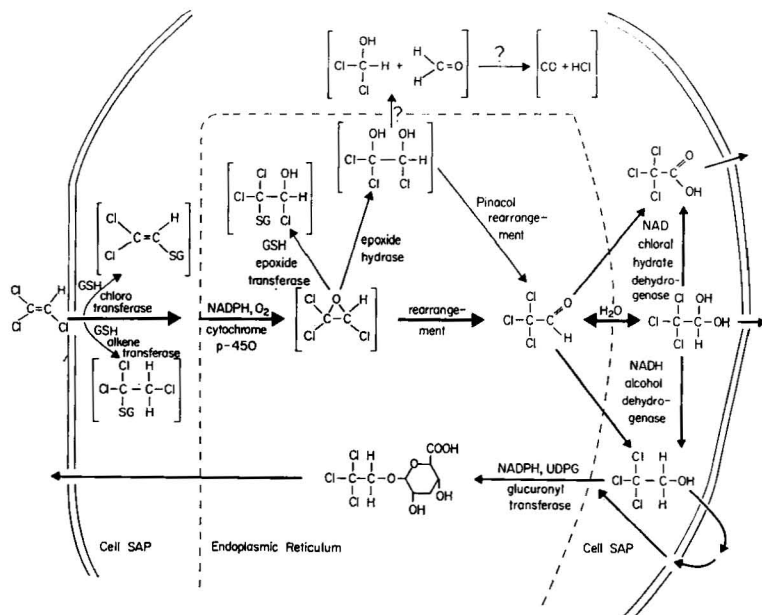


Fig 5. Biotransformation of trichloroethylene. The figure has been reproduced from Reynolds & Moslen (84) with approval from the authors and the publishing company.

the induction of cytochrome P-450 with phenobarbital and polychlorinated biphenyls (PCB) but not after treatment with 3-methylcholanthrene. These results indicate that only certain forms of cytochrome P-450 catalyze the formation of an epoxide from trichloroethylene (70). In addition animals that have been chronically exposed to trichloroethylene have a more rapid metabolism of the solvent (58). Following the exposure of rats to trichloroethylene, it has been possible to show covalent binding of metabolites to cellular macromolecules to the same extent as after exposure to carbon tetrachloride (10). In addition it has been possible to show a covalent binding of trichloroethylene metabolites to DNA in vitro (8).

Tetrachloroethylene is metabolized in a manner similar to that of trichloroethylene, but to a smaller extent. Trichloroacetic acid has been detected in the urine of exposed humans (59). Several urinary metabolites have been reported in mice and rats, namely, oxalic acid, traces of dichloroacetic acid and inorganic chlorine (27, 109). Rearrangement of the epoxide from tetrachloroethylene to trichloroacetylchloride, which could then react with different cell components, has been suggested as an explanation for the hepatotoxic effect of tetrachloroethylene (12, 59, 70). The pretreatment of rats with the enzyme inducer

Arochlor 1254 (PCB) increases the sensitivity to liver injury (70), evidently due to an increased formation of the reactive metabolite.

Other solvents

Solvents that are not aromatic or halogenated have, with a few exceptions, been considerably less investigated than the solvents already described. A short presentation of this group is given.

Alkanes

Unbranched hydrocarbons such as n-heptane and n-hexane have been shown to be substrates for the microsomal cytochrome P-450 system and are metabolized to alcohols with 2-heptanol and 2-hexanol, respectively, as the main products (39, 40). The alcohols formed can be further metabolized to carbon monoxide or be conjugated to glucuronic acid. In the case of n-hexane, formation of methyl-n-butyl ketone and 2,5-hexanedione has been shown to occur in vitro (23), in addition to the formation of 2-hexanol. Furthermore, 2,5-hexanedione and 5-hydroxy-2-hexanone have been detected in the serum of guinea pigs exposed to n-hexane (32). 2,5-Hexanedione is thought to be responsible for the neurotoxic effect of n-hexane and methyl-n-butyl ketone (24, 31, 90).

Alcohols

Primary alcohols are mainly oxidized to aldehydes and then to carboxylic acids, while the secondary alcohols are oxidized to ketones. Conjugation to glucuronic acid and sulfuric acid occurs (13, 29). After oxidation to carboxylic acids, primary alcohols are further transformed via β oxidation and via the citric acid cycle to carbon monoxide and water. The metabolic rate decreases in the following order: propanol $\approx$ 1-butanol > ethanol > isopropanol (29).

Isopropanol is transformed to acetone, which may then enter the normal metabolism of the body, possibly with the accumulation of ketone bodies, or may be excreted unchanged (73). The most important enzyme in the oxidation of alcohol is the liver alcohol dehydrogenase, a relatively nonspecific enzyme. Especially in connection with exposure to high doses of solvents, metabolism is also thought to occur via catalase and cytochrome P-450 (29, 73, 100).

The biotransformation of methanol is relatively well known and is shown in fig 6. The harmful effect of methanol on the eyes, as well as methanol-induced metabolic acidosis, has been shown to depend upon the accumulation of formic acid (62). Ethylene glycol is transformed to several different metabolites, including

glycol aldehyde, glycolic acid, glyoxal, glyoxalic acid, oxalic acid, glycine, and carbon dioxide. Investigations with monkeys have shown that ethylene glycol is eliminated mainly unchanged as glycolic acid (19). In this study and in an additional investigation on rats the authors claimed that glycolic acid is responsible for the acidosis and the acute toxic effects (19, 20). Other glycols, including the glycolic ethers (cellosolves) that are common in industry, have been investigated to a very small extent. However, *n*-butoxyacetic acid has recently been identified in urine from rats exposed to *n*-butoxyethanol (butylcellosolv) (55).

Ketones

With the exception of acetone, which has already been discussed, significant information is only available for methyl ethyl ketone, methyl isobutyl ketone and methyl-*n*-butyl ketone (31, 32). An analysis of metabolites in the serum of guinea pigs exposed to methyl ethyl ketone and methyl isobutyl ketone showed that these compounds are reduced to their respective alcohols (2-butanol and 4-methyl-2-pentanol, respectively), which are then conjugated with sulfuric acid or glucuronic acid, or enter the intermediary metabolism and are eliminated as carbon monoxide, or

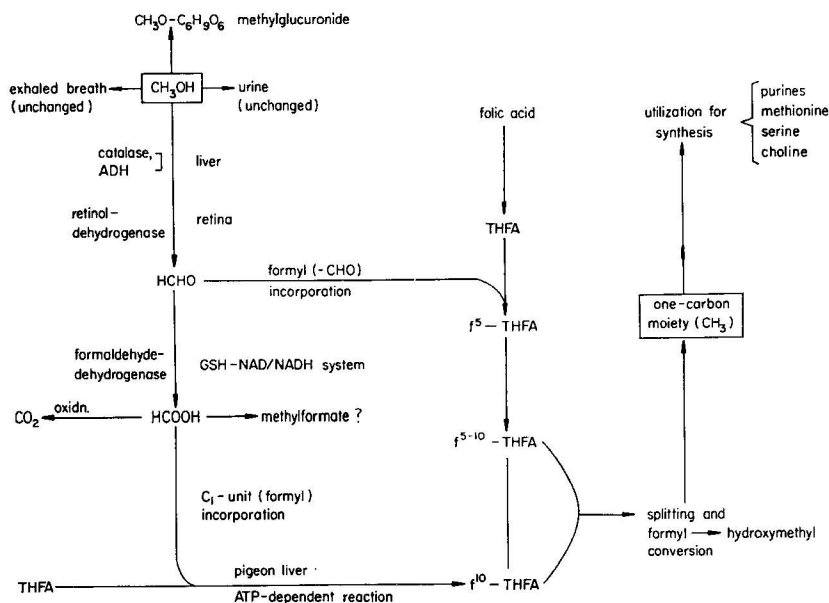


Fig 6. Metabolic fate of methanol and its interrelationship with single-carbon metabolism (THFA: tetrahydrofolic acid; $\text{f}^5\text{-THFA}$: 5-formyltetrahydrofolic acid; $\text{f}^{5-10}\text{-THFA}$: 5,10-methylenetetrahydrofolic acid; $\text{f}^{10}\text{-THFA}$: N¹⁰-formyltetrahydrofolic acid). The figure has been reproduced from DeBruin (29) with approval from the author and from the publishing company.

are hydroxylated, probably via cytochrome P-450, to 3-hydroxy-2-butanone and 4-methyl-4-hydroxy-2-pentanone, respectively (32).

Recently, the metabolism of methyl-n-butyl ketone has been investigated in detail, mainly due to its neurotoxicity (31). After the oral administration of 20 or 200 mg/kg (0.2 or 2.0 mmol/kg) of the radioactively labeled compound to rats, 6 % was eliminated unchanged, together with 38 % as carbon monoxide via expiration, 40 % was recovered in the urine, and 1.4 % in the feces during the 6 d following exposure. The largest quantity of residual radioactive compound was recovered in the blood and liver. As is evident from fig 7, methyl-n-butyl ketone may either be reduced to 2-hexanol, which is then excreted in the urine as a sulfate or a glucuronide or be α -oxidized to 2-keto-1-hexanol, which is probably further metabolized to carbon monoxide and norleucine, or (α -1)-oxidized to 5-hydroxy-2-hexanone, which may then be transformed to 2,5-hexanedione and a number of other metabolites.

The formation of 2,5-hexanedione, which is excreted in the urine, both unchanged and as a sulfate conjugate, may be regarded as a metabolic activation since this metabolite has a pronounced neurotoxicity. The initial hydroxylation that leads to the formation of this metabolite seems to be a cytochrome P-450-mediated reaction, since

pretreatment of this enzyme system with an inhibitor results in a significantly decreased formation of this metabolite in the urine.

Ethers and esters

Very little information is available about the metabolism of ethers and esters. If metabolism occurs it probably takes place via enzymatic cleavage of the ethers and via nonspecific esterases, eg, cholinesterases and pseudocholinesterases (41, 106). Recently, acetaldehyde has been demonstrated in the blood of patients after the administration of diethyl ether (6). Diethyl ether has earlier been regarded as resistant to metabolic transformation. Experiments with rats in vivo have shown that ethyl acetate is hydrolyzed to ethanol (92).

Carbon disulfide

Transformation of carbon disulfide is another example of metabolic activation where metabolism via cytochrome P-450 leads to formation of a reactive metabolite, in this case probably a singlet form of elementary sulfur. Experiments in vitro have shown that carbon disulfide can be metabolized to carbon dioxide via carbonyl sulfide under the formation of two reactive

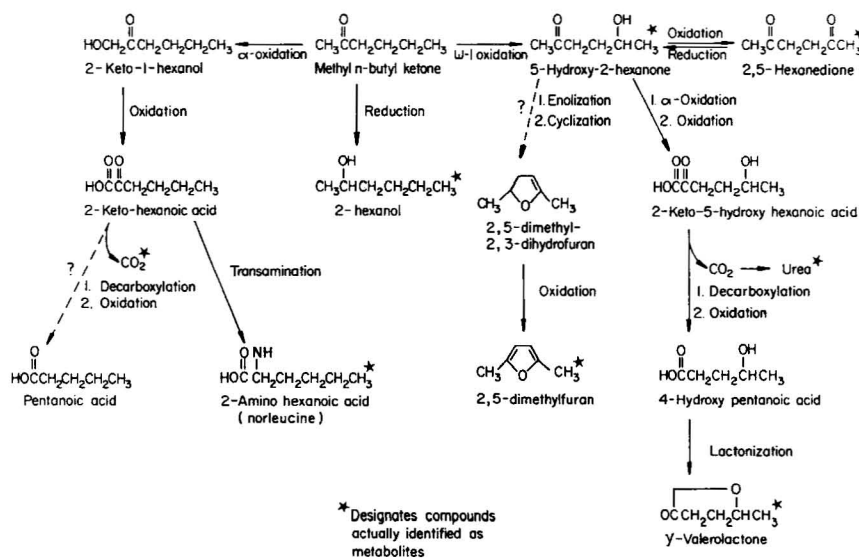


Fig 7. Proposed pathway for the metabolism of [1-¹⁴C]methyl-n-butyl ketone. The figure has been reproduced from DiVincento et al (31) with approval from the authors and from the publishing company.

sulfur atoms (25). Induction of cytochrome P-450 with phenobarbital in rats resulted in an increased sensitivity to liver injury following exposure to carbon disulfide (25, 38).

METABOLITES AS BIOLOGICAL EXPOSURE TESTS

One of the main objectives of occupational medicine is to prevent the development of occupational diseases. In this respect the biological monitoring of workers exposed to various industrial chemicals may play an important role by making it possible to detect excessive exposures. Biological monitoring has not yet reached an advanced stage of development, primarily because adequate biological exposure tests cannot be developed until sufficient knowledge has been obtained concerning the mechanisms of action and biotransformation of industrial chemicals. This knowledge is still very limited in the case of solvents.

Before the use of a biological parameter can be proposed for the routine monitoring of workers exposed to a chemical, certain prerequisites must be met. Some knowledge must be available concerning the health significance of the different levels of the parameter measured. The effect of various routes of administration (inhalation and percutaneous and peroral administration) on the biological parameter must be known. Certain knowledge must be available concerning the pharmacokinetics of the compound. A relationship must exist between the concentration of the chemical in the air and the measured biological parameter. The technique for measuring the parameter must be simple and must allow screening but at the same time give satisfactory precision and reproducibility. If all these prerequisites are satisfied, quantitations of metabolites offer important advantages as exposure tests over the monitoring of air levels at the place of work. A biological exposure test allows the integration of several factors of relevance for the judgement of the risk for development of illness (individual differences in rate of absorption, distribution, biotransformation and excretion). A short summary follows of the usual routine methods in

use for the determination of solvent metabolites in the urine of man.

Benzene

The excretion of phenol in the urine has been suggested as a test for benzene exposure. Usually a gas chromatographic assay is used. The use of phenol as a biological exposure test has however been criticized since ingestion of certain drugs may give increased concentrations of phenol in the urine (37). Another difficulty is that the normal level of phenol excretion (the background value) is subject to large individual variations (87).

Styrene

The presence of mandelic acid and phenylglyoxylic acid in the urine has been used as a biological exposure test for styrene [for references, see Lauwerys (56)]. Usually the assays are carried out with gas-liquid chromatography, even though colorimetric methods have also been used. Engström and her collaborators (35) found that the rate of excretion of mandelic acid is dependent upon the degree of styrene exposure and that the excretion of 2,300 mg of mandelic acid/g of creatinine (1.7 mol of mandelic acid/mol of creatinine) corresponds to about 100 ppm (425 mg/m³) of styrene. Härkönen and his collaborators (45) were not however able to find any correlation between the occurrence of symptoms in the central nervous system following exposure to styrene and the excretion of mandelic acid in urine.

Toluene

Hippuric acid in urine, which can be measured colorimetrically or by gas-liquid chromatography, has been used as a test for toluene exposure. Szadkowski and his collaborators (97) did not find any correlation between the rate of excretion of hippuric acid in the urine and the concentration of toluene in air or blood, and they recommended the determination of toluene in blood as the best method for surveilling workers exposed to toluene.

Wilczok & Bieniek (108), on the other hand, found that the excretion of hippuric acid in urine may be used as a reliable test for toluene exposure.

Xylene

Engström and her collaborators studied workers exposed to xylene and found that an excretion of 665 mg of methylhippuric acid/g of creatinine (0.38 mol of methylhippuric acid/mol of creatinine) in urine at the end of the workday corresponded to an exposure of 50 ppm (219 mg/m³) of xylene (36). The amount of methylhippuric acid in a morning sample at the end of the week, on the other hand, correlated to the mean exposure of the three preceding days.

Methylchloroform (1,1,1-trichloroethane), trichloroethylene and tetrachloroethylene

As exposure tests for methylchloroform, trichloroethylene and tetrachloroethylene, trichloroacetic acid (methylchloroform, trichloroethylene and tetrachloroethylene) and trichloroethanol (methylchloroform and trichloroethylene) in urine have been used. From the time of exposure to 70 h following the exposure, the amount of trichloroacetic acid and trichloroethanol excreted only corresponded to 0.5 and 2 %, respectively, of the absorbed amount of methylchloroform (68). The corresponding figures for trichloroethylene were 24 and 43 %, respectively (67). The authors concluded, however, that the amount of trichloroacetic acid in blood is the best biological exposure test for trichloroethylene (67). Only 1 % of the absorbed tetrachloroethylene is excreted in urine as trichloroacetic acid (69).

HIGH PRIORITY AREAS FOR FUTURE RESEARCH

The importance of the biotransformation of solvents is evident from the foregoing presentation. In most cases, toxic effects are only exerted following metabolic activation to reactive intermediates. Today knowledge about the nature of these metabolites and how they interact with various components in the cell is incom-

plete. For instance, we do not even know with certainty which are the reactive metabolites of the well-known toxic solvents benzene and carbon tetrachloride or how the toxic effect of these chemicals is exerted. For many solvents the main metabolites excreted in the urine are known, but intermediates and quantitatively less important metabolic routes have been insufficiently investigated. It is quite possible that these intermediary products and "minor pathways" are of major importance from a toxicologic point of view. For certain solvents, mainly those belonging to the groups glycolic ethers, ethers, esters, aliphatic hydrocarbons and ketones, information on biotransformation is almost completely missing.

Important factors affecting metabolism are sex and age, the influence of which has been insufficiently investigated. It is also important to investigate differences in metabolic capacity and metabolic patterns between different organs and the influence of the binding of solvents and their metabolites to plasma proteins. The importance of different routes of administration to further metabolism has to be taken into consideration since great differences in metabolism, both quantitatively and qualitatively, may exist following oral administration as compared to inhalation. The emphasis in future studies should be placed on inhalation studies and possibly also on percutaneous administration. The importance of enzyme induction in the liver and the lungs to the rate of metabolism and the formation of toxic intermediates should be investigated, as well as the capacity of the solvents to stimulate their own metabolism.

Another point that should be investigated is the dose-dependence of metabolism. This issue is important when the possibility that the metabolizing enzyme systems may be saturated is considered. In the rat such saturation occurs at 250 ppm (750 mg/m³) of vinyl chloride, 150 ppm (788 mg/m³) of trichloroethylene and 250 ppm (1,875 mg/m³) of carbon tetrachloride, respectively (10). Furthermore, the enzymes participating in the biotransformation of halothane are the most active at low concentrations of the substrate and become more and more inhibited at increasing concentrations of halothane (89).

Interactions between different solvents and between solvents and drugs or ethanol are very insufficiently known but are probably of great importance, since simultaneous exposure to several of these compounds is very common. It is, eg, known that aliphatic alcohols, among others, ethanol, may influence the metabolism of trichloroethylene and potentiate the hepatotoxic effect of chloroform and carbon tetrachloride (22). Recently it has also been shown that simultaneous exposure of rats to ethanol and vinyl chloride leads to a greater number of tumors than the administration of vinyl chloride only (81).

Knowledge about the absorption, distribution, and biotransformation of solvents, including reactive intermediates and their interaction with various components of the cell and factors that influence the metabolism of solvents, eg, in the form of inhibition or stimulation, is necessary for an understanding of the toxic mechanisms of action of solvents. Such an understanding is a prerequisite for a correct judgement of the risks following exposure to solvents. It would seem very important to increase the level of knowledge within the areas specified in view of the common occurrence of solvents and the suspicion that solvents may be, eg, neurotoxic, carcinogenic, teratogenic, and leukemogenic (46, 47, 50).

Examples of high priority areas for research concerning the biotransformation of solvents are the following:

1. Investigations on the capacity of different solvents to form reactive metabolites which can be bound covalently to proteins, lipids or DNA and of which enzyme systems that catalyze the activation. These studies may initially be carried out in vitro with radioactively labeled compounds and using cell fractions, isolated cells or isolated organs.
2. The identification of reactive metabolites and the investigation of how DNA and other macromolecules may be modified by these metabolites. The objective should be to try to correlate structure with toxicity.
3. The investigation of the capacity of different organs to activate different solvents. Special interest should be di-

rected towards the liver, the lungs, the bone marrow and the kidneys. For these studies cell fractions, isolated organs, and in vivo experiments can be used.

4. The correlation of biotransformation to morphological changes or other toxic effects in different organs in experimental animals.
5. Investigations on the importance of the induction or inhibition of activating enzyme systems to metabolism and investigations aimed at determining whether the solvents themselves cause these effects.
6. The investigation of whether simultaneous exposure to several solvents may affect their metabolism. It is also essential to investigate the possible occurrence of interaction effects with ethanol and common drugs, eg, oral contraceptives.
7. Studies on the role of glutathione and glutathione-S-transferases in the metabolism of chloroform, trichloroethylene, aromatic hydrocarbons and other solvents with purified enzyme preparations and in vivo.
8. The construction of pharmacokinetic models for solvents. It is especially important to investigate the occurrence of dose-dependent metabolism. If possible, comparative studies between experimental animals and man should be carried out.
9. Studies on the biotransformation of the solvents for which such information is lacking or is insufficient. This is especially important for common solvents such as methyl ethyl ketone, methyl isobutyl ketone, aliphatic hydrocarbons such as n-hexane and n-heptane, glycolic ethers (cellosolves), butanol, butyl acetate, ethyl acetate, components in white spirit, etc.
10. The development of practical tests on the metabolic capacity of different individuals, especially with regard to the existence of different types of cytochrome P-450. The aim is to use such test systems to obtain information about the capacity of different persons to metabolize solvents under certain work conditions and with a certain state of nutrition, and on the basis of this information identify risk groups.

11. The development of methods that will make it possible to use excreted urinary metabolites of solvents as exposure tests. As is evident from the summary, only a few methods are currently available, and these methods have questionable significance since they practically only allow group correlations between exposure and excretion, whereas individual correlations are considerably less reliable.

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