20

Toxic Effects of Solvent Exposure

20.1 TOXICOKINETICS, TOXICODYNAMICS, AND TOXICOLOGY

Tilman Hahn, Konrad Botzenhart, Fritz Schweinsberg
Institut für Allgemeine Hygiene und Umwelthygiene
Universität Tübingen, Tübingen, Germany

20.1.1 TOXICOKINETICS AND TOXICODYNAMICS

20.1.1.1 Exposure

Highest exposures can be found in workplace (e.g., evaporation of solvents) or during special processes (e.g., leaks of normally closed systems). Acute and severe solvent accidents often happen in workplaces (high solvent concentrations, intermittent high-level exposures, high duration of exposure). Apart from working sites, various other emission sources of solvents should be considered, e.g., consumer products.

The description of exposure parameters (type of solvents, concentrations, duration, routes of exposure) are important for the evaluation of toxicokinetics. Solvents and other chemicals are usually emitted as a mixture of various substances. Therefore, the risk assessment of emitted solvents is difficult to ascertain.[1,2] Solvent concentrations and duration of exposure vary in most cases (intermittent high-value peaks, periods of low exposure). The exposure is influenced essentially by surrounding occupational and environmental conditions, such as working climate, protective equipment and by individual parameters such as eating habits.

The exposure to solvents is regulated by relevant threshold limit values.[1,2] Exposure and exposure values can be controlled by defined methods (e.g., ambient and biological monitoring).

20.1.1.2 Uptake

Relevant uptake routes of solvents are absorption from the lung and percutaneous absorption. The intestinal uptake is usually caused by accidents or by intent. The absorption rate is influenced by various factors.

20.1.1.2.1 Inhalation

Inhalation is the most common pathway of solvent absorption, especially at working sites. The pulmonal absorption of solvents depends on the following parameters:[3-6]

- Exposure (concentrations and concentration fluctuations in the ambient air, exposure time, physical exertion). The alveolar concentration of solvents or the difference between air and blood concentration levels determine the diffusion process into alveolar blood vessels. Physical exertion influences lung parameters, especially ventilation, and consequently alveolar and blood concentrations.
- Lung parameters (pulmonary and alveolar ventilation, pulmonary perfusion, air-blood coefficient, blood-tissue coefficient). These coefficients describe the amount of solvents which can diffuse. The blood-tissue partition coefficient influences the tissue equilibrium concentrations. Solvents with stronger hydrophobic properties (e.g., toluene) reach equilibrium more rapidly because of a low tissue-blood coefficient. Intraindividual differences such as child/adult are also of significance.
- Physicochemical characteristics of solvents (solubility such as hydrophobic and hydrophilic properties, state such as liquid or gaseous and degree of volatility).

20.1.1.2.2 Dermal uptake

Dermal uptake of solvents requires skin contact and depends on the area of contact, skin thickness, dermal state (e.g., eczema and defects in the stratum corneum), exposure parameters (contact time, etc.) and solvent properties.[7,8]

The main barrier against percutaneous uptake of solvents are structures of the stratum corneum, especially intercellular lipids and fibrous keratin. Removal of lipids by polar solvents such as ethanol or hydration in the stratum corneum is associated with an increase of skin permeability. Defects or lack of stratum corneum that may occur in skin diseases, at particular skin locations such as hair follicles or glandula regions enhance the percutaneous movement of solvents. The absorption through mucosa membranes is facilitated because of the lack of the stratum corneum.

Skin defects or diseases can be provoked by solvents which cause irritation, cellular hyperplasia and swelling, or removal of lipids. Skin defects are provoked mainly by frequent use of solvents thus enhancing their absorption.

Other characteristics, which influence percutaneous absorption, are solvent concentration gradients, solvent partitioning (water/lipid partition coefficient) and permeability constants.

Lipophilic chemicals are absorbed most easily (for example, benzene). These can include liquid solvents or solvents having low vapor pressure.[9-11] Vapors absorbed by dermal uptake can significantly contribute to the body burden as a result of the whole body exposure: e.g. 1-2 % of xylene or toluene, up to 5-10 % 1-methoxypropane-2-ol.[10] For other substances, much higher skin absorption rates were measured after the whole body exposure: 2-methoxyethanol up to 55 %, 2-ethoxy-ethanol up to 42 %.[12]

It is important to consider that the dermal uptake of vapors is especially significant when using a gas-mask.[10] In addition to inhalation measurements, measurement of percutaneous absorption is an important method for assessing health or environmental risks.

Dermal absorption of solvents is shown in Table 20.1.1.

Table 20.1.1. Dermal uptake of solvents according to the German MAK-list.[2,64]

Benzene	Cyclohexanone	2-Hexanone	1,1,2,2-Tetrachloroethane
Bromomethane	Dimethylformamide	Methanol	Tetrachloroethene
2-Butanone	Dimethylsulfoxide	2-Methoxyethanol	Tetrachloromethane
2-Butoxyethanol	1,4-Dioxane	Methyl formate	Toluene
Carbon disulfide	2-Ethoxyethanol	Nitrobenzene	Toluidine(s)
2-Chloroethanol	Ethylbenzene	Nitrotoluene(s)	1,1,2-Trichloroethane
Chloromethane	Ethyl formate	Phenol	Trichloromethane
Cresol(s)	Ethylene glycol	iso-Propyl benzene	Xylene(s)
Cycolhexanol	n-Hexane	n-Propanol (from ACGIH[1])	

20.1.1.2 Metabolism, distribution, excretion

Specific toxicity of solvents is directly related to their metabolism which is predominantly catalyzed by cytochrome P-450 mixed-function oxidases in the liver or other tissues.

Relevant examples of specific metabolism are toxic epoxides of benzene (hemopoietic toxicity), n-hexane 2,5-hexanedione (peripheral neurotoxic effects), metabolites of ethylene glycol ethers (reproductive toxicity), and unidentified metabolites from trichloroethylene (renal-toxic effects).[13] It should be emphasized that only the metabolites of these solvents are associated with toxic effects.

Other relevant metabolic pathways result in detoxified substances, such as biotransformation processes in the liver – conjugation with glycine, glucuronic acid and sulphuric acid (e.g., via hydroxylation of toluene) or biotransformation by hydrolysis, oxidation and conjugation (e.g., glycol ethers).

It should be noted that metabolism processes vary according to the following conditions:[14]

- Species, sex, age, genetics, e.g., variability in enzymatic factors such as polymorphisms (cytochrome systems) or tissue repair mechanisms[15]
- Life style – diet, smoking, drug consumption, physical activity
- Saturation. Massive concentrations of solvents result in saturation of metabolic pathways. This is important with regard to detoxification
- Induction of enzymes. Specific induction of enzyme systems by chemicals (solvents as well as other chemicals such as drugs) may consequently provoke an increase or decrease of solvent toxicity
- Interactions may be involved in enhancing or reducing toxicity of solvents. For example Bloch et al.[16] showed that in cases of alcohol abuse an increase in the toxic effects of benzene and other lipophilic petroleum derivatives occurs. Also, it has been shown that benzene inhibits the metabolism of toluene.[17]

Solvents can be excreted via various pathways:

- Exhalation (unchanged)
- Urine tract and biliary tract (unchanged or metabolites, e.g. water-soluble conjugates)

20.1.1.3 Modeling of toxicokinetics and modifying factors

The complexity of toxicokinetic processes of solvents can be described in models, e.g., predicting exposure situations and distribution phenomena in the human body and quantifying these processes (e.g. dose-effect response relationships). This applies especially to simula-

tion of physiological and physicochemical parameters[18] or to assessing low exposures to complex chemical mixtures.[19]

20.1.2 TOXICOLOGY

20.1.2.1 General effects

General effects of solvents concern primarily acute exposures to high solvent concentrations. Despite some variations of symptoms, the resulting effects on the central nervous system (CNS) are rather stereotypical.[20]

Several solvents have depressant or narcotic effects, and hence, some solvents are used as anesthetics.[21] The main acute health hazards result from the narcotic effects. Their intensity is proportional to the solvent concentrations in brain tissue and is caused by the solvents themselves (physical and chemical interactions with neural membranes, nerve cells or neurotransmitters of the CNS).

General CNS dysfunctions after solvent exposure, are initially euphoria and disinhibition, higher exposures result in pre-narcotic symptoms such as dizziness, euphoria, disorientation and confusion, nausea, headache, vomiting, ataxia, paresthesia, increased salivation and tachycardia.[22,23] The symptoms are rapidly reversible when the solvents are removed.

In addition to the non-specific acute narcotic effects of solvents mentioned above, alterations of behavioral, cognitive and psychomotoric functions are typically found after short-term exposure to solvent levels close to the TLV. Overexposure leads to convulsions, coma and death. Typical changes are paresthesias, visual and auditory deficits, cognitive deficits (short-term and long-term memory loss), confusion, disorientation, affective deficits (nervousness, irritability, depression, apathy, compulsive behavior) and motor deficits (weakness in extremities, incoordination, fatigue, tremor).[24,25]

It is difficult to develop useful methods and models for testing these behavioral effects of solvents but for this purpose tests of attention and reaction, cognitive tests and other test systems are used.[26,27]

Acute CNS dysfunction diseases can show mild (organic affective syndrome), moderate or severe (acute toxic encephalopathy) symptoms.[28,29]

Unspecific irritations of skin and mucosa membrane structures can be caused by solvents. Various solvents are significant occupational irritants, e.g., solvents which cause irritant contact dermatitis.[30] Intact skin structures can be destroyed by solvents which dissolve grease and fat. Typically, the dermatitis is characterized by dryness, scaling and fissuring and is usually located on the hands. It is often caused by handling solvent-contaminated products or by cleaning procedures.[31,32]

Unspecific irritation of mucous membranes is often caused by solvent vapors, e.g., irritation of the eyes and various sections of the airways.

20.1.2.2 Specific non-immunological effects

Table 20.1.2 summarizes the main specific effects of solvents:[33-47]

- Hepatotoxicity
- Nephrotoxicity
- Reproductive toxicity
- Hemopoietic toxicity
- Neurotoxicity
- Ocular toxicity

Table 20.1.2. Examples for specific effects of selected solvents

Organ-system	Solvents	Symptoms
Liver	halogenated hydrocarbons (e.g., carbon tetrachloride, tetrachloroethane, chloroform), ethanol, 1,1,1-trichloroethane, trichloroethylene, bromobenzene, dimethylformamide	acute (necrosis, steatosis) and chronic (cirrhosis) hepatotoxic symptoms
Kidney	halogenated hydrocarbons (e.g., carbon tetrachloride), toluene, dioxane, diethylene glycol, ethylene glycol, glycol ethers, conjugates of trichloroethylene	acute tubular necrosis, glomerular and tubular dysfunctions (e.g., albuminuria, proteinuria), glomerulonephritis, note: modification of solvent effects caused by renal dysfunctions possible
Reproductive system	carbon disulfide, benzene, glycol ethers, nitrobenzene	disturbance of menstrual cycle; reduced sperm counts, embryotoxic effects
Hemopoietic system	benzene metabolites (e.g., benzoquinone, hydroquinone)	marrow depression, myelotoxic effects
Nerval system	n-hexane, ethanol, styrene, tetrachloroethylene	peripheral neuropathy (especially distal axons, axon swelling and degeneration, loss of sensibility, muscular atrophy, loss of tendon reflexes)
Eye	methanol	impaired vision

Note: the data shown come predominantly from data of occupational exposure.

20.1.2.3 Immunological effects

Various solvents have well-known allergic potentials. Allergic symptoms of the respiratory tract (rhinitis, tracheitis, bronchitis, asthma), allergic contact dermatitis and conjunctivitis can be provoked by solvents. The allergic effects of solvents can also contribute to other diseases such as MCS, autoimmune diseases.

Nowadays, solvents or by-products with allergic potential occur mainly at workplaces and, to a lesser degree, in consumer products. According to EG regulations, solvent ingredients of some consumer products, e.g., cosmetic products, must be labeled. It is often difficult to detect the causative solvent allergen (allergens which cause cross allergies, secondary products of solvents such as oxygenated terpenes, unknown allergens). Various specific test systems are available for carrying out individual test diagnoses: e.g., chamber tests,[48] skin tests such as patch-tests[49] and special applications of biological monitoring.

Solvent-induced allergies can occur at a variety of working sites, e.g., in shoe factories,[50] in electronic industries,[51] in synthetic chemical industries,[52] in metal industries[53] or in perfume and potter industries (oil of turpentine and other solvents).[54] Similar occurrence of solvents can be found in consumer products, e.g., in nail polishes (e.g., toluene).[55] Allergic solvent substances are listed in various catalogues and databases.[1,2,49]

Examples of allergic solvents are terpene products with high sensitivity potential, which can cause positive test reactions (patch-test) or even allergic diseases (contact sensitization and dermatitis). Allergic dermatitis can even be provoked by d-limonene in the air.[56] Terpenes and terpenoid substances are found especially in “natural products”, e.g., cosmetic products, foods, and plants (oilseed rape).[57,58]

Allergic potential of solvent products depends on the typical solvent structure. For example, in glycol ethers their allergic potential is proportional to the charge of interacting molecules.[59]

Allergic effects can also be associated with other skin conditions caused by solvents such as irritations. Multiple areas of skin damage, including solvent allergies, can change the skin structure and provoke severe skin disease.[60]

In addition to other substances (pesticides, food additives, dust, smoke, etc.), allergic effects of solvents are discussed as an initial cause of MCS.[61]

Organic solvents are associated with human autoimmune diseases, but defined pathomechanisms of these solvents have not yet been detected (role of solvents in the initiation or progression of autoimmune diseases).[62]

20.1.2.4 Toxic effects of solvents on other organisms

In addition to humans, microorganisms animals and plants are also exposed to solvents. The interaction between organisms and solvents are often specific. For example, the reactions elicited by certain solvents depend on the species and abilities of the particular organism affected.

Hydrophobic organic solvents, in particular, are toxic to living organisms, primarily because they disrupt cell membrane structure and mechanisms. Some living organisms especially certain bacterial species, are able to adapt to these solvents by invoking mechanisms such as accelerating repair processes (through changes in the rate of phospholipid biosynthesis), reduction of the diffusion rate of the solvent and active reduction of the intracellular concentration of the solvent. More information and examples are shown in Chapter 14.4.2.

20.1.2.5 Carcinogenicity

The term carcinogenicity is used for toxicants that are able to induce malignant neoplasms. Carcinogens can be effective at different stages of the carcinogenic process, e. g., initiation, promotion and progression. They may interact with other noxes and thereby enhance tumor development. Interactive carcinogenesis can be described as co- and syn-carcinogenesis. A co-carcinogen is defined as a non-carcinogenic compound that is able to enhance tumor development induced by a given carcinogen. In syn-carcinogenesis two or more carcinogens, each occurring in small amounts that are usually not sufficient to induce a tumor in a specific target organ, may interact to lead to tumor formation in that organ.

As with all carcinogens the carcinogenic potency of solvents has been assessed by short-term in vitro tests, e. g., Ames assay, by long-term tumor induction experiments in animals and - especially important for the evaluation of the carcinogenic action in humans - prospective and retrospective epidemiological studies, for solvent exposure mainly in work places.

From this data it is generally not possible to evaluate the carcinogenic action of solvent mixtures, which occur in the majority of exposure situations. It is also important to note, that for a number of reasons, e. g., very long latency period of tumor generation, accumulation of single hits in the target cells, significance of repair mechanisms it is not possible to define TLVs for carcinogens.

In accordance with the evidence available, different classes for chemical carcinogens have been developed by health authority organizations.[1,2,34-36] Examples of the classification of carcinogenic solvents are presented in Table 20.1.3.

Table 20.1.3. Carcinogenicity - Survey of selected solvents

Solvent	Organ-System	Category*				
		MAK	EG	ACGIH	IARC	NTP
Benzene	*hemopoietic system*	1	K1	A1	1	K
Bromomethane	upper gastrointestinal, tract and respiratory, tract (animals)	3	K3	n.c.**	3	n.l.**
Carbon tetrachloride	lymphatic system, liver (mice, rats), mamma (rats), suprarenal gland (mice)	3	K3	A2	2B	R
Epichlorohydrin	*lung, CNS*, forestomach (rats), nasal cavity, skin (mice)	2	K2	A3	2A	R
Chloroethane	uterus (mice)	3	K3	n.l.	3	n.l.
Cyclohexanone	suprarenal gland (rats)	3	n.c.	A4	3	n.l.
1,2-Dibromoethane	forestomach (mice), lung (mice, rats), nasal cavity, peritoneum, mamma, connective tissue (rats)	2	K2	A3	2A	R
1,2-Dichloroethane	*brain, lymphatic and hemopoietic system, stomach, pancreas*; lung, mamma, stomach (mice, rats), lymphatic system (mice)	2	K2	A4	2B	R
Dichloromethane	liver, lung (mice, rats), mamma (rats), lymphosarcomas (mice)	3	K3	A3	2B	R
1,2-Dichloropropane	liver (mice), mamma (rats)	3	K3	A4	3	n.l.
Dimethylformamide	*testes*	n.c.	n.c.	A4	3	n.l.
1,4-Dioxane	liver (rats, guinea pigs), biliary tract (guinea pigs), mamma , peritoneum (rats), nasal cavity (mice)	4	n.i.**	A3	2B	R
1,2-Epoxypropane	mamma, upper respiratory tract, thyroid gland (mice, rats)	2	K2	A3	n.l.	n.l.
Hexamethyl phosphoramide	nasal cavity, lung (rats)	n.l.	n.i.	A3	2B	R
2-Nitropropane	liver (rats)	2	n.l.	A3	2B	R
Nitrobenzene	lung, thyroid gland, mamma (mice), liver, kidney, uterus (rats)	3	K3	A3	2B	n.l.
2- Nitrotoluene	epididymis (rats)	2	K2	n.c.,BEI**	3	n.l.
Phenol	lymphatic system, hemopoietic system suprarenal gland, thyroid gland, skin (mice, rats)	3	n.c.	n.c.,BEI	3	n.l.
Tetrachloroethane	liver (mice)	3	K3	A3	3	n.l.
Tetrachloroethylene	*oesophagus, kidney, hemopoietic system, lymphatic system*; liver (mice), hemopoietic system (rats)	3	K3	A3	2A	R

Solvent	Organ-System	Category*				
		MAK	EG	ACGIH	IARC	NTP
Tetrachloromethane	stomach, liver, kidney, thyroid gland (rats, mice)	3	K3	A2	n.l.	n.l.
o-Toluidine	mamma, skin, bladder, liver, spleen, peritoneum, connective tissue (rats), vessels (mice)	2	n.i.	A3	n.l.	R
1,1,2-Trichlorethane	liver, suprarenal gland (mice)	3	K3	A4	3	n.l.
Trichloroethylene	*kidney*; liver, biliary tract, kidney, lung, cervix, testes, lymphatic system (rats, mice)	1	K3	A5	2A	n.l.
Chloroform	stomach, liver, kidney, thyroid gland (mice, rats)	4	K3	A3	n.l.	R
1,2,3-Trichloropropane	oral mucosa (mice, rats), uterus (mice), liver, pancreas, forestomach, kidney, mamma (rats)	2	n.i.	A3	2A	R

*Categories

MAK (German regulations)[2]

1: substances that cause cancer in humans and can be assumed to make a significant contribution to cancer risk. Epidemiological studies provide adequate evidence of a positive correlation between the exposure of humans and the occurrence of cancer. Limited epidemiological data can be substantiated by evidence that the substance causes cancer by a mode of action that is relevant to humans.

2: substances that are considered to be carcinogenic for humans because sufficient data from long-term animal studies or limited evidence from animal studies substantiated by evidence from epidemiological studies indicate that they can make a significant contribution to cancer risk. Limited data from animal studies can be supported by evidence that the substance causes cancer by a mode of action that is relevant to humans and by results of in vitro tests and short-term animal studies.

3: substances that cause concern that they could be carcinogenic for humans but cannot be assessed conclusively because of lack of data. In vitro tests or animal studies have yielded evidence in one of the other categories. The classification in Category 3 is provisional. Further studies are required before a final decision can be made. A MAK value can be established provided no genotoxic effects have been detected.

4: substances with carcinogenic potential for which genotoxicity plays no or at most a minor role. No significant contribution to human cancer risk is expected provided the MAK value is observed. The classification is supported especially by evidence that increases in cellular proliferation or changes in cellular differentiation are important in the mode of action. To characterize the cancer risk, the manifold mechanisms contributing to carcinogenesis and their characteristic dose-time-response relationships are taken into consideration.

5: substances with carcinogenic and genotoxic potential, the potency of which is considered to be so low that, provided the MAK value is observed, no significant contribution to human cancer risk is to be expected. The classification is supported by information on the mode of action, dose-dependence and toxicokinetic data pertinent to species comparison.

EG[65]

- **K1**: confirmed human carcinogen
- **K2**: compounds which should be considered as carcinogen
- **K3**: compounds with possible carcinogenic evidence

ACGIH[1]

- **A1**: confirmed human carcinogen
- **A2**: suspected human carcinogen
- **A3**: confirmed animal carcinogen with unknown relevance to humans
- **A4**: not classifiable as a human carcinogen
- **A5**: not suspected as a human carcinogen

IARC[34-36]

1: carcinogenic to humans
2A: probably carcinogenic to humans
2B: possibly carcinogenic to humans
3: not classifiable as to its carcinogenicity to humans

NTP[66]

K: Known to be a Human Carcinogen
R: Reasonably Anticipated to be a Human Carcinogen (RAHC)

**Notes:

italic: cancer in humans
n.c.: not classified as carcinogenic
n.i.: no information available
n.l.: not listed
BEI: not classified as carcinogenic but biological monitoring is recommended

20.1.2.6 Risk assessment

For risk assessment of solvent exposure, and in addition to factors for general risk assessment (age, gender, race, diet, physical activity, stress, physical noxes, etc.) it is important to consider:

- Occupational exposure (high doses) and environmental exposure (low doses) to solvents separately.
- The effect of exposure time, e. g., life long environmental low exposure or occupational intermittent high exposure.
- Exposure assessment (generally the most neglected aspect in risk assessment). This involves extensive ambient monitoring over a long period of time. Only a small amount of data on biological monitoring of solvents and/or metabolites (representing the "effective" dose) is available.
- The high volatility of solvents, e. g., VOCs and the fast biotransformation rate (in the environment and within the human body) for most of the solvents.
- Complex mixtures and numerous sources of environmental exposure.
- Especially for environmental solvent exposure: High-to-low-dose extrapolation for evaluation of adverse health effects may be misleading.
- Confounding factors, e.g., smoking and alcohol consumption, as adverse health effects which may dominate in cases of low solvent exposure.
- Risk in this context is defined in terms of the probability as occurrence of a particular adverse health effect, e. g. 1 in 10^6.
- Finally, as in general risk assessment, definition of a risk level that is acceptable.

20.1.3 CONCLUSIONS

- For solvent exposure at workplaces considerable amount of evidence for adverse health effects has been gathered.
- In this regard, specific and carcinogenic effects in particular have been discussed (see Table 20.1.2 and 20.1.3).
- For environmental solvent exposure only a few examples of adverse health effects have been documented.
- It is rather unlikely that potentially toxic environmental solvent exposures, e. g., benzene or halogenated hydrocarbons, can be prevented in the near future.

- Many suspicions, but only a small amount of scientific data demonstrate a correlation between "environmental diseases", e. g., sick building syndrome and solvent exposure.
- It has been hypothesized that - as a rule - exposure to mixtures of solvents at low non-toxic doses of the individual constituent represents no danger to health.[63]
- There exists overwhelming evidence of adverse health effects caused by accepted environmental noxes such as tobacco smoke and the consumption of alcoholic beverages.

REFERENCES

1 ACGIH, TLV´s and BEI´s, ACGIH, Cincinnati, 1998.
2 DFG, MAK- und BAT-Werte-Liste, VCH, Weinheim, 1999.
3 I. Astrand, *Scand. J. Work Environ. Health*, **1**, 199, 1975.
4 I. Astrand in **Occupational Health Hazards of Solvents**, A. England, K. Ringen, M.A. Mehlman, Eds., *Princeton*, NJ, 1986, pp. 141-142.
5 K.H. Cohr, in **Safety and Health Aspects of Organic Solvents**, V. Riihimäki, U. Ulfvarson, Eds., *Alan R. Liss*, N.Y., 1986, pp. 45-60.
6 J.J.G. Opdam, *Br. J. Ind. Med*., **46**, 831 (1989).
7 M.K. Bahl, *J. Soc. Cosmet. Chem*., **36**, 287 (1985).
8 M. Bird, *Ann. Occup. Hyg.*, **24**, 235 (1981).
9 J. Angerer, E. Lichterbeck, J. Bergerow, S. Jekel, G. Lehnert, *Int. Arch. Occup. Environ. Health*, **62**, 123 (1990).
10 I. Brooke, J. Cocker, I. Delic, M. Payne, K. Jones, N.C. Gregg, D. Dyne, *Ann Occup. Hyg*., **42**, 531 (1998).
11 G. Johanson, *Toxicol. Lett*., **43**, 5 (1988).
12 S. Kezic, K. Mahieu, A.C. Monster, F.A. de Wolff, *Occup. Environ. Med*., **54**, 38 (1997).
13 E.A. Lock, *Crit. Rev. Toxicol*., **19**, 23 (1988).
14 A. Lof, G. Johanson, *Crit. Rev. Toxicol*., **28**, 571 (1998).
15 H.M. Mehendale, *Toxicology*, **105**, 251 (1995).
16 P. Bloch, A. Kulig, M. Paradowski, T. Wybrzak-Wrobel, *Pol. J. Occup. Med*., **3**, 69 (1990).
17 O. Inoue, K. Seiji, T. Watanabe, M. Kasahara, H. Nakatsuka, S.N. Yin, G.L. Li, S.X. Cai, C. Jin, M. Ikeda, *Int. Arch. Occup. Environ. Health*, **60**, 15 (1988).
18 V. Fiserova-Bergerova, *Scand. J. Work Environ. Health*, **11**, 7 (1985).
19 S. Haddad, K. Krishnan, *Environ. Health Perspect*., **106**, 1377 (1998).
20 W.K. Anger in **Neurobehavioral Toxicology**, Z. Annau, Ed., *John Hopkins University Press*, Baltimore, MD, 1986, pp. 331-347.
21 A. Laine, V. Riihimäki in **Safety and Health Aspects of Organic Solvents**, V. Riihimäki, U. Ulfvarson, Eds., *Alan R. Liss*, N.Y., 1986, pp. 123-126.
22 E. Browning, **Toxicity and Metabolism of Industrial Solvents**, *Elsevier Publishing Co*., N. Y., 1965.
23 R.E. Gosselin, R.P. Smith, H.E. Hodge, **Clinical Toxicology of Commercial Products**, *Williams and Wilkins*, Baltimore, 1984.
24 E.L. Baker, *Ann. Rev. Public Health*, **9**, 233 (1988).
25 P. Grasso, M. Sharratt, D. M., Davies, D. Irvine, *Food Chem. Toxicol*., **22**, 819 (1984).
26 R.B. Dick, *Neurotoxicol. Teratol.,* **10**, 39 (1988).
27 W.K. Anger, *Neurotoxicology*, **11**, 627 (1990).
28 P. Arlien-Soborg, L. Hansen, O. Ladefoged, L. Simonsen, *Neurotoxicol. Teratol*., **14**, 81 (1992).
29 WHO, Organic solvents and the central nervous system, WHO European Office Copenhagen, (1985).
30 J.F.Fowler, *Dermatology*, **10**, 216 (1998).
31 K.E. Andersen in **Safety and Health Aspects of Organic Solvents**, V. Riihimäki, U. Ulfvarson, Eds., *Alan R. Liss*, N. Y., 1986, pp. 133-138, 1986.
32 C.G.T. Mathias, *Occup. Med. State of the Art Rev*., **1**, 205 (1986).
33 M. Hodgson, A.E. Heyl, D.H. Van Thiel, *Arch. Intern. Med*., **149**, 1793 (1989):
34 IARC, IARC Monographs on the evaluation of carcinogenic risks to humans. Some organic solvents, resin monomers and related compounds, pigments and occupational exposures in paint manufacture and painting, WHO, 47, IARC, Lyon, 1989.
35 IARC, IARC Monographs on the evaluation of carcinogenic risks to humans. Dry cleaning, some chlorinated solvents and other industrial chemicals, WHO, 63, IARC, Lyon, 1995.

36 IARC, IARC Monographs on the evaluation of carcinogenic risks to humans. Re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide. WHO, 71, IARC, Lyon, 1999.
37 DFG, Gesundheitsschädliche Arbeitsstoffe. Toxikologisch-arbeitsmedizinische Begründungen von MAK-Werten, VCH, Weinheim, 1999.
38 R.R. Lauwerys, A. Bernard, C. Viau, J.P. Buchet, *Scand. J. Work Environ. Health*, **11**, 83 (1985).
39 E.A. Lock, *Crit. Rev. Toxicol*., **19**, 23 (1988).
40 N.A. Nelson, T.G. Robins, F.K. Port, *Am. J. Nephrol*., **10**, 10 (1990).
41 H.J. Mason, A.J. Stevenson, G.M. Bell, *Ren. Fail*., **21**, 413 (1999).
42 O. Ladefoged, H.R. Lam, G. Ostergaard, E.V. Hansen, U. Hass, S.P. Lund, L. Simonsen, *Neurotoxicology*, **19**, 721 (1998).
43 A.M. Seppalainen, *Crit. Rev. Toxicol.*, **18**, 245 (1988).
44 P.S. Spencer, H.H. Schaumburg, *Scand. J. Work Environ. Health*, **11**, 53 (1985).
45 L.H. Welch, S.M. Schrader, T.W. Turner, M.R. Cullen, *Am. J. Ind. Med.*, **14**, 509 (1988).
46 I.J Yu, J.Y. Lee, Y.H. Chung, K.J. Kim, J.H. Han, G.Y. Cha, W.G. Chung, Y.M. Cha, J.D. Park, Y.M. Lee, Y.H. Moon, *Toxicol. Letters*, **109**, 11 (1999).
47 W.G. Chung, I.J. Yu, C.S. Park, K.H. Lee, H.K. Roh, Y.N. Cha, *Toxicol. Letters*, **104**, 143 (1999).
48 J.C.Selner, *Regul. Toxicol. Pharmacol*., **24**, 87 (1996).
49 K.E. Andersen, S.C. Rastogi, L. Carlsen, *Acta Derm. Venereol*., **76**, 136 (1996).
50 G. Mancuso, M. Reggiani, R.M. Berdodini, *Contact Dermatitis*, **34**, 17 (1996).
51 H.H. Tau, M. Tsu Li-Chan, C.L. Goh, *Am. J. Contact. Dermat*., **8**, 210 (1997).
52 T. Chida, T. Uehata, *Sangyo Igaku,* **29**, 358 (1987).
53 P.J. Coenraads, S.C. Foo, W.O. Phoon, K.C. Lun, *Contact Dermatitis*, **12**, 155 (1985).
54 J.T. Lear, A.H. Heagerty, B.B. Tan, A.G. Smith, J.S. English, *Contact Dermatitis*, **35**, 169 (1996).
55 E.L. Sainio, K. Engstrom, M.L. HenriksEckerman, L. Kanerva, *Contact Dermatitis*, **37**, 155 (1997).
56 A.T. Karlberg, A. DoomsGoossens, A., *Contact Dermatitis*, **36**, 201 (1997).
57 D.M. Rubel, S. Freeman, I.A. Southwell, *Australas J. Dermatol*., **39**, 244 (1998).
58 M. McEwan, W.H. McFarlane-Smith, *Clin. Exp. Allergy*, **28**, 332 (1998).
59 G. Angelini, L. Rigano, C. Foti, M. Grandolfo, G.A. Vena, D. Bonamonte, L. Soleo, A.A. Scorpiniti, A.A., *Contact Dermatitis*, **35**, 11 (1996).
60 J. van de Walter, S.A. Jimenez, M.E. Gershwin, *Int. Rev. Immunol*., **12**, 201 (1995).
61 D. Eis, *Allergologie*, **22**, 538 (1999).
62 J.J. Powell, J. Van-de-Water, M.E. Gershwin, *Environ. Health Perspect*., **197**, 667 (1999).
63 F.R. Cassee, *Crit. Rev. Toxicol*., **28**, 73 (1998).
64 **Römpp, Lexikon Chemie**, J. Falbe, M. Regitz, Eds., *Thieme*, Stuttgart (1999).
65 GISBAU, WINGIS, Bau-Berufsgenossenschaften (Professional Associations of the Building Industry in Germany), 1999.
66 U. S. Department of Health and Human Services, National Toxicology Program, The 8th Report on Carcinogens, 1998.

20.2 COGNITIVE AND PSYCHOSOCIAL OUTCOME OF CHRONIC OCCUPATIONAL SOLVENT NEUROTOXICITY

Jenni A Ogden
Department of Psychology, University of Auckland, Auckland, New Zealand

20.2.1 INTRODUCTION

Many organic solvents used in industry are neurotoxic, and may lead to a range of largely irreversible cognitive and psychological or psychiatric impairments in workers who are exposed over long periods of time, or who have had a peak exposure (an episode in which they were briefly exposed to a larger than normal level of solvent). The most vulnerable workers are those who work in the spray painting, boat building, printing, textile, plastic, agricultural and pharmaceutical industries. Often self-employed workers or those in small businesses are more at risk because the safety measures they take are not as closely monitored, and peer pressure to use safety equipment even when it is unwieldy, restrictive or expensive, is unlikely to be as strong as in large workshops. In addition they may be less well educated regarding the neurotoxic effects of the solvents they work with. The great majority of workers diagnosed with OSN are men, presumably because men make up the bulk of the workforce in trades and industries that use neurotoxic solvents.

The chronic, and often slow and insidious effects of occupational solvent neurotoxicity (OSN) include psychological and psychiatric symptoms, impairments in cognitive functioning, and negative psychosocial consequences. The Scandinavian countries are the research leaders in this field, and in recent years health professionals and industries in the United States and other major industrialized countries have become increasingly aware of the debilitating symptoms that can affect workers exposed to neurotoxins over a long time.[1] There have been allegations that OSN is often over-diagnosed by health professionals who are zealous believers, and that a significant number of workers who complain of OSN symptoms are malingering in the hope of obtaining financial compensation.[2] While these allegations almost certainly have some credibility, especially in countries such as the USA, where civil litigation has resulted in large settlements and the existence of OSN is now enshrined in legal precedent,[2] there is ample evidence that the OSN syndrome does exist and is a major health problem for workers in industries that utilize neurotoxic solvents. A number of research studies establishing the existence of OSN have been conducted in countries where there is only limited, if any, financial gain to be made from diagnosing OSN, including Hong Kong[3] and New Zealand.[4]

One of the primary difficulties researchers and health professionals face when trying to ensure that the symptoms they are observing are indeed the result of OSN, lies in the fact that the neurological damage resulting from chronic neurotoxin exposure tends to be diffuse, or may, for example, involve a neurotransmitter imbalance. It is therefore unlikely to be evident on a Computerized Tomograph (CT) or Magnetic Resonance Image (MRI) of the brain. A neurological examination is rarely helpful,[5] and in many cases the psychological and cognitive impairments are the only clear indicators of neurotoxicity. A neuropsychological assessment which utilizes a range of tests to assess cognitive abilities including attention, concentration, psychomotor speed, memory and visuospatial skills, along with a psychological interview or questionnaire assessing depression, irritability, mo-

tivation and fatigue, thus plays a major role in diagnosing chronic OSN.[6] The World Health Organization (WHO) and the Nordic and New Zealand Governments all require that a neuropsychological assessment be used in the diagnosis of solvent neurotoxicity.[7-9]

Many victims of OSN do not realize that their chronic fatigue, irritability, poor memory and other problems may be associated with the solvents in their workplace, and by the time they seek help from their doctor, psychologist or marriage guidance counsellor, the OSN symptoms are likely to be compounded and masked by other work and relationship problems (themselves possibly a consequence of the OSN symptoms).[6] Identification of OSN as the primary cause of the problems is therefore even more difficult, and proving cause and effect usually impossible. That OSN is a significant cause of the person's problems, can, however, often be established beyond reasonable doubt, provided that some guidelines are followed. The individual must clearly have been exposed to neurotoxins over a long period (usually set, rather arbitrarily, at 10 years or more of occupational exposure), or have suffered a peak exposure. Other major contributors to neurological impairment should be excluded (e.g., significant traumatic brain injury, or alcohol addiction), there should be no evidence of malingering, and the pattern of cognitive impairments and psychological symptoms should be typical of OSN.

20.2.2 ACUTE SYMPTOMS OF SOLVENT NEUROTOXICITY

Neurotoxic solvent exposure can result in some workers experiencing nausea, vomiting, loss of appetite, severe headaches, confusion, light-headedness and dermatitis. The solvent may be detectable on their breath and skin for hours and even days after they have left the solvent environment. Most of these symptoms resolve when they stop working with solvents but return when they come into contact with solvents again. Workers who suffer these acute symptoms do not necessarily go on to develop the chronic syndrome of OSN, perhaps in many cases because they are so disabled by the acute symptoms they stop working before irreversible damage occurs. Some workers who suffer acute symptoms do remain in the work environment, sometimes because of financial necessity, or because they do not realize the solvents are the cause of their problems.[10] Some workers who develop a chronic OSN syndrome have suffered from acute symptoms, but others have not. The reason for these individual differences is not clear.

20.2.3 CATEGORIZATION OF OSN

The 1985 International Solvent Workshop[11] proposed three types of OSN, as follows:

- Type 1 OSN: Characterized by subjective complaints of fatigue, irritability, depression and episodes of anxiety. No cognitive impairments are demonstrable on neuropsychological testing, and the psychological symptoms resolve on removal from the solvents. This is also known as the organic affective syndrome, or the neurasthenic syndrome.
- Type 2 OSN: A more severe and chronic form than Type 1 in which many of the symptoms and cognitive impairments are thought to be irreversible when the worker is removed from the solvent environment. It is also known as mild toxic encephalopathy. Type 2 has been divided further into two sub-types based on psychological symptoms (Type 2A) and cognitive impairments (Type 2B). Type 2A sufferers have a range of symptoms which may include sustained personality and mood disturbances, fatigue, poor impulse control and poor motivation. Type 2B symptoms include poor concentration, impairments of new verbal and visual

learning and memory, psychomotor slowing, and in more severe cases, executive (or frontal-lobe) impairments. These can include impoverished verbal fluency, difficulties with abstract thinking, and impairments in the ability to make plans and organize tasks logically. These cognitive symptoms must be demonstrable on neuropsychological tests following a solvent-free period. There is some research which indicates that this separation of Type 2 OSN into psychological and cognitive impairment profiles is largely unrealistic, as most workers with Type 2 OSN have symptoms of both types.[10,12] Type 2 OSN is the primary focus of this section given its largely irreversible nature and its frequency in the workplace.

- Type 3 OSN: This is the most severe form of OSN and signals an irreversible dementia with severe impairment across most cognitive and emotional domains. It is also known as severe toxic encephalopathy, and is fortunately rare in occupational situations. It is more likely to occur in long-term recreational solvent abusers.

20.2.4 ASSESSMENT OF OSN

There have been a few studies reporting specific symptoms caused by a specific solvent. The widely used industrial solvent trichloroethylene (TCE), has, for example, been reported to result in severe agitated depression, sometimes accompanied by violent behaviors towards self and others.[13] Toluene and TCE can cause peripheral neuropathy, and TCE can damage the trigeminal or fifth cranial nerve, resulting in a loss of sensation to the face, mouth and teeth.[1] It is, however, rare to be able to pinpoint a specific solvent as the cause of specific cognitive or psychological symptoms, and most research on occupational solvent neurotoxicity has been carried out on workers exposed to a mixture of solvents. A core neuropsychological battery has been developed by the WHO/Nordic Council,[8] and most other formal and informal batteries developed for the assessment of OSN include a similar range of tests, as these are the tests most sensitive to the common neuropsychological impairments of OSN.[9,14,15,16] Specific tests used in these batteries will not be listed here, as neuropsychologists qualified to administer, score, and interpret these tests can find specialist information in texts written on OSN assessment.[1]

The assessment of OSN may be initiated if a worker receives a poor score on a screening workplace questionnaire designed to assess the frequency of self-reported problems such as irritability and poor memory.[12] In other cases the worker comes to the attention of a health professional because of interpersonal or memory problems which concern the worker, family, or work colleagues. In New Zealand, in 1993 the Occupational Safety and Health Service (OSH) of the Government Department of Labour, established a panel of experts to develop national guidelines for the diagnosis of OSN.[4,9] Workers who are diagnosed as suffering from OSN are registered as part of the Notifiable Occupational Disease System. Other panels provide a similar function for other occupational diseases such as asthma and asbestos-related disorders. Following is a description of the procedures for diagnosing OSN that the New Zealand panel has developed and tested since 1993.[4,9]

Individuals, industries, industrial health workers, or general practitioners can notify a possible case of OSN to the panel. Occupational hygienists then attempt to measure the types and levels of solvents the worker has been potentially exposed to throughout his or her working life. This is easier if the worker is currently in the solvent environment, but estimates only can be made of solvent levels in previous workplaces, and of the workplace and worker's appropriate use of protective equipment over the years. If there is reason to suspect that the worker has been exposed to neurotoxic solvents for 10 years or more, or has suf-

fered peak exposures, the occupational physician will examine and interview the worker (and where possible a close family member) and make an initial assessment regarding the worker's symptoms. It is not uncommon at this interview stage for the worker, often a middle-aged tradesman not accustomed to talking about his cognitive or emotional problems, to break down in tears. Most health professionals experienced in assessing OSN are in no doubt that it is a real syndrome with devastating consequences for the worker and family.[6]

If the symptom complex generally fits with that typical of OSN, the symptoms are significant enough to be causing the worker or his family concern, and other possible causes have been explored and considered to be unlikely as the primary cause of the problems, the worker will proceed to a neuropsychological assessment. Whenever possible, this should take place following two or more weeks away from solvents. This is again a somewhat arbitrary time period, arrived at in an attempt to find a balance between the real time it takes for any acute effects of a mixture and range of solvents to resolve, and the amount of time (usually unpaid) an undiagnosed worker is willing or able to take away from his workplace. The assessment usually commences with a psychological assessment, which may include both an interview and standard questionnaires on mood, fatigue levels, motivation, memory problems in daily life and so on. Often, with the worker's permission, information is also obtained from family members and work colleagues. Not only does this allow an assessment of the problems the worker is experiencing at work and at home, but also gives the neuropsychologist some idea of the time course of these problems. Other possible confounding psychosocial factors are checked out at this point. Whilst factors such as a high use of alcohol, or a series of minor head injuries whilst playing sport 10 years previously, or a recent marriage breakup, may not negate the possibility of the worker being diagnosed as suffering from OSN, clearly these factors must be taken into account in making the diagnosis and the confidence that can be placed in that diagnosis, as well as when designing an intervention or rehabilitation program for the worker.

Having ascertained that the worker's exposure levels and psychological and subjective cognitive symptoms (e.g., complaints of memory problems) meet the criteria for possible OSN, a battery of carefully chosen neuropsychological tests is then given. This is often scheduled for a later session, given the distress that the worker may have expressed during the interview, and the high fatigue levels that are a common consequence of OSN. This battery should include one or more tests which can, along with education and occupational history, provide an estimate of the worker's cognitive ability level prior to working with solvents. Also included should be some tests which one would not expect to be impaired by solvents, such as well-established vocabulary (meanings of words). Tests which are included because of their sensitivity to OSN symptoms include tests of concentration and attention, new verbal and visuospatial learning and memorizing (old, well-established memories are rarely impaired), reaction time, psychomotor speed, and planning, organizational and abstraction abilities.

If the pattern of spared and impaired psychological and neuropsychological test results is typical of OSN, and other factors can be ruled out as the primary cause of this profile, the worker will be diagnosed as having OSN.[6,10] This pattern analysis provides one way of guarding against malingering, as the worker does not know which tests he or she should remain unimpaired on and which are commonly impaired following OSN. In addition, on many tests, it is very difficult or impossible for the malingerer to perform in a way that is consistent with true organic impairment, even if he or she has been coached on how to per-

form poorly on the tests. For example, if an individual was unable to remember any new visual stimuli (an extremely rare condition), when given a memory test where the worker is shown 50 photographs of unknown faces, and is then shown fifty pairs of faces and must choose from each pair the face which he or she has previously seen, he or she should obtain a score of approximately 50% (chance level) correct. If the score was considerably worse than that, malingering or exaggerating might reasonably be suspected. Tests which measure reaction or response times for increasingly complex tasks are also difficult to malinger successfully on as humans are not good at estimating response times in milliseconds, or even seconds.

A diagnosis of Type 2 OSN is based on score deficits (measured by the number of Standard Deviations (SD) below the worker's estimated premorbid ability level) on those tests commonly impaired by OSN. At least three neuropsychological test scores must fall more than 1 SD below the scores expected for that worker to be categorized as mild Type 2 OSN, three test scores below 2 SDs for moderate Type 2 OSN, and three or more test scores more than 3 SDs below the expected levels for moderate-severe Type 2 OSN.[4] The presence and severity of typical psychological symptoms are also taken into account, and in clear cases in which either psychological or cognitive symptoms are very dominant, this information informs a decision regarding Type 2A or Type 2B OSN. Whilst psychological symptoms are the reason most workers come to the attention of health professionals, because of the difficulty of measuring the severity of these symptoms and of attributing them to a neurological syndrome, only workers who demonstrate neuropsychological impairments on testing are positively diagnosed with OSN. The New Zealand experience has, however, demonstrated that the vast majority of workers with significant solvent exposure histories and severe psychological problems do demonstrate neuropsychological impairments, and *vice versa*.[10]

20.2.5 DO THE SYMPTOMS OF TYPE 2 OSN RESOLVE?

Occasionally after an extended period away from solvents (perhaps 6 months to a year), the worker's psychological symptoms resolve, and on re-testing it is found that his or her neuropsychological impairments have also resolved. In these cases the classification is changed to Type 1 OSN (resolved). A recent New Zealand study re-assessed 21 men with confirmed cases of OSN 6 to 41 (mean 27) months after ceasing exposure.[17] An exposure score was calculated for each worker by using the formula AxBxC, where A = years of solvent exposure, B = a weighting for the occupational group (where boat builders, spray painters and floorlayers had the highest weighting of 3), and C = a weighting reflecting the lack of safety precautions taken by the worker relative to other workers in the same job. Neuropsychological and psychological symptoms at the initial and follow-up assessments were categorized as mild, moderate or moderate-severe (using the system described above) by a neuropsychologist blind to the men's initial diagnosis or exposure history. Twelve men (57%) showed no improvement (or in one case a slight worsening) on cognitive and psychological assessment. Seven men showed some improvement on cognitive tests (but not to "normal" levels), only three of whom also improved on psychological assessment. A further two men showed an improvement in psychological functioning only. Men given a more severe OSN diagnosis at their initial assessment were more likely to improve than men with milder symptoms at the time of their first assessment. Possible explanations for this include the likelihood that some of the more severe symptoms on initial assessment were exacerbated by the lingering effects of acute solvent exposure, or that those with mild OSN were

misdiagnosed and their "symptoms" were due to some other cause or were "normal" for them, or that there were psychosocial difficulties present at the first assessment which exacerbated the organic symptoms and resolved with rehabilitation. The disturbing message is, however, that the symptoms of Type 2 OSN are often persistent, and in these cases probably permanent. Even in those individuals where improvement occurs, their symptoms rarely resolve completely.

There was no association between improvement on neuropsychological tests and either time between the two assessments or total time away from solvents. There was no correlation between the exposure score and severity at diagnosis or extent of recovery, and there was no association between a past history of peak exposures and either severity at initial diagnosis or change on neuropsychological assessment. A recent review[18] of studies looking at whether the degree of impairment is related to the dose severity concludes that although several studies have demonstrated significant dose response relationships, there are disturbing inconsistencies, with some studies showing no relationship,[19,20] and one study showing a dose response relationship in painters with levels of exposure considerably lower than the negative studies.[21] Methodological problems and differences and different research populations probably account for these inconsistent findings, and more research is clearly required.

20.2.6 INDIVIDUAL DIFFERENCES IN SUSCEPTIBILITY TO OSN

One possible reason for the inconsistent findings both across and within studies examining the relationship between exposure levels and OSN symptoms, may be that individuals have different susceptibilities to solvents. It is not uncommon to diagnose one worker with moderate Type 2 OSN, yet find no symptoms or serious complaints whatsoever in his workmate who has worked by his side in the same spray painting workshop for twenty years. On closer assessment it may be discovered that the affected worker sustained a number of minor head injuries in his younger football-playing days, or has smoked a marijuana joint every weekend for the past 20 years. Subclinical neuronal damage caused by previous insults, or even by normal aging, may make an individual more susceptible to OSN. Another possibility is that some people are biologically, and even genetically more susceptible to solvents. In this sense, OSN can be likened to the post-concussional syndrome following a mild to moderate traumatic brain injury.[6] Not only are the psychological and neuropsychological symptoms very similar, but for reasons which cannot be explained simply by lifestyle differences or malingering, individuals appear to differ widely regarding their susceptibility to developing a post-concussional syndrome. In illustration of this, a recent study reports varying outcomes from apparently equivalent head injuries in a group of athletes.[22]

20.2.7 PSYCHOSOCIAL CONSEQUENCES OF OSN, AND REHABILITATION

The common psychological and physical symptoms of OSN of fatigue, irritability, depression, sometimes aggression and violence, headaches, and hypersensitivity to noise and alcohol, along with memory difficulties, poor concentration, poor motivation, and slowed thinking, are a recipe for disaster in interpersonal relationships. Thus it is not uncommon for workers to be diagnosed and treated first for a psychiatric disorder (especially clinical depression) and for their marriages to break up, before they are even suspected of having OSN.[6,10] Once OSN is diagnosed, the prospect of losing their job is a grim one for most victims, most of whom are tradesmen in middle age or older who may have difficulty obtaining

or even training for another occupation, especially given their memory, motivation, and concentration problems.

Rehabilitation programmes[6,10] begin with psychoeducation for the worker and his family about the effects of solvents and the importance of protecting himself from exposure in the future. Family members can be taught strategies to reduce the stress on the victim, such as encouraging him to have a rest in the afternoon, and limit his alcohol intake, and by helping him avoid noisy environments such as parties and the family room in the early evening when children are irritable and hungry. Counseling and therapy for the victim and family can be helpful in assisting them to vent their anger at the unfairness of their situation, grieve for their lifestyle and cognitive abilities lost, and come to terms with a "different" person (whose memory may be permanently impaired, and concentration span and motivation lowered). Financial and practical assistance is more often than not of extreme importance, as it is difficult to find the motivation to work on one's psychological and family problems when one is worried about feeding and clothing the children. Antidepressant or anti-anxiety medications may be of assistance in severe cases of mood disorder. In some cases both the neurological damage and the psychological overlay can result in aggressive and violent behaviors not typical of the worker in his younger days. In these cases it is important to first attend to the safety of family members, and then to try and involve the worker in anger management programs, or other therapy with the goal of helping him understand how to control his aggressive or violent behaviors. Similarly, alcohol may be a problem given that it seems likely that neurotoxic solvents damage the pre-frontal lobes, thus resulting in a heightened susceptibility to intoxication. A rehabilitation program aimed at reducing alcohol intake will be important in this case.

Vocational counseling and training are important not only to guide the worker towards a new occupation where solvents are preferably absent, or where protection from solvent exposure is good, but it is also important for the victim's self-esteem and mood. Unfortunately, in many countries where unemployment is high, the prospects of finding a satisfying new career in middle-age are bleak. The task for the rehabilitation therapist in these sad cases is to encourage the worker to take up new hobbies and recreational activities, to spend more quality time with family and friends, and to try and live on a sickness benefit or unemployment benefit without losing self-respect.

REFERENCES

1 D.E.Hartman, **Neuropsychological Toxicology: Identification And Assessment of Human Neurotoxic Syndromes**. 2nd Ed. *Plenum Press*, New York, 1995.

2 P.R.Lees-Haley, and C.W.Williams, *J.Clin.Psychol*., **53**, 699-712 (1997).

3 T.P.Ng, S.G.Ong, W.K.Lam, and G.M.Jones, *Arch.Environ, Health*, **12**, 661-664 (1990).

4. E.W.Dryson, and J.A.Ogden, *N.Z. Med.J.*, **111**, 425-427 (1998).

5 J.Juntunen in *Neurobehavioral Methods In Occupational Health*, R.Gilioli, M.G.Cassitto, and V.Foa, Eds., *Pergamon Press*, Oxford, 1983, pp. 3-10

6 J.A.Ogden, **Fractured Minds. A Case-Study Approach To Clinical Neuropsychology**. *Oxford University Press*, New York, 1996, pp. 174-184; 199-213.

7 World Health Organization and Commission of the European Communities, Environmental Health Document 6: Neurobehavioral Methods In Occupational and Environmental Health: Symposium Report. WHO Regional Office for Europe and Commission of the European Communities, Copenhagen, 1985.

8 World Health Organization, Nordic Council of Ministers, Organic Solvents And The Central Nervous System, EH5, WHO, Copenhagen, 1985.

9 E.W.Dryson, and J.A.Ogden, Chronic Organic Solvent Neurotoxicty: Diagnostic Criteria. Department of Labour, Wellington, 1992.

10 J.A.Ogden, *N.Z. J. Psychol.,* **22**, 82-93 (1993).

11 E.L.Baker, and A.M.Seppalainen, *Neurotoxicology*, **7**, 43-56 (1986).
12 T.L.Pauling, and J.A.Ogden, Int. *J. Occup. Environ. Health*, **2**, 286-293 (1996).
13 R.F.White, R.G.Feldman, and P.H.Travers, *Clin. Neuropharm*., **13**, 392-412 (1990).
14 H.Hanninen in **Neurobehavioral Methods In Occupational Health**, R.Gilioli, M.G.Cassitto, and V.Foa, Eds., *Pergamon Press*, Oxford, 1983, pp. 123-129
15 L.A.Morrow, C.M.Ryan, M.J.Hodgson, and N.Robin, *J Nerv. Ment. Dis*., **179**, 540-545, (1991).
16 C.M.Ryan, L.A.Morrow, E.J. Bromet, *J. Clin. Exp. Neuropsychol*., **9**, 665-679, (1987).
17 E.W.Dryson, and J.A.Ogden, Organic solvent induced chronic toxic encephalopathy: Extent of recovery and associated factors following cessation of exposure. Submitted.
18 S.Mikkelsen, *Environ. Res.*, **73**, 101-112, (1997).
19 J.Hooisma, H.Hanninen, H.H.Emmen, and B.M.Kulig, *Neurotoxicol. Teratol*., **15**, 397-406, (1993).
20 A.Spurgeon, D.C.Glass, I.A.Calvert, M.Cunningham-Hill, and J.M.Harrington, *J. Occup. Environ. Med.*, **51**, 626-630, (1994).
21 M.L.Bleecker, K.I.Bolla, J.Agnew, B.S.Schwartz, and D.P.Ford, *Am. J. Ind. Med.*, **19**, 715-728, (1991).
22 S.N.Macciocchi, J.T.Barth, and L.M.Littlefield, *Clin. Sports Med.*, **17**, 27-36, (1998).

20.3 PREGNANCY OUTCOME FOLLOWING MATERNAL ORGANIC SOLVENT EXPOSURE

Kristen I. McMartin and Gideon Koren
The Motherisk Program, Division of Clinical Pharmacology and Toxicology, Hospital for Sick Children, Toronto, Canada

20.3.1 INTRODUCTION

Organic solvents are a structurally diverse group of low molecular weight liquids that are able to dissolve other organic substances.[1] Chemicals in the solvent class include aliphatic hydrocarbons, aromatic hydrocarbons, halogenated hydrocarbons, aliphatic alcohols, glycols, and glycol ethers. Fuels are a mixture of various hydrocarbons. They are generally ubiquitous in industrialized society, both at work and at the home. They may be encountered as individual agents or in complex mixtures such as gasoline. Incidental exposures may include vapors from gasoline, lighter fluid, spot removers, aerosol sprays and paints. These short duration and low level exposures may often go undetected. More serious exposures occur mainly in the industrial or laboratory settings during manufacturing and processing operations such as dry cleaning, regular working with paint removers, thinners, floor and tile cleaners, glue and as laboratory reagents. Gasoline sniffing or glue sniffing, albeit not occurring in the occupational setting, is another source of exposure to organic solvents during pregnancy.

Counseling pregnant women who are occupationally exposed to numerous chemicals (mostly organic solvents) is difficult because it is hard to estimate the predominant chemicals and their by-products. Even after identifying the more toxic agents, it is still difficult to assess the circumstances of exposure as for many chemicals one can measure neither airborne nor blood levels. Smelling the odor of organic solvents is not indicative of a significant exposure as the olfactory nerve can detect levels as low as several parts per million which are not necessarily associated with toxicity. As an example, the odor threshold of toluene is 0.8 parts per million whereas the TLV-TWA (threshold limit value-time weighted average) is 50 parts per million. In addition, reproductive information on many individual solvents is at best sparse, either limited to animal studies or nonexistent.

Many organic solvents are teratogenic and embryotoxic in laboratory animals depending on the specific solvent, dose, route of administration and particular animal species.[1] The various malformations described include hydrocephaly, exencephaly, skeletal defects, cardiovascular abnormalities and blood changes. Also, some studies suggest poor fetal development and neurodevelopmental deficits. In a portion of these studies exposure levels were high enough to induce maternal toxicity.

Organic solvents are a diverse, complex group and because exposure usually involves more than one agent and different circumstances, adequate human epidemiological studies are difficult to interpret. Many studies are subject to recall and response bias and are not always controlled for other risk factors such as age, smoking, ethanol, and concurrent drug ingestion. It is hard to prove or quantify the suspicion that organic solvents are a reproductive hazard. One may even expect that a ubiquitous exposure to solvents would by chance alone be associated with an increase in birth defects or spontaneous abortions, which may differ from one study to another. While fetal toxicity is biologically sensible in cases of intoxicated mothers, evidence of fetal damage from levels that are not toxic to the mother is scanty, inconsistent or missing.

This chapter will review the reproductive toxicology of organic solvents with particular focus on exposure during pregnancy. Firstly, examples of animal studies with regard to three organic solvents will be discussed. This will be followed by information obtained from human studies including: a meta-analysis of pregnancy outcome following maternal organic solvent exposure; results from the first prospective study by the Motherisk Program at the Hospital for Sick Children on gestational exposure during pregnancy; and finally, a proactive approach for the evaluation of fetal safety in chemical industries.

20.3.2 ANIMAL STUDIES

There are numerous experimental studies that examine the reproductive effects of organic solvents in animals. The reproductive effects of maternal organic solvent exposure will be summarized using three organic solvents as examples. The solvents discussed will be benzene, toluene and tetrachloroethylene.

Benzene

Watanabe and Yoshida[2] were the first to claim teratogenic effects of benzene after administration during organogenesis only. Groups of 15 mice were given single subcutaneous injections of 3 ml benzene/kg on one of days 11-15 of pregnancy. This dose caused leukopenia lasting 24-48 hours but had no effect on body weight in the dams. Litter size ranged form a average of 6.5-8.5 in the 4 treatment groups. Malformations were seen in most treated groups; cleft palate occurred in 5.5% of fetuses exposed on day 13 and in 1.0% of fetuses exposed on day 14 and agnathia or micrognathia was seen on 0.9%, 2.4% and 1.0% of fetuses exposed on days 11, 13 and 14 respectively. Extra 14th ribs were seen in 10-16% of fetuses in all treated groups. Fetuses from 5 dams treated on day 15 had no malformations but 24% had extra 14th ribs. In the absence of any control data it is not known if these represent significant increases in malformations and anomaly rates. Extra 14th ribs for example, can be a common skeletal variant in some strains of mice and rats.[9]

Matsumoto et al.[3] have given groups of 8-11 mice subcutaneous injections of 0, 2, or 4 ml of benzene/kg on days 8 and 9 or 12 and 13 of pregnancy. Fetuses were examined externally and for skeletal defects only; internal soft tissues were not examined. They claim that fetal weight was significantly decreased in both groups given 4 ml/kg and placental weight significantly reduced in those given 4 ml/kg on days 12 and 13 of pregnancy. However, re-

working of the data shows p values of >0.4 in all cases.[9] Sporadic malformations (cleft palate and open eye) did not differ significantly between treated and control groups, neither did the incidence of dead or resorbed embryos and fetuses. A small degree of retarded ossification was seen in fetuses from dams given 4 ml/kg.

Nawrot and Staples[4] investigated the effects of oral administration by gavage of 0.3, 0.5 or 1.0 ml/kg on days 6-15 of pregnancy or 1.0 ml/kg on days 12-15 of pregnancy in the mouse. After dosing on days 6-15, 0.5 and 1.0 ml/kg caused some maternal mortality and embryolethality. Fetal weight was significantly reduced at all 3 dose levels but no increase in malformations was seen. There were similar findings after dosing on days 12-15 except that resorptions occurred later in gestation. The study is reported in abstract only and no further details are given.

Murray et al.[5] exposed groups of 35-37 mice to 0 or 500 ppm benzene for 7 hr/day on days 6-15 of pregnancy. Acceptable teratological methods were used.[9] There was no evidence of maternal toxicity. There were no effects on implants/dam, live fetuses/dam, resorptions/dam or malformation rates. Fetal body weight was significantly reduced and delayed ossification significantly increased in fetuses from the benzene group.

Iwanaga et al.[6] demonstrated an increased postnatal susceptibility to benzene toxicity in mice exposed prenatally to benzene by injection of the dams with 4 ml benzene/kg on day 9 or 12 of gestation. At 10 weeks of age the offspring were injected with 5 daily doses of 0.1 ml benzene/kg and the effects on erythrocytes, leukocytes, body weight, thymus and spleen were more marked than in non-prenatally exposed controls.

There have been several inhalational studies on benzene in the rat. In an unpublished study summarized by Murray et al.,[5] teratogenic effects were observed at 500 ppm when rats were exposed to 0, 10, 50 or 500 ppm benzene for 7 hr/day on days 6-15 of pregnancy and a low incidence of exencephaly, kinked ribs and abnormal ossification of the forepaws was noted at 500 ppm. In another unpublished study quoted by Murray et al.[5] no teratogenicity but increased embryoloethality was seen after exposure to 10 or 40 ppm for 6 hours/day on days 6-15 of pregnancy in the rat.

Hudak and Ungvary[7] exposed groups of 19-26 rats to 0 or 313 ppm benzene for 24 hours/day on days 9-14 of pregnancy. Acceptable teratological methods were used.[9] There was no maternal mortality but maternal weight gain was significantly reduced. There were no significant effects on live fetuses/dam, resorbed or dead fetuses/dam or malformation rate. Mean fetal weight was significantly reduced and retarded ossification, abnormal fusion of sternebrae and extra ribs were all significantly increased in the benzene-exposed group.

Green et al.[8] exposed groups of 14-18 rats to 100, 300 or 2200 ppm benzene for 6 hours/day on days 6-15 of pregnancy, each benzene-exposed group having a concurrent 0 ppm control group. Maternal weight gain was significantly reduced in the 2200 ppm group, but not at lower exposure levels. There were no significant effects on implants/dam, live fetuses/dam, resorptions/dam or malformation rates. There was a significant 10% reduction in fetal weight in the 2200 ppm benzene group and skeletal anomalies were sporadically increased in benzene-exposed groups (missing sternebrae at 100 ppm, delayed ossification of sternebrae in female offspring only at 300 ppm and 2200 ppm and missing sternebrae at 2200 ppm). The authors suggest the higher number of affected female fetuses is in accordance with other observations on the increased susceptibility of females to benzene toxic-

ity.[9] In addition, they observed a non-significant low incidence of hemorrhages in all 3 benzene-exposed groups which were not seen in control fetuses.

In conclusion, embryolethal and teratogenic effects are not seen even at maternally toxic doses but significant fetotoxicity in terms of reduced body weight sometimes accompanied by increases in skeletal variants and delayed ossification is seen at doses which are not necessarily toxic to the dam. The absence of any such effects in a large number of adequately conducted studies reported in full suggests these observations may be of no biological significance. The role that benzene-induced maternal anemia may play in any adverse effects on the offspring is not known.[9]

Toluene

Euler[10] exposed mice to a mixture of toluene and trichloroethylene similar to that which has been used in the soling of shoes. The mixture was composed of 32 ppm (120 mg/m^3) toluene and 64 ppm (340 mg/m^3) trichloroethylene, equivalent to inhaling 157 mg/kg toluene and 406 mg/kg trichloroethylene in the mice. They inhaled the mixture for 10 days before mating or during part or the whole of pregnancy. Differences were noted between treated and control groups in pregnancy rates, length of pregnancy, damaged embryos, birth weights and neonatal mortality but the direction and magnitude of these differences is not stated. No groups were exposed to toluene alone.

Nawrot and Staples[4] gave mice 0.3, 0.5, or 1.0 ml toluene/kg orally by gavage on days 6-15 of pregnancy or 1.0 ml/kg on days 12-15 of pregnancy. There was no maternal toxicity except a decrease in maternal weight gain in those dosed on days 12-15. There was a significant increase in embrylolethality at all 3 dose levels and a significant reduction in fetal weight in the 0.5 and 1.0 ml/kg groups after dosing on days 6-15. Those dosed with 1.0 ml/kg on days 6-15 had a significant increase in numbers of fetuses with cleft palate which was not simply due to general growth retardation. Treatment on days 12-15 only had no adverse effects on the offspring. The study is reported in abstract only and no further details are given.

Teratological investigations on inhaled toluene in mice and rats have been carried out by Hudak et al.[7] Mice were exposed to 0, 133 or 399 ppm (500 or 1500 mg/m^3) toluene for 24 hr/day on days 6-13 of pregnancy. In the high dose group all 15 exposed dams died within the first 24 hr of exposure. No maternal deaths occurred in the 11 mice exposed to 133 ppm and there were no effects on implants/dam, live fetuses/dam, dead and resorbed fetuses/dam, malformations or anomaly rates, but fetal weight was significantly reduced by 10% in comparison with controls. It is not stated whether 133 ppm had any effect on maternal weight gain.[7]

In conclusion, similar to benzene, toluene does not appear to be teratogenic. It is fetotoxic, causing a reduction in fetal weight in mice and rats and retarded ossification and some increase in skeletal anomalies in rats at doses that are below those toxic to the dam as well as at toxic doses.[9] Embryolethality has also been seen with inhalation of very high concentrations lethal to some of the dams or following oral administration of non-toxic doses.[9]

Tetrachloroethylene

Schwetz et al.[11] exposed rats and mice to 300 ppm tetrachloroethylene for 7 h/day on days 6-15 of pregnancy. The dams were killed just before term and the fetuses examined by acceptable teratological methods but results are given on a per litter basis only. The number of treated animals in each case was 17 and the number of controls (air exposed) 30 for both rat and mouse studies.

Effects of tetrachloroethylene on the dams varied between species.[11] In the mouse relative liver weight was significantly increased and the absolute liver weight increased but not significantly and with no effect on maternal body weight. In the rat there was a non-significant decrease in absolute and relative liver weights and a significant 4-5% decrease in mean body weight. Food consumption was unaffected.

Effects on the embryo and fetus also differed.[11] In the mouse there was no effect on implantation sites, live fetuses or resorption rates but mean fetal weight was significantly reduced, 59% of litters containing runts (weight less than 3 standard deviations below the mean) compared with 38% of control litters. Whereas in the rat, resorption rate was significantly increased from 4% in controls to 9% in the exposed group, while fetal body was unaffected (mean slightly higher than controls).

In the mouse, examination for anomalies revealed an increase in delayed ossification of the skull bones (significant) and of the sternebrae (nonsignificant) as might be expected from the fetal weight data. There were also significant increases in the incidence of split sterenbrae and subcutaneous edema. No gross malformations were found. In the rat, gross malformations (short tail) were reported but the incidence did not differ significantly from that in controls. There were no other significant differences in soft tissue or skeletal abnormalities.[11]

The results of this study are difficult to assess, partly because no indication of the numbers of fetuses affected within affected litters is given and partly because of the uncertain nature of the "subcutaneous edema" reported.[9,11] Exposure to tetrachloroethylene and the concurrent controls were part of a large study on four different solvents. The incidence of subcutaneous edema in the mouse ranged from 8-59% of litters affected which seems very high and while the incidence in the tetrachloroethylene group was highest at 59%, it was as high as 45% in nonconcurrent controls (27% in concurrent controls).[11] In the rat, the incidence of this particular anomaly also varied enormously between groups from 0% (tetrachloroethylene group) to 28% (trichloroethylene group).[11] It is therefore important to know how strict were the criteria for designation of "subcutaneous edema" and in particular whether the designation was made before or after fixing, subcutaneous edema being a common fixative artifact.[9] However, the retardation of growth and ossification and the increased incidence of split sternebrae in fetal mice exposed to tetrachloroethylene were clear effects and in the absence of any effect on maternal body weight, suggest that tetrachloroethylene has some maternal hepatotoxicity but has no effect in the rat where there is no hepatotoxicity at 300 ppm.[11]

The results of a behavioral teratology study in the rat by Nelson et al. have been reported.[12] Rats were exposed to 0 or 900 ppm tetrachloroethylene for 7 hours/day on days 7-13 or 14-20 of pregnancy (9-16 rats per group). The dams were affected by this level, showing reduced food consumption and lower weight gain during exposure but histopathological examination of the maternal liver and kidney in dams sacrificed on day 21 of pregnancy revealed no abnormalities.[12]

Postnatally, offspring were tested for olfaction, neuromuscular ability, exploratory and circadian activity, aversive and appetitive learning.[12] There was evidence of impaired neuromuscular ability.[12] Offspring from dams exposed on days 7-13 were poorer than controls in ascent of a wire mesh screen during the second week of life and were poorer than controls on a rotorod test on one of the 3 days tested in the fourth week of life.[12] Offspring from dams exposed on days 14-20 performed less well in ascent of a wire mesh screen.

However, the latter group were consistently superior to controls on the rotorod later in development.[12] Both exposed groups were generally more active in open field tests than controls but only those exposed on days 14-20 of gestation differed significantly from controls.[12] Biochemical analyses of whole brain neurotransmitter levels showed no effects in newborns but significant reductions in acetylcholine levels at 21 days of age in both exposed groups of offspring and reduced dopamine levels at 21 days of age in those from dams exposed on days 7-13.[12] There were no significant differences between exposed and control groups on any other of the tests.[12] Exposure of offspring to 100 ppm on days 14-20 of gestation showed no significant differences from controls on any of the above behavioral tests.[12] It was not stated whether neurotransmitter levels were measured in this low-dose group.[9,12]

In view of these results, suggesting some fetotoxicity in the mouse but not the rat at 300 ppm and postnatal effects in the rat at 900 ppm but not 100 ppm, there is a need for further studies at low levels between 900 and 100 ppm to establish a more accurate no-effect-level.[9]

20.3.3 PREGNANCY OUTCOME FOLLOWING MATERNAL ORGANIC SOLVENT EXPOSURE: A META-ANALYSIS OF EPIDEMIOLOGIC STUDIES

[Adapted, by permission, from K.I. McMartin, M. Chu, E. Kopecky, T.R. Einarson and G. Koren, *Am. J. Ind. Med*., 34, 288 (1998) Copyright 1998 *John Wiley & Sons, Inc*. Reprinted by permission of Wiley-Liss, Inc. a division of John Wiley & Sons, Inc.]

Introduction

Evidence of fetal damage or demise from organic solvent levels that are not toxic to the pregnant woman is inconsistent in the medical literature. A mathematical method has been previously developed and utilized to help overcome bias and arrive at a single overall value that describes the exposure-outcome relationship; namely, meta-analysis.[15]

The risk for major malformations and spontaneous abortion from maternal inhalational organic solvent exposure during pregnancy is summarized using meta-analysis.[31] Besides being more objective than the traditional methods of literature review, it has the ability to pool research results from various studies thereby increasing the statistical strength/power of the analysis. This is especially useful in epidemiologic studies, such as cohort studies or case control studies since very often large numbers of subjects are required in order for any problem to be significantly addressed. This is particularly true for teratogenic studies where the frequencies of malformation are often very low.

Methods

A literature search was conducted to collect studies for the meta-analysis. Using Medline, Toxline and Dissertation Abstracts databases spanning 1966-1994, literature was identified concerning the problem in question. In addition, external colleagues were consulted (regarding unpublished studies) whose area of interest is in occupational exposure and reproductive toxicology. All references from the extracted papers and case reports were investigated. Standard textbooks containing summaries of teratogenicity data were consulted for further undetected references.

Inclusion criteria consisted of human studies of any language which were 1) case control or cohort study in design; 2) included maternal inhalational, occupational, organic solvent exposure; 3) had an outcome of major malformation and/or spontaneous abortion; and 4) included first trimester pregnancy exposure. Exclusion criteria consisted of animal studies, non-inhalational exposure, case reports, letters, editorials, review articles and studies

that did not permit extraction of data. For subgroup analysis, we also identified and analyzed cohort and case-control studies specifically involving solvent exposure. Major malformations were defined as malformations which were either potentially life threatening or a major cosmetic defect.[13] Spontaneous abortion was defined as the spontaneous termination of pregnancy before 20 weeks gestation based upon the date of the first day of the last normal menses.[14]

To obtain an estimate of the risk ratio for major malformations and spontaneous abortion in exposed versus unexposed infants, an overall summary odds ratio (ORs) was calculated according to the protocol established by Einarson et al.[15] Additionally, homogeneity of the included studies, power analysis and the extent of publication bias were also examined as described by Einarson et al.[15]

Results and discussion

The literature search yielded 559 articles. Of these, 549 in total were rejected for various reasons. The types of papers rejected were: animal studies (298), case reports/series (28), review articles (58), editorials (13), duplicate articles (10), not relevant (62), malformation not specified (29), spontaneous abortion not defined (31), unable to extract data (4), no indication of timing of exposure (16). Five papers were included into the major malformation analysis (Table 20.3.1) and 5 papers were included into the spontaneous abortion analysis (Table 20.3.2).

Table 20.3.1. Studies of teratogenicity of organic solvents meeting criteria for meta-analysis [Adapted, by permission, from K.I. McMartin, M. Chu, E. Kopecky, T.R. Einarson and G. Koren, *Am. J. Ind. Med.*, 34, 288 (1998) Copyright 1998 *John Wiley & Sons, Inc.* Reprinted by permission of Wiley-Liss, Inc. a division of John Wiley & Sons, Inc.]

Authors	Study type	Data collection	Malformation described
Axelsson et al.[16]	C	R	"serious malformations"
Tikkanen et al.[17]	CC	R	cardiac malformations
Holmberg et al.[18]	CC	R	CNS, oral clefts, musculoskeletal, cardiac defects
Cordier et al.[19]	CC	R	"major malformations"
Lemasters[20]	C	R	"major malformations"

CC=Case control; C=Cohort; R=Retrospective

A. Malformations

In total 5 studies describing results from organic solvent exposure were identified (Table 20.3.3). The summary odds ratio obtained was 1.64 (95% CI: 1.16 - 2.30) which indicates that maternal inhalational occupational exposure to organic solvents is associated with an increased risk for major malformations. The test for homogeneity yielded a chi square of 2.98 (df=4, p=0.56). When studies were analyzed separately according to study type, the chi square value from the test for homogeneity of effect for cohort studies was 0.52 (df=1, p=0.47) and for case control studies it was 0.01 (df=2, p=0.99). Their combinability remains justified on the basis of the lack of finding heterogeneity among the results.

Meta-analysis of both the cohort studies and case-control studies produced similar results, i.e., they demonstrate a statistically significant relationship between organic solvent exposure in the first trimester of pregnancy and fetal malformation. The summary odds ratio

for cohort studies was 1.73 (95% CI: 0.74 - 4.08) and 1.62 (95% CI: 1.12 - 2.35) for case-control studies.

Table 20.3.2. Studies of spontaneous abortion of organic solvents meeting criteria for meta-analysis. [Adapted, by permission, from K.I. McMartin, M. Chu, E. Kopecky, T.R. Einarson and G. Koren, *Am. J. Ind. Med.*, 34, 288 (1998) Copyright 1998 *John Wiley & Sons, Inc.* Reprinted by permission of Wiley-Liss, Inc. a division of John Wiley & Sons, Inc.]

Authors	Study type	Data collection
Windham et al.[21]	CC	R
Lipscomb et al.[22]	C	R
Shenker et al.[23]	C	P
Pinney[24]	C	R
Eskenazi et al.[25]	C	P

CC=Case control, C=Cohort, R=Retrospective, P=Prospective

Table 20.3.3. Results of studies comparing outcomes of fetuses exposed or not exposed to organic solvents. [Adapted, by permission, from K.I. McMartin, M. Chu, E. Kopecky, T.R. Einarson and G. Koren, *Am. J. Ind. Med.*, 34, 288 (1998) Copyright 1998 *John Wiley & Sons, Inc*. Reprinted by permission of Wiley-Liss, Inc. a division of John Wiley & Sons, Inc.]

Reference	Exposure		Congenital Defect		
			Yes	No	Total
Axelsson et al.[16]	organic solvents	yes	3	489	492
		no	4	492	496
		total	7	981	988
Tikkanen et al.[17]	organic solvents	yes	23	26	49
		no	546	1026	1572
		total	569	1052	1621
Holmberg et al.[18]	organic solvents	yes	11	7	18
		no	1464	1438	2902
		total	1475	1475	2950
Cordier et al.[19]	organic solvents	yes	29	22	51
		no	234	285	519
		total	263	307	570
Lemasters[20]	styrene	yes	4	68	72
		no	13	822	835
		total	17	890	907
TOTAL		yes	70	612	682
		no	2261	4100	6354
		total	2331	4712	7036

In this meta-analysis, major malformations were defined as "potentially life threatening or a major cosmetic defect".[13] In the general population there is a 1-3% baseline risk for major malformations. Estimate incidence via cohort studies indicated 2 studies with a total of 7 malformations in 564 exposures or 1.2% rate of malformations which falls within the baseline risk for major malformations.

Publication bias is the tendency for statistically significant studies to be submitted and accepted for publication in preference to studies that do not produce statistical significance.[15] This may be the case for solvent exposure and major malformations. Determining the extent of possible publication bias (file drawer analysis) is not unlike power analysis for nonsignificant results. Each provides some quantitative measure of the magnitude of the findings with respect to disproving them and requires judgment for interpretation. In order to perform a file drawer analysis effect sizes must be calculated from the summary statistic. Effect sizes represent the magnitude of the relationship between two variables. Unlike statistical significance, which is directly related to sample size, an effect size may be thought of as significance without the influence of sample size. In other words, effect size represents the "true" impact of an intervention. Cohen has determined that an effect size d=0.2 is considered small, 0.5 is medium and 0.8 is large.[15]

The result from this file drawer analysis indicates that one would have to obtain 2 articles with a small effect size (d=0.001) to bring the study's overall effect size (d=0.071) to a smaller effect size of 0.05. One of the acceptable studies achieved such a small effect size. The smallest effect size was d=0.000682.[16] It would therefore seem probable to have some studies stored away in file drawers with very small effect sizes (lack of statistical significance). Unfortunately, no statistical test yet exists to precisely determine such a probability and one must therefore exercise judgment.

There are some considerations to bear in mind when interpreting results of this meta-analysis:

1. Environmental exposure in pregnancy is seldom an isolated phenomenon, therefore, analysis of human teratogenicity data may require stratification for a number of factors depending on the intended focus of the analysis.

2. Organic solvents belong to many classes of chemicals. Not all of the studies have examined the exact same groups of solvents in terms of both extent and range of solvents as well as frequency and duration of exposure.

3. The malformations listed in each of the papers seems to reflect a diverse range of anomalies. One might expect to notice a particular trend in malformations between studies, however, this does not appear to be the case.

Certain factors should be kept in mind when evaluating the results such that a number of studies were case control in design. Certain factors inherent in this study design may affect the interpretation of their results, including recall of events during pregnancy, selection of samples based on volunteer reporting and a change in the knowledge over time regarding factors considered to significantly affect the fetus. Mothers of malformed children may understandably report exposure more often than mothers of healthy children. The recall of the exact name of the chemical, amount of exposure, starting and stopping date of exposure are also difficult to establish retrospectively. Recall may be affected by the method of questioning; when asked open ended questions, women may not recall details as well as when questioned with respect to specific chemical exposure. As a result, there could be systematic bias toward reporting exposure.

It is important to consider the criteria or "proof" for human teratogenicity as established by Shepard:[26]

1. Proven exposure to agent at critical time(s) in prenatal development. One of the inclusion criteria for this meta-analysis, with malformations as the outcome of exposure, was first trimester exposure to organic solvents.

2. Consistent findings by two or more epidemiologic studies of high quality including: control of confounding factors, sufficient numbers, exclusion of positive and negative bias factors, prospective studies if possible, and studies with a relative risk of six or more.

When this happens it is unlikely that methodological problems or systematic biases can influence the results of the studies conducted in different contexts and different study designs. The studies included in this meta-analysis usually controlled for such items as geographical location and date of birth, however, other potential confounding factors such as maternal age, alcohol, and smoking that could lead to subsequent problems in outcome presentation were not consistently reported.

In addition, this meta-analysis included studies that were contained within large databases spanning many years. The majority of information about occupational exposure in general during pregnancy originates from Scandinavia, namely, the Institute of Occupational Health in Helsinki. For example, Finland monitors spontaneous abortions through the spontaneous abortion registry. The registry contains all information about women who were hospitalized with spontaneous abortions covering approximately 90% of all spontaneous abortions in Finland. Finland also monitors births via the Finnish Register of Congenital Malformations. All new mothers in Finland are interviewed during their first prenatal visit, at 3 months post-delivery, at Maternity Care Centers located in every province throughout Finland.

When scanning the literature, there are no studies that prospectively examine occupational exposure to organic solvents during pregnancy and pregnancy outcome with regard to malformations. The studies are retrospective, either case-control or cohort in design. In contrast, however, there are a number of studies that prospectively examine occupational exposure during pregnancy and pregnancy outcome with regard to spontaneous abortion.

In all the studies there was an attempt to ascertain the occupational exposure by an industrial hygienist who blindly assessed the group exposure information. In addition, the individual studies included in the meta-analysis did not obtain an odds ratio or relative risk of 6.0 or more with a significant 95% confidence interval. The larger the value of the relative risk, the less likely the association is to be spurious. If the association between a teratogen is weak and the relative risk small (i.e., range 1.1-2.0), it is possible to think that the association is indeed due to unknown confounding factors and not to the teratogen under study. However, weak associations may be due to misclassification of exposure or disease. They may also indicate an overall low risk but the presence of a special subgroup at risk of teratogenesis within the exposed group.

3. Careful delineation of the clinical cases. A specific defect or syndrome, if present, is very helpful. If the teratogen is associated only to one or a few specific birth defects, the possibility of a spurious association becomes smaller. In this meta-analysis, the malformations were variable with no specific trend apparent.

4. Rare environmental exposure associated with rare defect.

5. Teratogenicity in experimental animals important but not essential.

6. The association should make biologic sense.

When a chemical or any other environmental factor caused a malformation in the experimental animals and/or the biological mechanism is understood, the observation of an association in humans becomes more plausible. Although the statistical association must be present before any relationship can be said to exist, only biological plausible associations can result in "biological significance".

The mechanisms by which many solvents exert their toxicity are unclear and may vary from one solvent to another. Halogenated hydrocarbons such as carbon tetrachloride may generate free radicals.[27] Simple aromatic compounds such as benzene may disrupt polyribosomes, whereas some solvents are thought to affect lipid membranes and to penetrate tissues such as the brain.[27]

In 1979 a syndrome of anomalies (hypertonia, scaphocephaly, mental retardation and other CNS effects) was suggested in two children in a small American Indian community where gasoline sniffing and alcohol abuse are common.[28] Four other children had similar abnormalities, however, in these cases it was impossible to verify gasoline sniffing. Also, it is unclear what was the contribution of the lead in the gasoline or the alcohol abuse in producing these abnormalities. It is important to remember that the mothers in many of these cases showed signs of solvent toxicity indicating heavy exposure. This is not the case in most occupational exposures during pregnancy. While fetal toxicity is biologically sensible in cases of intoxicated mothers, the evidence of fetal damage from levels that are not toxic to the mother is scanty and inconsistent.

7. Proof in an experimental system that the agent acts in an unaltered state.

8. Important information for prevention.

Several lists of criteria for human teratogenicity have included the dose (or concentration) response relationship.[1] Although a dose response may be considered essential in establishing teratogenicity in animals it is extremely uncommon to have sufficient data in human studies. Another criterion which is comforting to have but not very often fulfilled is biologic plausibility for the cause. Shepard states that at present there is no biologically plausible explanation for thalidomide embryopathy and that at least one half of all human teratogens do not fit this criterion.[26]

B. Spontaneous abortion

Estimates for clinically recognized spontaneous abortions as a proportion of all pregnancies vary markedly. In ten descriptive studies reviewed by Axelsson,[29] the proportion of spontaneous abortions varied from 9% to 15% in different populations. The variation depended not only on the characteristics of the population but on the methods used in the study, i.e., the selection of the study population, the source of pregnancy data, the definition of spontaneous abortion, the occurrence of induced abortions and their inclusion or otherwise in the data. The weaknesses of the studies using interviews or questionnaires pertain to the possibility of differential recognition and recall (or reporting) of spontaneous abortions and of differential response. Both exposure and the outcome of pregnancy may influence the willingness of subjects to respond to a study. One advantage of interview data is that it is more likely to provide information on early spontaneous abortion than medical records. However, the validity of information on early abortion which may be difficult to distinguish from a skipped or delayed menstruation has been suspect. Spontaneous abortions which have come to medical attention are probably better defined than self-reported abortions.

The feasibility of using medical records as a source of data depends on the pattern of use of medical facilities in the community and the coverage and correctness of the records.

Of concern is the potential selection bias due to differing patterns of use of medical services. The primary determinant for seeking medical care is probably gestational age so that earlier abortions are less likely to be medically recorded than later abortions.[29] The advantage of data on medically diagnosed spontaneous abortions, compared to interview data is that the former are independent of an individuals own definition, recognition and reporting.

In total, 5 papers describing results from organic solvent exposure were identified (Table 20.3.4). The summary odds ratio obtained was 1.25 (95% CI: 0.99 - 1.58). The test for homogeneity yielded a chi square=4.88 (df=4, p=0.300). When studies were analyzed separately according to study type, the chi-square value for homogeneity of effect for cohort studies was 4.20 (df=3, p=0.241). Meta-analysis of both cohort and case-control studies produced similar results, i.e., they do not demonstrate a statistically significant relationship between organic solvent exposure in pregnancy and spontaneous abortion. The summary odds ratio for cohort studies was 1.39 (95% CI: 0.95 - 2.04) and 1.17 (95% CI: 0.87 - 1.58) for case control studies. Their combinability seems justified on the basis of the lack of finding heterogeneity among the results.

Table 20.3.4. Results of studies comparing outcomes of fetuses exposed or not exposed to organic solvents. [Adapted, by permission, from K.I. McMartin, M. Chu, E. Kopecky, T.R. Einarson and G. Koren, *Am. J. Ind. Med.*, 34, 288 (1998) Copyright 1998 *John Wiley & Sons, Inc.* Reprinted by permission of Wiley-Liss, Inc. a division of John Wiley & Sons, Inc.]

Reference	Exposure		Spontaneous Abortion		
			yes	no	total
Windham et al.[21]	any solvent product	yes	89	160	249
		no	272	575	847
		total	361	735	1096
Lipscomb et al.[22]	organic solvent	yes	10	39	49
		no	87	854	941
		total	97	893	990
Schenker et al.[23]	organic solvents	yes	12	8	20
		no	16	21	37
		total	28	29	57
Pinney[24]	organic solvents	yes	35	228	263
		no	25	166	191
		total	60	394	454
Eskenazi et al.[25]	organic solvents	yes	4	97	101
		no	7	194	201
		total	11	291	302
TOTAL		yes	150	532	682
		no	407	1810	2217
		total	557	2342	2899

The overall ORs of 1.25 indicates that maternal inhalational occupational exposure to organic solvents is associated with a tendency towards a small increased risk for spontaneous abortion. The addition of one study of similar effect size would have rendered this trend statistically significant.

Traditionally, a power analysis would be conducted to determine the number of subjects or in this situation the number of "studies" that need to be added to produce a significant result. In order to perform a power analysis effect sizes must be calculated from the summary statistic. The result from this power analysis indicates that one would have to obtain 2 studies with a medium effect size (0.5) to bring this study's overall effect size (d=0.095) to a small effect size of 0.2. Similarly, 5 articles with an effect size of d=0.3 are needed to bring the study's overall effect size to 0.2. The largest effect size in the spontaneous abortion analysis was d=0.2. None of the acceptable studies achieved such a large effect size as 0.5. It may be improbable because one would expect that such results would undoubtedly have been published. Unfortunately, no statistical test yet exists to precisely determine such a probability and one must therefore exercise judgment.

This meta-analysis addresses the use of organic solvents in pregnancy. Organic solvent is a very broad term that includes many classes of chemicals. There may still exist rates of abortion higher than the value reported with certain groups of solvents. However, a detailed analysis of classes of solvents is in order to incriminate a particular solvent. Not all of the studies have examined the same groups of solvents in terms of both extent and range of solvents as well as frequency and duration of exposure. Hence it would be very difficult to obtain any clear estimate of risk for a given solvent given the limited number of studies available.

Conclusion

The meta-analysis examining organic solvent use in pregnancy did not appear to find a positive association between organic solvent exposure and spontaneous abortions (ORs = 1.25, confidence interval 0.99 - 1.58). The results from the meta-analysis examining organic solvent use in the first trimester of pregnancy and major malformations indicate that solvents are associated with an increased risk for major malformations (ORs = 1.64, confidence interval 1.16 - 2.30). Because of the potential implications of this review to a large number of women of reproductive age occupationally exposed to organic solvents, it is important to verify this cumulative risk estimate by a prospective study. Similarly, it is prudent to minimize women's exposure to organic solvents by ensuring appropriate ventilation systems and protective equipment.

Meta-analysis can be a key element for improving individual research efforts and their reporting in the literature. This is particularly important with regard to an estimate of dose in occupational studies as better reporting of the quantification of solvent exposure is needed in the reproductive toxicology literature.

20.3.4 PREGNANCY OUTCOME FOLLOWING GESTATIONAL EXPOSURE TO ORGANIC SOLVENTS: A PROSPECTIVE CONTROLLED STUDY

[Adapted, by permission, from S. Khattak, G. K-Moghtader, K. McMartin, M. Barrera, D. Kennedy and G. Koren, *JAMA*., **281**, 1106 (1999) Copyright 1999, *American Medical Association*]

The Motherisk Program at the Hospital for Sick Children was the first to prospectively evaluate pregnancy and fetal outcome following maternal occupational exposure to organic solvents with malformations being the primary outcome of interest.[30]

Methods

The study group consisted of all pregnant women occupationally exposed to organic solvents and counseled between 1987-1996 by the Motherisk Program at the Hospital for Sick Children. Details concerning the time of exposure to organic solvents were recorded for de-

termination of temporal relationship between exposure and conception. The details on chemical exposure were recorded, including occupation, type of protective equipment used, and other safety features, including ventilation fans. Adverse effects were defined as those known to be caused by organic solvents (e.g., irritation of the eyes or respiratory system, breathing difficulty, headache). Temporal relationship to exposure was investigated to separate these symptoms from those associated with pregnancy. One hundred twenty-five pregnant women who were exposed occupationally to organic solvents and seen during the first trimester between 1987and 1996. Each pregnant woman who was exposed to organic solvents was matched to a pregnant woman who was exposed to a nonteratogenic agent on age (+/- 4 years), gravidity (+/- 1) and smoking and drinking status.

The primary outcome of interest was major malformations. A major malformation was defined as any anomaly that has an adverse effect on either the function or the social acceptability of the child. The expected rate of major malformations is between 1% to 3%.

Results and discussion

Significantly more major malformations occurred among fetuses of women exposed to organic solvents than controls (13 vs 1; relative risk, 13.0; 95% confidence interval, 1.8-99.5). Twelve malformations occurred among the 75 women who had symptoms temporally associated with their exposure, while none occurred among 43 asymptomatic exposed women ($p<0.001$). (One malformation occurred in a woman for whom such information was missing.) More of these exposed women had previous miscarriage while working with organic solvents than controls (54/117 [46.2%] vs 24/125 [19.2%]; $p<0.001$). However, exposed women who had a previous miscarriage had rates of major malformation that were similar to exposed women who had no previous miscarriage.

The Motherisk protocol allowed us to record in a systematic manner all exposure data and other maternal and paternal medical details at the time of exposure during the first trimester of pregnancy and to follow up pregnancy outcomes prospectively in this cohort. The control group was assessed in an identical manner.

This prospective study confirms the results of our recent meta-analysis.[31] Women occupationally exposed to organic solvents had a 13 fold risk of major malformations as well as an increased risk for miscarriages in previous pregnancies while working with organic solvents. Moreover, women reporting symptoms associated with organic solvents during early pregnancy had a significantly higher risk of major malformations than those who were asymptomatic suggesting a dose-response relationship. Other factors, for example, type of solvent, might have accounted for the presence of symptoms in some women.

Although some human teratogens have been shown to cause a homogeneous pattern of malformation(s), in other cases no specific syndrome has been described.[32] No homogenous pattern of malformations is obvious from the prospective study. However, organic solvents although traditionally clustered together, are a diverse group of compounds that should not be expected to cause similar patterns of reproductive toxic effects. Although more prospective studies will be needed to confirm our results, it is prudent to minimize women's exposure to organic solvents during pregnancy. This is most important during the first trimester of pregnancy.

20.3.5 A PROACTIVE APPROACH FOR THE EVALUATION OF FETAL SAFETY IN CHEMICAL INDUSTRIES

[Adapted, by permission from K.I. McMartin and G. Koren, *Teratology*, **60**, 130 (1999) Copyright 1999 *John Wiley & Sons, Inc*. Reprinted by permission of Wiley-Liss, Inc. a division of John Wiley & Sons, Inc.]

Introduction

Women, their families and employers are concerned about potential fetal risks that may be associated with occupational exposure to chemicals. To be able to assess such risks in a particular plant, one has to quantify local exposure and contrast it with evidence-based literature data. There are, however, numerous obstacles that prevent such risk assessment from being routinely performed. In the reproductive literature there are few studies that actually quantify exposure levels. In the instance where authors attempt to quantify or stratify exposure, the exposure frequencies and the exposure doses are inconsistent between studies.

For many chemicals one can measure neither airborne nor blood levels. Smelling the odor of organic solvents is not indicative of a significant exposure as the olfactory nerve can detect levels lower than several parts per billion, which are not necessarily associated with toxicity. Odor thresholds for some solvents are far below several parts per million (ppm). Examples of some odor thresholds[33] include carbon disulfide (0.001 ppm vs. TLV-TWA (skin) [Threshold Limit Value-Time Weighted Average] 10 ppm), acetaldehyde (0.03 ppm vs. TLV-TWA 25 ppm), and ethyl mercaptan ($2x10^{-5}$ ppm vs. TLV-TWA 0.5 ppm).[34] In the workplace, exposure is usually to several chemicals that may change between working days or even within a single day. The amounts of chemicals absorbed are often unknown, and the circumstances of exposure may vary from workplace to workplace or even within the same operation.

Typically, investigations into fetal safety are induced by single or clusters of specific malformations, or by symptoms in exposed women. We recently reported a proactive consultation process where, for a selected chemical compound to which women working in the Products and Chemicals Divisions at Imperial Oil Limited (IOL) may be exposed, actual exposure data were contrasted with literature values and a risk assessment was constructed.[35]

Methods

An agent inventory list was used to analyze the component (the name of material or agent), exposure group, the number of employees within an exposure group, and the routine rating factor for routine work. Exposure group is defined as a group of employees who have similar exposures to chemical, physical, and/or biological agents when: 1) holding different jobs but working continuously in the same area (e.g., process workers), or 2) holding unique jobs in an area or moving frequently between areas (e.g., maintenance workers). The routine rating factor for routine work (work which is part of the normal repetitive duty for an exposure group) is defined as follows:

Rating Factor (RF)	*Definition*
0	No reasonable chance for exposure
1-5	Minimal, exposure not expected to exceed 10% of the occupational exposure limit (OEL)
6-9	Some daily routine exposures may be expected between 10% and 50% of the OEL
10-15	Some daily routine exposures may exceed 50% of the OEL

The rating factor (RF) can be assessed using industrial hygiene professional judgment or monitored data. NRRF is the non-routine rating factor for non-routine work defined as job task or activities which are done seasonally, occasionally or cyclical. The definitions listed for RRF apply.

For each component a listing was created with respect to individual chemicals, including rating factors, for female exposure in the Products and Chemicals Divisions. In addition, a literature search was performed for each chemical that incorporated female occupational exposure during pregnancy with human teratogenicity and spontaneous abortion as pregnancy outcomes. Teratogenicity and spontaneous abortion were chosen as the outcomes of interest as they represent the majority of endpoints examined in studies focusing on female occupational exposure during pregnancy.

Most of the selected female reproductive toxicology studies examined explicitly stated chemical exposure levels: either as parts per million, stratifying as to number of days of exposure, or as estimates of the percentage of the threshold limit values. Medline, Toxline, and Dissertation Abstracts databases were utilized to search for all research papers published in any language from 1966 to 1996. In total, 559 studies were obtained from the literature search. Of these, only 21 studies explicitly stated some sort of exposure level for the various chemicals. These chemical exposure levels in the literature and subsequent pregnancy outcomes were compared to IOL chemical exposure indices. The following is an example of one of the many chemical exposures encountered, namely exposure to toluene. For other compounds, Table 20.3.5 contrasts values in the literature with IOL indices of chemical exposure.

Table 20.3.5. Examples of IOL compound exposure indices contrasted to literature values. [Adapted, by permission from K.I. McMartin and G. Koren, *Teratology*, 60, 130 (1999) Copyright 1999 *John Wiley & Sons, Inc.* Reprinted by permission of Wiley-Liss, Inc. a division of John Wiley & Sons, Inc.]

Chemical	Reference	Literature Exposure Levels	IOL Exposure Levels
Aniline	Posluzhnyi[42]	"low exposure area"	"no reasonable chance for exposure to minimal exposure not expected to exceed 10% OEL" TLV-TWA: 2 ppm
Benzene	Mukhametova and Vozovaya[43]	"within or lower than the maximum permissible levels"	"no reasonable chance for exposure to some daily exposures may be expected between 10-50% OEL" TLV-TWA:10 ppm
Chloroform	Taskinen et al.[44]	<once a week >once a week	"no reasonable chance for exposure to minimal exposure not expected to exceed 10% OEL" TLV-TWA: 10 ppm
Dichloromethane	Taskinen et al.[44] Windham et al.[21]	>once a week <once a week >10 hrs a week <10 hrs a week	"no reasonable chance for exposure to minimal exposure not expected to exceed 10% OEL" TLV-TWA: 50 ppm

Chemical	Reference	Literature Exposure Levels	IOL Exposure Levels
Styrene	Saamanen[45] Harkonen[46]	70-100 ppm 20-300 ppm	"no reasonable chance for exposure to minimal exposure not expected to exceed 10% OEL" TLV-TWA: 50 ppm, TLV-STEL: 100 ppm
Toluene	Euler[10] Syrovadko[36] Ng et al.[39]	298 ppm 13-120 ppm 50-150 ppm	"no chance for exposure to some daily exposure exceeding 50% of the OEL" TLV-TWA: 50 ppm

IOL: Imperial Oil Limited, OEL: Occupational Exposure Limit

Results and discussion

Six studies were found that quantified toluene concentrations. The countries that reported these observations included Germany, Russia, Finland and Singapore. In general, IOL toluene levels are lower than those reported in the literature.

A few case reports of malformations in association with toluene exposure have appeared. Euler[10] reported 2 cases of multiple malformations where the anomalies were similar in children born to women who worked in shoemaking and were exposed to a soling solution containing toluene and trichloroethylene. The average concentration of toluene in the air was 298 ppm (1.12 mg/l) and of trichloroethylene 230 ppm (1.22 mg/l). No further details of these cases were given.

Toutant and Lippmann[28] reported a single case of adverse pregnancy outcome in a woman addicted to solvents (primarily toluene). The woman, aged 20 years, had a 14-year history of daily heavy solvent abuse. On admission to the hospital, she had ataxia, tremors, mild diffuse sensory deficits, short-term memory loss, blunted affect, and poor intellectual functioning compatible with severe solvent and/or alcohol abuse. The male child born at term was microcephalic with a flat nasal bridge, hypoplastic mandible, short palpebral fissures, mildly low-set ears, pronounced sacral dimple, sloping forehead and incoordination of arm movements with unusual angulation of the left shoulder and elbow. There was a poor sucking reflex and movements were jerky at 2-4 days of age, although this improved spontaneously. The authors of this report point out the similarities between this case and fetal alcohol syndrome and suggest that there may be an analogous "fetal solvent syndrome" or that excessive solvent intake may enhance the toxicity of alcohol.

Syrovadko[36] studied the outcome of pregnancy in a substantial number of women exposed to toluene. Toluene exposure averaged 55 ppm (range 13-120 ppm). The factory had its own maternity section where the women had their deliveries. Records of labor and newborns were examined for 133 women in contact with toluene and for 201 controls from the factory offices. There was no detectable effect on fertility. In the exposed group, records showed a mean pregnancy rate of 3.2/worker compared with 2.6/worker in the control group. There were no significant differences between exposed and control groups in the mortality or adverse effects on the newborn.

In the Finnish study of Holmberg[37] on central nervous system defects in children born to mothers exposed to organic solvents during pregnancy, 3 of the cases were exposed to toluene, or toluene in combination with other solvents. In one case with hydranencephaly and death 24 days after birth, there was exposure to toluene, xylene, white spirit and methyl ethyl ketone from rubber products manufacture. The second case had multiple abnormali-

ties of hydrocephalus, agenesis of the corpus callosum, pulmonary hypoplasia and diaphragmatic hernia, and died 2 hours after birth. In the second case, the mother was exposed to toluene while manufacturing metal products. The third case had lumbar meningomyelocele and survived. The mother was exposed to toluene and white spirit. Toluene air concentrations were not stated.

A case-referent study concerning selected exposures during pregnancy among mothers of children born with oral clefts was conducted in Finland.[38] The study covered the initial 3.5 years' material and was a more detailed extension of earlier retrospective studies concerning environmental factors in the causation of oral clefts, using cases accumulated from the Finnish Register of Congenital Malformations. More case mothers (14) than referent mothers (3) had been exposed to organic solvents during the first trimester of pregnancy. The mothers were considered "substantially" exposed if their estimated continuous exposure had been at least one-third of the current TLV concentration or if the estimated peak exposure had reached the TLV concentration, e.g., during home painting in confined spaces. Various solvents included: lacquer petrol, xylene, toluene, acetates, alcohols, denatured alcohol, methyl ethyl ketone, dichloromethane, turpentine, styrene, and aromatic solvent naphtha (C_4-C_{14} aromatics).

Ng et al.[39] examined the risk of spontaneous abortion in workers exposed to toluene. Rates of spontaneous abortions were determined using a questionnaire administered by personal interview to 55 married women with 105 pregnancies. The women were employed in an audio speaker factory and were exposed to high concentrations of toluene (mean 88 ppm, range 50-150 ppm). These rates of spontaneous abortion were compared with those among 31 women (68 pregnancies) who worked in other departments in the same factory and had little or no exposure to toluene (0-25 ppm) as well as with a community control group of women who underwent routine antenatal and postnatal care at public maternal health clinics. Significantly higher rates for spontaneous abortions were noted in the group with higher exposure to toluene (12.4 per 100 pregnancies) compared with those in the internal control group (2.9 per 100 pregnancies) and in the external control group (4.5 per 100 pregnancies). Among the exposed women, significant differences were also noted in the rates of spontaneous abortion before employment (2.9 per 100 pregnancies) and after employment in the factory (12.6 per 100 pregnancies).

Tikkanen et al.[17] performed a study to explore for possible associations between occupational factors and cardiovascular malformations. Information on the parents of 160 infants with cardiovascular malformations and 160 control parents were studied. The mother was considered "substantially" exposed to "organic solvents" if the estimated continuous exposure was at least one third of the ACGIH threshold limit value concentration or the estimated short term exposure reached the TLV concentration (while painting kitchen walls). Organic solvents were categorized as 1) "hydrocarbons", 2) "alcohols" and 3) "miscellaneous". Hygiene assessments of exposures were classified as i) "any exposure intensity" (at any period in pregnancy and in the first trimester only) and ii) "substantial exposure intensity" (at any period in pregnancy and in the first trimester only).

Of the 320 mothers, 41 case and 40 control mothers reported an exposure to organic solvents.[16] The hygiene assessment indicated some solvent exposure in 27 case and 25 control mothers. Twenty-one case and 16 control mothers had been exposed in the first trimester. Of these, substantial exposure to hydrocarbons occurred for 6 case and 2 control

mothers; one case and one control mother to toluene at work and five cases and one control mother to lacquer petrol while painting indoors at home for 1 to 2 days.

Lindbohm et al.[40] investigated the association between medically diagnosed spontaneous abortions and occupational exposure to organic solvents (case-control design). The study population was composed of women who were biologically monitored for solvents. The workers were classified into exposure categories on the basis of work description and the use of solvents as reported in the questionnaires and on measurements of biological exposure. Three exposure levels were distinguished: high, low, and none. The level of exposure was assessed on the basis of the reported frequency of solvent use and the available information on typical levels of exposure in that particular job, as based on industrial hygiene knowledge.

The feasibility of biological monitoring data for classification of exposure was limited because the solvent measurements describe only short-term exposure (from 2 hours to a few days) and only 5% of the workers had been measured during the first trimester of pregnancy. Therefore, the exposure classification was based mainly on the work task description and reported solvent usage. Exposure was defined as "high" if the worker handled the solvents daily or 1-4 days a week and the level of exposure was high according to biological exposure measurements or industrial hygiene measurements available at the Institute of Occupational Health. Exposure was defined as "low" if the worker handled solvents 1-4 days a week and the level of exposure according to the measurements of the Institute was low or if the worker handled solvents less than once a week. Otherwise, the level of exposure was defined as "none". After classification, the work tasks and the related exposures were listed by the level of exposure which was checked by an independent, experienced industrial hygienist. The final population for the analysis was restricted to the matched case-control sets who confirmed their pregnancy and reported in detail their occupational exposures during early pregnancy (73 cases and 167 controls).

The odds ratios for tetrachloroethylene and aliphatic hydrocarbons, adjusted for potentially confounding factors, increased with the level of exposure.[40] For toluene the reverse was the case. Aliphatic hydrocarbons had not been biologically monitored, but industrial hygiene measurements had been performed by the Institute of Occupational Health in two printing houses which contributed subjects to this study. In two of four measurements, the concentrations of white spirit in air exceeded, during the cleaning of the printing machine, the Finnish Threshold Limit Value (150 ppm). All the printers included in this study reported that their work included cleaning of the machine.

The association of tetrachloroethylene, toluene and aliphatic hydrocarbons with spontaneous abortions was also examined by detailed records of occupational task.[40] The odds ratio of spontaneous abortion for aliphatic hydrocarbons was increased among graphic workers [5.2 (1.3-20.8)] and painters [2.4 (0.5-13.0)] but not among other workers. However, in the latter group the proportion of highly exposed workers was only 30%, whereas it was 69% in the two former groups. The odds ratio was increased also among toluene-exposed shoe workers [odds ratio 9.3 (1.0-84.7)] and dry cleaners exposed to tetrachloroethylene [odds ratio 2.7 (0.7-11.2)].

The results of the study by Lindbohm et al.[40] support the hypothesis of a positive association between spontaneous abortion and exposure to organic solvents during pregnancy and suggest that exposure, especially to aliphatic hydrocarbons, increases the risk of abortion. The highest risk for aliphatic hydrocarbons was found among graphic workers who

were employed as offset printing workers or printing trade workers. They used the solvents for cleaning the printing machines and as diluent for printing ink. In cleaning the machines, exposure to mixtures of nonaromatic mineral oil distillates with 0-15% aromatic compounds may reach a high level for a short period.[40] The workers were also exposed among other things to toluene, 1,1,1-trichloroethane, thinner, and xylene. Although the data suggest that the findings are due to aliphatic hydrocarbons, combined solvent effects cannot be excluded because of the multiple exposures to different solvents.[40]

The mean measured level of blood toluene among the shoe workers was slightly higher (0.51 mmol/L, 13 morning samples) than the mean among the other toluene-exposed workers (0.38 mmol/L, 10 morning samples).[40] The shoe workers also reported use of toluene more frequently than the other toluene-exposed workers. Industrial hygiene measurements had been performed in three of the five work places of the shoe workers. The concentration of toluene in air varied from 1 ppm to 33 ppm. Other solvents detected were acetone and hexane. In two of the three shoe factories from which industrial hygiene measurements were available, relatively high levels of hexane (33-56 ppm) were measured. Hexane, being an aliphatic compound, may have contributed to the excess of spontaneous abortions.[40]

Comparison with IOL levels

The routine rating factor and non-routine rating factor from the Products and Chemical Divisions range from 00 to 11 indicating no reasonable chance for exposure to some daily exposures exceeding 50% of the OEL. The TLV-TWA for toluene is 50 ppm.[34] The Euler case reports documented air concentrations of 298 ppm for toluene and 230 ppm for trichloroethylene.[10] Both of these air concentrations exceed current standards, but no further details of these cases were given. Syrovadko reported a toluene exposure of 55 ppm (range 13-120 ppm), again, exceeding current standards.[36]

Holmberg et al.[38] and Tikkanen[17] considered workers "substantially" exposed if their estimated continuous exposure had been at least one-third of the current TLV concentration or if the estimated peak exposure had reached the TLV concentration. Similarly, Ng[38] described high concentrations of toluene (mean 88, range 50-150 ppm) exceeding current standards. All these exposure levels for toluene exceed the current threshold limit value. IOL toluene exposure levels are considerably lower than any value reported in the literature.

Lindbohm et al.,[40] for two of four air measurements, reported concentrations of white spirit exceeded the Finnish Threshold Limit Value (150 ppm) during the cleaning of the printing machine. Industrial hygiene measurements were performed in three of the five work places of the shoe workers. The concentration of toluene in air varied from 1 ppm to 33 ppm. Other solvents detected were acetone and hexane. In two of the three shoe factories from which industrial hygiene measurements were available, relatively high levels of hexane (33-56 ppm) were noted.

The routine rating factor and non-routine rating factor from the Products and Chemicals Divisions for hexane isomers range from 00 to 05 indicating no reasonable chance for exposure or minimal exposure not expected to exceed 10% of the occupational exposure limit (OEL). The routine rating factor and non-routine rating factor from the Products and Chemicals Divisions for n-hexane range from 00 to 07 indicating no reasonable chance for exposure or some daily exposures between 10% and 50% of the OEL. The TLV-TWA of n-hexane is 50 ppm or 176 mg/m^3.[34] The TLV-TWA of other hexane isomers is 500 ppm or 1760 mg/m^3 and the TLV-STEL is 1000 ppm or 3500 mg/m^3.[34] In comparison with the pre-

vious hexane levels reported in the literature, IOL hexane exposure levels are substantially lower.

In mice levels of inhalation exposure to toluene have included 100 to 2,000 ppm at various times during gestation as well as at various durations of exposure (6-24 hours/day).[41] Growth and skeletal retardation were noted at lower levels (133 ppm and 266 ppm, respectively) when such exposures were of a 12-24 hour duration for at least half of the gestation period.[41] Human levels of inhaled toluene exposure that would be comparable would be those obtained by chronic abusers (5,000-12,000 ppm). The only noted malformations were an increase in the frequency of 14th ribs, which was noted at 1,000 ppm on days 1-17 of gestation for 6 hours/day. As Wilkins-Haug[41] notes this has been the highest exposure studied in the mouse model and is comparable to the inhaled toluene exposure which produces euphoria in humans (500 ppm).

In 1991 we were approached by the medical department of Imperial Oil Limited to develop a proactive approach of risk evaluation of their female workers. The paradigm developed and used by us could be extrapolated to any other chemical operation. Its advantage is in its proactive nature, which aims at informing workers and preventing potential fetal risks, while also preventing unjustified fears which may lead women to quit their jobs or, in extreme cases, even consider termination of otherwise wanted pregnancies.

Upon comparing the occupational literature that presented any quantifiable chemical exposure dose or estimate of dose for any chemical with the IOL routine rating factors in the Products and Chemicals Divisions, we could conclude that IOL chemical exposure levels overall were lower than those reported in the literature. Of utmost importance is the need in published occupational reports for at least some industrial hygiene documentation, namely improved reporting of a quantifiable chemical exposure dose (for example, as implemented and currently utilized by IOL) and ideally a standard and consistent way of reporting this in the occupational literature.

20.3.6 OVERALL CONCLUSION

The Motherisk program is an information and consultation service for women, their families and health professionals on the safety/risk of exposure to drugs, chemicals, radiation and infection during pregnancy and lactation. Chemical exposure in the workplace is a common source of concern among our patients and health professionals.

Occupational exposure to organic solvents during pregnancy is associated with an increased risk of major fetal malformations. This risk appears to be increased among women who report symptoms associated with organic solvent exposure. Although more prospective studies will be needed to confirm our results, it is prudent to minimize women's exposure to organic solvents during pregnancy. This is most important during the first trimester of pregnancy. Moreover, symptomatic exposure appears to confer an unacceptable level of fetal exposure and should be avoided by appropriate protection and ventilation. Health care professionals who counsel families of reproductive age should inform their patients that some types of employment may influence reproductive outcomes.

Of utmost importance is the need in published occupational reports for some industrial hygiene documentation. Specifically, improved reporting of a quantifiable chemical exposure dose (for example, as implemented and currently utilized by IOL) and ideally a standard and consistent way of reporting this in the occupational literature pertaining to human reproductive toxicology.

REFERENCES

1 J. Schardein, in **Chemically Induced Birth Defects**, *Marcel Dekker,* New York, 1985, pp. 645-658.
2 G. Watanabe and S. Yoshida, *Acta. Medica. Biol. Niigata.*, **12**, 285 (1970).
3 W. Masumoto, S. Ijima and H. Katsunuma, *Congenital Anomalies*, **15**, 47 (1975).
4 P.S. Nawrot and R.E. Staples, *Teratology*, **19**, 41A (1979).
5 F.J. Murray, J.A. John, L.W. Rampy, R.A. Kuna and B.A. Schwetz, *Ind. Hyg. Ass. J*., **40**, 993 (1979).
6 R. Iwanaga, T. Suzuki and A. Koizumi, *Jap. J. Hyg.*, **25**, 438 (1970).
7 A. Hudak and G. Ungvary, *Toxicology*, **11**, 55 (1978).
8 J.D. Green, B.K.J. Leong and S. Laskin, *Toxicol. Appl. Pharmacol*., **46**, 9 (1978).
9 S.M. Barlow and F.M. Sullivan, in **Reproductive Hazards of Industrial Chemicals**, *Academic Press*, London, 1982.
10 H.H. Euler, *Arch. Gynakol*., **204**, 258 (1967).
11 B.A. Schwetz, B.M.J. Leong and B.J. Gehring, *Toxicol. Appl. Pharmacol*., **32**, 84 (1975).
12 B.K. Nelson, B.J. Taylor, J.V. Setzer and R.W. Hornung, *J. Environ. Pathol. Toxicol*., **3**, 233 (1980).
13 O. Heinonen, in **Birth Defects and Drugs in Pregnancy**, *PSG Publishing*, Littleton, MA, 1977, pp. 65-81.
14 F.G. Cunningham, P.C. McDonald and N. Gant in Williams Obstetrics, Appleton and Lange, Norwalk, Connecticut, 1989, pp. 489-509.
15 T.R. Einarson, J.S. Leeder and G. Koren, *Drug. Intell. Clin. Pharm*., **22**, 813 (1988).
16 G. Axelsson, C. Liutz and R. Rylander. *Br. J. Ind. Med*., **41**, 305 (1984).
17 J. Tikkanen and O. Heinonen, *Am. J. Ind. Med*., **14**, 1 (1988).
18 P.C. Holmberg, K. Kurppa, R. Riala, K. Rantala and E. Kuosma, *Prog. Clin. Biol. Res.*, **220**, 179 (1986).
19 S. Cordier, M.C. Ha, S. Ayme and J. Goujard, *Scand. J. Work Environ. Health*., **18**, 11 (1992).
20 G.K. Lemasters, An epidemiological study of pregnant workers in the reinforced plastics industry assessing outcomes associated with live births, University of Cincinnati, Cincinnati, 1983.
21 G.C. Windham, D. Shusterman, S.H. Swan, L. Fenster and B. Eskenazi, *Am. J. Ind. Med*., **20**, 241 (1991).
22 J.A. Lipscomb, L. Fenster, M. Wrensch, D. Shusterman and S. Swan, *J. Occup. Med*., **33**, 597, (1991).
23 M.B. Schenker, E.B. Gold, J.J. Beaumont, B. Eskenazi, S.K. Hammond, B.L. Lasley, S.A. McCurdy, S.J. Samuels, C.L. Saiki and S.H. Swan, Final report to the Semiconductor Industry Association. Epidemiologic study of reproductive and other health effects among workers employed in the manufacture of semiconductors, University of California at Davis, 1992.
24 S.M. Pinney, An epidemiological study of spontaneous abortions and stillbirths on semiconductor employees, University of Cincinnati, Cincinnati, 1990.
25 B. Eskenazi, M.B. Bracken, T.R. Holford and J. Crady, *Am. J. Ind. Med*., **14**, 177, (1988).
26 T.H. Shepard, *Teratology*, **50**, 97, (1994).
27 Y. Bentur in **Maternal Fetal Toxicology**, G. Koren, Ed., *Marcel Dekker*, New York, 1994, pp. 425-445.
28 C. Toutant, and S. Lippmann, *Lancet*, **1**, 1356, (1979).
29 G. Axelson, R. Rylander, *Int. J. Epidemiol.*, **13**, 94, (1984).
30 S. Khattak, G. K-Moghtader, K. McMartin, M. Barrera, D. Kennedy and G. Koren, *JAMA*., **281**, 1106 (1999).
31 K.I. McMartin, M. Liau, E. Kopecky, T.R. Einarson and G. Koren, *Am. J. Ind. Med*., **34**, 288 (1998).
32 G. Koren, A. Pastuszak and S. Ito, *N. Engl. J. Med*., **338**, 1128 (1998).
33 M.J. Ellenhorn and D.G. Barceloux, **Medical Toxicology: Diagnosis and Treatment of Human Poisoning**. *Elsevier Science Publishing Company Inc.*, New York, 1988, pp. 1412-1413.
34 Anonymous, Threshold limit values for chemical substances and physical agents and biological exposures, American Conference of Governmental Industrial Hygienists, Cincinnati, 1993, pp. 12-35.
35 K.I. McMartin and G. Koren, *Teratology*, **60**, 130 (1999).
36 O.N. Syrovadko, *Gig Tr Prof Zabol.*, **21**, 15 (1977).
37 P.C. Holmberg, *Lancet*, **2**, 177, (1979).
38 P.C. Holmberg, S. Hernberg, K. Kurppa, K. Rantala, and R. Riala, *Int. Arch. Occup. Environ. Health*, **50**, 371 (1982).
39 T.P. Ng, S.C. Foo, and T. Yoong, *Br. J. Ind. Med.*, **49**, 804, (1992).
40 M.L. Lindbohm, H. Taskinen, M. Sallmen, and K. Hemminki, *Am. J. Ind. Med*., **17**, 447, (1990).
41 L. Wilkins-Haug, *Teratology*, **55**, 145, (1997).
42 P.A. Podluzhnyi, *Gig. Sanit*., **1**, 44, (1979).
43 G.M. Mukhametova, and M.A. Vozovaya, *Gig. Tr. Prof. Zabol.*, **16**, 6 (1972).
44 H. Taskinen, M.L. Lindbohm, and K. Hemminki, *Br. J. Ind. Med*., **43**, 199 (1986).
45 A. Saamanen, Styreeni, Institute of Occupational Health, Helsinki, 1991.
46 H. Harkonen, S. Tola, M.L. Korkala, and S. Hernberg, *Ann. Acad. Med. Singapore*, **13**, 404 (1984).

20.4 INDUSTRIAL SOLVENTS AND KIDNEY DISEASE

NACHMAN BRAUTBAR
University of Southern California, School of Medicine, Department of Medicine, Los Angeles, CA, USA

20.4.1 INTRODUCTION

Industrial solvents are used extensively in the industry, as well as modern living. The principle class of components are the chlorinated and non-chlorinated hydrocarbons. The various types of commonly used hydrocarbons are presented in Figure 20.4.1.

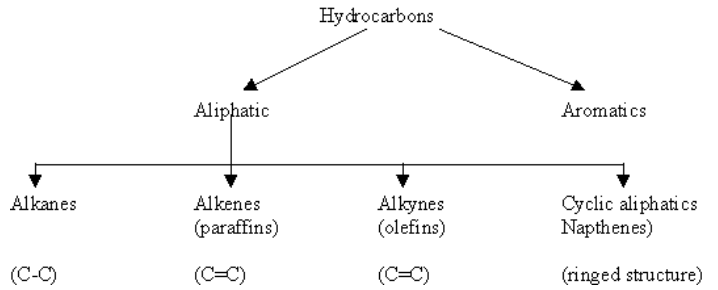


Figure 20.4.1. General classification of hydrocarbons based on general structure.[Adapted, by permission, from A.T. Roy, *Nephron*, **58**, 385, 1991.]

Solvents are absorbed into the human body through several routes including 1) inhalation through the lungs, 2) absorption through the skin, 3) ingestion (in rare cases). The main route of absorption is commonly pulmonary, the lung, and this depends on several factors including the frequency of breathing, diffusion of solvent vapors across the alveolar membrane, partial pressure of solvent vapor in inspired air and blood, and solubility of the solvent in blood as the result of to air partition coefficient, and blood flow through the lungs.[1,2,3] Once in the circulation, 25% of the cardiac output which is about 1200 cc of blood per minute passes through the kidneys. Therefore it is no surprise that with this amount of blood passing through the kidney and carrying solvents (from either industrial inhalation, skin absorption, and on rare occasions ingestion) the effects of solvents on the kidney has become a practical clinical one.

Since inhaled hydrocarbons are readily absorbed into the blood stream and become lipophilic and readily pass across the lipid membranes. In addition to reaching the kidney, the solvents reach the brain (as does the most ancient solvent, alcohol) and enter the blood brain barrier in high concentration.

Skin absorption is the second most important route for solvent entry into the body and at times is much more significant than inhalation. The reason is that absorption of organic solvent vapors by inhalation at the threshold limit value is insignificant and is less than 2% of the amount absorbed via inhalation under the same exposure conditions.[3] In contrast, solvents may be absorbed through the skin in significant amounts even at below the threshold limit value.[3] Factors that effect the skin absorption of solvents include the composition of

the skin, whether the skin is healthy or not (there is increased absorption if the skin has reduced cellular membrane), and the lipid solubility of the solvent.

As far as the gastrointestinal tract, commonly this is not a significant route of absorption. Solvents absorbed via the gastrointestinal tract are removed immediately by the liver through the first-pass metabolism. If the amount of solvents and quantity of solvents ingested is increased significantly and exceeds the capacity of the liver to metabolize the solvents, then the gastrointestinal tract route will become significant.[4,5,6]

The distribution of an organic solvent in the human body depends upon its partial pressure in the arterial blood and the solubility of the solvent in the tissue, as well as the blood flow rate through the tissue.[7] Data on tissue distribution of various solvents are limited at best.

The metabolism of solvents depend on the solvent. Alcohols are metabolized via alcohol dehydrogenase, whereas other organic solvents are mainly metabolized by the cytochrome P-450-dependent enzymes. These enzymes may be found in the liver, kidneys, lungs, gastrointestinal tract, gonads, adrenal cortex, and other body organ tissues. The metabolism of solvents has been described extensively,[8,9] and the reader is referred to those writings.

The metabolites of the organic solvents are eliminated via the kidneys through urine excretion and to some extent, by exhalation of the unchanged original solvent. Commonly the parent solvent is eliminated by the kidneys and this amounts to less than 1%. The metabolites are the main source of excretion of the metabolized parent solvent.

In the last several decades, there have been several studies in experimental animals, case reports in humans, case studies in humans, and epidemiological studies in humans on the effects of solvents on the kidney, both acutely and chronically. The scope of this chapter is the clinical chronic effects of solvents on the kidney (chronic nephrotoxicology).

20.4.2 EXPERIMENTAL ANIMAL STUDIES

The toxic effects of organic solvents on the kidneys has been studied in several experimental species, especially mice and rats. Damage to the kidney has been shown in these experimental animals in the form of acute damage to various parts of the nephron, especially the tubules. This has usually been described as tubular degeneration with regenerative epithelium, deposits of mineral crystals and of intralobular proteins, and interstitial inflammation.[8,10-15] Several studies have shown glomerular damage in experimental animal[16,17] and have suggested that long-term solvent exposure alters the immune system and leads to the glomerulopathy with mesangial IgA deposits.

While the exact mechanism is not known and various mechanisms have been postulated, it is reasonable to accept a mechanistic approach which takes into account genetic, environmental, susceptibility (such as pre-existing diseases including hypertensive kidney disease and diabetes), direct tubular toxicity, permeability changes and immunosuppression.

20.4.3 CASE REPORTS

The earlier documentation of chronic renal disease and hydrocarbon exposure consists of case reports, and this data was summarized by Churchill et al.[18] describing Goodpasture's syndrome in 15 adults, epimembranous glomerulonephritis in 5 adults, and subacute proliferative glomerulonephritis in one adult. The hydrocarbon exposures were for solvents in 12 patients, gasoline in 4, gasoline-based paint in 3, jet fuel, mineral turpentine and un-

specified in 1 case report. These case reports were previously summarized by us in a previous publication[19] and are represented in the following table.

Table 20.4.1. Case series report of glomerulonephritis and hydrocarbon exposure. [Data from reference number 19]

Investigator	n	Diagnosis	Agent
Sperace[20]	2	Goodpasture's	Gasoline
Heale, et al.[21]	1	Goodpasture's	Gasoline
Klavis and Drommer[22]	1	Goodpasture's	Gasoline-based paint spray
Beirne and Brennan[23]	5 1	Goodpasture's RPGN	Degreasing and paint Solvents and jet fuel
D'Apice, et al.[24]	2	Goodpasture's	Gasoline mineral turpentine
Kleinknecht, et al.[25]	2	anti-GBM nephritis	Organic solvent vapors
Daniell, et al.[26]	1	anti-GBM nephritis	Stoddard solvent
Von Scheele, et al.[27]	1	subacute GN	Paint solvent
Ehrenreicht, et al.[28]	4	epimembranous GN	Solvents
Cagnoli, et al.[29]	1	epimembranous GN	?

GBM = Glomerular basement membrane; RPGN = rapidly progressive glomerulonephritis; GN= glomerulonephritis

While these studies represent case reports, they suggest an association between exposure and the development of chronic glomerular disease.

20.4.4 CASE CONTROL STUDIES

Several case-control studies have examined the role of organic solvent exposure in a population of patients with glomerulonephritis. A total of 14 case control studies examining human exposure to solvents and glomerulonephritis have been conducted and are documented here in Table 20.4.2.[30]

Table 20.4.2. Glomerulonephritis and organic solvents: Case-control studies summarized. [Data from reference number 30]

Investigator	Increased risk factor	Investigator	Increased risk factor
Lagrue, et al.[31,32]	4.9*, 5.2*	Nuyts, et al.[39]	1.1*
Bell, et al.[33]	increased	Zimmerman, et al.[40]	increased
Ravnskov, et al.[34]	3.9*	Ravnskov[41]	increased
Ravnskov, et al.[35]	2.8*	Finn, et al.[42]	3.6*, 3.2*
Porro, et al.[36]	3.9*	Van der Laan[43]	1.1
Yaqoob, et al.[37]	aliphatic 15.5, halogenated 5.3, aromatic-oxygenated 2.0	Harrison, et al.[44]	8.9*
Yaqoob, et al.[38]	increased	*P<0.05, statistically significant	

The study by Lagure, et al,[31,32] showed significantly increased risk of solvent related glomerulonephritis of 4.9. That this increased risk of glomerulonephritis follows a dose-response relationship was shown in the study of the populations examined by Ravnskov, et al.,[34,35] Bell, et al.,[33] Porro, et al.,[36] Yaqoob, et al.,[37,38] Nuyts, et al.,[39] and demonstrates: 1) temporal relationship between exposure to solvents and the development of kidney disease, 2) a dose-response relationship, strongly showing the causal link between solvent exposure and glomerulonephritis. The study by Nuyts, et al.,[39] examined a large population of 272 patients with chronic renal failure and assessed several occupational exposures, among those were hydrocarbons. The increased risk of chronic kidney disease in the form of renal failure in patients exposed to solvents was 5.45. The study of Askergren et al.[45] looked into kidney functions in patients exposed to various organic solvents, specifically excretion of red blood cells in the urine in 101 patients exposed to solvents as compared to 39 non-exposed controls. Those who were exposed to organic solvents significantly excreted more cells than the ones who were not exposed. These studies showed the role for organic solvent exposure in the development of damage to the glomerules since excretion of red blood cells represents damage to the glomerules rather than tubules. That exposure to solvents is associated with glomerular damage rather than tubular damage fits with the various case reports and case-control studies and further suggest a plausible causal connection between exposure to industrial solvents and glomerular damage leading later on to chronic glomerulonephritis. The study by Bell et al.[33] studied 50 patients who had organic solvent exposure and biopsy-proven proliferative glomerulonephritis. They have shown that none of these patients had evidence of any other systemic disease or preexisting infection, and compared those with 100 control subjects matched for age, sex and social class. This study is important since exposure assessment was done and showed significantly greater exposure scores in patients with glomerulonephritis compared to the control subjects. Furthermore, the degree of exposure was significantly higher in those patients who have more severe glomerulonephritis than those who have less severe glomerulonephritis, further indicative of a dose response relationship. This is a study which demonstrates significant statistical association, as well as dose response relationship between solvent exposure and kidney damage in the form of glomerular lesion and end-stage glomerulonephritis, ranging from mild to chronic severe glomerulonephritis. The study by Daniell et al.[26] evaluated the risk of developing glomerular lesion associated with hydrocarbon exposure and showed a does-response relationship and variations in disease severity in relation to the exposure intensity. They showed an increase risk of developing glomerular nephritis, ranging from 2.8 to 8.9 fold increase as compared to the non-exposed population. There was clear temporal relationship between the exposure, absence of any other causes, a dose-response relationship which further validated the observations of Bell et al.[33] and conclude that intense or long-term exposure (low-level but long-term or short-term and high levels) to commonly used industrial solvents played a causal role in the development of glomerular damage and chronic glomerulonephritis.

In a comprehensive study, Yaqoob et al.[46] performed a population study which looked into 3 groups of healthy men working in 3 different areas of a major car manufacturing plant. They have studied 3 groups, Group 1 included 112 paint sprayers exposed to a paint-based mixture of hydrocarbons, Group 2 which was composed of 101 transmission shop workers with exposure to petroleum-based mineral oils, and Group 3 which was comprised of 92 automated press operators with minimal background exposure to lubricating

oils and who acted as internal controls. The 3 groups studied were comparable in age, duration of employment, duration of hydrocarbon exposure, and other factors. The cumulative exposure to hydrocarbons was evaluated. The hydrocarbon exposure scores were significantly higher in Groups 1 and 2, as compared to Group 3 (which served as an internal control and epidemiologically is a good working population control group, since this method takes into account the healthy worker). The principal hydrocarbons used throughout the period of time of the study were toluene, xylene, and n-butyl alcohol in paints and various petroleum fractions in the mineral oils. The study evaluated markers of kidney dysfunction in the subjects chronically exposed to hydrocarbons at the described work site. The authors concluded that paint exposure in the long-term is associated with renal impairment and micro-proteinuria without elevation in serum creatinine (which indicates that the kidney functions from a creatinine clearance point of view are still intact, and are less sensitive as a biological marker of glomerular damage) is a feature of workers chronically exposed to petroleum based mineral oils. The investigators also reported significant urinary excretion of protein which also indicated early glomerular damage in susceptible individuals. The authors concluded from these studies that chronic hydrocarbon exposure can be associated with renal impairment. They further concluded that the significance of the early markers of renal damage can predict progressive deterioration in renal functions. These data indicate that chronic hydrocarbon exposure may be associated with early and sub-clinical renal dysfunction leading to a chronic glomerulonephritis.

Porro et al.[36] performed a case referent study and they looked into a group of 60 patients with chronic glomerulonephritis established by biopsy, with no evidence of any other systemic diseases, and was compared to 120 control subjects who were not exposed to solvent vapors. Exposure assessment was based on scores from questionnaires. Exposure was significantly higher in the case group studies than in the reference control group for both total and occupational solvent exposure. They further found that the odds ratio of chronic glomerulonephritis for patient's occupationally exposed to solvents was 3.9 and using a logistic regression model and they showed a dose-response effect of occupational exposure to solvents and glomerulonephritis. Histological studies of the 60 patients with chronic glomerulonephritis ruled out other systemic disease and demonstrated the whole-spectrum of glomerular diseases, the most common one is IgA nephropathy. When the sub-group of patients with IgA nephropathy and their matched controls were separately examined, the cases appeared to be significantly more exposed than the patients with other non-glomerular diseases such as kidney stones. Based on their findings, the investigators concluded that their results are in agreement with the hypothesis that the onset of glomerulonephritis could be related to a non-acute exposure to solvents even of light intensity.

The work of De Broe et al.[47] looked into occupational renal diseases and solvent exposure. They have concluded that the relation between hydrocarbon exposure and glomerulonephritis seems to be well-defined from an epidemiological point of view. They further show, in a case-control study of a group of patients with diabetic nephropathy, that hydrocarbon exposure was found in 39% of the patients with that particular form of kidney disease. They find that this was in agreement with the findings of Yaqoob et al.[37] who found higher levels of hydrocarbon exposure in patients with incipient and overt diabetic nephropathy than in diabetic patients without clinical evidence of nephropathy. These data indicate a particular sensitivity of patients with diabetic kidney toward the damaging effects of the hydrocarbons. The findings of these investigators are agreement with the study of

Goyer,[48] who showed that existing renal diseases, particularly hypertensive and diabetic nephropathies, are clear risk factors predisposing to abnormal accumulation and excess blood levels of any nephrotoxic drugs and chemicals, as well as solvents. Indeed this observation makes a lot of scientific and clinical sense, since it is known that the ability of the kidney to excrete the breakdown metabolites of various materials including industrial solvents is reduced with any incremental reduction of kidney function, and there would certainly be more accumulation of these breakdown products, as well as the parental solvents in the kidney tissue, and as such, it makes sense that these individuals with underlying kidney disease such as hypertensive kidney disease, diabetic kidney disease, or interstitial kidney disease which may not yet be clinically overt, are at a significantly increased risk of developing chronic kidney disease as a result of the documented damaging effects of solvents on the kidney.

20.4.5. EPIDEMIOLOGICAL ASSESSMENT

The epidemiological diagnostic criteria for most cases of end-stage kidney disease is deficient since no etiologic information is available in the majority of the cases. Fewer than 10% of the end-stage renal disease cases are characterized etiologically.[49] Clinically, many patients are classified histologically such as glomerulonephritis, but little effort is made to look for toxic factors. Indeed, the majority of the clinicians seeing patients with end-stage kidney disease are not trained to look into occupational, environmental, or toxicological issues and end-stage renal disease. Many patients are listed as having hypertensive end-stage kidney disease and are presumed therefore to be "idiopathic" in origin, however, these cases may very well be the result of other industrial and/or environmental factors, among them, solvent exposure. Many of the problems in the epidemiological analysis is the result of a great reserve capacity of the kidney that can function relatively adequately despite slowly progressive damage. End-stage kidney disease is typically not diagnosed until considerable kidney damage has already occurred at the time when the patient seeks clinical attention. Furthermore, kidney biopsy and post-mortem examination, almost always find small kidneys, inadequate to help in the histopathological assessment, and therefore the etiology is either missed or is misclassified as "idiopathic" or "unknown".

Indeed the study by Stengel. et al.[50] looked at organic solvent exposure and the risk of IgA nephropathy. These investigators have shown that the risk of IgA nephropathy is highest among the most exposed group to oxygenated solvents. The study by Yaqoob et al.[37] showed an increased risk factor of 15.5 for development of glomerulonephritis in patients exposed to aliphatic hydrocarbons and a risk factor of 5.3 in patients exposed to halogenated hydrocarbons. These epidemiological data further supports observations made in the case reports, case studies and experimental animal studies. The epidemiological studies by Steenland et al.[51] had evaluated the risks and causes of end-stage kidney disease and concluded that regular exposure to industrial solvents played a significant role in the development of chronic end-stage kidney disease.

Based on the current literature from experimental animal studies, case reports, case-control studies, and epidemiological studies, one can conclude that the studies show:

1. Biological plausibility.
2. A temporal relationship between exposure to industrial solvents and the development of chronic kidney disease (glomerulonephritis).
3. A dose-response relationship.
4. Consistency of association.

5. Statistical association in the majority of the studies.

These criteria fulfill the Bradford-Hill criteria,[52] and establish the basic criteria required for causation.

20.4.6. MECHANISM

Immune-mediated mechanisms play a major role in the pathogenesis of glomerular disease, in general. In the vast majority of the cases, antigen-antibody reaction and immune complexes form in the kidney, mainly around the glomerular capillary wall and mesangium. Cellular antigens, both endogenous such as DNA and tumor antigens, as well as exogenous such as viral antigen hepatitis B and C, drugs, and bacteria have been shown to be causative factors in human glomerular immune-mediated diseases. The most common pathological process described in association with solvent exposure and chronic glomerular nephritis has been that of IgA nephropathy, Good Pasture's syndrome, and proliferative glomerulonephritis.

Unlike acute renal failure caused by hydrocarbons, where the renal damage is secondary to the nephrotoxins and mainly cause damage of the proximal tubule acute renal failure, the glomerular chronic renal failure, appears to be immunologically mediated. Among others, genetic factors may be involved in the pathogenesis of hydrocarbon induced nephropathy. It has been suggested that the propensity to develop this autoimmune disease depends on a combination of a genetic component and predilection, and environmental component.[24] Individuals susceptible to glomerular or tubular injury by hydrocarbons may develop chronic kidney disease through three possible mechanisms. The first mechanisms is direct tubular toxicity which is commonly the cause of acute renal failure. While it is true that the initial injury of acute renal failure is directed toward the tubule of the nephron, glomerulonephritis may be the result of an autoimmune reaction to the tubulotoxins.[53,54] The second mechanism mainly involves immunosuppression. Ravnskov,[53] in a review of the pathogenesis of hydrocarbon associated glomerulonephritis, suggested that hydrocarbons are immunosuppressives and this effect is noted in several locations in the immunological cascade. This includes leukocyte mobility and phagocytosis suppression such as shown in the benzene effects in mice.[55] This suppression of the normal immune response by hydrocarbons may play a role in the pathogenesis of immune-mediated glomerular lesions. The third mechanism involves alteration in membrane permeability. Good Pasture's syndrome is mediated by antibodies reactive with the glomerular basement membrane and alveolar basement membranes. Antibodies in experimental models can usually bind to alveolar basement membranes in vitro by indirect immunofluorescence. Experimental studies suggested that hydrocarbons alter the permeability of pulmonary capillaries, thereby allowing anti-glomerular basement membranes to bind to the alveolar basement membranes.[56] This etiology is further supported by the observation that differential sensitivity to exposures due to genetic factors since DR3 and DR4 antigens are more frequent in patients with toxic nephritis than in the general population.[57,58] The study by Zimmerman et al.[40] have shown that in 6 of 8 patients with Good Pasture's syndrome had extensive occupational exposure to solvents ranging from 4 months to 10 years. The results of this study suggested that interaction between the inhaled hydrocarbons and the lung and kidney basement membranes could induce autoantibodies to these membranes. Goyer[48] suggested an autoimmune mechanisms responsible for glomerular lesions following chronic exposure to solvents. Based on case studies and case reports, it is proposed that chronic exposure to low levels of solvents in susceptible individuals induces an initial cell injury sufficient to damage cell membranes and to

provide the antigen triggering the immune response, accelerating a cascade of a reaction ending with glomerulonephritis.

REFERENCES

1 Morrison, RR, and Boyd RN, **Organic Chemistry**, 5th Edition, Morrison RT, Boyd RN, Eds, *Allyn & Bacon*, Boston (1987).
2 Domask, WG, **Renal Effects Of Petroleum Hydrocarbons**, Mehlman, MA, Hemstreet GP III, Thorpe JJ, Weaver NK, Eds, *Princeton Scientific Publishers*, Princeton, 1-25.
3 Pederen, LM., *Pharmacol Toxicol*, **3**, 1-38 (1987).
4 Ervin ME, **Clinical Management of Poisoning and Drug Overdose**, Addad LM, Winchester JF, Eds, *Saunders*, Philadelphia, 771 (1983).
5 Wolfsdorf J, *J Pediatr*, **88**, 1037 (1976).
6 Janssen S, Van der Geest S, Meijer S, and Uges DRA, *Intensive Care Med*, **14**, 238-240 (1988).
7 Smith TC, and Wollman H, **The Pharmacological Basis of Therapeutics**, Goodman A, Gilman L, Eds, *MacMillan*, New York, 260-275 (1985).
8 Clayton GD, and Clayton EE (Eds), **Patty's Industrial Hygiene and Toxicology**, *Wiley*, New York, 26 (1981) and 2C (1982).
9 World Health Organization and Nordic Council of Ministers, Organic Solvents and the Central Nervous System, Environmental Health 5, Copenhagen and Oslo, 1-135 (1985).
10 Browning E, **Toxicity & Metabolism of Industrial Solvents**, *Elsevier Publishing Company*, Amsterdam-London-New York (1965).
11 Mehlman MA, Hemstreet GP III, Thorpe JJ, and Weaver NK, Series: Advances in Modern Environmental Toxicology: Volume VII- **Renal Effects of Petroleum Hydrocarbons**, *Princeton Scientific Publishers*, Princeton (1984).
12 Carpenter CP, Geary DL Jr Myers, et al, *Toxicol Appl Pharmacol*, **41**, 251-260 (1977).
13 Gibson JE, and Bus JS, *Ann NY Acad Sci,* **534**, 481-485 (1988).
14 Thomas FB, and Halder CA, Holdsworth CE, Cockrell By, **Renal Heterogeneity and Target Cell Toxicity**, Back PH, Lock EA, Eds, Chichester, 477-480 (1985).
15 Halder CA, Van Gorp GS, Hatoum NS, and Warne TM, *Am Ind Hyg Assoc J*, **47**, 164-172 (1986).
16 Coppor, et al, *American Journal of Kidney Disease*, **12**, 420-424 (1988).
17 Emancipator SN, *Kidney International,* **38**, 1216-1229 (1990).
18 Churchill DN, Fina A, and Gault MH, *Nephron*, **33**, 169-172 (1983).
19 Roy AT, Brautbar N, and Lee DBN, *Nephron*, **58**, 385-392 (1991)
20 Sperace GA, *Am Rev Resp In Dis*, **88**, 330-337 (1963).
21 Hele WF, Matthisson Am, and Niall JF, *Med J Aust*, 355-357 (1969).
22 Klavis G, and Drommer W, *Arch Toxicol*, **26**, 40-50 (1970).
23 Beirne GJ, and Brennan JT, *Arch Environ Health*, **25**, 365-369 (1972).
24 D'Apice AJF, et al, *Ann Intern Med*, **88**, 61-62 (1980).
25 Kleinknecht D, et al, *Arch Intern Med*, **140**, 230-232 (1980).
26 Daniell WE, Couser WG, and Rosenstock L, *JAMA*, **259**, 2280-2283 (1988).
27 Von Scheele C, Althoff P, Kempni V, and Schelin H, *Acta Med Scan*, **200**, 427-429 (1976).
28 Ehrenreicht T, Yunis SL, and Churg J, *Environ Res*, **24**, 35-45 (1977).
29 Cagnoli L, et al, *Lancet,* 1068-1069 (1980).
30 Brautbar N and Barnett A, *Environmental Epidemiology and Toxicology*, **2**, 163-166 (1999).
31 Lagrue G, et al, *J Urol Nephrol*, **4-5**, 323-329 (1977).
32 Lagrue G, et al, *Nouv Press med*, **6**, 3609-3613 (1977).
33 Bell GM, et al, *Nephron*, **40**, 161-165 (1985).
34 Ravnskov U, Forsbergg B, and Skerfving S, *Acta Med Scand,* **205**, 575-579 (1979).
35 Ravnskov U, Lundstrom S, and Norden A, *Lancet,* 1214-1216 (1983).
36 Porro A, et al, *Br J Ind Med*, **49**, 738-742 (1992).
37 Yaqoob M, Bell GM, Percy D, and Finn R, *Q J Med,* **301**, 409-418 (1992).
38 Yaqoob M, et al, *Diabet Med*, **11**, 789-793 (1994).
39 Nuyts GD, et al, *Lancet*, **346**, 7-11 (1994).
40 Zimmerman SW, Groehler K, and Beirne GJ, *Lancet*, 199-201 (1975).
41 Ravnskov U, *Acta Med Scand*, **203**, 351-356 (1978).
42 Finn R, Fennerty RG, and Ahmad R, *Clin Nephrol*, **14**, 173-175 (1980).
43 Van der Laan G, *Int Arch Occup Envion Health*, **47**, 1-8 (1980).

44 Harrison DJ, Thompson D, and MacDonald MK, *J Clin Pathol*, **39**, 167-171 (1986).
45 Askergren A, A*cta Med Scand*, **210**, 103-106 (1981).
46 Yaqoob M, et al, *Q J Med*, **86**, 165-174 (1993).
47 De Broe ME, D'Haese PC, Nuyts GD, and Elseviers MM, *Curr Opin Nephrol Hypertens*, **5**, 114-121 (1996).
48 Goyer RA, *Med Clin of North America*, **74**, 2, 377-389 (March 1990).
49 Landrigan P, et al, *Arch Environ Health*, **39**, 3, 225-230 (1984).
50 Stengels B, et al, *Int J Epidemiol,* **24**, 427-434 (1995).
51 Steenland NK, Thun MJ, Ferguson BA, and Port FK, *AJPH*, **80**, 2, 153-156 (1990).
52 Hill AB, The Environment and Disease: Association or Causation? President's Address. Proc Royal Soc Med, 9, 295-300 (1965).
53 Ravnskov U, *Clin Nephrol*, **23**, 294-298 (1985).
54 Tubbs RR, et al, *Am J Clin Pathol*, **77**, 409 (1982).
55 Horiguchi S, Okada H, and Horiuchi K, *Osaka City Med J*, **18**, 1 (1972).
56 Yamamoto T, and Wilson CB, *Am J Pathol*, **126**, 497-505 (1987).
57 Emery P, et al, *J Rheumatol*, **11**, 626-632 (1984).
58 Batchelor JR, et al, *Lancet, i*, 1107-1109 (1984).

20.5 LYMPHOHEMATOPOIETIC STUDY OF WORKERS EXPOSED TO BENZENE INCLUDING MULTIPLE MYELOMA, LYMPHOMA AND CHRONIC LYMPHATIC LEUKEMIA

Nachman Brautbar
University of Southern California, School of Medicine, Department of Medicine, Los Angeles, CA, USA

20.5.1 INTRODUCTION

Benzene, is an aromatic hydrocarbon and historically has been produced during the process of coal tar distillation and coke production, while today benzene is produced mainly by the petrochemical industry. Based on the National Institute of Occupational Safety and Health (NIOSH), in the United States, it has been estimated that in 1976 two million Americans were exposed occupationally to benzene.[1] Worldwide production of benzene is approximately 15 million tons[2] and the production in the United States is estimated to be increasing at least 3% annually,[3] approaching 6 million tons of benzene produced in the United States in 1990 and 6.36 million tons produced in 1993.[2] Benzene has been described as a clear, colorless, non-corrosive, and flammable liquid with a strong odor.

Benzene is used as an excellent solvent and degreasing agent, and as a basic aromatic unit in the synthetic process of other chemicals.[4] Exposure to benzene in the occupational setting most commonly occurs in the chemical, printing, rubber, paint, and petroleum industry. Among other sources of exposure to benzene, non-occupational exposures in the form of cigarette smoking and exposure to gasoline and its vapors during fueling motor vehicles.[5]

20.5.2 ROUTES OF EXPOSURE

The major route of exposure to benzene is inhalation through the lungs of benzene vapors, however skin absorption of benzene has been shown to be significant depending on the circumstances of the exposure such as time of contact between the benzene and the skin.[1,6] Benzene absorption, as other solvents, through the skin is enhanced if the skin condition is altered by either disease or loss of skin or cracking of the skin.[4] The dermal absorption of benzene deserves much more attention than previously described in various texts of toxicol-

ogy and occupational medicine, as well as environmental and industrial health. Dermal absorption of benzene in workers who use either toluene containing benzene or other solvents containing benzene, is a significant factor in calculating dosimetry and absorption of benzene and can be calculated utilizing standard accepted methodology. A recent study by Dr. Brenner et al.[6] described chronic myelogenous leukemia due to skin absorption of benzene as a contaminant of other solvents. The investigators in that study[6] concluded that the total benzene absorbed dose via skin and inhalation was equivalent to an accumulated vapor exposure of 196.4 + 42 ppm-years. Dermal exposure accounted for 97% of the total absorbed dose of benzene. Inhalation of benzene from occupational, smoking and ambient non-occupational sources accounted for only 3% of the benzene dose. The authors presented the reports of dermal absorption of benzene in the following table.

Table 20.5.1. Summary of benzene dose expressed as equivalent ppm-years. [Adapted, by permission, from D. Brenner, *Eur. J. Oncol.*, 3(4), 399-405, 1998.]

Case	Solvent	Dermal	Occupational inhalation	Cigarette smoke	Ambient inhalation	Total
1997	Toluene, MEK, Acetone	170.4	19.2	0.05*	0.29	189.9
1998 Case 1	Mineral spirits	41.1	17.8	0.1*	0.23	59.3
1998 Case 2	Refinery process streams	19.0	4.6	1.4	0.4	196.4

*Second hand cigarette smoke

Therefore, workers who are exposed to solvents containing benzene should be evaluated for skin absorption dosimetry, in addition to other sources such as inhalation, to address the range of levels of exposure.

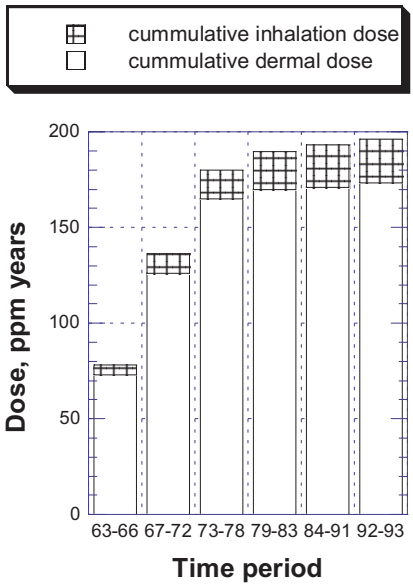


Figure 20.5.1. Cumulative Dose of benzene over 30 years. [Adapted, by permission, from D. Brenner, *Eur. J. Oncol.*, **3**(4), 399-405, 1998.]

Therefore, dermal absorption of benzene, especially in connection with benzene as a byproduct in other solvents, is of extreme importance in dosimetry analysis.

Once benzene reaches the blood, it is metabolized mainly in the liver. The metabolic products are excreted in the urine within 48 hours from absorption. Several metabolites have been found in the urine after benzene exposure, among those are phenol, quinone, hydroquinone, and muconic acid.[7] The liver utilizes the cytochrome P450 and oxidization system for the metabolism of benzene.[8,9]

Specific cellular toxic effects from benzene have been described and those include, among others, the central nervous system (doses of over 100 ppm), liver, kidney, skin, immunological, and carcinogenic. The various toxicological effects of benzene will not be discussed in this chapter

since the scope of this chapter is the hematopoietic effects, the reader is referred to other sources.[3]

20.5.3 HEMATOPOIETIC EFFECTS OF BENZENE

Benzene is a proven human carcinogen. The toxicity of benzene has been known since the 19th Century when aplastic anemia was first reported.[4,10] Indeed the causal link between benzene and bone marrow toxicity in the form of hematoxicity and bone marrow suppression was described already in 1897.[11] In 1928, Delore et al. described leukemia as a result of benzene exposure.[12] In 1932 Lignac[13] reported lymphoblastoma in association with benzene exposure. Several studies have reported the association between exposure to benzene and hematopoietic toxicity and leukemia.[14,15] Acute myeloid leukemia has been the most frequent form of leukemia found to be related to benzene exposure. Other forms of leukemia have been described in association with benzene exposure, such as erythroleukemia, thrombocytopenia, acute myeloid leukemia, myelodysplastic syndrome, acute lymphoblastic leukemia, chronic lymphocytic leukemia, and Hodgkin's and non-Hodgkin's lymphoma. As a result of the high toxicity of benzene the American Petroleum Institute in their paper on benzene exposure in 1948 have concluded that the only safe level of exposure to benzene is no exposure at all.[16] The language utilized was as follows, "In as much as the body develops no tolerance to benzene, and as is there is a wide variation in individual susceptibility, it is generally considered that the only absolutely safe concentration for benzene is zero."

The following hematological conditions have been described in association with benzene.[17-40]

1. Acute myelogenous leukemia.
2. Erythroleukemia.
3. Aplastic anemia.
4. Acute monocytic leukemia.
5. Chronic myelogenous leukemia.
6. Myelofibrosis and myeloid metaplasia.
7. Thrombocythemia.
8. Acute lymphoblastic leukemia.
9. Chronic lymphocytic leukemia.
10. Lymphomas and related disorders.
11. Multiple myeloma
12. Myelodysplastic syndrome.

20.5.4 CARCINOGENIC EFFECTS OF BENZENE

Several well conducted epidemiological scientific studies and data have provided the epidemiological basis for benzene as a hematopoietic and lymphopoietic cancer. In his paper entitled "Benzene Health Effects", Mehlman described a wide range of the hematotoxicity of benzene.[41] Nilsson et al.[42] described leukemia, lymphoma and multiple myeloma in seamen exposed to benzene in tankers. In this study, an increased incidence of lymphatic and hematopoietic malignancies was described and, while it is true that the cargo vapors from gasoline and other light petroleum products and chemicals have been studied, benzene exposure during loading, unloading and tank cleaning operations was concluded to be the likely source of the carcinogenic exposure. Rinsky et al.[43] described various hematological malignancies in their study of benzene exposure and showed that the overall standardized

mortality ratio for leukemia and multiple myeloma were increased significantly. The investigators of this study concluded that there is a quantitative association between benzene exposure and development of leukemia. Wong[44] evaluated a mortality study of chemical workers occupationally exposed to benzene and found a significantly increased risk for lymphohematopoietic malignancies. Linet et al.[45] studied hematopoietic malignancies and related disorders among benzene exposed workers in China and showed a wide spectrum of hematopoietic malignancies.

Song-Nian Yin et al.[46] in a cohort study of cancer among benzene exposed workers in China, studied workers employed in a variety of occupations and showed a statistically significant increased deaths among benzene exposed subjects for leukemia, malignant lymphoma, neoplastic diseases of the blood, and other malignancies. The rates were significantly elevated for the incidence of lymphohematopoietic malignancies risk ratio of 2.6, malignant lymphoma risk ratio of 3.5, and acute leukemia risk ratio 2.6. A significant excess risk was also found for aplastic anemia and myelodysplastic syndrome. These investigators concluded that employment in benzene exposure occupations is associated with a wide spectrum of myelogenous and lymphatic malignant diseases and related disorders of the hematopoietic lymphatic system.

Hayes et al.[47] in one of the largest epidemiological studies on benzene exposure, showed a wide spectrum of hematological neoplasms and their related disorders in humans. The risk for these conditions is elevated at average benzene exposure levels of less than 10 ppm. These investigators further concluded that the pattern of benzene exposure appears to be important in determining the risk of developing specific diseases. Wong[48] studied a cohort of 7,676 male chemical workers from seven plants who were occupationally exposed continuously or intermittently to benzene for at least 6 months, and compared them to a group of male chemical workers from the same plant who had been employed for at least 6 months during the same period but were never occupationally exposed to benzene and showed a significantly increased risk of lymphohematopoietic malignancies.

In experimental animals benzene has been shown to be associated, in rats, with cancers of zymbal gland, oral cavity, nasal cavities, skin, forestomach, mammary gland, Harderian gland, preputial glands, ovary, uterus, angiosarcoma of liver, hemolymphoreticular neoplasia, lung cancers and leukemia.[49] The ability of benzene metabolites to effect lymphocytic growth and function in vitro, is shown to correlate with the oxidation capacity and concentration of the metabolites at the target site. Benzene also effects macrophages, as well as lymphocytes.[36,50,51] Kalf and Smith[52,53] have shown that benzene exposure reduces the ability of marrow stromal cells to support normal stem cell differentiation. From these experimental animal studies, and in vitro studies a wide range of bone marrow effects of benzene metabolites is shown. It has been concluded that the hematotoxicity of benzene depends on the breakdown metabolites and can effect the stem cell at any point in time, for instance myeloid, erythroid, macrophages, lymphocytic stem cells, and therefore benzene has been named as a pleural potential stem cell toxicant.[54]

In addition to carcinogenic effects, animal studies have shown the effects of benzene exposure on the immune system. Reid et al.[55] showed a significant decrease in splenic cell proliferation in mice exposed to benzene for 14 days. Experimental animal studies also reported reduced circulating white blood cells, as well as changes in spleen morphology and weight in various experimental animal studies.[54] These experimental animal studies further support the observation from 1913 by Winternits and Hirschfelder[56] that rabbits exposed to

benzene showed an increased susceptibility to pneumonia and tuberculosis. The experimental animal data and the epidemiological studies clearly show that 1) benzene is a carcinogen for the lymphohematopoietic system, 2) benzene has a direct effect on the immune system, 3) benzene has a direct effect on the early development of the blood cells, and 4) benzene is a pluripotent hematological carcinogen.

20.5.5 RISK ASSESSMENT ESTIMATES

The United States EPA has used several databases in their estimates for benzene exposure and risk. (Environmental Protection Agency, 5.0 Benzene, 5.1. Chemical and Physical Properties, EPA, 1988) The data utilized by the EPA to assess the risk included the study by Rinsky et al. in 1981[57] where the duration of exposure was at least 24 years and exposure levels are between 10 to 100 ppm (8 hour TWA) with a statistically significant increase incidence of leukemia. The study of Ott et al.[57] in 1978 showed levels of anywhere from 2 to 25 ppm (8 hour TWA) with increased incidence of leukemia; and Wong et al.[57] 1983, where the exposure was at least 6 months, levels were from 1 ppm to 50 ppm, and there was a statistically significant increase in the incidence of leukemia, lymphatic and hematopoietic cancers.

The International Agency for Research on Cancer (IARC) has classified benzene as a Group 1 carcinogen. [58] A Group 1 carcinogen is defined as an agent that is carcinogenic to humans. This classification is based on sufficient evidence for carcinogenicity in humans. IARC based this conclusion on the fact that numerous case reports and follow-up studies have suggested a relationship between exposure to benzene and the occurrence of various types of leukemia. In addition, IARC considers the evidence for carcinogenicity to animals to be sufficient. No unit risk was determined by IARC for benzene.

The regulatory agencies in the estimated risk of increased benzene related cancers rely mainly on the study by Rinsky, 1987, which concluded that the mean annual cumulative exposure level of less than 1 ppm accumulated over 40 years working life-time would not be associated with increased death from leukemia. This epidemiological study showed an exponential decrease in the risk of death from leukemia which could be achieved by lowering occupational exposure to benzene. According to the model derived in this study, a worker occupationally exposed to benzene at an average exposure level of 10 ppm for 40 years would have an increased risk of death form leukemia 154.5. If the average exposure was lowered to 1 ppm, that excess risk would decrease to 1.7. At 0.1 ppm times 40 years cumulative exposure the risk be virtually equivalent to background risk,[26] Infante et al.[59] have shown a relative risk of 5.6 with an estimated cohort exposure of 10 to 100 ppm over 8.5 years average, and Vigliani[60] showed a relative risk of 20 estimated cohort exposure to 200 to 500 ppm over 9 years average, and Aksoy showed a relative risk of 25 with an estimated cohort exposure of 150 to 210 ppm over 8.7 years average.[20,40] These studies clearly show that the risk of developing lymphohematopoietic cancers is significant, and that benzene is carcinogenic from an epidemiological point of view at very low levels of 0.1 ppm.

20.5.6 LEVELS OF EXPOSURE

Regulatory levels of exposure to various chemicals, among them solvents and benzene, have been a subject for constant pressure from industry manufacturers on one hand, regulatory agencies, health care, and patients on the other hand. The most common question asked is “Is there a safe level of exposure to benzene?” The answer to that question has been given by the American Petroleum Institute in their paper on benzene, 1948,[16] and their statement

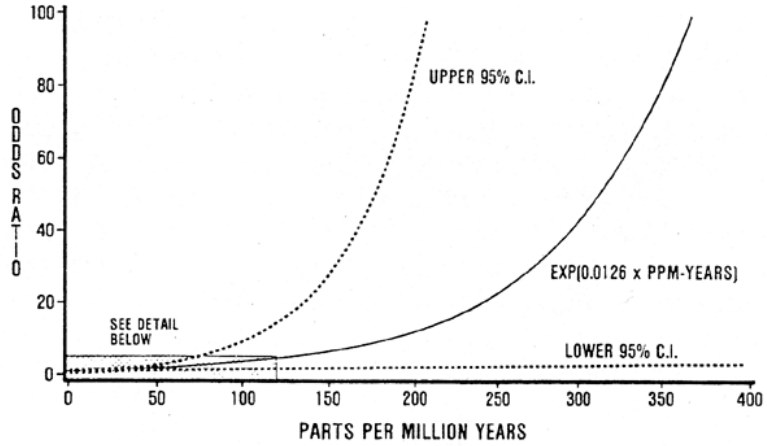


Figure 20.5.2. Extrapolations of levels of exposure to benzene. [Adapted, by permission, from R.A. Rinsky, *New England J. Med.*, 1987.]

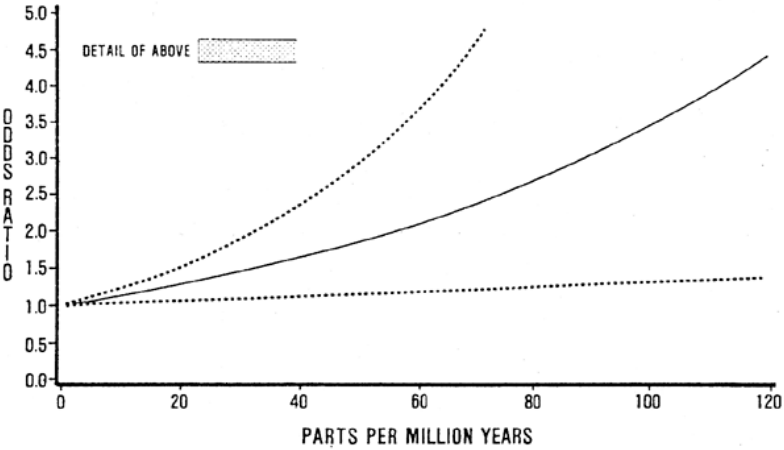


Figure 20.5.3. Extrapolations of levels of exposure to benzene. [Adapted, by permission, from R.A. Rinsky, *New England J. Med.*, 1987.]

that the only safe level of exposure to benzene is no exposure at all. Rightly so, the paper based that opinion on the fact that the body develops no tolerance to benzene and there is wide variation in individual susceptibility and therefore the only absolutely safe concentration for benzene is zero. This approach has been confirmed from a point of view of epidemiological studies and experimental animal studies showing benzene to cause cancer in experimental animals, and case reports and epidemiological studies in humans. Based on epidemiological studies and extrapolation from experimental animal studies, IARC's position is that a linear regression line should be applied for the dose response crossing the zero point for low level exposure of benzene. The EPA concurred that at low levels of exposure (since no epidemiological studies are available at low levels of exposure) linear dose response is indicated. The modalities of exponential dose response relationship for low levels

is not applicable here based on the most recent EPA and IARC positions.[57,58] This linear, non-threshold model assumes that every increment of dose is accompanied by a commensurate increment in the excess cancer risk. The use of this toxicological model allows extrapolation of risks from relatively high dose levels, where cancer responses can be measured, to relatively low dose levels, where such risks are too small to be measure directly through epidemiological studies.[61] Figures 20.5.2 and 20.5.3 demonstrate the extrapolation from high levels of exposure to low levels of exposure utilizing the linear modality.

Indeed, in its most recent publication the U.S. EPA, 10/14/98,[62] further supports that approach and the panel members who evaluated the data felt that for the leukemogenic effects of benzene the linear model is consistent with the spirit of the proposed cancer risk assessment guidelines.

20.5.7 CELL TYPES: HEMATOLYMPHOPROLIFERATIVE EFFECTS OF BENZENE

The hematopoietic cell type toxicity from benzene have been described in animal data, case reports, case studies, animal data, and epidemiological data. Essentially, Wong in his OSHA testimony[63] concludes that for the continuously exposed group the lymphohematopoietic cancer risk ratio was 3.2 with a statistical significance of $p<0.05$, the risk ratio for non-Hodgkin's and other lymphopoietic cancer, i.e., all lymphopoietic cancers minus Hodgkin's disease, for the continuously exposed group was 3.77. The data demonstrated a statistical significant dose response relationship between cumulative exposure to benzene and mortality from all lymphopoietic cancer combined with leukemia. Wong further stated that it would be appropriate to combine lymphoma and leukemia in some of the analyses. This approach has also been recommended recently by other investigators[64] and therefore the agency conducting the hearing felt that the analysis based on the revised grouping of lymphopoietic cancers with Hodgkin's disease separated out was appropriate.[63] In the documents submitted to the OSHA hearing, Wong concluded that a dose response relationship for all lymphatic and hematopoietic cancers has been demonstrated.

Based on the case reports, case studies, and epidemiological studies, the sub-classification to cell types is indicated from a medical point of view to treat various hematopoietic diseases and cancers with the various appropriate treatments per each type of cell injured, however the data from the experimental data and clinical analysis of benzene cases clearly show that benzene causes damage to the stem cell and therefore it is a pluripotent toxin, causing a wide range of lymphohematopoietic malignancies.

20.5.8 EPIDEMIOLOGICAL STUDIES

Wong,[48] studied a cohort of 7,676 male chemical workers from seven plants who were occupationally exposed continuously or intermittently to benzene for at least 6 months, and compared them to a group of male chemical workers from the same plant who had been employed for at least 6 months during the same period but were never occupationally exposed to benzene. When the group with no occupational exposure was used for direct comparison, the continuously exposed group experienced a relative risk from lymphohematopoietic cancer of 3.2 with a statistical significance of $p<0.05$. That paper further concluded that the medical problems are replete with reports documenting the transition from certain lymphomas and multiple myelomas to leukemias. It was concluded that the transitions or progressions from lymphoma to leukemia are further complicated by the historical changes in nomenclature and in diagnostic overlap between the 2 disorders. It was felt,

based on the work of others, that the major clone in chronic myelocytic leukemia affected cells capable of becoming lymphocyte, granulocyte, and erythrocyte differentiations leading to the conclusion that transformation events occur at an early multipotent stem cell level.

Nilsson et al.[42] investigated Swedish seamen, 20-64 years of age, who had been exposed to cargo vapors for at least 1 month on chemical or product tankers, had an increased risk of lymphatic and hematopoietic malignancies odds ratio of 2.6 with 95% confidence interval, with a significant exposure response relation. The odds ratio was increased for non-Hodgkin's lymphoma at 3.3 with 95% confidence interval and was statistically significant. Rinsky et al.[43] studied a cohort of 11,065 white men with at least 1 ppm per day of cumulative exposure to benzene. They have demonstrated that there was a statistical significant increase in death from all lymphatic and hematopoietic neoplasms, 15 observed versus 6.6 expected standard mortality ratio which is 227, 95% confidence interval, further demonstrating that benzene is toxic to all cell types.

Hayes et al.[47] studied a cohort of 74,821 benzene exposed and 35,805 unexposed workers from 1972 until 1987 in 12 cities in China. By and large this is the largest and most significant cohort of benzene workers studied and published. The investigators found that 1) benzene exposure is associated with a spectrum of hematological neoplasms, 2) workers with 10 or more years of benzene exposure had a risk ratio of developing non-Hodgkin's lymphoma of 4.2 with 95% confidence interval, and the development of this neoplasm was linked most strongly to exposure that had occurred at least 10 years before the diagnosis, and 3) the risk for the combination of acute non-lymphocytic leukemia and related myelodysplastic syndromes was significantly increased among those with more recent benzene exposure. These studies confirm the previous studies proving that the damage from benzene is to all cell type.

Linet et al.[45] studied hematopoietic malignancies and related disorders among benzene exposed workers in China and showed a wide range of hematopoietic malignancies. Yin et al.[46] examined a large cohort of benzene workers and concluded that benzene exposed workers have a statistically significant excess death due to leukemia, risk ratio of 2.3 with 95% confidence interval; malignant lymphoma, risk ratio of 4.5 with 95% confidence interval; and non-neoplastic diseases of the blood.

In summary, these epidemiological studies published in the peer-reviewed scientific literature and relied on by scientific and governmental agencies clearly show 1) significant statistical association between benzene exposure and lymphohematopoietic cancers of all cell types, 2) an increased risk and/or increased standard mortality rate over a factor of 2 in patients exposed to benzene with the development of non-Hodgkin's lymphoma, leukemias, and other lymphohematopoietic malignancies, and 3) benzene is carcinogenic with a linear dose response demonstrating no threshold.

20.5.9 SOLVENTS AND BENZENE

Solvents commonly used in the industry have been shown to contain benzene. Elkins, et al.[65] found that from time to time analyzed solvents for benzene content showed anywhere from 1% to 2% benzene. In that paper, which was published in 1956, the authors state that the TLV value from a regulatory point of view, at that time, was 35 ppm compared with 100 ppm previously. According to their calculations, they found that a benzene content below 3.5% will be necessary, for instance in solvents containing naphtha, hexane, and toluene, otherwise the permissible level for benzene vapor will be exceeded over 35 ppm, which

we now know is extremely and significantly higher than the standard allowed today from a regulatory point of view. At the request of the petrochemical companies, the authors decided to reevaluate the content of benzene in solvents and for this purpose a total of 8 samples of low boiling petroleum naphtha were obtained. After utilizing methodology which included, among others, mass spectrometry for benzene content, the authors concluded that, in general, the benzene content of solvents ranged from 1% to 4% in volume. They have further shown that in the air of one plant where hexane with a relatively low benzene content (1.5%) was used as a solvent in a fabric-spreading operation, a benzene vapor concentration of 1 ppm was found. Since exposure to benzene is cumulative, if a worker is exposed to hexane containing 1.5% benzene, with both inhalation and skin contact, the cumulative exposure over a certain period of time increases the risk of developing benzene related cancers as described by the Rinsky model. The study by Pagnotto,[66] looked at and analyzed 32 naphtha solvents. The benzene concentration ranged from 1.5% to 9.3% by weight. Excessive benzene exposure was found at 3 out of 4 plants during their operations on a daily basis. On one occasion the concentration of benzene vapor was as high as 125 ppm (extremely high), and the urinary phenol excretion of the workers in these 3 plants were the highest that these investigators report ranging from 370 to 917 mg per liter of urine. These study indicate that solvents which contain benzene, even at levels of 1.5% per volume, can be associated with significant atmospheric exposure to benzene, shown as causing human exposure with significant excretion of phenol in the urine indicative of heavy benzene exposure. The investigators recommended additional ventilation, and on a follow-up visit the benzene exposure was found to have been reduced to about 70 ppm with urinary phenols of less than 70 mg per liter, still significantly elevated and considered a significant risk. These investigators also looked at blood examinations of 47 men at these plants. Five employees showed lower hemoglobin. One man showed a low hemoglobin at the age of 28, having been employed for 3 years in the environment preparing mixes for the saturating machine. While leukemia was suspected due to bone marrow disease, the patient was treated with iron and recovered. The authors conclude that excessive benzene exposures is consistently found on saturators using naphtha containing more than 3% benzene. This study further shows the importance of assessing benzene concentration is other solvents, from a dosimetry point of view.

The manuscript entitled A Recommended Standard for Occupational Exposure to Refined Petroleum Solvents from the U.S. Department of Health, Education and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, July 1977,[67] recommended standards to be applied to occupational exposure of workers to the following refinery petroleum solvents: petroleum ether, rubber solvent, varnish maker's and painter's naphtha, mineral spirits, Stoddard solvents, and kerosene are all included in the term refined petroleum solvents. According to these standards petroleum ether and rubber solvents which contain 1.5% benzene, varnish maker's and painter's naphtha which contain 1.5% benzene, mineral spirits which contain 13-19% aromatics, Stoddard solvent which contains 0.1% benzene, 140 Flash Aliphatic Solvent which contains 0.7% benzene, kerosene. NIOSH indicated that some of the refined petroleum solvents contain aromatic hydrocarbons including, in some cases, benzene. Standards were applied, among others, to reduce the benzene exposure. Among others, the use of respirators and skin protective devices were required to protect from the effects of the solvents, as well as the benzene component.[67] In his testimony in front of the Occupational Safety and Health

Administration,[68] Proctor testified that, among others, refining operations are continuously changing. Many refineries obtain crude of differing characteristics from various producing areas which sometimes must be processed individually due to crude incompatibility and produce requirements. This means the operation of a crude fractionation unit is altered frequently; a single crude run may be as short as 2 days. Consequently, the crude tankage, crude fractionation units, and all downstream processing units frequently contain benzene. Proctor further stated that it should be clear by now that benzene is a naturally occurring compound in crude oil and is also found in the catalytic and cracking process, and therefore will always be a contaminant of these solvents. Benzene levels in gasoline today are running about 1.1% on the average across the nation but occasionally may reach 4% on individual samples. Reduction of benzene levels in gasoline is technically possible through employment of a number of physical processing schemes to the various gasoline component streams. He further testified that any attempt of physical separation of hydrocarbons, such as distillation, solvent extraction, or adsorption, the separation is not 100% complete. Therefore, some residuals of benzene will always be present in the remaining fraction. Therefore he recommended that benzene should be converted to cyclohexane by hydrogenation which would require an expensive catalyst, expensive high pressure reaction vessels, and consumption of valuable hydrogen. The testimony further indicates that it is believed that it is almost physically impossible to reduce these streams below 0.1% benzene. The 1978 OSHA[69] indicate that "The record establishes that there is a wide variation in the benzene content of petroleum solvents used in the rubber, paints, coatings, adhesives, sealants, and other downstream industries. As reported by Smith, reporting on behalf of MCA,[69] the benzene content of petroleum solvents of all types generally range from under 0.1% to 4%. Data submitted by downstream industries confirms that benzene is present in virtually all petroleum solvents, at levels which approach and even exceed 3.5% in some cases." It was stated that in the rubber industry, solvent benzene content appears to range from 0.1% to 0.7% or slightly higher. Similarly, solvents used by adhesive manufacturers show broad variations from less than 0.1% to 3.5%.[69] Representatives of the paint industry report variations from under 0.1% to as high as 3.7%.[69] Smith in his testimony emphasized that solvent benzene content is likely to vary substantially among supplies, among different plants of the same supplier, and among deliveries from the same plant. Because refinery processes are not designed to precisely control benzene content, variations will inevitably occur.

These data clearly show that benzene contents in solvents are difficult to control and vary depending on the sources, processes and therefore solvent exposure must take into account the level of benzene concentration in these solvents. These data, taken together with the most recent study of Brenner et al.[6] show that industrial toluene solvent does contain benzene and contributed significantly to the exposure via the skin to benzene. One must remember the importance of benzene exposure through the use of solvents produced through the petrochemical refining processes.

20.5.10 GENETIC FINGERPRINT THEORY

Benzene and its metabolites have long been known to cause chromosomal aberrations of various types of cell cultures of exposed humans. (To be discussed in Chapter 20.6 in this book entitled as Benzene Exposure and Sister Chromatoid Changes.) While it is true that genetic changes have been described and frequently effect chromosomes 5 and 7, and others, there is no scientific evidence that these are required for the diagnosis of benzene exposure related cancers. Specifically, many cases of patients who have been exposed to

benzene and have developed hematopoietic malignancies do not have changes in chromosomes, therefore the chromosomal changes cannot be used as a "genetic fingerprint". Indeed, Irons' publication in the Journal of Toxicology and Environmental Health[70] concluded that the significance of these chromosomal alterations with respect to bone marrow damage or leukemogenesis of benzene is unclear. It is not possible today to determine whether leukemia is caused by benzene based on changes in chromosomes, specifically chromosomes 5 and 7. Smith from the University of California at Berkley, who is a leading authority in the biological markers of benzene exposure, opined that the data which supposedly suggest that one must have changes in chromosomes 5 and 7 to assume benzene causation is unreliable and obsolete.[71] In summary, while it is true that benzene and related products have been described with changes in chromosomes, DNA adducts, and cell cycle, by no means can they not be used as a diagnostic tool to address benzene causation or not.

REFERENCES

1 NIOSH, Revised Recommendation for Occupational Exposure Standards for Benzene, Cincinnati, OH, DHEW Publications (NIOSH), 76-76-137-A (1976).
2 Fishbein L, *Scan J Work Environ Health*, **8**, Supplement 1, 5-16 (1992)
3 ATSDR Toxicological Profile for Benzene- Update, U.S. Department of Health & Human Services, Atlanta, Georgia (1996)
4 Browning E. **Toxicity and Metabolism of Industrial Solvents**, Amsterdam, *Elsevier Publishing*, Chapter 1 (1965)
5 Wallace, LA, *Environ Health Perspect,* **82**, 165-169 (1999)
6 Brenner D, et al., *Eur J Oncol,* **3**(4), 399-405 (1998)
7 Henderson RF, et al., *Environ Health Perspect*, 82, 9-17 (1989).
8 Schneider CA, et al., *Toxiocology & Applied Pharmacology*, **54**, 323-331 (1980)
9 Schneider R, Damitriadis E, and Guy R, *Environ Health Perspect*, **8**, 31-35 (1989)
10 Goldstein BD, *Adv Mod Environ Toxicol*, **4**, 51-61 (1983)
11 Santesson CG, *Arch Hyg,*, **31**, 336-349 (1897)
12 Delore P and Borgomano C, *Journal Medicine Lyon*, **9**, 227-233 (1928)
13 Lignac GOE, *Krankeitsforsch*, **9**, 426-453 (1932)
14 Vigliani EC, and Saita G, *New England Journal of Medicine*, **271**, 872-876 (1974)
15 Aksoy M, Erdem S, and Dincol G, *Blood*, **44**, 837-841 (1974)
16 API Toxicological Review, Benzene, September, (1948)
17 Maltoni C, **Myths and Facts in the History of Benzene Carcinogenicity : in Advances in Modern Environmental Toxicology**, Volume IV, M. Mehlman (Ed). *Princeton Scientific Publishing Company,* Princeton, 1-15 (1983)
18 NIOSH, Criteria for a Recommended Standard. Occupational Exposure to Benzene. U.S. Department of Health, Education and Welfare, Public Health Service, Center for Disease Control, Cincinnati, OH, Pub. No.74-137 (1974)
19 NIOSH, Occupational Safety and Health Guidelines for Benzene: Potential Human Carcinogen. U.S. Department of Health and Human Services, Public Health Services, Cincinnati, OH, Pub No. 89-104, Supp II (1988)
20 Aksoy M, *Environ Res*, **23**, 181-190, (1980)
21 Aksoy M, *Environ Health Perspect*, **82**, 1931-198 (1989)
22 USEPA, Ambient Water Quality Criteria for Benzene, Environmental Criteria and Assessment Office, Cincinnati, OH, EPA 440/5-80-018, NTIS PB81-117293 (1980)
23 Goldstein BD and Snyder CA, *Environ Sci Res*, **25**, 277-289 (1982)
24 IARC and IACR, In: IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Some Industrial Chemicals and Dyestuffs. IARC, Lyon, France, 28, 183-225 (1982)
25 Aksoy M, et al., *Br J Ind Med*, **44**, 785-787 (1987)
26 Rinksy RA, et al., *N Engl J Med*, **316**, 1044-1050 (1987)
27 Goldstein BD, **Benzene Toxicity, In: Occupational Medicine**: State of the Art Reviews, 3(3), 541-554, NK Weaver (ed), *Hanley and Belfus, Inc*, Philadelphia PA (1988)
28 Paci E, et al., *Scand J Work Environ Health*, **15**, 313-318 (1989)
29 Rinksy RA, *Environ Health Perspect,* **82**, 189-192 (1989)

30 Ciranni R, Barale R, and Adler ID, *Mutagenesis*, **6**, 417-421 (1991)
31 Cox LA, *Risk Anal,* **11**, 453-464 (1991)
32 Ruis MA, Vassallo J, and Desouza CA, *J Occup Med*, **33**, 83 (1991)
33 Midzenski MA, et al., *Am J Ind Med*, **22**, 553-565 (1992)
34 ATSDR Toxicological Profile for Benzene- Update, U.S. Department of Health & Human Services, Atlanta, Georgia (1993)
34 Medinsky MA, Schlosser PM and Bond JA, *Environ Health Perspect*, **102** Suppl 9:119-124 (1994)
35 Travis LB, et al., *Leuk Lymphoma*, **14**(1-2), 92-102 (1994)
36 Niculescu R and Kalf GF, *Arch Toxicol*, **69**(3),141-148 (1995)
37 Wintrobe MM, Lee GR and Boggs DR, **Wintrobe's Clinical Hematology**, 554, *Lea and Febiger*, Philadelphia, PA (1981)
38 Schottenfeld D and Fraaumeni JF Jr, **Cancer: Epidemiology and Prevention,** *Saunders*, Philadelphia, PA (1982)
39 Zoloth SR, et al., *J Natl Cancer Inst.*, **76**(6),1047-1051 (1986)
40 Mehlman MA (ed.) **Advanced in Modern Environmental Toxicology**, Volume IV, *Princeton Scientific Publishing Company*, Princeton, NJ (1983)
41 Mehlman MA, *American Journal of Industrial Medicine*, **20**, 707-711 (1991)
42 Nilsson RI, et al., *Occupational & Environmental Medicine*, **55**, 517-521 (1998)
43 Rinsky A, et al., *New England Journal of Medicine*, **316**, 17, 1044-1050 (April 23, 1987)
44. Wong O, *British Journal of Industrial Medicine*, **44**, 382-395 (1987)
45 Linet MS, et al., *Environmental Health Perspectives*, **104**, Supplement 6, 1353-1364 (December 1996)
46 Yin SN, et al., *American Journal of Industrial Medicine*, **29**, 227-235 (1996)
47 Hayes RB, et al., *Journal of the National Cancer Institute*, **89**, 1065-1071 (1997
48 Wong O, *British Journal of Industrial Medicine*, **44**, 365-381 (1987)
49 Huff JE, et al., *Environ Health Perspec.*, **82,** 125-163 (1989)
50 Lewis JG, Odom B and Adams DO, *Toxicol Appl Pharmacol*, **92**, 246-254 (1988)
51 Thomas DJ, Reasor MJ and Wierda D, *Toxicol Appl Pharmacol*, **97**, 440-453 (1989)
52 Kalf GF, *Crit Rev Toxicol*, **18**, 141-159 (1987)
53 Smith MT, et al., *Environ Health Perspect*, **82**, 23-29 (1989)
54 Gist GL and Burg JR, *Toxicol and Industrial Health*, **13**(6), 661-714 (1997)
55 Reid LL, et al., *Drug Chem Toxicol,* **17**(1):1-14 (1994)
56 Winternitz MC and Hirschfelder AD, *J Exp Med*, **17**, 657-664 (1913)
57 USEPA, 5.0 Benzene, 5.1 Chemical and Physical Properties, EPA (1988)
58 International Agency for Research on Cancer (IARC), On the evaluation of carcinogenic risk of chemicals to humans: overall evaluations of carcinogenicity: An updating of IARC monographs 1 to 42, IARC, Lyon, France, Suppl 7 (1987)
59 Infante PF, et al., *Lancet*, **2**:76:1977
60 Vigliani EC and Forni A, *J Occup Med*, **11**, 148 (1969)
61 McMichael AJ, Carcinogenicity of benzene, toluene, and xylene: Epidemiological and Experimental Evidence, IARC, Environmental carcinogens methods of analysis and exposure measurement, Volume 10-**Benzene and Alkylated Benzens**, Fishbein L and O'Neill IK (eds) *IARC Scientific Publications*, No. 85 (1988)
62 USEPA, Attachment to IRIS file for benzene, 10/14/98, Response to the Peer-Review Draft Carcinogenic Effects of Benzene: An Update, EPA/600/P-97/001A, June 1997
63 Statement Submitted to the OSHA Benzene Hearing by Otto Wong, ScD., FACE., Environmental Health Associates, Inc, 03/04/86.
64 Decoufle P, Blattner WA and Blair A, *Environ Res*, **30**, 16-25 (1983)
65 Elkins HB, and Pagnotto LD, *Archives of Industrial Health*, **13**, 51-54 (1956)
66 Pagnotto LD, et al., *Industrial Hygiene Journal*, 417-421 (December 1961)
67 NIOSH. A Recommended Standard for Occupational Exposure to Refined Petroleum Solvents from the U.S. Department of Health, Education and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health (July 1977)
68 Testimony of R.S. Proctor in regards to Proposed Revised Permanent Standard for Occupational Exposure to Benzene, OSHA Docket No. H-059A, (July 11, 1977)
69 Post-Hearing Comments and Records Designations in OSHA Docket H-059 In re: Proposed Amendment to the Permanent Standard for Occupational Exposure to Benzene (June 12, 1978)
70 Irons R, *Toxicology and Environmental Health*, **16**, 673-678 (1985)
71 Kathleen Lavender, et al., v Bayer Corporation, et al., Civil Action No. 93-C-226K, in the Circuit Court of Marshall County, West Virginia

20.6 CHROMOSOMAL ABERRATIONS AND SISTER CHROMATOID EXCHANGES

Nachman Brautbar
University of Southern California, School of Medicine, Department of Medicine, Los Angeles, CA, USA

Several technologies have developed in the last 10 years to look at chromosomal changes and DNA changes caused by environmental exposures, as well as a marker of environmental exposures. The use of chromosomal translocation as a biological marker of exposure in humans have become an important tool in the research, as well as in some instance a marker of exposure. Several methodologies have utilized and include structural chromosomal aberrations, sister chromatoid exchanges (SCEs) and micronuclear changes. These are markers of changes in the cellular genetic materials, and represent damage induced by chemicals. These methodologies are viewed as cytogenetic assays, and by themselves cannot provide a diagnosis, but they complement other methodologies which include gene mutation analysis, and DNA changes. Among the important uses of cytogenetics as a biomarker is the relationship between chromosomal aberrations secondary to chemicals and carcinogeneses. Since the scope of this chapter is not addressing mechanisms of carcinogeneses, the reader is referred to other sources.[1]

Studies in patients with acute non-lymphocytic leukemia whose bone marrow analyzed for chromosomal changes have shown that 50% of them had changes in chromosomes 5, 7, 8 and 21.[2] Mitelman et al.[3] have looked at patients with acute non-lymphocytic leukemia and looked at the occupational history. They have studied a group of 56 patients. Twenty-three out of the 56 patients had a history of exposure to chemical solvents, insecticides and petroleum products. They have further shown that in males with acute non-lymphocytic leukemia the frequency of exposure to petroleum products was as high as 36%. In their study, Mitelman et al.[3] found a striking differences between the chromosomal findings in non-exposed versus exposed groups. In the non-exposed group only 24.2% of the patients had chromosomal aberrations in their bone marrow cells, while 82.6% of the exposed patients had chromosomal aberrations. The authors concluded, based on these studies that the difference between the exposed and non-exposed group strongly indicates that the karyotypic pattern of the leukemic cells were, in fact, influenced by the exposure. The authors further suggested that the prognosis in those patients with normal chromosomes was significantly better than those with abnormal chromosomes, something which has been suggested by previous investigators.[4,5,6] Based on their observations and others,[7-11] the authors suggested that certain chromosomal regions possible being specifically vulnerable to the chromosome damaging actions of different chemicals which are carcinogens. Indeed, the changes of chromosomes 5, 7, 8 and 21 in workers exposed to different chemical solvents, among them also petrol and pesticides, supports this hypophysis. Studies in cultured lymphocytes from 73 workers in chemical laboratories and the printing industry were found to have a significantly increased frequency of chromatoid and sister-chromatoid breaks in comparison to 49 control subjects.[12] The authors suggested that the observed cytogenetic changes is reasonably assumed to be the result of strong factors in the working environment which induced chromosome breaks and sister-chromatoid exchange. The considerable vari-

ation in cytogenic changes between subjects suggested varying degrees of exposure to mutagenic agents. Brandt et al.[13] studied the effects of exposure to organic solvents and chromosomal aberrations. Ten patients had a history of daily handling of organic solvents for at least one year preceding the diagnoses of non-Hodgkin's lymphoma. All have been exposed to a variety of solvents to include aromatic and aliphatic compounds. Forty-four patients for only a shorter period of time worked with organic solvents, and therefore served as the control group. There was a statistically significant increase in chromosomal changes in the exposed group compared to the non-exposed group. The authors suggested based on their results, that at diagnosis of non-Hodgkin's lymphoma, patients with a history of significant occupational exposure to organic solvents tends to have a larger number of chromosomal aberrations in the lymphoma cells. Furthermore, certain aberrations may be characteristic for the exposed patients. They have concluded that the over representation of certain chromosome aberrations in non-Hodgkin's lymphoma patients occupationally exposed to organic solvents supports the concept that these exposure may be relevant for the subsequent development of non-Hodgkin's lymphoma. Their data are in agreement with the studies published previously, indicating that workers handling organic solvents and other petroleum products have an increased frequency of chromosomal aberrations in lymphocytes.[12,14,15,16] Indeed, association between exposure to solvents and other chromosomal changes in the cells have been studied by other investigators, describing chromosomal changes in acute non-lymphocytic leukemia.[3,17,18,19] In cultured cells, Koizumi[20] examined the effects of benzene on DNA syntheses in chromosomes of cultured human lymphocytes. They have shown an increased incidents of chromatoid gaps and breaks in cultures treated with benzene, compared to those who were untreated with benzene. They also showed changes in DNA metabolism in the form of inhibition of syntheses in those samples which were treated with benzene. This observation was confirmed by other investigators[21] who have shown an increased chromosomal aberrations in cultured human leukocytes exposed to benzene. From the in vitro to the in vivo experimental animals, it was shown that treated rats with benzene, were found to have increased chromosomal changes taken from the bone marrow as compared to the non-treated animals.[22] It was of interest that the degree of chromosomal changes that were induced by benzene, were similar to that induced by toluene[23] (probably secondary to the benzene in toluene). Experimental rabbits treated with benzene also showed a significant amount of bone marrow chromosomal changes persisting up to 60 days after the end dosing with benzene.[24]

A patient who has developed aplastic anemia after exposure to benzene, was shown to have significant chromatoid fragments.[25] A cytogenic study which was carried out later,[26] on a patient who developed leukemia after 22 years of continuous exposure to a high concentration of benzene, showed that later in the process there were changes in 47 chromosomes in the bone marrow. Sellyei et al.[27] studied patients who developed pancytopenia after having been exposed for 18 months to benzene. Significant chromosomal changes were detected even 7 years after remission from the anemia and the presentation of leukemia. In line with these changes, Forni et al.[28] have studied 25 subjects with a history of hematopoietic abnormalities and benzene exposure, and compared these to 25 matched controls. They have shown that 18 years after clinical and hematological symptoms chromosomal aberrations were increased as compared to the control group. In 1965, Tough et al.[29] have studied chromosomes of workers exposed to benzene for periods varying from 1 to 18 years. They have also shown a small but significant increase in chromosomal changes com-

pared to a control group. These same investigators looked at workers exposed to benzene levels from 25 to 120 ppm, and found that they had significant chromosomal aberrations as compared to the normal population (which has a general background exposure to benzene levels). The study of Forni et al.[14] showed significant chromosomal aberrations in those patients who were exposed mainly to benzene, and not to those who were exposed to toluene only. Hartwich et al.[30] looked at 9 healthy refinery workers who were exposed to low levels of benzene, and also found significantly increased chromosomal changes compared to the control group. The National Research Council Advisory Center and Toxicology Study[31] concluded that close correlation between occupational exposure to benzene and persistence of chromosomal aberrations can be discussed only when there is an association between benzene induced hematopoietic disease and chromosomal aberrations, however, the absence of chromosomal changes, cannot be a determinant in the temporal relationship between exposure to benzene and hematopoietic diseases.[31] While some studies suggested that the chromosomal changes require heavy exposure to benzene, the study by Picciano, 1979,[32] looked at chromosomal changes in 52 workers exposed to a mean benzene concentration of less than 10 ppm compared to non-exposed controls. There was a statistical significant increase in chromosomal aberrations in exposure as low as less than 10 ppm. Furthermore, these same investigators[33] reported a dose response increase in the aberrations when the exposed workers were divided into smaller groups by the exposure levels (less than 1 ppm, 1-2.5 ppm, and 2.5-10 ppm). Drivers of petrol tankers and crew members of gasoline tankers, ships and petrol station attendants were studied for chromosomal changes.[15] The degree of exposure to benzene of the three groups was estimated to be at a mean of 0.4 ppm, and the crew members were estimated to be at 6.56 ppm while engaging in handling of gasoline. The frequency of chromosomal and chromatoid aberrations in the petrol tanker drivers was significantly greater than in those of petrol attendants and the crew members. The effects of long-term benzene exposure from the incidents of chromosomal changes were studied in 16 female workers who were exposed to a maximum of 40 ppm benzene between 1-20 years.[34] The cytogenetic study was conducted 6 months after benzene was eliminated from the work environment, and they have found no significant increase in chromosomal changes. Clare et al.[35] looked at chromosomal changes in the peripheral lymphocytes of workers after a single, one exposure to benzene. Exposure levels were described as high after a spillage of a large amount of benzene during the loading of a ship. Three months after the incident, chromosomal analysis showed no significant abnormalities. The authors concluded that there was no evidence of lasting chromosomal damage in the peripheral blood lymphocytes of these exposed workers. Golomb et al.[18] reviewed the literature and reported the results in regards to exposure to benzene and chromosomal changes. They have stated that they studied exposure data on 74 patients with acute leukemia. They describe that 75% of the exposed patients had an abnormal karyotype, whereas only 43% of the patients characterized as non-exposed had an abnormal karyotype. While it is true that these findings are in agreement with previous studies[18] they still could not explain the 43% of the patients who were not exposed, and still had abnormal chromosomal changes. This is a very important observation, since some investigators in the field claimed that the "absence of chromosomal changes" in benzene exposed individuals negates the clinical causative diagnosis of benzene induced hematopoietic disease. Essentially, all of the studies show that benzene can cause chromosomal changes, but does not cause it in all the patients, and the absence of chromosomal changes cannot and does not rule out the ex-

posure to benzene as a causative factor. In this same paper, Golomb et al.[18] looked at the chromosomal changes of patients treated with chemotherapeutic agents for other malignancies. Essentially, they looked at a secondary leukemia developing as a result of alkylating agents. For some reason, they have proposed that losses of part or all of chromosomes numbers 5 and 7 are the specific change resulting from mutageneses, leukemogeneses associated with various chemicals including insecticides, petroleum products and alkylating agents.

While this interpretation is compatible with the various animal studies, as well as observations in patients, there is certainly a lack of scientific connection between the benzene exposed chromosomal changes, and the chromosomal changes reported in patients treating with alkylating agents.

Smith, in a recent paper[36] suggested that oxidation of benzene to multiple metabolites plays a role in producing benzene induced toxicity of DNA damage in bone marrow, and adds further weight to the hypophysis that multiple metabolites are involved in benzene toxicity. They also described DNA changes which have been shown to be cause-point mutation. The investigators measured mutation frequency in 24 workers heavily exposed to benzene, and 23 matched controls. They found that benzene caused a highly significant increase in one variant of mutation, suggesting that benzene produces gene duplicating mutations, but no gene inactivating ones. They suggested that the most-likely consequence of aberrant recombination caused by benzene metabolites is the production of stable chromosomal translocation. Indeed, there are several chromosomal abnormalities shown in leukemic cells. This includes Philadelphia chromosome which results from reciprocal translocation between chromosome 9 and 22, and has been associated with chronic myeloid leukemia, and reciprocal translocation between chromosomes 8 and 21. From these studies it is concluded that benzene is a genotoxic carcinogen, but that other genetic phenomena may mediate benzene induced hematopoietic toxicity. Based on the available data up to date, it is proposed[36] that benzene is a carcinogen that does not produce cancer through simple gene mutations, but rather through a separate class of carcinogens (metabolites of benzene) that act by a similar mechanism.

In summary, the studies in experimental animals, in vitro, and patients show that benzene and a wide range of organic solvents are associated with changes in chromosomes and DNA adducts. While these changes may be helpful in epidemiological studies, the absence or presence of genetic changes or DNA adducts, cannot be used in a specific case to rule out or establish causation. The biomarkers described in this chapter in the form of genetic biomarkers, can be helpful in identifying individual susceptibility, and in some cases understanding of the mechanism of the disease process. They have a significant number of limitations, and these include measurements, errors and confounding factors.

REFERENCES

1 **Biomarkers: Medical and Workplace Applications**, Medelson ML, Moor LC and Peeters JP (eds), J*ohn Henry Press,* Washington, D.C., (1998)
2 Rowley JD and Potter D, *Blood*, **47**:705, (1976)
3 Mitelman F, Brandt L and Nilsson PG, *Blood*, **52**(6):1229-1237, (December 1978)
4 First International Workshop on Chromosomes in Leukaemia: Chromosomes in acute non-lymphocytic leukaemia. *Br J Haematol*, **39**:311, (1978)
5 Golomb HM, Vardiman J and Rowley JD, *Blood*, **48**:9, (1976)
6 Nilsson PG, Brandt L and Mitelman R, *Leukemia Res*, **1**:31, (1977)
7 Mitelman F, et al., *Science*, **176**:1340, (1972)

8 Mitelman F, The Rous sarcoma virus story: Cytogenetics of Tumors induced by RSV in German J (ed): **Chromosomes and Cancer**, New York, *Wiley*, page 675, (1974)
9 Levan G and Levan A, *Hereditas*, **79**:161, (1975)
10 Levan G and Mitelman F, *Hereditas*, **84**:1, (1976)
11 Sugiyama T, et al., *J Natl Cancer Inst*, **60**:153 (1978)
12 Funes-Cravioto F, et al., *The Lancet,* 322-325, (August 13, 1977)
13 Brandt L, et al., *European Journal of Haematol*, **42**:298-302, (1989)
14 Forni A, Pacifico E, and Limonta A, *Arch Environ Health*, **22**:373-378, (1971)
15 Fredga K, Reitalu J, and Berlin M, Chromosome studies in workers exposed to benzene. In: Berg K (ed), **Genetic Damage in Man Caused by Environmental Agents**, New York, *Academic Press*, 187-203, (1979)
16 Hogstedt B, et al., *Hereditas*, **94**:179-184, (1981)
17 Mitelman F, et al., *Cancer Genet Cytogenet*, **4**:194-214, (1981)
18 Golomb HM, et al., *Blood*, **60**(2):404-411, (1982)
19 Fourth International Workshop on Chromosomes in Leukemia, 1982: The correlation of karyotype and occupational exposure to potential mutagenic/carcinogenic agents in acute nonlymphocytic leukemia. *Cancer Genet Cytogenet*, **11**:326-331, (1984)
20 Koizumi A, et al., *Jap J Ind Health*, **12**:23-29, (1974)
21 Morimoto K, *Jap J Ind Health,* **17**:106-107, (1975)
22 Philip P and Jensen MK, *Acta Pathol Microbiol Scand, Section A*, **78**:489-490, (1970)
23 Dobrokhotov VB, *Gig Sanit,* **37**:36-39, (1972)
24 Kissling M and Speck B, *Helv Med Acta*, **36**:59-66, (1971)
25 Pollini G and Colombi R, *Med Lav*, **55**:244-255, (1964)
26 Forni A and Moreo L, *Eur J Cancer,* **3**:251-255, (1967)
27 Sellyei M and Kelemen E, *Eur J Cancer*, **7**:83-85, (1971)
28 Forni AM, et al., *Arch Environ Health*, **23**:385-391, (1971)
29 Tough IM and Court Brown WM, *Lancet*, **I**, 684, (1965)
30 Hartwich G and Schwanitc G, *Dtsch. Med. Wschr*, **87**:45-49, (1972)
31 National Research Council Advisory Centre on Toxicology, Washington D.C., Health Effects of Benzene: A Review. Prepared for the Environmental Protection Agency. Report No. NAS/ACT/P-829, (June, 1976)
32 Picciano DJ, *Environ Res*, **19**:33-38, (1979)
33 Picciano DJ, Monitoring Industrial Populations by Cytogenetic Procedures. In: Infante PF and Legator MS (eds). Proceedings of the Workshop on Methodology for Assessing Reproductive Hazards in the Workplace, U.S. Government Printing Office, Washington DC, pages 293-306, (1980)
34 Watanabe T, et al., *Environ Health*, **46**:31-41, (1980)
35 Clare MG, et al., *Br J Ind Med*, **41**:249-253, (1984)
36 Smith MT, *Environ Health Persp*, **104**(6):1219-1225, (December 1996)

20.7 HEPATOTOXICITY

Nachman Brautbar
University of Southern California, School of Medicine
Department of Medicine, Los Angeles, CA, USA

20.7.1 INTRODUCTION

Solvents which are inhaled or gain access to the blood circulation via skin absorption or at times ingestion largely are metabolized by the liver. The liver has a complex mechanism composed of the cytochrome P450 enzyme, and other enzymes related to conjugation pathways such as glutathione conjugation. This is represented schematically in Figure 20.7.1

It is therefore no surprise that in the occupational setting as well as non-occupational setting, liver damage anywhere from transient to subacute to chronic, and at times terminal liver damage has been described.

The circumstances of exposure to hepatotoxic agents are divided to:

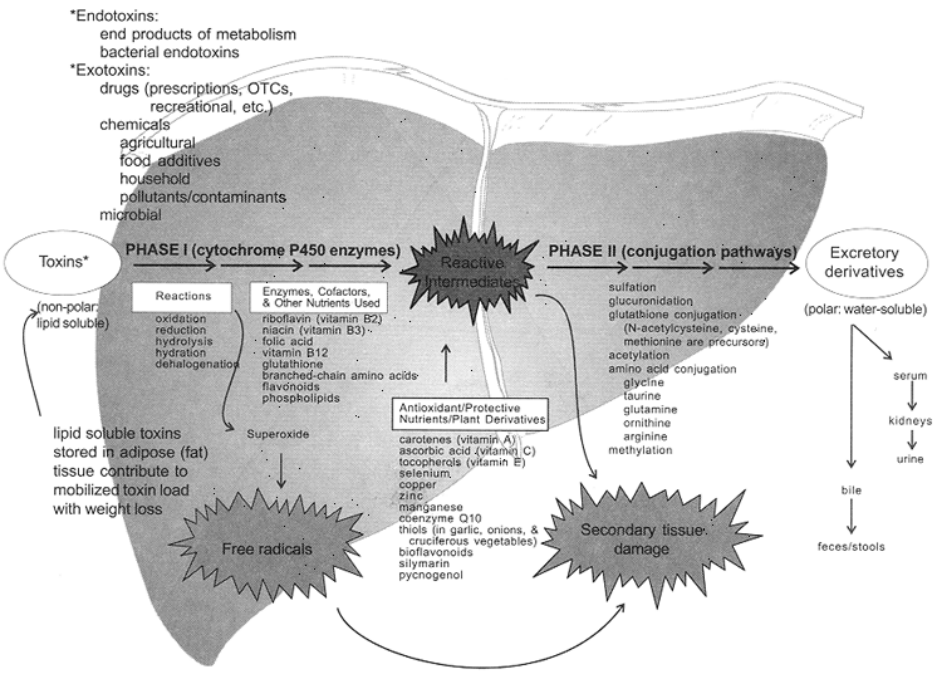


Figure 20.7.1. The phases of detoxification. [Adapted, by permission, from DS Jones, Institute for Functional Medicine, Inc., 1999]

1. Occupational: Either through a routine daily inhalation or skin absorption of solvents which have been shown to be toxic to the liver (accidental exposure).

2. Domestic during either accidental or intentional suicidal exposure, ingestion in foods or as a toxic contaminant of food, exposure to toxic agents such as in the form of glue sniffing.

3. Environmental, most commonly exposure through contaminated water with solvents (drinking water contamination) or through atmospheric pollution such as release to the environment from plants utilizing solvents.

Historically the first cases attributing chloroform to liver toxicity were described in 1887, 1889 and 1904.[2,3,4] The role of carbon tetrachloride and liver injury has been originally described in 1967 and 1973.[5,6] In general, the understanding of hepatotoxicity is extremely complex, and the reader is referred to the outstanding text by Hyman J. Zimmerman.[7] A typical example of how metabolism and toxicity of a water takes place is the aromatic chemical such as benzene attached to bromine. The effect on the liver has been originally studied by Mitchell in 1975[8] who have shown that a change in the rate of the metabolism of this compound is required to create its toxic products. While bromobenzene and carbon tetrachloride share a similar place of metabolism in the liver, the toxicity of bromobenzene and carbon tetrachloride are different, since the bromobenzene toxicity is related to the metabolic capacity of the liver, while that of carbon tetrachloride is not.

Several factors contribute to the handling of the solvents by the liver and affect the final toxicity, including species differences. For instance, rats are vulnerable to a wide variety of toxic agents such as carbon tetrachloride and bromobenzene due to the ability of the liver

to convert these agents to their respective toxic metabolites.[6,9] Among other mechanisms responsible for the species differences are liver blood flow, protein binding, and the points of binding intracellularly. Genetic factors in humans are of extreme importance. Genetic factors are most-likely responsible for the various levels of adverse effects of alcohol in different individuals due to induced activity of detoxification enzymes in the liver in some and lack of those or reduced activity in others. Another important factor is age. The effect of age on the susceptibility has been shown in experimental animals. For instance, the neonatal rats are less susceptible to carbon tetrachloride and bromobenzene toxicity as compared to adult animals.[9] In humans, liver necrosis after the administration of Halothane was rare in children, but more common in more elderly patients. Factors such as sex and endocrine status have also influence and different toxic effects of solvents in this case. Nutritional status is a major factor in the effects of solvents on the liver. For instance, protein malnutrition leads to reduced activity of cytochrome P450. Increasing the percentage of fat in the liver has been shown to increase the susceptibility of toxicity to such agents as carbon tetrachloride. Several studies have looked into the histopathological injury of some solvents and solvent-like agents and the liver, and are shown in Table 20.7.1.

Table 20.7.1. Partial list of agents that produce hepatic necrosis in experimental animals. [Adapted, by permission, from HJ Zimmerman, Hepatotoxicity, 1978]

	Site of necrosis				
	Centrizonal	Midzonal	Peripheral Zone	Massive	Steatosis
Bromobenzene	+				(+)
Bromotrichloromethane	+				+
CCl_4	+				+
Chlorobenzenes	+			(+)?	
Chloroform	+				+
Dichloropropane	+				+
Dinitrobenzene	+			(+)	+
Dinitrotoluene	+			(+)	+
Ethylene dichloride	+				+
Methylene chloride	(+)			(+)	(+)
Naphthalene	+				+

The specific mechanism of hepatotoxicity of many solvents are unknown, however, the knowledge have been gathered from experimental studies now available for the reader for review.[10,11,12,13]

Due to the lack of specific information for many solvents, I have decided to discuss in this chapter some of the most typical ones which have been used in the past heavily, or are used currently.

Table 20.7.2. Some occupational solvents that produce acute and chronic liver disease

Carbon tetrachloride	Halothane	Ethyl alcohol
Chloroform	Trichlorodiphenyl	A mixture of solvents such as
Trichloroethylene	Trichloroethene	toluene and xylene
Tetrachloroethylene	Trinitrotoluene	Dichloromethane
Dinitrobenzene		

Tables 20.7.3 and 20.7.4 describe two major phases in the liver metabolism and detoxification of drugs, foreign agents and solvents.

Carbon tetrachloride was found to cause liver injury in man and in experimental animals.[14] Carbon tetrachloride is a known and potent liver toxicant, and therefore has been studied extensively in experimental animals. Recknagel[6] and Reynolds[15] have shown that single doses will lead to areas of necrosis in the liver within a few minutes. This has been shown to be associated with changes in liver enzymes which are known to indicate liver damages.[10,11] Prolonged exposure to carbon tetrachloride has been shown to lead to liver cirrhosis and to liver cancers. In order to become toxic the carbon tetrachloride has to undergo metabolic changes in the liver.[6,16] The lesions described initially in animals have been shown in humans poisoned with carbon tetrachloride.[14,17] It has also been shown that alcohol enhances the susceptibility to carbon chloride toxicity.[18] Several factors play a role in the susceptibility to toxicity by carbon tetrachloride, among them are sex, age, diet, underlying preexisting liver dysfunction and alcoholism. Over the years in both clinical and experimental studies and observations, it has been shown that carbon tetrachloride induced liver damage is divided to fatty metamorphosis, and independently liver necrosis. Fat starts to accumulate in experimental animals as early as one hour after administration of a high dose of carbon tetrachloride. Liver necrosis occurs as early as 6 to 12 hours, and a maximum of 24 to 36 hours.

The concept of steatosis (fat accumulation in the liver) is a common one for looking at the effect of solvents (those which are known to be toxic to the liver). The fat accumulation is the result of abnormal transport of lipids and as a result, accumulation of lipids in the liver. Therefore clinically industrial exposure to hepatotoxic solvents is associated with liver steatosis, among others.

Necrosis, which is the second most common effect of liver damage of solvents toxic to the liver, is the result of destruction of the cell architecture as well as biochemical pathways. It has been shown in many experimental studies that the toxicity of carbon tetrachloride (and some of the other solvents which are toxic to the liver and other organs, such as benzene and the hematopoietic system) requires several reactions, in order to produce the toxic metabolites which are causing the damage to the liver. Most studies point to the responsibility of cytochrome P450 system.[6] The metabolite responsible for the liver damaging effect of carbon tetrachloride is a C Chloride III which is formed from carbon tetrachloride.[6,19] There is however also information that non-metabolized carbon tetrachloride contributes to the injury, especially that of the cell membrane,[20,21] something which is logical since solvents are a mechanistic injury to various tissues is through the effects on the cell membrane which is either dissolved or damaged by the solvent. A consolidation of the data available and views on the pathogenesis of carbon tetrachloride liver damage has been eloquently described by Zimmerman.[7]

Table 20.7.3. Phase I reactions. [Adapted, by permission, from HJ Zimmerman, Hepatotoxicity, 1978]

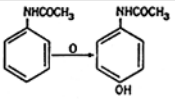
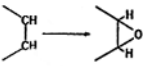
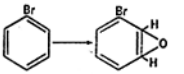
I. OXIDATIONS	
A. MFO-dependent	
Aromatic hydroxylations	
Aliphatic hydroxylations	
Primary alcohol	$RCH_2CH_2CH_3 \xrightarrow{O} RCH_2CH_2CH_2OH$
Secondary alcohol	$RCH_2CH_2CH_3 \xrightarrow{O} RCH_2CH(OH)CH_3$
Tertiary alcohol	$RCH_2CH(R')CH_3 \xrightarrow{O} R{-}CH_2{-}C(R')(OH){-}CH_3$
Epoxidation	
Arene oxide formation	
Oxidative dealkylation	
N—dealkylation	$R{-}NH{-}CH_3 \longrightarrow [R{-}NH{-}CH_2OH] \longrightarrow RNH_2 + HCHO$
O—dealkylation	$R{-}O{-}CH_3 \longrightarrow [R{-}O{-}CH_2OH] \longrightarrow ROH + HCHO$
S—dealkylation	$R{-}S{-}CH_3 \longrightarrow [R{-}S{-}CH_2OH] \longrightarrow RSH + HCHO$
N—Oxidation	
Primary amine	$RNH_2 \longrightarrow RNHOH$
Secondary amine	$R_2NH \longrightarrow R_2NOH$
Tertiary amine	$R{-}N(R'')R' \longrightarrow R{-}N(R'')(R')=O$
S—oxidation	$S \longrightarrow S{=}O$
Deamination	$RCH(NH_2){-}CH_3 \longrightarrow [R{-}C(OH)(NH_2){-}CH_3] \longrightarrow R{-}C(=O){-}CH_3 + NH_3$
Desulfuration	$RSH \longrightarrow ROH$
Dechlorination	$CCl_4 \longrightarrow [CCl_3] \longrightarrow CHCl_3$
B. Amine oxidase	$RCH_2{-}NH_2 \longrightarrow RCHO + NH_3$
C. Dehydrogenation	
Alcohols and aldehydes	$CH_3CH_2OH \longrightarrow CH_3CHO \longrightarrow CH_3COOH \longrightarrow CO_2 + H_2O$
II. REDUCTION REACTIONS	
Azo reductions	$RN{=}NR' \longrightarrow RNH{-}NHR' \longrightarrow RNH_2 + R'NH_2$
Nitro reductions	$RNO_2 \longrightarrow RNO \longrightarrow RNHOH \longrightarrow RNH_2$
Carbonyl reductions	$RC(=O){-}R' \longrightarrow RCH(OH){-}R'$
III. HYDROLYSIS REACTIONS	
Esters	$RCOOR' \longrightarrow RCOOH + R'OH$
Amides	$RCONH_2 \longrightarrow RCOOH + NH_3$

Table 20.7.4. Phase II (conjugation) reactions. [Adapted by permission, from HJ Zimmerman, Hepatotoxicity, 1978]

Type of conjugation	Endogenous substance	Transferring enzyme and location	Type of xenobiotics & metabolites conjugated
Glucuronidation	UDP-glucuronic acid	UDPG-transferase (microsomes)	Phenols; alcohols; carboxylic acids; primary amines; hydroxylamines; sulfonamides, etc.
Dihydrodiol formation	Water	(Epoxide) hydrase (cytosol)	Epoxides and arene oxides
GSH conjugation	GSH	GSH-S-transferase (cytosol)	Epoxides; arene oxides; halides; nitro groups; hydroxylamines, etc.
Glycine conjugation	Glycine	Acyl CoA-glycine transferase (mitochondria)	CoA derivatives of carboxylic acids
Sulfate conjugation	PAP-sulfate	Sulfotransferase (cytosol)	Phenols; alcohols; aromatic amines
Methylation	S-adenosyl-methionine	Transmethylase (cytosol)	Catechols; phenols; amines; histamine

The carcinogenic effect of carbon tetrachloride will not be discussed in this chapter, and the reader is referred to other texts.[22]

20.7.2 INDIVIDUAL VARIABILITY AND HEPATOTOXICITY OF SOLVENTS

The issue of individual variability based on various factors as described above is important, especially in medical monitoring and risk assessment in occupationally exposed patients. The fact that workers in industrial environments are not the same and are subject for differences such as body build, underlying kidney function differences (genetic or acquired), exposure to other solvents or other liver toxicants may effect the results. A recent study[23] evaluated a population response to solvent exposure. These investigators have shown that body fat is the most important body compartment for fat soluble solvents. Body fluids and physical work load effect the blood flow, alveoli ventilation and therefore will effect the amount of solvent inhaled as well as absorbed through the blood and delivered to the liver. They have developed the physiological model which takes into account variability in the form of exposure, physical overload, body build, liver function and renal function. Other factors which have been taken into account are solubility in blood and tissue. Investigators suggested that such a model should be useful in improving our understanding of the complex and multifactorial system and to generate a hypothesis, and to improve our assessment of occupational exposure. This has significance from a clinical toxicology point of view. A patient who has an increased body fat will be at a higher risk of solvent toxicity. If this same patient also has a habit of heavy alcohol consumption the risk for solvent liver toxicity is significantly increased. Epidemiological studies are commonly not designed to evaluate the individual hepatotoxicity of solvents and therefore the issue of cause and effect must be viewed taking into account individual variability, other risk factors, and medical common sense, following the well established criteria by practitioners of medicine.

Chloroform is another haloalkane which has been typically used as an example to understand and study the toxic effects on the liver. Studies in experimental animals in 1866[24] have shown that the chloroform causes liver toxicity. In 1923 Meyer et al.[14] have shown that

toxicity of chloroform to the liver in humans. Chloroform was used years ago as an anesthetic, and has been used successfully, however, due to its' toxicity the use of it has been abandoned. Acute exposure and toxicity has been associated with liver necrosis, liver steatosis, and chronic exposure has been associated with liver cirrhosis. The mechanism of injury most-likely is the result of metabolic changes of chloroform by the liver. Different effects and level of toxicity between carbon tetrachloride and chloroform is most-likely the result from the solubility in lipid and water, and the mechanisms by which these two agents are metabolized and then cause liver toxicity. Table 20.7.5 summarizes liver damage described in the literature as a result of halogenated aliphatic hydrocarbons.

Table 20.7.5. Lesions produced by halogenated aliphatic hydrocarbons. [Adapted, by permission, from HJ Zimmerman, Hepatotoxicity, 1978]

Steatosis and centrizonal necrosis	Steatosis only	Slight steatosis or no injury
CCl_4	CH_2ClBr	CH_3Cl
CI_4	CH_2Cl_2	CH_3Br
CCl_3Br	CHCl=CHCl (cis)	CH_3I
$CHCl_3$	$CCl_2=CCl_2$	CCl_2F_2
CHI_3	$CH_3CH_2CHClCH_3$	CHCl=CHCl (trans)
$CHBr_3$		CH_3CH_2Cl
$CHCl_2CHCl_2$		CH_3CH_2I
CH_2ClCH_2Cl		CH_3CH_2Br
CH_2BrCH_2Br		$CH_3CH_2CH_2CH_2Cl$
CH_3CCl_3		
$CHCl_2CCl_3$		
$CHCl=CCl_2$		
$CH_3CHClCH_3$		
$CH_3CHClCH_2Cl$		

20.7.3 NON-HALOGENATED SOLVENTS

While the halogenated hydrocarbons discussed here include carbon tetrachloride, chloroform, 1,1,1-trichloroethene, trichloroethylene are significantly hepatotoxic, the literature on the toxicity of the non-halogenated hydrocarbons is a combination of positive and negative studies. Several studies looking into the hepatotoxicity of both aliphatic solvents such as kerosene, hexane and aromatics such as xylene, toluene and styrene have reported mixed results. Xylene is an aromatic hydrocarbon which is used heavily in the industry, as well as medical technology as a solvent.

Xylene commonly has been reported with impurities in varying amounts which include ethyl benzene, prime ethyl benzene, phenol, benzene and toluene.[25] To evaluate the effects of xylene in experimental animals, Toftgard et al.[26] studied rats who were exposed for three days by inhalation to xylene and to a mix of xylene isomers. Hepatic cytochrome P450 concentration increased as well as C reductase activity, and NADPH cytochrome C reductase activity. Furthermore, xylene and its isomers were able to modify the metabolism of other potentially toxic substances. In addition to these biochemical changes, the investigators found that xylene increased liver body weight, most-likely secondary to proliferation of the endoplastic reticulum. These studies show that at these levels xylene induced cytochrome P450 activity and NADPH cytochrome C reductase activity, but was not associated with significant pathological abnormalities. On the other hand, these same investiga-

tors, concluded that the capacity of xylene and xylene isomers to induce hepatic cytochrome P450 suggests the possibility of synergistic toxic effects from simultaneous exposure to xylene and substances metabolically activated by these cytochromes P450. Therefore, exposure to a mixture of solvents which also include xylene will increase the toxicity and xylene is a synergistic liver toxicant. For instance, the formation of 2-hexanoyl a metabolic precursor of 2,5-hexanedione, which is the main metabolite found in urine of workers exposed to n-hexane, is increased following xylene treatment.[27]

20.7.4 SOLVENT MIXTURES

Various solvent mixtures have been reported as hepatotoxic, Fishbien et al.[28,29] reported abnormal liver functions in chemical workers exposed to a mixture of solvents. It was suggested that bile acids as indicators of hepatic function will be utilized as markers of injury. Franco et al.[30] examined a group of workers exposed to organic solvents, and used the criteria addressing exposure to solvent mixtures for over two years, daily ethanol consumption less than 50 grams, no history of hepatic disease, no drug intake in the previous three months. Workers were exposed to between 6 and 9 solvents, mostly toluene, xylene, acetone, methyl acetate, and butanol ethyl acetate. The mean levels of liver enzyme activities in the exposed and the control group were similar. The mean serum bile acid contents was statistically and significantly increased in the exposed group compared to the controlled group. The authors concluded that the observation of higher serum bile acid levels in the group of workers currently exposed to organic solvents might be explained by a change in hepatocyte function, and that the commonly followed parameters of liver enzymes may be insensitive to these preliminary initial changes in liver function. Conventional liver function tests seem to be rather insensitive to early detect liver damage from solvents exposure. Early detection is crucial, but is probably missed since the standard liver functions tests are not sensitive enough to detect early liver damage from solvents. What that means is that by the time the patient is seen by the doctor with liver fibrosis or necrosis and solvent toxicity, it is already in advanced stages. Joung-Dar-Chen et al.[31] evaluated the effects of solvents exposure on liver functions, specifically looking at gamma glutamyl transferase activity. They have studied the effects of xylene and toluene. The median air concentration was evaluated in the exposed workers who used mixed solvents in the process of spray booth car painting. These investigators showed that gamma glutamyl transferase activities increased independently with both an increased consumption of alcohol and exposure to a mixture of solvents. They have concluded that an increase in GGT activity may be a form of enzyme induction rather than a marker for cellular damage. Kurpper et al.[32] examined the effect of mixed organic solvents on liver enzymes activity in car painters, and found that at the exposure level of that study (at that time was 1/2 the level recommended by the regulatory agencies) the liver enzyme activities of car painters were not effected by exposure to mixed solvents. The continuation of the previous study by Franco et al.[33] examined serum bile acid concentrations as a marker of liver functions in a group of workers exposed to organic solvents. They have shown a significantly increased concentration of serum bile acids with normal liver enzymes, and concluded that this indicated a very sensitive and early marker of liver function abnormality in patients exposed to mixed solvents, and might be explained as an early sign of liver dysfunction. While it was not possible to state which solvent caused what type of abnormality, the authors concluded that conventional liver function tests seem to be rather insensitive for early liver disease detection, and normal measurement does not rule out the existence of subclinical disease, and therefore, an elevation of serum bile acid indicates

early potential hepatotoxicity from mixed organic solvents. From a clinical point of view this study suggests that exposure to mixture of solvents in an industrial setting may cause liver damage which is subclinical and initially undetected, unless liver enzymes and bile acids are measured. It seems likely that this initial stage of liver damage in patients exposed to solvent mixtures is commonly missed.

20.7.5 TRICHLOROETHYLENE

The liver is a target organ toxicity for trichloroethylene in experimental animals. Data in humans are limited.[34] Case reports describe trichloroethylene induced hepatitis and liver necrosis.[35] Guzelian et al. described both hepatic necrosis and fatty metamorphasis.[36] As early as 1962, the hepatotoxicity of trichloroethylene has been studied in humans. Trichloroethylene has been found to cause liver damage after both acute and chronic exposures.[37,38,39,40,41] Several studies reported a history of pathological changes including individual or focal necrosis after treatment of experimental animals with trichloroethylene.[42] In addition to these histopathological changes Berman also found a dose response relationship to the histopathological changes. Since trichloroethylene is commonly present as a contaminant in ground water (from degreasing, paint thinning and plastic metal processes) Barton has evaluated risks assessment of trichloroethylene and liver toxicity,[43] and showed that exposure was associated with continuous response in the form of liver toxicity. There was a connection between increased liver weight over body weight and liver toxicity, this effect also appears to be a sensitive indicator of liver toxicity. The authors concluded that trichloroethylene is toxic to the liver, based on their analysis of their findings, and that the use of liver enzymes by themselves may miss the early signs of toxicity to the liver by trichloroethylene. From these studies on trichloroethylene, and the studies on solvent mixtures described above, it seems reasonable to conclude that the early subclinical stages of solvent hepatotoxicity are commonly missed. When patients present to the doctor they will already be in more advanced stages such as liver steatosis and/or chronic hepatitis, and at times liver fibrosis.

The toxicity of trichloroethylene is dependent upon metabolism and induction of cytochrome P450. Trichloroethylene is metabolized through chloral hydrate to compounds including trichloroacetic acid and dichloroacetic acid which alter intercellular communication, induce peroxisome proliferation and may promote tumor production.[44] Significant variability in trichloroethylene metabolism in 23 human haptic microsomal samples was reported by Lipscomb et al.[44] It was also demonstrated that the trichloroethylene metabolism is dependent on enzymatic activities of the cytochrome system, and they conclude that their data indicates that humans are not uniform in their capacity for CPY dependent metabolism of trichloroethylene and increased activity may increase susceptibility to trichloroethylene induced toxicity in humans. These observations are compatible with the variability reaction which is depending on nutritional factors, enzyme induction factors, hormonal factors and interaction with other environmental chemicals, prescription medications and general health conditions, and explains the variable reports as far as trichloroethylene and level of liver toxicity in the various individuals studied.

In a predisposed individual (for example, a patient who is on medications or alcohol) it is highly likely that exposure to trichloroethylene will be a substantial factor in the genesis of a wide variety of liver diseases.

20.7.6 TETRACHLOROETHYLENE

Tetrachloroethylene is synthetic chemical used for dry cleaning fabrics, and has also been named as perchloroethylene and tetrachloroethene. The liver is the target organ in humans, mainly in reports of accidental exposure to a high concentration. Meckler et.al.[45] has shown liver damage in a woman exposed occupationally to tetrachloroethylene fumes documented by a liver biopsy. Other investigators also have shown elevation of liver enzymes, jaundice and enlarged liver.[46,47] Experimental animal studies also have shown liver damage by inhalation of tetrachloroethylene.[48,49,50,51] Liver necrosis occurred in experimental mice exposed to 100 and 200 ppm of tetrachloroethylene for 103 weeks.[52] Experimental animals exposed orally to tetrachloroethylene have been shown to develop liver changes similar to those produced by inhalation studies, and mice are more sensitive than rats to tetrachloroethylene induced liver toxicity. Humans exposed by oral routes to tetrachloroethylene except for heavy doses commonly have not shown significant changes other than obstructive jaundice and enlarged liver reported in an infant exposed to tetrachloroethylene via breast milk.[53] Issues of carcinogenicity will not be addressed in this chapter, and the interested reader is referred to the toxicological profile for tetrachloroethylene.[54] It is highly likely that tetrachlorethylene is a hepatotoxic agent in high doses, and probably in low doses in susceptible individuals with either other environmental exposures, prescription medications, alcoholism, nutritional and/or genetic factors, and preexisting disease of the liver.

20.7.7 TOLUENE

Industrial use of toluene is wide, commonly in paint, paint thinners, fingernail polish, lacquers, adhesives, rubbers and in the printing letter industry. Toluene is extensively metabolized by the liver; however, the liver does not appear to be a primary target for toluene toxicity. A study of printing factory workers who were exposed to toluene at a concentration of less than 200 ppm, showed minimal changes of liver enzymes.[55] The cohorts included 289 men, of which 8 showed elevated liver enzymes, and 6 of them had enlarged livers. Seven of those patients had liver biopsies which showed some centrally lobular and periportal fat accumulation, and Kupffer cell hyperplasia. The study by Svensson et al.[56] has looked at 47 rotogravure workers occupationally exposed to toluene at a concentration of 80 ppm for 3-39 years, and showed a significant elevation of liver enzymes, finding of chemical hepatitis. Seiji et al.[57] has examined a group of 157 female shoemakers who were exposed to toluene at levels of 7-324 ppm from 2-14 months, and showed no significant elevation of commonly measured liver functions. Another study that looked at 47 Swedish paint industry workers who were exposed for more than a 10 year period of time to organic solvents which included xylene, toluene, isobutanol, n-butanol, mineral spirits, methyl acetates, dichloromethane, methyl ethyl ketone and isopropanol did not show any changes in liver enzymes.[58] However, this study cannot be relied upon as a specific study, since the cohort size was small, and there were multiple exposures to multiple solvents, and therefore, the study had only limited power to detect the effects of toluene on the liver of exposed workers. Experimental animals exposed to toluene at concentrations of 533 to 800 ppm for 7 days showed increased liver weights, but no significant morphological changes by microscopy. Electron microscopical examination revealed ultrastructural changes which were compatible with changes in the cytochrome P-450 concentrations. Others have shown no effect on liver size or liver functions.[59,60,61,62] Overall the data seems to suggest that toluene may cause liver damage in certain industries, and especially in synergism with other sol-

vents. It is highly likely that, in predisposed individuals, toluene can cause liver damage from chemical hepatitis to necrosis and fibrosis.

20.7.8 DICHLOROMETHANE

Dichloromethane also called dichloromethane, is a colorless liquid that has a mild sweet odor. It is used widely in the industry as a solvent and a paint stripper. It is commonly found in spray painting operations, automotive degreasing, in cleaners and in household products. Stewart et al.[63] showed no changes in liver enzymes in patients exposed for a period of 6 weeks to levels of dichloromethane via inhalation from 50-500 ppm. On the other hand, Ott et al.[64] has shown an elevation in bilirubin in workers exposed to dichloromethane up to 475 ppm. In experimental animals dichloromethane exposure has been associated with fatty changes of the liver and elevated liver enzymes. Norpoth et al.[65] have shown hepatic microsomal enzyme elevation at 500 ppm of dichloromethane exposure for 10 days, and others have shown significant fatty changes of the liver upon exposure of mice and rats for 100 days to 75-100 ppm of trichloroethylene.[66,67,68] When exposure to dichloromethane continues for 2 years there was increased evidence of pathological changes and fatty liver changes.[69,70,71] The overall weight of the data supports a hepatotoxic effect of dichloromethane on the liver.

20.7.9 STODDARD SOLVENT

Stoddard solvent is a widely used organic solvent synthetically made, and comes from the refining of crude oil. It is a petroleum mixture made from distilled alkanes, cycloalkanes (naphthenes), and aromatic compounds. In addition, it goes by other names such as Varsol 1, Texsolve S and others. It is commonly used as a paint thinner, as solvents in some types of photocopier toners, printing ink, adhesives, dry cleaning and as a general cleaner and degreaser. Twelve men exposed to 610 mg per cubic meter of vaporized Stoddard solvents for a period of 6 hours revealed no changes in serum glucose, triglycerides, cholesterol or urate.[72] Dossing et al.[73] described painters who were exposed to non-specified levels of Stoddard solvents and other chemicals for chronic periods, and elevated levels of serum alanine aminotransferase, but other functions were normal and normal liver biopsies. Flodin et al.[74] has studied a group of patients exposed to a variety of solvents, including Stoddard solvents and showed normal liver function tests, but an elevated gamma glutamyl transferase. Hane et al.[75] has shown that a group of painters exposed to Stoddard solvents had no significant abnormality of liver enzymes. Studies in experimental animals showed minimal fatty changes of the liver, Jenkins et al.[76] as did the studies by Carpenter and Phillips.[77,78,79] The data from experimental animals and humans suggests a potential hepatotoxic effect of Stoddard solvents, but additional studies and a case by case evaluation is required.

20.7.10 1,1,1-TRICHLOROETHANE

1,1,1,-Trichloroethane is a colorless solvent which is manmade. It is produced by industry and is used in commercial products. It is used as a solvent, and is heavily used in glue and paint, as well as a degreaser and metal parts manufacturing. It is also used in some household products such as spot cleaners, glues and aerosol sprays. It is commonly found in soil and water as a contaminant. Brief single exposures to very high levels of 1,1,1-trichloroethane and a moderate high concentration have been shown to cause elevation of urobilinogen in patients.[80] This type of finding indicates reduced bile excretion and some intrinsic liver damage. Stewart et al.[81] showed in patients accidentally exposed to a high concentration of 1,1,1-trichloroethane increased levels of urinary urobilinogen for ap-

proximately 4 days following the exposure. While looking at enzyme levels in the blood after exposure to 1,1,1-trichloroethane, there was no evidence of elevated serum enzyme levels.[80,82,83,84] Case studies of people exposed to a high concentration of 1,1,1-trichloroethane did not show elevated liver enzymes.[81,85] Histopathological examination of the liver of patients who died following inhalation of a high concentration of 1,1,1-trichloroethane showed minimal changes, mainly those of mild fatty changes of the liver.[86,87] Kramer et al.[88] studied humans at low levels of exposure and found minor changes in liver enzymes. Experimental animal studies showed mild histopathological changes and effects on liver enzymes.[89,90] Truffert et al.[91] showed that intermittent duration and intermittent exposure to a low concentration of 1,1,1-trichloroethane produced a 67% increase in the synthesis of DNA of the livers of exposed rats, and concluded that the DNA synthesis measurements may be a more sensitive indicator of liver damage than just measurements of liver enzymes. McNutt et al.[92] showed histological damage following exposure to 1,1,1-trichloroethane with hepatocyte necrosis. The most commonly reported effects of 1,1,1-trichloroethane on the liver in experimental animals is increased fat accumulation.[93,94,95] The function of duration of exposure played an important role in experimental rats, and was seen in those who were exposed for 7 hours, but was not seen in those exposed for 2 hours in high levels. Savolainen et al.[96] showed that exposure to a moderate concentration in experimental animals caused decreased microsomal cytochrome P450 enzyme activity. Overall, the animal studies and human studies suggest an effect of 1,1,1-trichloroethane on the liver, but the severity appears to be related to the dose and duration of the exposure.

20.7.11 SUMMARY

In summary, from the available data, it is clear that exposure to solvents and hepatotoxicity must be evaluated in context of the individual variability, exposure to mixture of solvents, and synergistic toxicity.

REFERENCES

1 Jones DS, ed, **Detoxification: A Clinical Monograph**, *Institute for Functional Medicine, Inc*., 1999
2 Ungar, *Viertelsjarisch f. gericht. Med.*, **47**:98 (1887)
3 Ostertag R, *Virchows Arch.*, **118**:250 (1889)
4 Stiles HJ and McDonald S, *Scott Med Surg J*, **15**:97 (1904)
5 Recknagal RO, *Pharmacol Rev*, **19**:145 (1967)
6 Recknagel RO and Glinde EA, *CRC Crit Rev Toxicol*, **2**:263 (1973)
7 Zimmerman HJ, **Hepatotoxicity: The Adverse Effects of Drugs and Other Chemicals on the Liver**, *Appleton-Century-Crofts*, New York, 1978
8 Mitchell JR, et al. In **Concepts in Biochemical Pharmacology**, Part 3, JR Gillette, JR Mitchell and PS Randall (eds), *Springer-Verlag*, Berlin, 383-419 (1975)
9 Mitchell JR, et al., *Drug Met Disp*, **1**:418 (1973)
10 Rouiller CH, In **The Liver**, Volume II, CH Rouiller (ed), *Academic Press*, New York, 335-476 (1964)
11 Von Oettingen WF, **The Halogenated Hydrocarbons of Industrial and Toxicological Importance**, *Elsevier*, Amsterdam (1964)
12 Browning E, **Toxicology and Metabolism of Industrial Solvents**, *Elsevier*, Amerstdam (1965)
13 Von Oettingen WF, The Halogenated Aliphatic, Olephinic Cyclic, Aromatic and Aliphatic-Aromatic Hydrocarbons Including the Halogenated Insecticides. Their Toxicity and Potential Dangers. U.S. Dept. HEW, U.S. Govt Printing Office, Washington, D.C. (1955)
14 Meyer J and Pessoa SB, *Am J Trop Med*, **3**:177 (1923)
15 Reynolds ES, *Biochem Pharmacol*, **21**:2255 (1972)
16 Slater TF, *Nature* (Lond), **209**:36 (1966)
17 Jennings RB, *Arch Pathol*, **55**:269 (1955)
18 Klatskin G, **Toxic and Drug Induced Hepatitis. In Diseases of the liver**, 4th Ed, L Schiff (ed), *JB Lippincott*, Philadelphia, 604-710 (1975)

19 Slater TF, **Free Radical Mechanisms in Tissue Injury**, *JW Arrowsmith, Ltd*, Bristol, 118-163 (1972)
20 Le Page RN and Dorling PR, *Aust J Exp Biol*, **49**:345 (1971)
21 Rufeger U and Frimmer M, *Arch Pharmacol*, **293**:187 (1976)
22 Agency for Toxic Substances and Disease Registry (ATSDR), Toxicological Profile for Carbon Tetrachloride, Atlanta, Georgia (1997)
23 Droz PO, Wu MM, Cumberland WG and Berode M, *Br J Ind Med*, **46**(7):447-460 (July 1989)
24 Nothnagel, *Berlin Klin Wchnschr*, **3**:31 (1866)
25 International Agency for Research on Cancer (IARC) Monographs on the Evaluation of Carcinogenic Risks to Humans, Volumed 47:125-156; IARC, Lyon, France Volumes 43-48 (1987-1990)
26 Toftgard R and Nilsen OG, *Toxicology*, **23**(2-3):197-212 (1982)
27 Frommer U, Ullrich V and Orrenius S, *FEBS Lett*, **41**(1):14-16 (Apr 15, 1974)
28 Sotaniemi EA, et al., *Acta Med Scand*, **212**:207-215 (1982)
29 Fisbien A, et al., *Lancet*, **1**:129 (1983)
30 Franco G, et al., *Br J Ind Med*, **46**:141-142 (1989)
31 Chen JD, et al., *Brit J Ind Med*, **48**:676-701 (1991)
32 Kurppa K and Husman K, *Scand J Work Environ Health*, **8**:137-140 (1982)
33 Franco G, et al., *Int Arch Occup Environ Health,* **58**:157-164 (1986)
34 Davidson IW and Beliles RP, *Drug Metabolism Review*, **23**:493-599 (1991)
35 Bond GR, *Clinical Toxicology*, **34**(4):461-466 (1996)
36 Guzelian PS, Disorders of the Liver, In **Principles and Practice of Environmental Medicine**, Aylce Bezman Tacher (eds), *Plenum Medical Book Company*, New York, 319-333, (1992)
37 Baerg RD and Kimberg DV, *Annals of Internal Medicine*, **73**:713-720 (1970)
38 Clearfield HS, *Digestive Digest,* **15**:851-856 (1970)
39 Fielder RJ, Loweing RK and Shillaker RO, *Toxic Rev*, **6**:1-70 (1982)
40 Litt I and Cohen M, *New England Journal of Medicine*, **281**:543-544 (1969)
41 Sax N, **Dangerous Properties of Industrial Chemicals**, 4th Edition, *Van Nostrand Reinhold Co*, Toronto, Canada, 1186 (1975)
42 Berman E, et al., *J Tox Environ Health,* **45**(2):127-143 (June 1995)
43 Barton HA, et al., *Regulatory Toxicology & Pharmacology*, **24**:269-285 (1986)
44 Lipscomb JC, et al., *Toxicology & Applied Pharmacology*, **142**:311-318-1997
45 Meckler LC and Phelps PK, *J Am Med Assoc*, **197**:662-663 (1966)
46 Coler HR and Rossmiller HR, *AMA Arch Ind Hyg Occup Med*, **8**:227-233 (1953)
47 Hake CL and Stewart RD, *Environ Health Perspect*, **21**:231-238 (1977)
48 Kyline B, et al., *Acta Pharmacol Toxicol*, **20**:16-26 (1963)
49 Kyline B, Sumegi I and Yllner S, *Acta Pharmacol Toxicol*, **22**:379-385 (1965)
50 Carpenter CP, *J Ind Hyg Toxicol*, **19**:323-336 (1937)
51 Rowe VK, et al., *AMA Arch Ind Hyg Occup Med*, **5**:566-579 (1952)
52 National Toxicology Program (NTP) - Technical Report Series No. 311, Toxicology and Carcinogenesis Studies of Tetrachloroethylene (Perchloroethylene) (CAS No. 127-18-4) in F344/N rats and B6C3F1 mice (inhalation studies), Research Triangle Park, NC, U.S. Dept of Health & Human Services, Public Health Service, National Institute of Health, NIH Publication No. 86-2567 (1986)
53 Bagnell PC and Ellenberger HA, *Can Med Assoc J*, **117**:1047-1048 (1977)
54 Agency for Toxic Substances and Disease Registry (ATSDR), Toxicological Profile for Tetrachloroethylene, Atlanta, Georgia (1993)
55 Guzelian P, Mills S and Fallon HJ, *J Occup Med*, **30**:791-796 (1988)
56 Svensson BG, et al., *Br J Ind Med*, **49**:402-408 (1992)
57 Seiji K, et al., I*nd Health*, **25**:163-168 (1987)
58 Lundberg I and Hakansson M, *Br J Ind Med*, **42**:596-600 (1985)
59 Bruckner JV and Peterson RG, *Toxicol Appl Pharmacol*, **61**:27-38 (1981)
60 Bruckner JV and Peterson RG, *Toxicol Appl Pharmacol*, **61**:302-312 (1981)
61 Kjellstrand P, et al., *Acta Pharmacol Toxicol,* **57**:242-249 (1985)
62 National Toxicology Program (NTP) - Technical Report Series, Toxicology and Carcinogenesis Studies of Toluene (CAS No. 108-88-3) in F344/N rats and 86C3F mice (inhalation studies), Research Triangle Park, NC, U.S. Environmental Protection Agency, U.S. Dept of Health & Human Services, No. 371. PB90-256371 (1990)
63 Stewart RD, et al., Methylene Chloride: Development of Biologic Standard for the Industrial Worker by Breath Analysis. Report of the National Institute of Occupational Safety and Health, Cincinnatti, OH, by the Medical College of Wisconsin, Milwaukee, Wisconsin, NTIS No. PB83-245860 (1974)

64 Ott MG, et al., *Scand J Work Environ Health*, **9**(Supple 1):17-25 (1983)
65 Norpoth K, Witting U and Springorum M, et al., *Int Arch Arbeitsmed*, **33**:315-321 (1974)
66 Haun CC, et al., Continuous Animal Exposure to Low Levels of Dichloromethane. In: Proceedings of the Third Annual Conference on Environmental Toxicology. Wright Patterson Air Force Base, OH: Aerospace Medical Research Laboratory, 199-208, AMRL-TR-72-130, (1982)
67 Kjellstrand P, et al., *Acta Pharmacol Toxiocl (Copenh)*, **59**:73-79 (1986)
68 Weinstein RS and Diamond SS, Hepatotoxicity of dichloromethane (methylene chloride) with continuous inhalation exposure at a low dose level. In: Proceedings of the Third Annual Conference on Environmental Toxicology. Wright Patterson Air Force Base, OH: Aerospace Medical Research Laboratory, 209-220, AMRL-TR-72-130, (1972)
69 Burek JD, et al., *Fund Appl Toxicol*, **4**:30-47 (1984)
70 Nitschke KD, et al., *Fundam Appl Toxicol*, **11**:48-59 (1988)
71 National Toxicology Program (NTP) - Technical Report Series No. 306, Toxicology and Carcinogenesis Studies of Dichloromethane (Methylene Chloride) (CAS No. 75-09-2) in F344/N rats and B6C3F1 mice (inhalation studies), Research Triangle Park, NC, U.S. Dept of Health & Human Services, Public Health Services, Centers for Disease Control, National Institute of Health, (1986)
72 Pederson LM and Cohr KH, *Acta Pharmacol Toxicol,* **55**:317-324 (1984)
73 Dossing M, Arlien-Soborg P and Petersen LM, *Eur J Clin Invest*, **13**:151-158 (1983)
74 Flodin U, Edling C and Axelson O, *Am J Ind Med*, **5**:287-295 (1984)
75 Hane M, et al., *Scand J Work Environ Health*, **3**:91-99 (1977)
76 Jenkins LJ, et al., *Toxicol Appl Pharmacol*, **18**:53-59 (1971)
77 Carpenter CP, et al., *Toxicol Appl Pharmacol*, **32**:246-262 (1975)
78 Carpenter CP, et al., *Toxicol Appl Pharmacol*, **32**:282-297 (1975)
79 Phillips RD and Egan GF, *Fundam Appl Toxicol*, **4**:808-818 (1984)
80 Stewart RD, et al., *Am Ind Hygn Assoc J*, **22**: 252-262 (1961)
81 Stewart RD, *J Am Med Assoc*, **215**:1789-1792 (1971)
82 Stewart RD, et al., *Arch Environ Health*, **19**:467-472 (1969)
83 Dornette WHL and Jones JP, *Anesthesia and Analgesia*, **39**:249-252 (1960)
84 Torkelson TR, et al., *Am Ind Hygn Assoc J*, **19**:353-362 (1958)
85 Wright MF and Strobl DJ, *J Am Osteopath Assoc,* **84**:285-288 (1984)
86 Caplan YH, Backer RC and Whitaker JQ, *Clin Toxicol*, **9**:69-74 (1976)
87 Hall FB and Hine CH, *J Forensic Sci*, **11**:404-413 (1966)
88 Kramer CG, et al., *Arch Environ Health*, **33**:331-342 (1978)
89 Carlson GP, *Lif Sci (United States)*, **13**:67-73 (1973)
90 Gehring PJ, *Toxicol Appl Pharmacol*, **13**:287-298 (1968)
91 Truffert L, et al., *Arch Mal Prof Med Trav Secur Soc*, **38**:261-263 (1977)
92 McNutt NS, et al., *Lab Invest*, **32**:642-654 (1975)
93 Takahara K, *Okayama Igakkai Zasshi*, **98**:1099-1110 (Japanese) (1986)
94 Adams EM, et al., *Am Med Assoc Arch Ind Hyg Occup Med*, **1**:225-236 (1950)
95 Herd PA, Lipsky M and Martin HF, *Arch Environ Health*, **28**:227-3 (1974)
96 Savolainen H, et al., *Arch Toxicol*, **38**:229-237 (1977)

20.8 SOLVENTS AND THE LIVER

David K. Bonauto
Occupational Medicine, University of Washington
Seattle, Washington, USA
C. Andrew Brodkin
Department of Internal Medicine and Department of Environmental Health,
University of Washington, Seattle, Washington, USA
William O. Robertson
Washington Poison Center, University of Washington
Seattle, Washington, USA

The toxic effects of organic solvent compounds on the liver are dependent on the intensity and duration of exposure, route of exposure, the intrinsic toxicity of the specific compound, as well as individual susceptibility factors.[1] There are a number of pathologic manifestations of solvent induced hepatotoxicity, including inflammation, fat accumulation in the liver (steatosis), hepatocellular necrosis and carcinogenesis. Functional disturbances in liver physiology have also been associated with solvent exposure.

The purpose of this chapter is to review the known hepatotoxicity of commonly used industrial solvents.[2] A brief review of normal anatomic and physiologic function of the liver will be provided as a background for understanding histopathologic and biochemical changes associated with solvent toxicity. The final segment includes a discussion of solvents known to cause liver injury with a review of the available medical evidence suggestive of human hepatotoxicity of solvents at present day exposure levels. Solvent induced hepatotoxicity is almost exclusively encountered in an occupational setting and thus this review will focus on evidence culled from that setting.

20.8.1 NORMAL ANATOMIC AND PHYSIOLOGIC FUNCTION OF THE LIVER

The liver is the largest internal organ and is involved in many physiologic processes including nutrient homeostasis, synthesis and excretion of bile, lipid metabolism and lipoprotein and protein synthesis.[3] Most importantly for purposes of this chapter, the liver is the site of the biotransformation of a wide variety of endogenous and exogenous toxins.[4] The ability of the liver to biotransform various chemicals is due to the multiple different enzyme systems contained within the hepatocytes.[3,5] One such enzyme system is the cytochrome p 450 enzyme system. It consists of a large group of enzymes which biotransform many different substances by either oxidation or reduction to facilitate excretion from the body. Specifically different members of the cytochrome p 450 family catalyze reactions involving aromatic and aliphatic hydroxylation, epoxidation, dehalogenation, dealkylation, N-, S-oxidation as well as O-, N-, S- dealkylation reactions.[3,5]

The diverse metabolic activities of the liver make it susceptible to solvent induced injury, particularly from reactive intermediates which damage cellular macromolecules. The microscopic anatomy of the liver provides an explanation for this susceptibility. The basic unit of the liver is the hepatic lobule which consists of a central vein surrounded radially by sinusoids of liver cells (hepatocytes). Portal triads consisting of a hepatic artery, a hepatic vein and a bile canniliculus are located at the periphery. Liver cells closest to the vascular

supply or the portal triad, zone one, are more resistant to oxidative stress, while hepatocytes near the central vein, zone three or centrizonal region, are most susceptible to solvent induced injury.

20.8.1.1 Factors Influencing Solvent Hepatotoxicity

Bioavailability: The physical and chemical properties of a solvent and its toxicokinetics determine its availability to hepatic tissues. The primary route of absorption of most solvents which cause hepatotoxicity into human biological systems is via the lung. Therefore, the greater the volatility of the solvent, the greater its concentration in the air, and subsequently the larger the potential dose.[4] While accidental or intentional ingestion of solvents is reported in the medical literature, it is an uncommon route of exposure in the occupational setting. Dermal absorption should be considered a significant route of exposure for most solvent compounds based on their lipid solubility. The degree of exposure can often be modified by the use of personal protective equipment such as gloves or a respirator and engineered exposure controls such as building ventilation.

The lipid solubility of solvents also favors their deposition of into lipid rich organ systems such as the liver. The toxicity of a particular solvent may be enhanced by its long residence time in the liver.[6]

Genetic and environmental factors: While some solvents are directly hepatotoxic, frequently biotransformation of solvents by hepatic mixed function oxygenases, such as the cytochrome p-450 system, result in toxic intermediates.[7] A variety of genetic and environmental factors inhibit or induce the activity of these hepatic enzyme systems, effecting the biotransformation and resulting toxicity. Genetic factors thought to determine the activity or even the presence of an enzyme within an organism center around human polymorphisms or variations in the genetic code.[7] As the activities of the liver enzymes are changed so will the rate of formation of the metabolite thus increasing or decreasing the toxicity of the foreign substance.[3] Environmental factors which determine the activity of liver enzyme systems include co-exposure to other drugs and toxins or characteristics of the individual particularly disease states which induce or inhibit the activity of liver biotransformation enzymes.[1,3,7-9] Individual characteristics such as age, nutritional status, pregnancy or disease states such as diabetes or obesity may also change the activity of cytochrome p-450 enzymes.[7,9] The assessment of an individual's susceptibility to exposure should attempt to account for these variables in determining risk.

20.8.1.2 Microscopic, Biochemical and Clinical Findings Associated with Liver Injury due to Solvents

Hepatotoxic manifestations associated with acute solvent exposure are dose dependent. Acute cytotoxic injury of a solvent directly or by its metabolites causes an alteration in the normal physiologic function leading to ballooning fatty change and ultimately cellular necrosis. If the dose is minimal and doesn't exceed the 'regenerative capacity' of the liver, inflammatory changes generally resolve within two weeks to several months. Metabolic derangement results in the accumulation of fats in the liver, termed steatosis.[10] A less common form of acute cytotoxic injury is related to cholestatic injury with disruption of normal biliary flow.[11] Severe long term exposures can lead to fibrosis or scarring and cirrhosis which distort the hepatic architecture and lead to altered liver function.

Current research focuses on the effect of low doses of solvents on the liver, with concern that low grade prolonged solvent exposure could lead to chronic injury and eventual impairment.[5,12]

Tests used to evaluate and screen for liver injury can be divided into three general categories: serum biomarkers of disease, tests of hepatic clearance, and anatomic evaluation.[11] The hepatic enzymes most commonly screened for related to hepatocellular necrosis and inflammatory changes are aspartate aminotransferase (AST), and alanine aminotransferase (ALT). Elevation of these enzymes in the setting of significant exposure is indicative of hepatotoxic injury, though alternative causes such as alcohol and viral hepatitis should be excluded. Importantly, serum hepatic transaminase levels indicate hepatocellular necrosis or inflammation, but may not indicate more subtle metabolic alterations in hepatic function. Measures of other hepatic enzymes, gamma glutamyl transpeptidase (GGT), alkaline phosphatase (Alk Phos), total and direct bilirubins may also be suggestive of solvent induced hepatotoxicity. Specifically if hepatic excretion of bile, is diminished, the resulting intrahepatic cholestasis is associated with elevations in GGT, Alk Phos and serum bile acids. Significant elevations of bilirubins leads to the clinical observation of jaundice or yellowing of the skin. However, pathologic obstruction of the biliary tract is not a common finding in solvent induced hepatotoxicity.[5]

Clearance tests of liver function assess a number of physiologic activities including hepatic uptake, hepatic metabolism, and hepatic excretion. Typical clearance tests of liver function include indocyanine green (ICG), antipyrine clearance test and ^{14}C aminopyrine breath test. These tests give an estimation of the ability of the liver to extract and detoxify exogenous toxins (xenobiotics). Measuring the excretion of endogenously produced serum bile acids is an additional measure of hepatic clearance and has been used as a sensitive measure of early solvent hepatotoxicity.[13,14]

Anatomic evaluation of solvent hepatotoxicity centers on physical examination of the liver, radiologic study and liver biopsy. Physical exam is nonspecific as to the cause and characterization of the disease. Radiologic studies such as ultrasound can identify hepatobiliary disease and liver parenchymal disease, namely steatosis and fibrosis. Steatosis and fibrosis are noted on ultrasound by a change in the echogenicity of the liver. While liver biopsy is the 'gold standard' for anatomic evaluation of the liver, the invasiveness of the test, the morbidity and discomfort of the procedure, and its cost make it prohibitive for routine screening. It is usually reserved for definitive diagnostic and prognostic purposes. Algorithmic strategies for screening for liver injury and evaluation of abnormal results have been reported in several references.[11,12,15,16]

20.8.2 HEPATOTOXICITY ASSOCIATED WITH SPECIFIC SOLVENTS

The following section presents specific classes of organic solvents strongly associated with hepatotoxicity in human populations or animal studies. While there is more limited evidence of hepatotoxicity related to inhalational and dermal exposure to aliphatic hydrocarbons, ketones, alcohols, aldehydes, esters and ethers, potential hepatotoxicity related to these agents must be assessed on an individual basis with regard to concentration, duration, and bioavailability of exposure.[5,11] Variations in individual susceptibility must also be considered with regard to concurrent use of alcohol, mixed solvent exposure, underlying liver diseases (e.g., viral hepatitis, hemochromatosis, hypertriglyceridemia and diabetes) as well as demographic differences in hepatic metabolism.[11] Given these limitations, the organic solvents of primary concern with regard to hepatotoxicity are the haloalkanes, haloalkenes,

dimethylformamide, and nitroparaffins.[5,11] Other agents such as styrene have been associated with hepatotoxicity in some studies.[17,18] Potential interactive effects of solvent mixtures should always be considered in the assessment of hepatotoxicity, even if composed of solvents not commonly associated with hepatotoxicity.

20.8.2.1 Haloalkanes and haloalkenes

Some of the most extensively studied and most concerning hepatoxins are the haloalkane solvents. Major haloalkanes encountered industrially, with documented animal and human hepatotoxicity, are carbon tetrachloride, chloroform, 1,1,2,2-tetrachlorethane, methyl chloroform and 1,1,2-trichloroethane, tetrachloroethylene, and trichloroethylene.[5,6,19] The relative hepatotoxicity of each is correlated inversely with the carbon chain length, and carbon halogen bond energy and correlated directly with the number of halogens on the molecule and the atomic number of the halogen.[5,20] Some of these solvents have been eliminated from common industrial use due to their deleterious environmental and human effects, though they may still be encountered in specific processes and regions (e.g., developing countries). Carbon tetrachloride is the most extensively studied and serves as a model for hepatotoxicity for other haloalkanes.[21,22]

20.8.2.2 Carbon tetrachloride

Carbon tetrachloride hepatotoxicity has been reported since the early twentieth century.[23] The toxicological literature is extensive with regard to carbon tetrachloride hepatotoxicity in animals. [21,23] Human toxicological information derives primarily from accidental or intentional ingestion in humans or by inhalational exposure in groups of workers.[21] The industrial use of carbon tetrachloride has declined precipitously, due to the recognized health effects and regulatory policy.[21] Historically, it was used as a solvent in the manufacture of industrial chemicals, in the dry cleaning industry and even as an antiparasitic medication.[5] Presently the main means of exposure is in research laboratory settings, or as low level environmental contaminant.[21] Because it is so volatile, the main mode of carbon tetrachloride exposure in occupational setting is via inhalation, although exposure by the dermal route also occurs.

Animal and human susceptibility to carbon tetrachloride hepatotoxicity is dependent on many different factors. There is substantial interspecies variation in carbon tetrachloride induced hepatotoxicity in animals due to differences in metabolic pathways among species.[21] Based on animal models, hepatotoxicity in humans is most likely mediated from the trichloromethyl radical formed from the metabolism of carbon tetrachloride by hepatic cytochrome p 450 2E1.[24] Animal studies suggest differential hepatotoxicity based upon the animal's age and gender, with greater toxicity demonstrated in adult rats compared to newborns,[25,26] and males compared with females.[5] Cytochrome p-450 enzyme systems are present in the human fetus suggesting a potential for in utero liver toxicity.[27] Human gender differences in the metabolism of carbon tetrachloride have not been demonstrated despite potential sex steroid influences on the cytochrome p-450 system.[28]

The hepatotoxic effects of carbon tetrachloride are more severe in the setting of alcohol consumption.[21,29,30] Animal studies demonstrate that the temporal relationship between ethanol ingestion and carbon tetrachloride exposure determines the severity of toxicity.[31-33] Maximal hepatotoxicity is derived from ethanol ingested eighteen hours preceding exposure to carbon tetrachloride,[32,33] whereas exposure to ethanol three hours prior to carbon tetrachloride exposure leads to minimal hepatotoxicity.[33] The mechanism for this interaction is

the induction of cytochrome p-450 enzymes leading to greater formation of toxic intermediates.[34] In contrast, exposure to ethanol immediately preceding carbon tetrachloride exposure leads to competitive inhibition of carbon tetrachloride metabolism.[34] Several other alcohols (e.g., isopropanol,[32,35] t-butyl alcohol[36]) and ketones[37] potentiate the effect of carbon tetrachloride hepatotoxicity. Exposures to other haloalkanes[38] or haloalkenes[39] potentiate carbon tetrachloride hepatotoxicity while carbon disulfide exposure is 'protective' of carbon tetrachloride hepatototoxicity.[40] Dietary factors, medications, chronic diseases and persistent halogenated environmental contaminants such as PCB's and DDT have been shown to modulate or exacerbate the hepatotoxicity of carbon tetrachloride.[5,21]

Cellular disruption leading to hepatocellular necrosis results from damage to cellular macromolecules by trichloromethyl radicals.[24] Cellular disruption involves alteration of calcium homeostasis,[41] impaired oxidative phosphorylation,[42] and trichloromethyl radical binding to cellular proteins, nucleic acids, and induction of lipid peroxidation.[6,21] Histologically there is preferential necrosis of zone three hepatocytes in the liver acinus so called centrizonal necrosis as well as zone three steatosis.

As with other halogenated hydrocarbons, carbon tetrachloride is an intrinsic hepatotoxin with adverse effects occurring at predictable exposure levels. The American Conference of Governmental and Industrial Hygienists (ACGIH) Threshold Limit Value (TLV), for carbon tetrachloride, based upon animal and human exposure data where limited adverse health effects are observed, is 5 ppm over an 8 hour time weighted average and a 40 hour work week for carbon tetrachloride.[43] In human population studies, elevations in hepatic transaminase levels occur at carbon tetrachloride concentrations averaging 200 ppm, with small but significant elevations of ALT, AST, Alk Phos and GGT occurring at exposure levels below the TLV.[44-46]

Carbon tetrachloride also affects many other organ systems, specifically the central nervous system, the gastrointestinal tract, the liver and the kidney.[5,6] Hepatic manifestations of carbon tetrachloride include serum AST and ALT elevations as early as three hours following exposure. Clinical evidence of hepatic disease occurs approximately twenty four hours following exposure, and is manifest in half the cases as jaundice accompanied by hepatic enlargement. In severe poisonings, progressive hepatic injury leads to coma and death within a week of exposure. Fortunately, non lethal exposures are often associated with significant clinical recovery in two to three weeks. Treatment is limited to supportive care, in a hospital setting. Chronic exposures to carbon tetrachloride have been associated with hepatic fibrosis and cirrhosis in animals and documented as well in several case reports in humans.[47-50]

20.8.2.3 Chloroform

Medical and industrial use of chloroform has also declined significantly.[51] Today, industrial use is limited to the manufacture of refrigerants and fluoropolymers.[51] Chloroform metabolism involves the same cytochrome p-450 2E1 as carbon tetrachloride but with oxidation of chloroform to trichloromethanol with spontaneous formation of phosgene via the elimination of hydrochloric acid.[3] In turn, phosgene reacts with hepatic lipids and microsomal proteins and depletes cellular glutathione, a cellular antioxidant.[7,51] Factors potentiating chloroform hepatotoxicity include ethanol and other alcoho1s,[52-54] hypoxia,[53] ketones,[55] fasting state,[56,57] concomitant chronic medical disease and chronic medication use, or those with repeated exposures to chloroform.[5] The pattern of human liver injury associated from chloroform poisoning is centrilobular necrosis and steatosis.[23]

The ACGIH has set a TLV of 10 ppm over an 8 hour time weighted average and a 40 hour work week for chloroform. Because chloroform is a potential carcinogen, the lowest possible exposure is recommended. Occupational hepatotoxicity below the ACGIH TLV has been demonstrated, with evidence of adverse effects between 2 and 10 ppm.[51,58]

Clinical manifestations of chloroform toxicity involve multi-organ system effects including damage to the central nervous system, the kidney and lung as well as the liver.[59] Fulminant toxic hepatitis appears within one to three days following exposure, with death at approximately one week in severe poisonings.[57] In nonfatal cases, hepatic inflammatory changes,with hepatomegaly and transaminitis can occur within hours.[57] Ingestion or significant inhalational exposure should be managed in a closely monitored hospital setting.

20.8.2.4 Dichloromethane

Dichloromethane is commonly used as a degreaser and a paint stripper. It is metabolized in the liver by the cytochrome p-450 pathway to produce carbon monoxide.[60] An independent pathway of metabolism occurs via conjugation with glutathione.[60] Animal experimentation has demonstrated hepatotoxicity at near lethal concentrations of dichloromethane.[61,62] Dichloromethane potentiates carbon tetrachloride hepatotoxicity in rat livers.[38] Short term exposure to both ethanol and dichloromethane demonstrate an antagonistic relationship, while chronic exposure potentiates hepatotoxicity.[63]

Cases of human hepatotoxicity to dichloromethane have been reported.[62,64] Workers in an acetate fiber production plant, exposed to 140 to 475 ppm of dichloromethane, with concomitant exposures to acetone and methanol, were observed to have elevated bilirubin and ALT levels relative to workers exposed to acetone alone.[64] Bilirubin elevations were dependent on the level of dichloromethane exposure.[64] Other studies have shown no significant effects in the range of 5 to 330 ppm of dichloromethane.[65] Chronic exposure (greater than 10 years) to dichloromethane levels greater than 475 ppm was not associated with significant elevations in liver function tests.[66] There is minimal evidence of human hepatotoxicity of dichloromethane less than the ACGIH TLV of 50 ppm over an 8 hour time weighted average.[43]

20.8.2.5 Trichloroethanes

There are two isomers of trichloroethane, namely methyl chloroform and 1,1,2-trichloroethane. Animal hepatotoxicity to 1,1,2-trichloroethane is documented in the literature[67] with potentiation of toxicity in association with acetone,[68] isopropyl alcohol[69] and ethanol.[70] Hepatotoxicity, with steatosis, necrosis, elevated serum enzymes, and increased liver weight have been observed in animal models exposed to 1000 ppm of methyl chloroform.[71] Human studies consist of case reports documenting hepatotoxicity, with elevated serum transaminases and fatty liver disease related to 1,1,1-trichloroethane exposure.[72,73] Epidemiologic evidence suggests little hepatotoxicity related to this agent at exposure levels <350 ppm.[74,75]

20.8.2.6 1,1,2,2-Tetrachloroethane

Though rarely used in current practice, this solvent was an important cause of hepatotoxicity in the past. Its hepatotoxic potential was first identified during its use in the first World War.[5] Animal hepatotoxicity with fatty degeneration of the liver has been documented in multiple species.[76] Human inhalational exposures manifest in liver enlargement, jaundice, steatosis with subsequent liver failure in severe poisonings.[77,78] Subacute exposure periods of weeks to months is generally required for hepatic injury.[77] Liver regeneration oc-

curs after nonfatal exposures.[77] The mechanism of hepatotoxicity has not been elucidated in humans but the reactive metabolites 1,1-dichloroacetyl chloride with binding to hepatic macromolecules may play a role.[76] Metabolism of 1,1,2,2-tetrachloroethane is potentiated by fasting and ethanol in rats.[79,80] There is little documentation of any precise inhalational exposure levels necessary to cause hepatotoxicity.

20.8.2.7 Tetrachloroethylene and trichloroethylene

This widely used dry cleaning agent and degreasing agent is associated with hepatotoxic effects.[81,82] Cases of human hepatotoxicity to tetrachloroethylene at exposure levels greater than 100 ppm have been reported in the literature.[83,84] Humans exposed to tetrachloroethylene at dosages up to 150 ppm for durations of one to five 8 hour shifts had no difference in hepatic enzyme levels from baseline levels.[85] Studies of workers chronically exposed to concentrations of tetrachloroethylene less than 50 ppm showed no difference in liver enzyme levels, relative to groups of workers who did not have the exposure.[82] However, dry cleaning workers chronically exposed to low levels of tetrachloroethylene at less than 25 ppm had evidence of an alteration in hepatic echogenicity relative to non-exposed workers.[80] This is suggestive evidence that steatosis may occur at levels below the ACGIH TLV, without associated alterations in serum hepatic enzymes. The long term effects of exposures have not been well characterized.

Wide spread use of trichloroethylene occurs in the dry cleaning industry and industrially as a degreasing agent. Historical use as an anesthetic generally suggests little acute hepatotoxicity.[86,87] Longer term exposures in an occupational setting are associated with elevations in serum transaminases, with variable findings in epidemiologic studies.[88-90] Exposures below the ACGIH TLV of 50 ppm in workers using trichloroethylene as a cleaning agent found elevated levels of serum bile acids.[45,91] Hepatotoxicity is potentiated by alcohol,[92] isopropanol and acetone.[69] The long term effects of subclinical exposures are not known.

20.8.2.8 Other halogenated hydrocarbons

Vinyl chloride, a gas at normal temperature and pressure, has solvent properties at high pressures; its industrial use as a monomer in the manufacture of polyvinylchloride and hepatotoxicity with chronic exposure make it an important public health risk. Vinyl chloride is associated with angiosarcoma,[93,94] a rare highly malignant hepatic tumor, hepatic fibrosis,[6] hepatocellular injury[95] and hepatoportal sclerosis, a form of noncirrhotic portal hypertension.[96,97] Appearance of angiosarcoma and hepatoportal sclerosis occurred in workers after decade long exposures.[94,98] Measures to limit both occupational and environmental exposures have been instituted to decrease potential hepatic outcomes, with effective screening programs using indocyanine green clearance tests.[6]

Haloalkanes other than the chloroalkanes, especially those with structural homology to known hepatotoxic chloroalkanes, should be considered potentially hepatotoxic despite little industrial use as solvents.[5,20] Case reports of bromoethane and hydrochlorofluorocarbon poisonings with hepatotoxicity have been reported in the literature.[99-102]

20.8.2.9 Styrene and aromatic hydrocarbons

Styrene is not only used as a monomer in the production of polystyrene but also as a reactive solvent in the manufacture of unsaturated polyester resins.[103] The hepatic metabolism of styrene involves the formation of the reactive intermediate styrene 7,8-oxide.[104] In rat models, styrene 7,8-oxide binds to hepatic macromolecules and lipids causing hepatocellular in-

jury.[105,106] Epidemiologic investigations of workers exposed to high concentrations (greater than 50 ppm) of styrene have shown elevations in GGT, AST, ALT,[107-109] and serum bilirubin levels.[110] At the ACGIH TLV of 50 ppm or less, evidence of transaminase and GGT elevations[111,112] are lacking but elevated levels of serum bilirubins[113] and bile acids[17,110] have been demonstrated. There is no evidence of alterations in hepatic echogenicity at exposure levels less than 50 ppm.[18]

Toluene, benzene and xylenes are generally considered to have limited hepatotoxicity.[6,114-118] Exposure to xylene is reported to cause mild steatosis.[5] Exposure to a mixture of solvents, inclusive of xylene and toluene have been reported to produce elevated serum bile acids.[13]

20.8.2.10 N-substituted amides

Two important N-substituted amides are dimethylformamide and dimethylacetamide. Dimethylformamide is used in the fabrication of synthetic textiles such as rayon. Its hepatotoxicity has been well demonstrated in occupational settings.[119-121] Evidence of dose dependent alcohol intolerance and subjective gastrointestinal symptoms (abdominal pain, anorexia and nausea) have been described.[122] Objective clinical and biochemical signs include elevations of transaminases, AST and ALT, hepatomegaly and abnormal liver biopsy findings demonstrating hepatocellular necrosis and steatosis.[120,123] Workers with acute toxicity related to DMF have more severe symptoms and higher transaminase levels than workers with toxicity related to chronic exposures.[124] Of significance, symptoms may occur under the ACGIH TLV of 10 ppm. Dermal absorption is a main exposure pathway in addition to inhalation.[119-121]

Dimethylacetamide is used as a solvent in the manufacture of plastics and as a paint remover. Occupational poisoning and hepatotoxicity to extreme concentrations of dimethylacetamide (DMA) are reported in the medical literature.[125] Decreases in hepatic clearance measures and alterations in hepatic transaminases with hepatomegaly have been reported at lower doses.[126] Like dimethylformamide, DMA is readily absorbed through the skin. Chronic exposures in workers exposed to low air concentrations of DMA of less than 3 ppm and with biological monitoring assessments to measure dosages by dermal absorption demonstrated little evidence of hepatotoxicity by clinical chemistries.[127]

20.8.2.11 Nitroparaffins

The well known hepatotoxicity of nitroaromatic compounds such as trinitrotoluene lends suspicion to the hepatotoxicity of the nitroparaffins.[114] Nitromethane and nitroethane produce steatosis in animal models, but there is limited evidence of hepatotoxicity of these agents in humans.[114] Evidence for the hepatoxicity of 2-nitropropane has been raised by case reports and case series of occupational fatalities in settings of severe exposure.[128,129] In these cases the lack of appropriate industrial hygienic measures such as adequate ventilation, and personal protective equipment contributed to the severity of the exposures.[130] Autopsies of the fatal cases revealed hepatocellular necrosis and fatty infiltration of the liver.[128] No significant evidence of hepatotoxicity has been demonstrated below the ACGIH TLV of 10 ppm.[131] Medical surveillance of workers exposed to less than 25 ppm of 2-nitropropane have not shown alterations in liver chemistries.[132]

20.8.2.12 Other solvents and mixed solvents

Suggestive evidence for hepatotoxicity of many compounds exist in the literature.[5] Two solvents with some suspicion for hepatotoxic potential in humans are tetrahydrofuran and 1,4-dioxane, both solvents used in industry.[133-135] Cases of tetrahydrofuran induced hepatotoxicity have been reported in the literature.[133] Tetrohydrofuran's inhibition of the cytochrome p-450 enzyme system lends biologic credibility to it being a hepatotoxin.[134] 1,4-dioxane is reported to be hepatotoxic but epidemiologic evidence in human populations for this is limited.[135]

Rarely do solvents exist in isolation and thus evaluation of hepatotoxicity must consider the effects of mixtures of solvents.[136] Alterations in the hepatotoxic potential of a chemical may exist, especially when the biotransforming enzymes are modulated or effected by various components of the mixture. Usual mechanisms for the potentiation of toxicity by alcohols, ketones may be altered when solvents are mixed. In such settings hepatotoxicity may occur below recommended levels.[137]

Much remains unknown regarding the hepatotoxic effects of compounds. For this reason, vigilance regarding the potential adverse hepatic effects of chemicals is appropriate. Maintaining active surveillance for solvent induced hepatotoxicity is important in protecting workers' health, and will further our knowledge of the hepatotoxic effects of solvents. With emerging knowledge, occupational and environmental standards can be refined to further protect the health of workers and the public.

REFERENCES

1 H. Zimmerman in **Schiff's Diseases of the Liver**, E. Schiff, M. Sorrell and W. Maddrey, Eds., *Lippincott-Raven Publishers,* Philadelphia, 1999, pp.973 -1064.

2 J. Rosenberg in **Occupational and Environmental Medicine**, 2nd ed., J. LaDou. Ed., *Appleton and Lange*, Stamford, Conn., 1997, pp. 359-386.

3 A. Parkinson in **Casarrett and Doull's Toxicology; The Basic Science of Poisons**, 5th ed., C. Klaasen, Ed., *McGraw-Hill*, New York, 1996, pp. 113-186.

4 M. Ellenhorn, S. Schonwald,G. Ordog, and J. Wasserberger eds., **Ellenhorn's Medical Toxicology: Diagnosis and Treatment of Human Poisoning**. *Williams and Wilkins.* Baltimore, 1997.

5 H. Zimmerman, **Hepatotoxicity: The Adverse Effects of Drugs and Other Chemicals on the Liver**. 2nd Ed., *Lippincott, Williams and Wilkins, Philadelphia*, 1999.

6 N. Gitlin in **Hepatology; A Textbook of Liver Disease**. 3rd ed., D. Zakim and T. Boyer, Eds., *WB Saunders Co.*, Philadelphia, 1996, pp. 1018-1050.

7 J. Raucy, J. Kraner, and J. Lasker, *Crit. Rev. Toxicol*., **23**, 1 (1993).

8 P. Watkins, *Semin. Liver Dis*., **10**, 235 (1990).

9 D. Vessey in **Hepatology; A Textbook of Liver Disease**. 3rd ed., D. Zakim and T. Boyer, Eds., *WB Saunders Co*., Philadelphia, 1996, pp. 257-305.

10 G. Lundqvist, U. Flodin, and O. Axelson, *Am. J. Ind. Med.*, **35**, 132 (1999).

11 C. Redlich and C. Brodkin in **Textbook of Clinical Occupational and Environmental Medicine**, L. Rosenstock and M. Cullen, Eds., *WB Saunders*, Philadelphia, 1994, pp. 423-436.

12 C. Tamburro and G. Liss, *J. Occup. Med*., **28**, 1034 (1986).

13 G. Franco, R. Fonte, G. Tempini, and F. Candura, *Int. Arch. Occup. Environ. Health*, **58**, 157 (1986).

14 G. Franco, *Br. J. Ind. Med*., **48**, 557 (1991).

15 D. Herip, *Am. J. Ind. Med*., **21**, 331 (1992).

16 R. Harrison, *Occupational Medicine: State of the Art Reviews*, **5**, 515 (1990).

17 C. Edling and C. Tagesson, *Br. J. Ind. Med.*, **41**, 257 (1984).

18 C. Brodkin, J. Moon, D. Echeverria, K. Wang, and H. Checkoway, ICOH 2000 Congress, (Abstract In Press).

19 R. Harbison, **Hamilton and Hardy's Industrial Toxicology**. 5th ed., *Mosby*, St. Louis, 1998.

20 G. Plaa and W. Hewitt in **Toxicology of the Liver**, G. Plaa and W. Hewitt, Eds., *Raven Press,* New York, 1982, pp. 103-120.

21 O. Faroon, J. Riddle, Y. Hales and W. Brattin, **Toxicological Profile for Carbon Tetrachloride**. Agency for Toxic Substances and Disease Registry (ATSDR), *US Department of Health and Human Services*, Atlanta, 1994.
22 R. Recknagel, *Pharmacol. Rev*., **19**, 145 (1967).
23 W. Von Oettingen, **The Halogenated Hydrocarbons of Industrial and Toxicological Importance**, *Elsevier*, Amsterdam, 1964.
24 R. Recknagel and E. Glende, Jr., *Crit. Rev. Toxicol.*, **2**, 236 (1973).
25 M. Dawkins, *J. Pathol. Bacteriol*., **85**, 189 (1963).
26 S. Cagen and C. Klaasen, *Toxicol. Appl. Pharmacol*., **50**, 347 (1979).
27 G. Mannering, *Fed. Proc.*, **44**, 2302 (1985).
28 J. Gustaffson, *Annu. Rev. Physiol.,* **45**, 51 (1983).
29 M. Manno, M. Rezzadore, M. Grossi, and C. Sbrana, *Hum. Exp. Toxicol.,* **15**, 294 (1996).
30 P. New, G. Lubash, L. Scherr, and A. Rubin, *JAMA*, **181**, 903 (1962)
31 H. Ikatsu, T. Okino, and T. Nakajima, *Br. J. Ind. Med*., **48**, 636 (1991).
32 G. Traiger and G. Plaa, *Toxicol. Appl. Pharmacol*., **20**, 105 (1971).
33 H. Cornish and J. Adefuin, *Am. Ind. Hyg. Assoc. J*., **27**, 57 (1966).
34 T. Castillo, D. Koop, S. Kamimura, G. Triadafilopoulos, and H. Tsukamoto, *Hepatology*, **16**, 992 (1992).
35 D. Folland, W. Schaffner, H. Ginn, O. Crofford, and D. McMurray, *JAMA*, **236**, 1853 (1976).
36 R. Harris and M. Anders, *Toxicol. Appl. Pharmacol*., **56**, 191 (1980).
37 G. Plaa, *Fundam. Appl. Toxicol.*, **10**, 563 (1988).
38 Y. Kim, *Fundam. Appl. Toxicol*., **35**, 138 (1997).
39 D. Pessayre, B. Cobert, V. Descatoire, C. Degott, G. Babany, C. Funck-Brentano, M. Delaforge and D. Larrey, *Gastroenterology*, **83**, 761 (1982).
40 Y. Masuda and N. Nakayama, *Biochem. Pharmacol*., **31**, 2713 (1982).
41 E. Glende and R. Recknagel, *Res. Commun. Chem. Pathol. Pharmacol*., **73**, 41 (1991).
42 G. Christie and J. Judah, *Proc. Roy. Soc. Lond. B*., **142**, 241 (1954).
43 American Conference of Governmental Industrial Hygienists (ACGIH), Threshold Limit Values(TLVs) for chemical substances and physical agents and Biological Exposure Indices (BEIs), American Conference of Governmental Industrial Hygienists, Cincinnati, 1996.
44 R. Barnes and R. Jones, *Am. Ind. Hyg. Assoc. J.*, **28**, 557 (1967).
45 T. Driscoll, H. Hamdan, G. Wang, P. Wright, and N. Stacey, *Br. J. Ind. Med.*, **49**, 700 (1992).
46 J. Tomenson, C. Baron, J. O'Sullivan, J. Edwards, M. Stonard, R. Walker, D. Fearnley, *Occup. Env. Med*., **52**, 508 (1995).
47 G. Cameron and W. Karunatne, *J. Pathol. Bacteriol*., **42**, 1 (1936).
48 C. Poindexter and C. Greene, *JAMA*, **102**, 2015 (1934).
49 N. Gitlin, *S.A. Med. J*., **58**, 872 (1980).
50 T. Paerez, *Hepatology*, **3**, 112 (1983).
51 S. Chou, W. Spoo, **Toxicological Profile for Chloroform**. Agency for Toxic Substances and Disease Registry (ATSDR), *US Department of Health and Human Services*, Atlanta, 1997.
52 S. Kutob and G. Plaa, *J. Pharmacol. Exposure Therap*., **135**, 245 (1962).
53 K.Hutchens and M. Kung, *Am. J. Med*., **78**, 715 (1985).
54 S. Ray and H. Mehendale, *Fundam. Appl. Toxicol*., **15**, 429 (1990).
55 J. Brady, D. Li, H. Ishizaki, M. Lee, S. Ning, F. Xiao, and C. Yang, *Toxicol. Appl. Pharmacol*., **100**, 342 (1989).
56 D. McMartin, J. O'Connor Jr., and L. Kaminsky, *Res. Commun. Chem. Pathol. Pharmacol.*, **31**, 99 (1981).
57 S. Winslow and H. Gerstner, *Drug Chem. Toxicol*., **1**, 259 (1978).
58 L. Li, X. Jiang, Y. Liang, Z. Chen, Y. Zhou, and Y. Wang, *Biomed. Environ. Sci.*, **6**, 179 (1993).
59 Occupational Safety and Health Administration(OSHA), Occupational Safety and Health Guidelines for Chloroform, available at http://www.osha.slc.gov/SLTC/ healthguidelines/chloroform/recognition.html, 2000.
60 R. Snyder and L. Andrews in **Casarrett and Doull's Toxicology; The Basic Science of Poisons**, 5th ed., C. Klaasen, Ed., *McGraw-Hill*, New York, 1996, pp. 737-771.
61 K. Mizutani, K. Shinomiya, and T. Shinomiya, *Forensic Sci. Int.*, **38**, 113 (1988).
62 Occupational Safety and Health Administration, OSHA Federal Register: Occupational Exposure to dichloromethane-62: 1494-1619, available at http://www.osha-slc.gov/FedReg_osha_data/FED19970110.html, 2000.
63 M. Balmer, F. Smith, L. Leach, and C. Yuile, *Am. Ind. Hyg. Assoc. J.*, **37**, 345 (1976).
64 M. Ott, L. Skory, B. Holder, J. Bronson, and P. Williams, *Scand. J. Work Environ. Health*, **9** S(1), 1 (1983).

65 H. Anundi, M. Lind, L. Friis, N. Itkes, S. Langworth, and C. Edling, *Int. Arch. Occup. Env. Health*, **65**, 247 (1993).
66 K. Soden, *J. Occup. Med.,* **35**, 282 (1993).
67 Syracuse Research Corporation, Toxicological Profile for 1,1,2-Trichloroethane, Agency for Toxic Substances and Disease Registry (ATSDR), US Department of Health and Human Services, Atlanta, 1989.
68 J. MacDonald, A. Gandolfi, I. Sipes, and J. MacDonald, *Toxicol. Lett*., **13**, 57 (1982).
69 G. Traiger and G. Plaa, *Arch. Environ. Health*, **28**, 276 (1974).
70 C. Klaasen and G. Plaa, *Toxicol. Appl. Pharmacol*., **9**, 139 (1967).
71 M. Williams and F. Llados, Toxicological Profile for 1,1,1-Trichloroethane. Agency for Toxic Substances and Disease Registry (ATSDR), US Department of Health and Human Services, Atlanta, 1995.
72 M. Hodgson, A. Heyl, and D. Van Thiel, *Arch. Intern. Med.*, **149**, 1793 (1989).
73 C. Cohen and A. Frank, *Am. J. Ind. Med*., **26**, 237 (1994).
74 C. Kramer, H. Imbus, M. Ott, J. Fulkerson, and N Hicks, *Arch. Environ. Health*, **38**, 331 (1978).
75 R. Stewart, H. Gay, A. Schaffer, D. Erley, and V. Rowe, *Arch. Environ. Health*, **19**, 467 (1969).
76 L. Smith and J. Mathews, Toxicological Profile for 1,1,1,2-Tetrachloroethane, Agency for Toxic Substances and Disease Registry (ATSDR), US Department of Health and Human Services, Atlanta, 1994.
77 H. Coyer, *Ind. Med.*, **13**, 230 (1944).
78 R. Gurney, *Gastroenterology*, **1**, 1112 (1943).
79 T. Nakajima and A. Sato, *Toxicol. Appl. Pharmacol*., **50**, 549 (1979).
80 A. Sato, T. Nakajima, and Y. Koyama., *Br. J. Ind. Med.*, **37**, 382 (1980).
81 C. Brodkin, W. Daniell, H. Checkoway, D. Echeverria, J. Johnson, K. Wang, R. Sohaey, D. Green, C. Redlich, D. Gretch, and L. Rosenstock, *Occup. Env. Med*., **52**, 679 (1995).
82 P. Gennari, M. Naldi, R. Motta, M. Nucci, C. Giacomini, F. Violante, and G. Raffi, *Am. J. Ind. Med.*, **21**, 661 (1992).
83 G. Saland, *N.Y.S. J. Med*., **67**, 2359 (1966).
84 L. Meckler, and D. Phelps, *JAMA*, **197**, 662 (1966).
85 R. Lauwerys, J. Herbrand, J. Buchet, A. Bernard, and J. Gaussin, *Int. Arch. Occup. Environ. Health*, **52**, 69 (1983).
86 G. Smith, *Br. J. Ind. Med*., **23**, 249 (1966).
87 R. Defalque, *Clin. Pharmacol. Ther*., **2**, 665 (1961).
88 R. McCunney, *Br. J. Ind. Med.*, **45**, 122 (1988).
89 G. Bond, *J. Toxicol. Clin. Toxicol*., **34**, 461 (1996).
90 T. Nagaya, N. Ishikawa, H. Hata, and T. Otobe, *Int. Arch. Occup. Environ. Health*, **64**, 561 (1993).
91 M. Neghab, S. Qu, C. Bai, J. Caples, and N. Stacey, *Int. Arch. Occup. Environ. Health*, **70**, 187 (1997).
92 G. Müller, M. Spassowski, and D. Henschler, *Arch. Toxicol.*, **33**, 173 (1975).
93 J. Creech Jr., and M. Johnson, *J. Occup. Med.*, **16**, 150 (1974).
94 F. Lee, P. Smith, B. Bennett, and B. Williams, *Gut*, **39**, 312 (1996).
95 S. Ho, W. Phoon, S. Gan, and Y. Chan, *J. Soc. Occup. Med*., **41**, 10 (1991).
96 P. Smith, I. Crossley and D. Williams, *Lancet*, **2** (7986), 602 (1976).
97 P. Bioulac-Sage, B. LeBail, P. Bernard, and C. Balabaud, *Semin. Liver Dis.*, **15**, 329 (1995).
98 W. Lelbach, *Am. J. Ind. Med*., **29**, 446 (1996).
99 A. Van Haaften, *Am. Ind. Hyg. Assoc. J*., **30**, 251 (1969).
100 P. Hoet, M. Graf, M. Bourdi, L. Pohl, P. Duray, W. Chen, R. Peter, S. Nelson, N. Verlinden, and D. Lison, *Lancet*., **350**, 556 (1997).
101 D. Anders and W. Dekant, *Lancet*, **350**, 1249 (1997).
102 G. Rusch, *Lancet*, **350**, 1248 (1997).
103 P. Pfaffli and A. Saamanen in **Butadiene and Styrene: Assessment of Health Hazards**, *IARC Scientific Publications* No 127, M. Sorsa, K. Peltonen, H. Vainio, and K. Hemmiki Eds. International Agency for Research on Cancer, Lyon, 1993, pp. 15 - 33.
104 J. Bond, *Crit. Rev. Toxicol*., **19**, 227 (1989).
105 J. Marniemi in **Microsomes and Drug Oxidations**, V. Ullrich ed., *Pergamon*, New York, 1977, pp. 698-702.
106 J. Van Anda, B. Smith, J. Fouts, and J. Bend, *J. Pharmacol. Exp. Ther*., **211**, 207 (1979).
107 O. Axelson O and J. Gustavson, *Scand. J. Work Environ. Health*, **4**, 215 (1979).
108 G. Triebig, S. Lehrl, D. Weltle, K. Schaller, and H. Valentin, *Br. J. Ind. Med*., **46**, 799 (1989).
109 A. Thiess, and M. Friedheim, *Scand. J. Work Environ. Health*, **4** (S2), 220 (1978).
110 R. Vihko in **Biological Monitoring And Surveillance Of Workers Exposed To Chemicals**, A. Aitio, V. Riihimäki, and H. Vainio Eds., *Hemisphere Publishing Corp*, Washington, D.C., 1984, pp. 309-313.

111 W. Lorimer, R. Lilis, W. Nicholson,, H. Anderson, A. Fischbein, S. Daum, W. Rom, C. Rice, and I. Selikoff, *Environ. Health Perspect*., **17**, 171 (1976).
112 H. Harkonen, A. Lehtniemi, and A. Aitio, *Scand. J. Work Environ. Health*, **10**, 59 (1984).
113 C. Brodkin, Personal Communication.
114 H. Zimmerman and J. Lewis, *Gastroenterol. Clin. North. Am*., **24**, 1027 (1995).
115 L. Low, J. Meeks, and C. Mackerer, *Toxicol. Ind. Health*, **4**, 49 (1988).
116 P. Guzelian, S. Mills, and H. Fallon, *J. Occup. Med*., **30**, 791 (1988).
117 R. Morris, *J. Occup. Med.*, **31**, 1014 (1989).
118 C. Boewer, G. Enderlein, U. Wollgast, S. Nawka, H. Palowski, and R. Bleiber, *Int. Arch. Occup. Environ. Health*, **60**, 181 (1988).
119 V. Scailteur and R. Lauwerys, *Toxicology*, **43**, 231 (1987).
120 C. Redlich, W. Beckett, J. Sparer, K. Barwick, C. Riely, H. Miller, S. Sigal, S. Shalat and M. Cullen, *Ann. Int. Med*., **108**, 680 (1988).
121 A. Fiorito, F. Larese, S. Molinari, and T. Zanin, *Am. J. Ind. Med*., **32**, 255 (1997).
122 S. Cai, M. Huang, L. Xi, Y. Li, J. Qu, T. Kawai, T. Yasugi, K. Mizunuma, T. Watanabe, and M. Ikeda, *Int. Arch. Occup. Environ. Health,* **63**, 461 (1992).
123 L. Fleming, S. Shalat, and C. Redlich, *Scand. J. Work Environ. Health*, **16**, 289 (1990).
124 C. Redlich, A. West, L. Fleming, L. True, M. Cullen, and C. Riely, *Gastroenterology*, **99**, 748 (1990).
125 G. Marino, H. Anastopoulos, and A. Woolf, *J. Occup. Med.*, **36**, 637 (1994).
126 G. Corsi, *Med Lav*, **62**, 28 (1971).
127 G. Spies, R. Rhyne Jr., R. Evans, K. Wetzel, D. Ragland, H. Turney, T. Leet, and J. Oglesby, *J. Occup. Med.*, **37**, 1102 (1995).
128 C. Hine, A. Pasi, and B. Stephens, *J. Occup. Med*., **20**, 333 (1978).
129 R. Harrison, G. Letz, G. Pasternak, and P. Blanc, *Ann. Int. Med.,* **107**, 466 (1987).
130 D. Hryhorczuk, S. Aks, and J. Turk, *Occup Med: State of Art Reviews*, **7**, 567 (1992).
131 T. Lewis, C. Ulrich, and W. Busey, *J. Environ. Pathol. Toxicol*., **2**, 233 (1979).
132 G. Crawford, R. Garrison, and D. McFee, *Am. Ind. Hyg. Assoc. J*., **46**, 45 (1985).
133 R. Garnier, N. Rosenberg, J. Puissant, J. Chauvet, and M. Efthymiou, *Br. J. Ind. Med*., **46**, 677 (1989).
134 D. Moody, *Drug Chem. Toxicol*., **14**, 319 (1991).
135 C. DeRosa, S. Wilbur, J. Holler, P. Richter, and Y. Stevens, *Toxicol. Ind. Health*., **12**, 1 (1996).
136 F. Tomei, P. Giuntoli, M. Biagi, T. Baccolo, E. Tomao, and M. Rosati, *Am. J. Ind. Med*., **36**, 54 (1999).
137 E. Sotaniemi, S. Sutinen, S. Sutinen, A. Arranto, and R. Pelkonen, *Acta Med. Scand*., **212**, 207 (1982).

20.9 TOXICITY OF ENVIRONMENTAL SOLVENT EXPOSURE FOR BRAIN, LUNG AND HEART

Kaye H. Kilburn
School of Medicine, University of Southern California
Los Angeles, CA, USA

This chapter considers the neurobehavioral effects of environmental exposures to organic solvents. Much information applicable to environmental or community exposures usually at home came from animal experiments, brief human exposures in chambers and prolonged workplace exposures. The mode of entry of solvent chemicals into the body is almost always by inhalation not by contact or ingestion.[1] While inhalational exposures to single chemicals occur in the community mixtures are usual making measurements more complex. Effects from animal experiments, and human exposures in chambers, and workplace exposures are usually consistent and help predict environmental effects. The major categories of environmental exposures to solvents are from petroleum refining to consumer use indoors, Table 20.9.1. Sometimes adverse human effects are from surprisingly small environmental doses, an order of magnitude or two lower than those needed for workplace

effects. One possible explanation is greater sensitivity of measurements but many of the methods were adapted from studies of workers.[2]

Table 20.9.1. Sources of environmental exposure to solvents

Processes	Chemicals	Media	Example
Losses during refining and chemical production	MTBE	air	Seymour, IN, Santa Maria, CA
Losses from use in industry	TCE + toluene	water surface, ground water, air, water	Phoenix, AZ, Motorola, Printers, Baton Rouge, LA, Abuse-glue sniffers
Leaks and spills during transportation (pipeline, truck, rail, ship)	toluene, xylene, PAH	air	Avila Beach, CA, Livington Parish, LA
Combustion: a. Fires as incidents, b. Incineration of fuel air pollution c. Incineration of garbage	hydrocarbon particles	air	Wilmington, CA, San Bernardino, CA, Los Angeles, Houston, TX, Mexico City Oak Ridge, TN, Walker, LA
Contaminated sites	TCE	water, ground water	800 national hazardous acid pits
Outgassing indoors of forest products-like particle board, carpets, drapes, adhesives	TCA	air	Indoor air incidents, Sick building syndrome

+ - other chlorinated solvents; TCA - trichloroethane; MTBE - methyl ter butyl ether, additive in gasoline; PAH - polyaromatic hydrocarbons, example benzo(a)pyrene

Table 20.9.2. Differences in environmental and occupational toxicology

	Occupational	Environmental
Subjects health age, years selection positive	 healthy 18-60 selected for employment attenuated by losses of sick	 chronic illness 0-100 unselected sick collect
Duration in a week total	 40 hours years	 168 hours lifetime
Source	raw materials processes	leaks and spills (gasoline) outgassing of consumer goods fuel combustion
Chemical exposure	one or few	many
Monitoring exposure	area or personal	rarely possible
Environmental transformation of agents	unusual	frequent

Differences between exposure at work and in the community are important, Table 20.9.2. Most worker groups were younger and healthier, met job-entry criteria, have more reserve function so are less likely to manifest damage. Workers have had selective attrition of affected or less fit people to accentuate the difference.[3] In contrast community people are unselected and include more susceptible groups: infants, children, the aged and the unwell.[1] Some differences in exposure are obvious, work exposure is rarely longer than 40 of the weeks 168 hours. This time away allows work acquired body burdens of chemicals (and their effects) to diminish or disappear while workers are at home. In contrast, home exposures may be continuous or nearly so.[4] At work the time that elapsed between exposure and effect is short, making measurement of the dose of a toxic agent easy. It is less obvious what should be measured in community exposures.

Good detective work is needed to specify the chemicals to search for and measure in air, water or soil. Community effects may take years to be recognized as a problem. Opportunities for pertinent environmental measurements were overlooked and have disappeared with time, often simply evaporated. Thus measurement of relevant doses are seldom possible and dose-response curves can rarely be constructed. The logical surrogates for dose such as distance and direction from a chemical source and duration are rarely satisfactory.[1] Thus plausible estimates of dose are needed to focus the association of measured effects and the chemicals that are probably responsible.

The realistic starting place is people's symptoms-complaints that indicate perception of irritation from chemicals.[3] These serve as sentinels to alert one to a problem but cannot be interpreted as impairment or damage without measurements of brain functions. The inability to characterize exposure should not postpone or prevent adequate investigation for adverse human health effects. It is intuitive and ethical to suggest that absent of adverse human effects should be the only reason for stopping inquiry. People's complaints and upset moods (anxiety, depression, anger, confusion and fatigue) frequently reflect or parallel impairment. The question then becomes how to measure effects on the brain to decide whether it is damaged and if so how much, Table 20.9.3.

Table 20.9.3. Useful tests of evaluation of brain damage from solvents

Tests	Part of brain measured
Simple reaction time & visual two choice reaction time	retina, optic nerve and cortex integrative radiation to motor cortex
Sway-balance	inputs: ascending proprioceptive tracts, vestibular division 8th cranial nerve, cerebellum, vision, visual integrative and motor tracts
Blink reflex latency	sensory upper division trigeminal nerves (V), pons, facial nerves (VII)
Color confusion index	center macular area of retina, with optic cones, optic nerve, optic occipital cortex
Visual fields	retina-optic nerve-optic cortex occipital lobe
Hearing	auditory division of 8th cranial nerve
Verbal recall memory	limbic system of temporal lobe, smell brain
Problem solving culture fair & digit symbol	cerebral cortex: optic-occipital and parietal lobe cortex

Tests	Part of brain measured
Vocabulary	long-term memory, frontal lobes
Information, picture completion & similarities	long-term memory, frontal lobes
Pegboard performance	optic cortex to motor cortex
Trail making A & B	(eye-hand coordination)
Fingertip number writing	parietal lobe, sensory area of pre-Rolandic fissure
Profile of mood states	limbic system for emotional memory

The effects of ethyl alcohol are familiar to most people so I will start with this best studied of mind altering solvents. Measurements of alcoholic patients in the mid-twentieth century at New York's Bellevue Hospital helped David Wechsler formulate his adult intelligence scale, 11 tests that measure attention, problem solving, concept juggling and memory including vocabulary.[5] Many other tests were devised to estimate intelligence, how the mind works as defined by AR Luria and others.[6] Ward Halstead assembled and created function tests to measure the effects of traumatic damage to the brain by wartime missiles and by neurosurgery, prefrontal lobotomy.[7] Application of these tests, by Reitan,[8] helped differentiate the organic brain disorders from schizophrenia and other mental illnesses. Thus the starting place for testing became brain diseases recognized by the neurologist using simple bedside qualitative tests. The tests were not used to detect impairment before it was clinically recognized. The first steps were Benjamin Franklin's recollections of his own brain poisoning by lead while he was a printer and Lewis Carroll's mad hatter, from mercury used in felting beaver hair. The next steps were taken in Nordic countries in the 1960's.[9]

Carbon disulfide was the first solvent studied and had adverse effects observed by Delpech in 1863. Neuropsychiatric abnormalities were described 13 years later by Eulenberg in workers in the rubber and viscose rayon industries.[2] A Finnish psychologist, Helen Hanninen tested 100 carbon disulfide exposed workers in 1970, 50 were poisoned, 50 exposed and compared them to 50 unexposed.[10] She found intelligence, tasks of attention, motor skill vigilance and memory were impaired in clinically poisoned and exposed men compared to unexposed. Digit symbol substitution from the Wechsler's scale[5] showed the most effect of exposure. Additional studies of spray painters in the 1970's and compared to computer augmented tomography (CT) scans of the brain and function tests. Symptomatic painters after 20 years or more of exposure had brain atrophy associated with impairment.[11-14]

The key to progress in this field was sensitive tests to measure brain function, Table 20.9.3. Fortunately, the Finnish, Danish and Swedish occupational-environmental health centers units included cooperative neurologists, neurophysiologists and psychologists who did not defend disciplines to limit activities. The obvious reality that the nervous system regulates and controls many essential functions helped select measurements to assess vision, hearing, vibration, odor perception, balance, reaction time including automatic responses that are measured as blink reflex latency,[15,16] heart rate variation[17] and peripheral nerve conduction and Hoffmann's (H) reflex.[18] Tests must be sensitive and reliable, easily understood and economical of time, taking 3 to 4 hours with rest periods.

Sensitivity's main dimensions are time and mapping. For example, balance is measured using the classic Romberg stance (1850) standing feet together with eyes ahead open and then closed and using a force (displacement) platform or even simpler the position of the head from a sound emitter secured to a headband and recorded by two microphones to inscribe the distance swayed and the speed, in centimeters per second.[19] From three performances for 20 seconds with the eyes closed alternating with the eyes open the minimal speed of sway is selected. The inscribed path, the map, may provide more information but how to interpret this is unclear.

Eye-hand choice reaction time is tested as the speed to cancel by tapping a keypad, a 4 inch letter that appears on the screen of a laptop computer.[20] Twenty trials repeated twice and the median time of last 7 trials in each run is recorded. Simple, same letter, reaction time takes 1/4 of a second, 250 ms while choice between 2 letters takes twice as long, 500 ms. Many tests are faster in women, most deteriorate with aging after 25 years and for people with more years of educational attainment scores are higher.[21]

Vision is measured by mapping for color perception which is a central retinal cone function. This consists of placing 15 pale colors in a spectral array, the Lanthony desaturated hue test. Retinal rod function which is light perception was mapped for the central 30º of each visual field at 80 points using an automated perimeter recording to a laptop computer.[22] This standardized and speeded up the fields that had been done by the tangent screen and a skilled operator for 100 years.

It was logical to consider the 12 nerves of the head, cranial nerves as the scaffold for organizing tests and for reviewing brain functions that are adversely affected by chemicals. Smell (Nerve I, olfactory), is tested by recognition of familiar odors and of threshold concentration for detecting them. Smell disorders include loss and disturbed perception. Nerve II, the optic was described above. Nerves III, IV and VI move the eyes and rarely show effects of chemicals. An exception is the optokinetic effects of styrene. In contrast the faces sensory nerve, the trigeminal, number V and motor nerve, the facial, number VII are needed to blink and are tested by blink reflex latency which is measured electromyographically in milliseconds (10 to 15 ms) after stimulation by a tap, that is mechanical or an electrical impulse. Blink is slowed by exposures to chlorinated solvents like trichloroethylene (TCE), by chlorine and by arsenic. Nerve VIII has hearing and vestibular (balance) divisions which are tested by audiometry and by sway speed for balance. Nerve IX, the glossopharyngeal innervates the throat and is needed for the gag reflex and baroreceptor. Nerve X, the vagus X is evaluated by recording variations of heart rate with breathing. Nerves XI, spinal accessory is tested as strength of neck muscles and XII tongue's hypoglossal nerve by speech.

Using these tests implies comparing scores observed to a standard, an expected value. Ideally that would be to the same subject which is rarely possible, although it works for before and after exposures of workers. The next best comparisons are to suitable unexposed normal subjects who can be called controls.[21] We developed over several years a national sample of unexposed people, tested their performance and calculated expected values using prediction equations with coefficients for age, sex, education and other factors such as height and weight that affected some tests. Thus individual observed values for each subject are compared to predicted values (observed/predicted x100) equal percent predicted. Frequently, we needed to be sure that comparison groups of apparently unexposed control people were normal because adverse effects are widespread. Next from the standard deviations

of the mean, each tests confidence intervals were developed that included 95% of values, excluded as abnormal approximately 5% of unexposed subjects on each test that defined abnormal precisely. For these tests they were values outside the mean plus 1.5 x standard deviations (sd) that defined normal. The next concern what was the best summary for each subject. The number of abnormalities was best adjusting balance and vision above other tests and given grip strength, blink reflex and color discrimination 0.5 for right and left sides of the body.

The attributes of plausible association leading to attribution of effect include temporal order, strength of association, exposure intensity and duration, specificity, consistency of findings and coherence and plausibility.[1] As noted earlier the fact of exposure or suspected exposure may be the only certainty about exposure so its plausibility is important based on chemical properties, experiments and studies of workers. Consistency with results of occupational exposures and animal experiments is helpful. Koch's 4 postulates developed to judge causation of infectious agents (1, organism present in every case; 2, grown in pure culture; 3, produces the disease when inoculated and 4, recovery and growth in pure culture) are usually inapplicable. This reality is discomforting to some interpreters of the new observations.

The next section reviews the neurobehavioral affects of solvents found in the environment in the order of importance.[2,4] We begin with trichloroethylene (TCE) and related short chain chlorinated agents.[22] Next are ring compounds toluene including related xylene and styrene with comments on creosols or phenols. The chlorinated ring compounds follow: dichlorophenol and polychlorinated biphenyls and their highly neurotoxic derivatives, the dibenzofurans. Other straight chain solvents leading off with n-hexane move through white solvent (paint thinner) and solvent mixtures.

Before studies of effects of TCE on many brain functions came the measurement of blink reflex latency in 22 people exposed at home to solvents rich in TCE at Woburn, MA. They showed significant delay of blink but no other functions were measured.[16] In France about this time workers exposed to TCE had similar delays of blink.[24] Earlier experimental exposure of 12 subjects to TCE at 1,000 parts per million (ppm) for 2 hours in a chamber had produced rapid flickering eye movements when following figures on a rotating drum (optokinetic nystagmus), a lowered fusion limit.[22] Thus TCE induced dysfunction of several cranial nerves VI (with III, IV) and V and VII. Nystagmus normalized after a washout and recovery time. Blink is the easier and quicker measurement.[16]

A community within Tucson, AZ of over 10,000 people who depended on well-water for drinking and bathing had developed many complaints and had excesses of birth defects and cancers that associated with TCE in their water. The source was metal cleaning that included stripping off protective plastic coatings, from demothballing aircraft stored on the desert with TCE. This had dumped vast quantities of TCE on the porous, desert floor that drained into the shallow Santa Cruz River aquifer. Testing of 544 people from this water exposure zone showed increased blink reflex latency, impaired balance, slowed simple and choice reaction times, reduced recall, poor color discrimination, and impaired problem solving in making designs with blocks, digit symbol substitution and Culture Fair (consisting of 4 subtests: selection of designs for serial order, for difference, for pattern completion and refining defined relationships).[25,26] Also peg placement in a slotted board and trail making A (connecting 25 numbers in ascending sequence and B connecting numbers alternating with letters).[27] TCE concentrations at the well heads and distribution pipes to homes were

measured to calculate with duration of exposure, lifetime peak levels, lifetime averages, and cumulative exposure. Possible relationships of neurobehavioral test scores to these surrogates for dose were searched by regression analysis. No relationships were found for these dose surrogates which was disappointing.

TCE dominated the mixture of chlorinated aliphatic solvents in air and water of northeast Phoenix around two Motorola microchip manufacturing plants that began production in 1957. Neighbor's complaints of adverse health effects started amelioration effects in 1983. An underwater TCE solvent plume spread west and south of the plants in the Salt River aquifer. Test wells showed concentrations of TCE from 50 ppm to 1.4%. Also air dispersal was important for direct exposure as TCE escaped into the air from the plant. It drained into dry wells, sewers and into a canal running northwest through the neighborhood. In 1993, 236 exposed adults were compared to 161 unexposed ones from a town 80 km northwest across the mountains at a higher elevation. The exposed group showed delayed blink reflex, faster sway speed, slowed reaction time, impaired color discrimination and reduced cognitive function and perceptual motor speed and reduced recall. Airway obstruction was shown by pulmonary function testing. Adverse mood state scores and frequencies of 32 symptoms were also increased.[4,28]

Remedial efforts directed at dumping and ground water had not reduced the effect suggesting either these were ineffective or impairment was permanent and had developed after 1983. Additional groups of subjects on plume but not in the lawsuit were not different from clients so there was no client bias. Phoenix residents off the plume had only abnormal slowing of blink interpreted as due to TCE and abnormal airway obstruction compared to the unexposed population of Wickenburg, AZ. Airway obstruction was attributed to Phoenix wide air pollution. Proximity within 1.6 km seemed to increase impairment.

In 1998 retesting of 26 people from original groups showed improvement with faster blink reflexes but worse airway obstruction that had persisted (ref. 4 and unpublished). The improvement to normal in blink paralleled that seen in chlorine exposed people 3 years after exposure and first evaluations that were abnormal.[4] The perceptual motor tests, trail making A and B and peg placement were improved, as were cognitive function measured as Culture Fair and verbal recall. We deduced that diminished TCE releases from Motorola after 1993 allowed recovery of cranial nerves V and VII so blink latency decreased, accompanied by some improvement in vigilance and tracking for the better scores. A possible reversal of effect is so important that these observations should be verified in other groups.

Workers welding and grinding on jet engines in a repair shop were an unusual way to focus attention on the Gerber-Wellington aquifer in Oklahoma.[28] Our attention was temporarily on metals in alloys but when testing of 154 workers showed impaired balance, slowed choice reaction times and impaired color discrimination compared to 112 unexposed subjects, the priority became effects on the brain. These worker's cognitive function, perceptual motor and recall were all abnormal using the same tests as in Phoenix. We probed for their exposures after observing these effects and found that these workers had used TCE, trichloroethane, methanol and Freon FC-113 in metal cleaning. The 112 control subjects had not worked with solvents or TCE, but many people, both workers and control groups, lived on and drew well water from the Gerber-Wellington aquifer that is contaminated with TCE. Mapping the blink reflex latencies in the control group showed the people with normal blink lived outside the aquifer. The aquifer was TCE contaminated (national priority

list 1990) Thus here we had an example of a probable environmental exposure to TCE with a superimposed occupational one.[4]

TCE leakage had produced these same effects in Joplin, MO neighbors of a company manufacturing ball bearings and cleaning with this solvent. The companies decision to clean and reuse TCE rather than dump it ameliorated the effects.[4] In San Gabriel and San Fernando Valleys in California similar observations confirm adverse effects from TCE contamination of groundwater and air. Clearly the observations were replicated and each time TCE was associated with reasonable timing and proximity. More than half of the federal superfund sites in the US are contaminated with TCE suggesting 800 potential replications.[29] Experience with several patients have shown me that the effects of dichloroethylene and of 1,1,1-trichloroethane are indistinguishable from those of TCE.

Toluene is the most toxic and best studied of the aromatic ring compound solvents. Both acute and chronic effects were observed by 1961 from inhaling "huffing" toluene[30,31] or lacquer thinner,[32] especially in children sniffing airplane glue.[30] Chronic impairment was shown shoemakers[33] and rotogravure printing workers[34] using neurobehavioral testing. Toluene exposed experimental animals, mainly rats and mice showed enhanced motor activity, abnormal movements, altered sleep patterns and electroencephalograph (EEG) changes from an integrative brain loop, the hippocampus.[2] Occupational exposures produced memory disturbances, poorer performance on block design assembly, embedded figures, visual memory and eye-hand coordination. CT scans showed some generalized brain swelling[34] that correlated with impaired psychological functions.[35] Women working in electronic assembly had environmental air levels of toluene of 88 ppm compared to 13 ppm for controls and comparable differences in blood levels. These workers were less apt at placing pegs in a grooved board, at trail making, digit symbol, visual retention and reproduction and verbal memory.[36] They were tested during the day after being away from exposure for at least 16 hours.

Protracted sniffing of solvents alone or in glue has produced intention tremor and titubating gait[30] consistent with cerebellar degeneration which continued after 5 years with ataxia, EEG slowing and cerebral atrophy.[31] Polyneuropathy was observed in 2 glue sniffers in Japan whose exposures were to n-hexane and toluene.[37] Many such descriptions[2] outweigh one epidemiological study that found no differences in performance when comparing 12 glue sniffing boys, ages 11 to 15, mean 13.8 years and 21 controls, ages 11 to 15, mean 12.6 years. Four non-standard tests and the Benton visual retention and design reproduction test were used but the exposed group was 1.2 years older and should have outperformed younger controls whose skills were less developed.[38]

Some published data are difficult to interpret. For example, 26 men were exposed in tanks and holds of two merchant vessels being painted (solvents) and sprayed with malathion 20% and pyrethrin 1.5%, with piperonyl butoxide in toluene. They showed losses of concentration, unawareness of danger and unconsciousness at toluene levels estimated as 10,000 to 12,000 ppm and up to 30,000 ppm below waist level.[39] Additive effects of the neurotoxic insecticides were not discussed.

Effects of toluene in 52 men and paint solvents in 44 men were contrasted with unexposed men. Painters had impaired reading scores, trails B, visual search, block design, grooved pegboard, simple reaction time and verbal memory.[40] Toluene exposed men had only abnormal reading scores reduced significantly, although scores on all tests were lower.

Levels of toluene were less than 200 ppm for 4 years prior to this study, although above 500 ppm earlier.

The axiom that environmental exposures are "never" to pure chemicals is matched by another that the mixtures are frequently so complex as to defy description. The few observations suggest that effects seen from mixtures may be due to one or two specific neurotoxic agents. Judgment must be exercised to curb bias and accept the most plausible attribution as the above studies illustrate.

Studies of a population exposed in Louisiana to toluene rich solvents and other chemicals distilled from a site for 17 years were contrasted to unexposed people living 55 km to the east.[41] The Combustion site accepted 9 million gallons of used motor oil in 1975-1976 and 3 to 4 million gallons from 177 to 1983. Tons of liquid chemical waste from over 100 chemical factories was consigned to this site including toluene, xylene, styrene and benzene, many chlorinated aliphatics solvents like TCE and chlorinated aromatics including PCBs and dibenzofurans. Lead, cadmium, mercury and other metals were present in samples of sludge in ponds after the site closed in 1983 but they were rich in toluene, benzene and other aromatics. Modeling based on toluene and benzene and using standard Environmental Protection Agency assumptions and a windrose showed symmetrical spread eastward. Excesses of leukemia in school children, cancers and neurobehavioral symptoms in the about 5,000 neighbors of the site led to neurobehavioral testing for impairment in 131 adult subjects within 2 km of the site and 66 adult controls from voter registration rolls of a town 50 km east. The exposed group matched controls for age but were 1.4 years less educated.

There were adverse effects from exposures while living within 2 km from the site for 4 to 17 years.[41] that were shown by slowed simple and choice reaction times and abnormal sway speeds. Cognitive function in Culture Fair and block design was decreased and pegboard and trail making A and B were diminished, as was recall of stories. Profile of mood states (POMS) scores were 2.5 fold increased with low vigor and high depression, tension, confusion, anger and fatigue. Thirty of 32 symptoms inquiring about chest complaints, irritation, nausea and appetite associated, balance, mood, sleep, memory and limbic brain were significantly more frequent in exposed people and the other two were rare in both groups. When differences were adjusted for age, color discrimination and similarities became abnormal were added and trail making A became normal.

The second study was designed to answer how large an area-population was affected, was direction important and were abnormalities related to the duration of exposure.[42] I examined 408 subjects selected to fill 3 distances outward to 1.6, 3.2 and 4.8 km in 8 compass octants, thus 24 sectors. The same tests were given by the same staff and results replicated the earlier study. Regression analysis of each test against distance showed no significant coefficient and comparison of inner and other sectors found no differences, thus there was no evidence of a diminished effect from distance. There were no effects of direction. A possible lessening of effect for durations of exposure of less the 3 years was seen only for peg placement and trail making B scores. Distance, direction and duration as surrogates for exposure did not influence impairment as measured.

We concluded that the periphery of effect was beyond 4.8 km meaning a health impact area larger than 75 km^2. There was no gradient of effect from the distilling plant outward suggesting airborne spread and mixing had produced even dosing from a large "cloud".[4] Peoples migration inside the exposure zone did not influence effects. Bias of examiners

was unlikely. Was the control group suitable? Their average measurements and the distribution were like three other groups in different parts of the country. The possibility of confounding exposures was considered from two sites that were beyond 4.8 km and to the south. Unfortunately, the resources were unavailable to extend testing beyond 4.8 km to find the rim of Combustion's effect on people and detect effects of other nearby sites.

Xylene is a solvent for paints, lacquers and adhesives and is a component of gasoline. In human volunteers in exposure chambers xylene at 70 ppm for 2 hours had no effect on reaction time or recall memory but levels of 100 to 400 ppm for 2 hours impaired body balance, memory span, critical flicker fusion and cause eye irritation.[43-45] Alcohol and 1,1,1-trichloroethane had adverse effects on balance that show synergism with xylene.[46] and increased the latencies for visual and auditory evoked potentials.[47] Occupational studies have focused on psychiatric symptoms in photogravure workers who also showed headache, nausea, vomiting and dizziness.[48] Only one study showed impairment for recall memory attributed to xylene but workers were also exposed to formaldehyde.[49]

Xylene toxicity has received less study than that of toluene, but appears considerable less which supports attributed the neurotoxicity to toluene of mixtures of xylene, benzene and toluene with straight chain hydrocarbons such as gasoline.

Styrene's major use is in reinforced fiberglass plastics in constructing boats and bathtubs and showers and in styrene-butadiene rubber.[2] Small amounts are used in polystyrene foam cups and packing materials. Styrene inhalation increased locomotion activity in rats and grip strength at the highest 700 to 1,400 ppm concentrations.[2] Studies of workers showed hearing loss (increased high frequency hearing thresholds at 16 kHz).[50] Color discrimination is also reduced.[51] Other observers found abnormal hearing and by posturography-larger sway areas and poor rotary visual suppression-inhibition or vestibulatory nystagmus.[52] In 25 studies of workers[2] some showed slowing of reaction time, poor performance on block design, short-term memory, EEG abnormalities and neuropathy.

These relatively mild effects made me predict less than the severe impairment than observed in 4 women from a factory making styrene-fiberglass shower-bathtubs. Two sprayed styrene and the other 2 who had developed skin and airway symptoms on initial exposure did lay-up and assembly. Five weeks after her first exposure one woman became lightheaded and dizzy, felt hot and her vision blacked out. On testing reaction times were slow, sway speed was increased. Problem solving was impaired as was verbal recall and POMS scores were elevated. She left work stopping exposure. Ten days later, on a trip to the mountains 4,000 feet above sea level she collapsed and became unconscious. Retesting showed constricted visual fields and worse performance of the above tests. Testing on the second woman who had developed asthma showed multiple blind spots in her visual fields, diminished problem solving ability, grip strength, excessive fingertip writing errors and failure to recall stories after 30 minutes. A third woman also had asthma and severe airway obstruction showed abnormal balance with eyes open and closed, diminished hearing, bilaterally constricted visual fields and decreased vibration sense.

The fourth woman had a skin rash and red welts that had kept her away from direct contact with epoxy and styrene. She had abnormal color discrimination, decreased vibration sensation, a blind spot in the retina of the left eye and decreased recall of stories. She was the least impaired although her POMS score and symptom frequencies were increased.

Air sampling was not permitted, concentrations of styrene are unknown and contributions of other chemicals to this exposure cannot be excluded. However, exposures to formaldehyde and phenol are unlikely as these workers did not "lay-up" fiberglass resin. Inhalation of sprayed styrene is the most attribution for the neurobehavioral impairments. The impairment exceeded that found in a review of boat building and other studies but tests were more sensitive and the styrene levels may have been higher. We encourage more neurobehavioral evaluations of styrene spraying workers using such sensitive tests.

Polychlorinated biphenyls (PCBs), the ultimate (poly)chlorinated solvents are 2 membered ring compounds that when heated to 270° produce dibenzofurans (DBFs) that are 1,000 or more times as neurotoxic.[53] Initial evaluations were of a few PCB exposed individuals and 14 firemen exposed to DBFs who showed severe impairment measured after a medical schools power plant transformers cooked and exploded. Most of the firemen could not pass the physical, balance and truck driving requirements to return to duty and were retired on disability.[4,54]

A community study explores effects of environmental exposures. PCBs were used as pump lubricants in natural gas pipelines running north from Texas and Louisiana from 1950 to the middle 1970's. One pumping station was at Lobelville, TN and at least 16 other US communities had them.[4] Ninety-eight adult village dwellers were compared to 58 unexposed subjects from 80 km east or 35 km north. The exposed people were the most abnormal group I have studied. They had abnormal simple and choice reaction times, balance, hearing, grip strength and the visual function of color discrimination, contract sensitivity and visual field performance. The cognitive functions of Culture Fair, digit symbol were abnormal as were vocabulary, information, picture completion and similarities. Story recall was diminished and peg placement and trail making A and B and fingertip number writing errors were decreased. Other possible associations were ruled out and there were no other causes of impairment. This exposure had caused the most severe neurobehavioral impairment for these people that I have observed.[4] It exceeded that from distilling chemical waste rich in toluene, from TCE and from other solvents.

n-hexane by inhalational or through the skin causes peripheral nerves to die-back. Glue sniffing exposure frequently combines n-hexane and toluene. Twenty-five percent of workers using glue in shoes and leather goods with n-hexane, 40 to 99.5% had symptomatic polyneuropathy, slowed nerve conduction and neurological signs.[55] Abnormal findings increased with age and durations of exposure and were accompanied by lower limb weakness and pain, abnormal sensations (parenthesis) in the hands and muscles spasm. In another shoe plant exposure group upper extremity nerve conduction was slowed, frequently after 5 years of exposure.[56] Sensormotor distal neuropathy characterized 98 of 654 workers in the Italian shoe industry, 47 had decreased motor conduction velocity with headache, insomnia, nausea and vomiting irritability and epigastric pain.[57] Most workers improved when removed from exposure.[56] In Japan beginning in 1964 several studies found polyneuropathy in polyethylene laminating printers[58] and makers of sandals and slippers.[59] A major metabolite of n-hexane and of methyl butyl ketone is 2,5-hexanedione that is more neurotoxic than these precursors causing swelling of nerve axons and accumulations of neurofilaments in mid-portions of peripheral nerves.[60] Methyl ethyl ketone studied in workers lengthened choice reaction time and motor nerve conduction and decreased vibration sensation signs of neuropathy. These effects were also seen in glue sniffers.[2]

The focus on polyneuropathy in 14 studies[2] has usurped studies of central nervous system (CNS) functions except for one showing increased latencies of visual and auditory evoked potentials.[61] Neurophysiological and psychological assessments[62] show narcotic effects that match those in animals.[2] Until restricted from foods n-hexane was used to extract oil from soybean meal and exposed US workers. They had headache, dysesthesia, insomnia, somnolence and memory loss but testing for appraise brain damage was not done.

Gasoline is a mixture of aliphatic straight and branched chains and aromatic hydrocarbons with toxicity attributable to toluene, xylene and perhaps hexane and additives including methyl ter butyl ether and tri-orthocresyl phosphate.[63]

Effects of the lungs of inhaled solvents simplify to the consideration of agents affecting airway cells that include n-hexane and PCBs. Both cause proliferation and transformation of distal airway lining cells to produce mucus and obstruct airways[64-66] and cause inflammatory cells to pour into the lungs distal alveolar spaces interfering with for gas exchange.

Cardiac effects of solvents are of three types A, alterations in rhythm B, cardiomyopathy and C, hypertension directly and via renal changes as in interstitial nephritis, glomerulonephritis and Goodpasture's syndrome.[67]

Alterations in heart rhythm have been attributed to anesthesia with TCE and were serious, especially when administered in soda lime CO_2 absorbing anesthesia machines to stop the use of TCE for anesthesia in the 1960's.[4] Rhythm disturbances have also been observed in some groups of workers exposed to TCE. Knowing this we did electrocardiograms (ECG's) on the Tucson TCE exposed population and found no arrhythmias. A loss of respiratory variation in heart rate has been associated with exposure to organic solvents, including carbon disulfide, acrylamide and alcohol but not toluene and with diabetes mellitus and syndromes of autonomic nervous system dysfunction.[17,68,69] Later freons, volatile chlorofluorocarbons were associated with arrhythmias and withdrawn from use to propel therapeutic aerosols used for asthma.[70] Cardiomyopathy, heart muscle dysfunction and enlargement have been associated with alcohol ingestion. Two epidemics were ascribed to cobalt used to color beer.[9] But in the most common cardiac muscle disorder from alcohol, cobalt is not incriminated.

Hypertension has been associated with solvent exposure in workers, an association that needs further study. Associations with hypertension were absent in the authors studies of TCE, toluene rich waste and PCBs discussed earlier.

Workers who used methylene chloride in making acetate film had sleepiness and fatigue and decreased digit symbol substitution scores and lengthened reaction time.[71] Use of methylene chloride in closed spaces has been fatal with brain edema, elevated blood levels of carboxyhemoglobin and caused temporary right hemispheric paralysis and/or unconsciousness.[2] Chronic exposure has been associated with dementia, headache, dizziness and disturbed gait.[72]

Chloromethane exposures from foam production caused tremor and decreased attention and ability to do arithmetic.[73] Environmental exposures from leaks in refrigerating systems[2] caused deaths, convulsions, myoclonus and personality changes. One fishing boat exposure of 15 men left profound neurological residuals, fatigue, depression and alcohol intolerance.[2] The effects resemble those of methyl bromide poisoning.

Methanol has profound and specific toxic effects on the optic nerve and vision causing central blind spots and ingestion of methanol for the intoxicating effects of ethanol has

caused blindness.[74] Its metabolism to formic acid suggests the possibility that of other central nervous system effects.

White spirit is a mixture of straight and branched chain paraffins, naphthalenes and alkyl aromatic hydrocarbons is used widely as a paint solvent. Ten studies of painters, mostly in Nordic countries, have shown increased neurobehavioral symptoms and several showed decreased performance on psychological tests.[11-14] Longitudinal studies showed an almost doubled risk for neuropsychiatric disability pension in painters compared to construction workers. Several such studies support the concept of neuropsychiatric impairment and disability linked to the painting trade in many countries.[75-78] Several women in my consulting practice had profound neurobehavioral impairment after entering their homes during spray painting including unconsciousness which suggest there may be a considerable problem from environmental exposures.

Many industrial painters exposures are to solvent mixtures. Those painting airplanes where dust and hence fume exposure is limited by strict cleanliness, which means good air hygiene for the workers, have little trouble compared to symptoms, impairment, disability and brain atrophy with dementia in car and refrigerator painters. These groups supplied the clear evidence of solvent effects in workers in Nordic countries that established how to assess human subject's neurobehavioral status and detect impairment that were discussed early in this chapter. Many cross sectional studies showed adverse effects, excessive neuropsychiatric symptoms and several longitudinal studies show greatly increased likelihood of receiving a pension for neuropsychiatric disability.[2,78-80]

Chemical companies fight the concept that chemicals damage human subjects. They are more combative and better defended than are bacteria and other infectious agents. In the past 25 years companies learned from asbestos litigation, the bankruptcy of Johns Manville Company and the banning of asbestos to contest observations and their scientific basis and frequently hire scientists to support their position of null effects-not harm and sponsor environmental meeting and advertise their concern and sense of responsibility. They avoid or shift responsibility for damage to the victim or community and the social security system. The necessary banning of PCBs and chlordane enforced their strategy of "controversy" even about incontrovertible facts. Perhaps, they count on having the 50 years that tobacco companies enjoyed before having to accept responsibility for adverse effects of tobacco smoking.

REFERENCES

1 K Kilburn, and R Warshaw, **Epidemiology of adverse health effect from environmental chemicals**, *Princeton Scientific Publishing Co.*, 1995, pp 33-53.
2 P Arlien-Soberg, **Solvent Neurotoxicity**, *CRC Press*, Boca Raton, 1992.
3 J Angerer, *Scan. J. Work. Environ. Hlth*., **11** (Suppl 1),45(1985).
4 K Kilburn, **Chemical Brain Injury**, *John Wiley & Sons*, New York, 1998.
5 D Wechsler, **Adult Intelligence Scale Manual**, (revised), *The Psychological Corporation*, New York, 1981.
6 AR Luria, **Higher Cortical Function in Man**, *Travistock*, London, 1966.
7 W Halstead, **Brain and Intelligence**, *The University of Chicago Press*, Chicago, 1947.
8 R Reitan, *Percept. Motor Skills,* **8**, 271(1958).
9 D Hunter, **Diseases of Occupations**, 4th Edition, *Little Brown*, Boston, 1969.
10 H Hanninen, *Brit. J. Indust. Med.*, **28**, 374(1971).
11 B Knave, B Kolmodin-Hedman, H Persson, and J Goldberg, *Work Environ, Health*, **11**, 49(1974).
12 S Elofsson, F Gamberale, T Hindmarsh, et al., *Scand. J. Work Environ. Health*, **6**, 239(1980).
13 O Axelson, M Hane, and C Hogstedt, *Scand. J. Work Environ. Health*, **2**, 14(1976).
14 P Arlien-Soberg, P Bruhm, C Gyldensted, and B Melgaard, *Acta Neurol. Scand.*, **60**, 149(1979).
15 R Feldman, J Chirico-Post, and S Proctor, *Arch. Environ. Health*, **43**, 143(1988).

16 K Kilburn, J Thornton, and B Hanscom, *Electromyograph Clin. Neurophysiol.*, **38**, 25(1998).
17 E Matikainen, and J Juntunen, **Neurobehavioral Methods in Occupational and Environmental Health**. *World Health Organization*, Copenhagen, 1985, pp. 57-60.
18 S Oh, **Clinical Electromyography; nerve conduction studies**, 2nd Edition, *Wilkins and Wilkins*, Baltimore, 1993, pp.89.
19 K Kilburn, and R Warshaw, *Occup. Environ. Med*., **51**, 381(1994).
20 J Miller, G Cohen, R Warshaw, J Thornton, and K Kilburn, *Am. J. Indust. Med*., **15**, 687(1989).
21 K Kilburn, J Thornton, and B Hanscom, *Arch. Environ. Health*. **53**, 257(1998).
22 B Kylin, K Axell, H Samuel, and A Lindborg, *Arch. Environ. Health*, **15**, 48(1967).
23 K Kilburn, *Neurotoxicology*, **21**, (2000).
24 L Barrett, S Garrel, V Danel, and J Debru, *Arch. Environ. Health*, **42**, 297(1987).
25 R Cattell, S Feingold, and S Sarason, *J. Educational Psych.*, **32**, 81(1941).
26 R Cattell, *J. Consulting Psych*., **15**, 154(1951).
27 K Kilburn, and R Warshaw, *J Toxicol. Environ. Health*, **39**, 483(1993).
28 K Kilburn, *Environ. Res.*, **80**, 244(1999).
29 National Research Council, **Public Health and Hazardous Waste, Environ. Epid**., *National Academy Press*, Washington DC, 1991.
30 D Grabski, *Am. J. Psychiatry*, **118**, 461(1961).
31 J Knox, and J Nelson, *New Eng. J. Med.*, **275**, 1494(1966).
32 L Prockop, M Alt, and J Tison, *JAMA*, **229**, 1083(1974).
33 T Matsushita, Y Arimatsu, A Ueda, K Satoh, and S Nomura, *Ind. Health*, **13**, 115(1975).
34 J Juntunen, E Matikaninen, M Antti-Poika, H Suouanta, and M Valle, *Acta Neurol. Scand*., **72**, 512(1985).
35 H Hanninen, M Antti-Poika, and P Savolainen, *Int. Arch. Occup. Environ. Health*, **59**, 475(1987).
36 S Foo, J Jeyaratnam and D Koh, *Brit. J. Indust. Med.*, **47**, 480(1990).
37 T Shirabe, T Tsuda, A Terao, and S Araki, *J. Neurol. Sci*., **21**, 101(1974).
38 J Dodds, and S Santostefano, *J. Pediatr*., **64**, 565(1964).
39 E Longley, A Jones, R Welch, and Lomaev, *Arch. Environ. Hlth*., **14**, 481(1967).
40 N Cherry, H Hutchins, T Pace, and H Waldron, *Brit. J. Indust. Med*., **42**, 291(1985).
41 K Kilburn, and R Warshaw, *Neurotoxicol. Terato*., **17**, 89(1995).
42 K Kilburn, *Environ. Res*., **81**, 92(1999).
43 C Carpenter, E Kinkead, D Geary Jr, L Sullivan, and J King, *Toxicol. Appl. Pharmacol.*, **33**, 543(1976).
44 K Savolainen, V Riihimaki, and M Linnoila, *Int. Arch. Occup. Environ. Hlth.*, **44**, 201(1979).
45 K Savolainen, V Riihimaki, Omuona, and J Kekoni, A Laine, *Arch. Toxicol., Suppl.*, **5**, 96(1982).
46 K Savolainen, V Riihimaki, O Muona, J Kekoni, R Luukkonen, and A Laine, *Acta Pharmacol. Toxicol.,* **57**, 67(1985).
47 A Seppalainen, K Savolainen, and T Kovala, *Electroencephalo. Clin. Neurophysiol.*, **51**, 148(1981).
48 D Klaucke, M Johansen, and R Vogt, *Am. J. Induct. Med.*, **3**, 173(1982).
49 K Kilburn, R Warshaw, and J Thornton, *Arch. Environ. Health*, **42**, 117(1987).
50 H Muijser, E Hoogendijk, and J Hooisma, *Toxicol*., **49**, 331(1988).
51 F Gobba, and A Cavalleri, In: **Butadiene and Styrene: Assessment of Health Hazards**, *IARC Scientific Publications* No. 127, Lyon, France, 1993.
52 C Moller, L Odkvist, B Larsby, R Tham, T Ledin, and L Bergholtz, *Scand. J. Work. Environ. Hlth*., **16**, 189(1990).
53 O Hutzinger, G Choudhry, B Chittim, and L Johnston, *Environ. Health Perspect.*, **60**, 3 (1985).
54 K Kilburn, R Warshaw, and M Shields, *Arch. Environ. Health*, **44**, 345(1989).
55 E Buiatti, S Cecchini, O Ronchi, P Dolara, and G Bulgarelli, *Brit. J. Ind. Med.*, **35**, 168(1978).
56 I Aiello, G Rosati, G Serra, and M Manca, *Acta Neurol.,* **35**, 285(1980).
57 S Passero, N Battistini, R Cioni, F Giannini, C Paradiso, F Battista, F Carboncini, and E Sartorelli, *Ital. J. Neurol. Sci.*, **4**, 463(1983).
58 S Yamada, *Jpn. J. Ind. Health,* **6**, 192(1964).
59 Y Yamamura, *Folia Psychiatr. Neurol. Jpn*., **23**, 45(1969).
60 P Spencer, and H Schaumburg, *J. Neurol. Neurosurg. Psychiatr*., **38**, 771(1975).
61 A Seppalainen, C Raitta, and M Huuskonen, *Electroencephalogr. Clin. Neurophysiol*., **47**, 492(1979).
62 S Sanagi, Y Seki, K Sugimoto, and M Hirata, *Int. Arch. Occup. Environ. Health*, **47**, 69(1980).
63 T Burbacher, *Environ. Hlth. Perspect*., **101**(Suppl 6),133 (1993).
64 R Warshaw, A Fischbein, J Thornton, A Miller, and I Selikoff, *Ann. NY Acad. Sci.*, **320**, 277(1979).
65 P Houch, D Nebel, and S Milham Jr, *Am. J. Indust. Med*., **22**, 109(1992).
66 N Shigematsu, S Ishimaru, and R Saito, *Environ. Res.*, **16**, 92(1978).

67 N Benowitz, **Cardiotoxicity in the workplace, Occup. Med**., Vol 7, No. 3, *Hanley & Belfus, Inc*., Philadelphia, 1992.
68 Y Kuriowa, Y Shimada, and Y Toyokura, *Neurology*, **33**, 463-467(1983).
69 K Murata, S Araki, K Yokoyama, K Yamashita, F Okajima, and K Nakaaki, *NeuroToxicol.*, **15**, 867-876(1994).
70 W Harris, *Arch. Intern. Med.*, **131**, 1621(1973).
71 N Cherry, H Venables, H Waldron, and G Wells, *Brit. J. Ind. Med*., **38**, 351(1981).
72 B Friedlander, T Hearne, and S Hall, *J. Occup. Med.*, **20**, 657(1978).
73 J Repko, P Jones, L Garcia, E Schneider, E Roseman, and C Corum, **Behavioral and neurological effects of methyl chloride,** CDC-99-74-20, *National Institute for Occupational Safety and Health*, 1976.
74 W Grant, **Toxicology of the Eye**, *Charles C Thomas Publisher,* Springfield, Illinois, 1974.
75 S Mikkelsen, *Scand. J. Soc. Med*., (Suppl. **16**), 34,(1980).
76 H Rasmussen, J Olsen, and J Lauritsen, *J. Occup. Med.*, **27**, 561(1985).
77 K Lindstrom, *Scand. J. Work Environ. Health*, **7** (Suppl. 4), 48(1981).
78 J Olsen, and S Sabroe, *Scand. J. Soc. Med*., **44** (Suppl. 16), 44,(1980).
79 K Lindstrom, H Riihimaki, and K Hanninen, *Scand. J. Work Environ. Health*, **10**, 321(1984).
80 T Riise, and B Moen, *Acta Neurol. Scand*., **77** (Suppl. 116), 104(1988).
Note: New general source for neurotoxicity for solvents. R Feldman, **Occupational and Environmental Neurotoxicology**, *Leppincott-Raven*, Hagerstown, Maryland, 1999.