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# XYLENE - ITS HEALTH HAZARDS AND BIOCOMPATIBLE SUSBTITUTES

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## ABSTRACT

Xylene is a hazardous chemical, a dimethylbenzene that occurs naturally in petroleum and coal tar. It has extensive use in different industries and histopathology. It is potentially toxic and flammable in nature. Histotechnicians are routinely exposed to this hazardous chemical. Because xylene is used so pervasively in histopathology, it has always been a concern for pathologists and laboratory workers, as its regular and prolonged exposure have serious health effects. Considering its toxicity, different biocompatible xylene substitutes have been evaluated. This review focuses on the health hazards of xylene and its different biocompatible substitutes.

**KEYWORDS:** Xylene, Health, Hazards, Biocompatible, Substitutes.

## INTRODUCTION

Xylene (dimethylbenzene) is an aromatic hydrocarbon occurring naturally in petroleum and coal tar or is produced in chemical industries from petroleum. It also forms during forest fires to a small extent. It is a colorless, flammable liquid with a sweet odor having a chemical formula of $C_6H_4(CH3)_2$. The term ‘xylene’ is derived from Greek word 'xylon' meaning ‘Wood’ as it is found in crude wood spirit.[1,2]

Xylene is used in many industries such as printing, rubber, leather industries, paint and varnishes, plastics, and synthetic fiber, coating materials of fabrics and papers. It is also found in small amounts in airplane fuel, gasoline, and cigarette smoke.[1] In dentistry, it is used in endodontic retreatment as a guttapercha solvent.[3] In histopathology, xylene is used as a de-alcoholization agent of choice during tissue processing, also used in staining and mounting of tissue sections, in spite of its toxicity to laboratory personnel and the danger it

poses to the environment.[4] Its high solvency factor allows maximum displacement of alcohol and renders the tissue transparent, enhancing paraffin infiltration. Its excellent dewaxing and clearing capabilities contribute brilliant staining.[3]

Technical and commercial grades of xylenes often contain substantial amounts of ethylbenzene (10-50%), and perhaps minor amounts of other solvents as well. Mixtures of xylenes and ethylbenzene are occasionally termed mixed xylenes. Thus most occupational exposure to xylenes results in exposure to ethylbenzene also.[1,2,5]

Xylene was used as a safe alternative to dangerous chemicals such as aniline oil, benzene, chloroform, dioxane, and toluene in the histology laboratory in 1950s. But this proved to be an example of a failed substitution. By the late 1970s, there were great concerns about its safety with evidence that its acute neurotoxicity was greater than that of benzene or toluene.[6]

This review focuses on the health hazards of xylene and its biocompatible substitutes. The literature search was performed using Pubmed and Google scholar search engines with search terms such as xylene, health hazards, exposure, substitutes, natural and biocompatible. All the original research articles as well as both systematic and simple review articles, case reports, safety guidelines, health safety reports available in English literature were included in the study.

**Exposure to Xylene**

Workers who routinely come in contact with xylene-containing solvents in the workplace are the population most likely to be exposed to high levels of xylene.[1] One can also come in contact with xylene through automobile exhaust and a variety of consumer products such as cigarette smoke, paints, varnish, rust preventives and shellac. Hazardous waste disposal sites and spills of xylene into the environment are also possible sources of exposure. Xylene is sometimes released into water and soil as a result of the use, storage, and transport of petroleum products and that can also cause exposure.[1] Xylene is present in many household solvents, air fresheners, stainless steel cleaners, floor polishers, and gasoline.[6] In the histology laboratory, the histo-technicians are exposed to xylene during tissue processing, dewaxing sections before staining, clearing them before coverslipping, while cleaning tissue processors and recycling.[6]

Exposure to xylene can occur via inhalation, ingestion, eye or skin contact. Xylene is most likely to enter the body by inhalation of xylene vapors and it is rapidly absorbed by lungs. The amount of xylene retained ranges from 50 to 75% of the amount of inhaled xylene. Physical exercise increases the amount of xylene absorbed by the lungs. Absorption of xylene in the gut after eating food or drinking water containing it is both rapid and complete. Absorption of liquid xylene through the skin also occurs rapidly following direct contact with xylene, but absorption of xylene vapor through the skin is only about 12% of the amount absorbed by the lungs. Xylene passes into the blood soon after entering the body.[1] It is primarily metabolized in the liver by oxidation of a methyl group and conjugation with glycine to yield methyl hippuric acid (MHA), which is excreted in the urine. Smaller amounts are eliminated unchanged in the exhaled air.[7,8] Small amounts of breakdown products of xylene have appeared in the urine of people as soon as 2 hours after breathing air containing xylene. Usually, most of the xylene that is taken in leaves the body within 18 hours after exposure. There is a low potential for accumulation. About 4–10% of absorbed xylene may be stored in fat, which may prolong the time needed for xylene to leave the body.[1] It is stored in adipose tissue as it is soluble in it. It has a half life of 1 to 6 days in the subcutaneous fat.[9]

According to Occupational Safety and Health Administration (OSHA), the permissible exposure limit of xylene is 100 parts of xylene per million parts of air (ppm) averaged over an eight-hour work shift (TWA, time-weighted average) in a 40-hour work week and 200 ppm for 10 min as a short-term limit.[1,2] Monitoring xylene vapor became a practice in some work places and TWA exposure limits of OSHA were followed. But this approach does not reflect its incorporation into the employee's system as measured by the concentration of its major metabolite in urine, MHA, with a biological exposure index (BEI) limit of 20 mg/dL recommended by the American Conference of Governmental Industrial Hygienists (ACGIH).[6] However, the BEIs are not to be used for the diagnosis of an occupational illness but as an indicator of exposure to significant concentrations of the chemical substances if the workers show values of the analyte at/above the value of its BEI.[10]

The amount of biomarker of xylene exposure in urine can be analysed using techniques such as High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC). HPLC-Tandem Mass Spectrometry can be used for simultaneous measurement of biomarkers of various chemicals in urine samples.[10]

**Health Hazards of Xylene**

Xylene causes health effects from both acute (<14 days) and also chronic (>365 days) exposure. The type and severity of health effects depends on several factors, including the amount of chemical one is exposed to, duration of exposure, individual response and the route of exposure.[3,10] Various animal studies have proved that excessive exposure to xylene can cause toxicity to multiple tissues such as the nervous system, the liver, the skin, and the lung. The cell toxicity of xylene has been linked to the induction of mitochondrial uncoupling and oxidative stress.[11]

In humans, acute inhalational exposure to mixed xylene at 200 ppm for 3-5 minutes may result in irritation of the nose and throat.[12] It may also cause impaired short-term memory, impaired reaction time, performance decrements in numerical ability, and alterations in equilibrium and body balance.[1] Severe neurotoxicity, loss of cochlear hair cells and/or hearing deficits were observed in rats, mice, and gerbils following acute- or intermediate-duration inhalation exposure to the various xylene isomers at concentrations in excess of 1,000 ppm.[1] Morley et al., reported a case of human death following acute inhalation exposure to xylene.[13] Chronic occupational exposure to an unspecified concentration of vapors of mixed xylene through inhalation can cause nose and throat irritation, labored breathing and impaired pulmonary function.[14,15]

Abu Al Ragheb et al., reported a case of death following accidental or intentional ingestion of xylene leading to pulmonary congestion and edema, respiratory failure secondary to depression of the respiratory center in the brain causing death.[16] Recchia et al., reported a case of temporary comatose state for 26 hours in a person following accidental ingestion of xylene.[17]

Dermal contact with xylene is likely in many occupational situations. In histology technicians, nausea, decreased pulmonary function, dyspnea, flushing, chest pains, and palpitations have been reported, but these studies also involved exposure via inhalation route.[14,18] In hand immersion studies in human subjects, acute exposure to xylene elicited transient symptoms of skin erythema (irritation), vasodilation of the skin, dryness and scaling of the skin.[19,20] A case report of urticaria in a female cytology worker on exposure to xylene vapors had been published.[21]

Irritation to the eye may occur from contact with xylene vapor or xylene liquid, that may cause photophobia, redness of the conjunctiva, and partial loss of the conjunctival and corneal epithelia.[1]

The various health effects due to xylene exposure have been documented in the literature. There is limited human data on the genotoxic and carcinogenic effects xylene.[1]

A number of theories exist for the mechanisms by which xylene exerts its toxic effects on the various systems of the body. The pulmonary, gastric and ocular effects of xylene are attributed to the irritant nature of the chemical.[22, 23] Xylene interferes with proteins present in the neuronal membrane, which are essential for normal neuronal function. Liposolubility of xylene in neuronal membrane may directly interact with the proteins in membranes or may disrupt the lipid environment in which the membrane proteins function. It is suggested that methyl benzaldehyde, which is produced by oxidation of xylene by brain microsomal enzymes, is responsible for the toxicity of xylene.[23] Animal studies have reported changes in the levels of various neurotransmitters and lipid composition in several areas of brain following acute- and intermediate-duration exposure to xylene. However, it is not clear whether these represent direct effects of xylene or are secondary changes resulting from nonspecific central nervous system depression.[24,25] The mechanism for xylene's toxic effects on the kidneys is unknown, but may be related to formation of reactive metabolites and subsequent irritation or direct membrane fluidization. Nephrotoxicity due to xylene may involve induction of apoptosis through the activation of mitochondrial caspase-9 and caspase-3, typical features of apoptosis, DNA fragmentation and upregulation of Bax protein compared to Bcl-2 protein (increasing the ratio of Bax/Bcl-2), were observed.[1,26,27,28] Franchini et al. reported that the urinary β-glucuronidase levels in humans exposed to xylene are high that indicates a faster turnover of the renal cells due to toxicity of the toxic metabolites of xylene.[29]

Dermal absorption is also a major route of xylene exposure. Higher levels of MHAs in the urine in workers with eczema in hands have been reported. Percutaneous absorption of xylene in such atopic individuals is exaggerated due to disruption of the epithelial barrier as a result of removal of ceramide of the corneal layer of the skin epithelium. In animal studies, treatment with xylene led to increased levels of inducible nitric oxide synthetase and tumor necrosis factor-alpha (TNF-alpha), a pro-inflammatory cytokine, in skin, as well as plasma levels of interleukin-1-alpha (IL-1alpha) and the histopathological effects of xylene exposure

included swelling and disruption of the stratum corneum, granulocyte infiltration of the epidermis, and separation of the epidermis and dermis, with evidence of local inflammation (accumulation of mast cells, plasma cells).[30,31,32]

**Substitution of Xylene**

In the field of histopathology technicians are occupationally exposed to xylene as it forms an integral part of pathological laboratory as a clearing agent of tissue samples.[22] Considering the hazardous effects of xylene, several studies have been conducted in search of a safer alternative to xylene.

A solution can be considered as a clearing agent, if it rapidly penetrates into tissues to clear them.[33] Clearing agent is used as an intermediate solvent that is fully miscible with both ethanol and paraffin wax.[34] The viscosity of the solution plays an important role in easy penetration into tissues. A less viscous solution penetrates faster to that of high viscous solutions.[33] This solvent will displace the ethanol in the tissue, then, this in turn will be displaced by molten paraffin wax.[34] Different biocompatible substances have been evaluated to substitute xylene and thereby to eliminate the hazards associated with it.

**Essential Oils**

An essential oil, also known as volatile or ethereal oil, is a liquid that is distilled mostly by steam or water from different parts of the plant such as leaves, stems, flowers, bark, and roots. These essential oils are used as natural flavouring agents for food, as fragrances in perfume, and in medicine as well as in alternative medicines such as aromatherapy. Some of these oils used as an alternative for clearing are discussed below.[33]

**Coconut oil:** Sermadi et al., used coconut oil as a clearing agent and found that the tissues were more translucent, less rigid (with no interference in impregnation and cutting), and had less shrinkage as compared to the tissues cleared with xylene. There was no difference in staining quality and tissue architecture. The only drawback, they found with coconut oil, is its tendency to get solidified at a lower temperature, which could be overcome by performing the clearing procedure in an incubator, maintaining the required temperature.[4]

**Bleached palm oil:** Udonkang M et al., conducted a study with bleached palm oil-and found that bleached palm oil-processed tissues (at 60$^{o}$C) were as good as the xylene-processed

tissues in terms of transparency, production of serial sections and quality of histological staining. It is safe and economical.[35]

**Cedarwood oil:** Cedarwood oil is perhaps the most well known natural wood oil for clearing tissues. It causes no damage to the tissue. But it takes significantly longer time to process and is expensive than the usually used alternatives.[33] Indu et al., compared the efficacy of cedarwood oil and xylene in hematoxylin & eosin (H&E) staining procedures. They found that tissue cleared with cedarwood oil were shown to give much favorable staining results (adequate nuclear staining in 90% of sections and adequate cytoplasmic staining in 93.33% sections cleared with cederwood oil). They suggested that cedarwood oil can be considered as an uncompromised alternative to xylene as a clearing agent.[36]

**Pine oil:** Pine oil has similar colour, viscosity properties with that of xylene and is very economical as compared to xylene. It is more viscous than xylene but when subjected to heat they showed equal penetration to that of xylene. Swamy et al., reported that pine oil is superior in its physical and clearing properties, superior in translucency and causes less shrinkage of tissue compared to that of xylene. It also preserves tissue cellular architecture with clear distinction between nucleus and cytoplasm and the overall staining quality is equivalent to that of xylene.[34]

**Other essential oils:** Swamy et al., evaluated carrot oil, olive oil, and rose oil and found that they have the equivalent penetrative ability as that of xylene, when subjected to heat. Carrot and rose oils showed less shrinkage of tissue compared to xylene. Translucency of the tissues cleared in carrot and rose oils was similar to tissues cleared in xylene. Tissue cellular architecture was preserved in all the sections cleared with different oils and a clear distinction was observed between nucleus and cytoplasm. The overall staining quality was almost equivalent to that of xylene.[34]

Andre et al., evaluated a mixture of peanut oil, soyabean oil, coconut oil and cotton oil to be used as clearing agent. They found that that this mixture was a poor alternative.[37] Ramussen et al., evaluated a mixture of coconut oil and olive oil and noticed incomplete impregnation, leading to problems in section cutting.[38]

**Mineral oil:** Premalatha BR et al., evaluated the commonly available refined mineral oil (RMO, commercial nonstick hair oil with 80%-RMO, 20%-coconut oil) as a xylene substitute

and found that optimal results of deparaffinization were obtained at 90°C and the quality of H&E staining was at par with that of xylene. The stability and longitivity of H&E staining for over a period of 6 months were found to be stable and unaltered. They also cited compatibility of the procedure with high quality DNA extraction using PCR.[39]

Buesa et al., evaluated and found a mixture of ethanol, isopropyl alcohol and mineral oil as an efficient de‑alcoholization agent and an alternative for xylene.[6]

**Other substitutes**

Kunhua et al., suggested that good maintenance of cell morphology, structure and distinct nuclear and cytoplasmic staining with H&E can be achieved with a novel non-toxic xylene substitute of White oil No. 2 and 14% N-Heptane (SBO). This agent was equally effective for use in various special stain procedures, immunostaining and the staining results were comparable or superior to those of xylene.[11]

Ananthaneni et al., reported the efficacy of diluted lemon water (95%) along with 1.5% dish washing solution (DWS) as deparaffinising agent compared to xylene.[40] Pandey et al., also reported that liquid diluted DWS is a safe and efficient alternative to xylene and alcohol in deparaffinization and routine H&E staining procedure.[41]

Considering the serious adverse effects of xylene, many attempts have been made to replace this agent with safer alternatives and the development of more novel, more effective and safer substitutes is increasingly necessary.[11]

## CONCLUSION

Elimination of hazardous chemicals with effective, biocompatible substitutes is very important to move forward in the path of green chemistry and to create an eco-friendly safer working environment in histology laboratories. Xylene is a known toxic chemical. To create a xylene-free safer working environment, the onus lies on the pathologists and also on everyone related to histology and their commitment to accept the change.

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