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Effect of methyl substitution of benzene on the percutaneous absorption and skin irritation in hairless rats

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Abstract

The permeation rate and skin retention of benzene and methylbenzenes were assessed in vitro using hairless rat skin. The effects of unocclusive dermal exposures of these chemicals (15 μl every 2 h for 8 h a day for 4 days) on the transepidermal water loss (TEWL), erythema and skin histopathology were measured in CD hairless rats. The expression of IL-1α and TNF-α in the skin and blood were measured at the end of dermal exposures. The flux of benzene was about 1.5-, 2.5- and 80-fold higher than toluene, xylene and tetramethyl benzene isomers (TMB), respectively, and the values were inversely correlated with molecular weight ($r^2 = 0.7455$) and log octanol–water partition coefficient ($r^2 = 0.7831$). The retention of chemicals in stratum corneum (SC) was in the order of TMB > xylene > toluene ≈ benzene. The TEWL and erythema data demonstrated that the irritation was in the following order: TMB > xylene > benzene. The histo-pathological examination showed that xylene and TMB induced granulocyte infiltration, swelling of the epidermis, and extensive disruption and damage of stratum corneum. Likewise, the expression of IL-1α in the blood and TNF-α in the skin after dermal exposures was higher for TMB followed by xylene and benzene compared to control. In conclusion, the aromatic hydrocarbon chemicals induced cumulative irritation upon low-level repeat exposures for a 4-day period and the irritation increased with the number of methyl groups of benzene. The affinity of the chemical to SC and their gradual accumulation in the skin in the present study is the reason for the differences in the skin irritation profiles of different aromatic chemicals. Our ultimate goal is to develop a biologically based model that connects skin retention of chemical to the skin irritation response. The findings of the present study will be helpful in understanding the role of these chemicals in the jet fuel and various petroleum based fuels in inducing skin irritation response.

Keywords: Skin permeation; Skin retention; Skin irritation; Aromatic hydrocarbons; TEWL; Cytokine

1. Introduction

The skin is the major potential route for the absorption of hazardous materials encountered in the work place. Toxicity at the site of contact with chemicals is much more common than systemic toxicity due to

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Table 1
Physical properties of aromatic hydrocarbons used in the present study

Chemical	Molecular formula	Molecular weight	Melting point (°C)	Boiling point (°C)	log K_p	Water solubility (mg/L)
Benzene	C_6H_6	78.12	5.5	80.0	2.13	1790
Toluene	C_6H_6–CH_3	92.14	−94.9	110.6	2.73	526
Xylene	C_6H_6–$(CH_3)_2$	106.17	−25.2	144.5	3.12	178
Tetramethyl benzene	C_6H_6–$(CH_3)_4$	134.22	−23.7	198.0	4.0	27.9

dermal absorption (Mathur and Khanna, 2002). The aromatic hydrocarbons are important components of jet fuel, petroleum and its refined products in terms of having higher dermal toxicological potential than aliphatic hydrocarbons (Chou et al., 2003). The most important aromatic hydrocarbons in these fuels are benzene and various methyl substituted benzenes. Benzene–toluene–xylene (BTX) mixture is a major constituent in gasoline. Benzene and methylbenzenes are used as constituents in motor fuels, as a solvent for fats, waxes, resins, oils, inks, paints, plastics, and rubber. These are also used as chemical intermediates and in the manufacture of detergents, explosives, pharmaceuticals, and dyestuffs. Individuals employed in the industries that manufacture or use benzene and methylbenzenes may be exposed to the high levels of these chemicals. Aromatic hydrocarbons have the ability to cause immunotoxicity, neurotoxicity, birth defects or melanoma (Hsieh et al., 1991; Ritchie et al., 2001; Moszczynski and Lisiewicz, 1982; Xiao et al., 1999). These chemicals penetrate the skin in significant quantities and repeated exposures can damage the skin tissue leading to molecular changes and contact dermatitis (Gunasekar et al., 2003; Kezic et al., 2001; Lynch et al., 1978). The skin penetration of benzene and methylbenzenes has been fairly well studied in vitro as well as in vivo in experimental animals and humans (Wester and Maibach, 2000; Blank and McAuliffe, 1985; McDougal et al., 1990; Tsurata, 1982; Tsurata et al., 1987). However, there is little information in the literature on the effect of chemical structure on the percutaneous absorption and skin irritation potential of aromatic hydrocarbons. This information will be very helpful in understanding the role of individual components of hydrocarbon-based fuels on the skin permeation and irritation and molecular responses of the fuel. Therefore, we have conducted a systematic investigation to determine the effect of different methyl substitutions of benzene ring (as given in Table 1) on: (1) the skin permeation rate of chemicals; (2) retention of chemical in different skin layers; (3) skin irritation potential (transepidermal water loss, Draize scoring and histological changes in the skin) of the chemicals; (4) expression of molecular markers (IL-1α, TNF-α) upon dermal exposures in hairless rats. Further, we attempted to relate the skin levels of these chemicals to their irritation potential upon unocclusive dermal exposures.

2. Materials and methods

2.1. Materials

Radiolabeled benzene (UL-^{14}C, 5.0 mCi/g), toluene (4-^{3}H, 2.4 mCi/g), xylene (methyl-^{3}H, 2.4 mCi/g) and ^{3}H tetramethyl benzene (ring-^{3}H, 2.4 mCi/g) were procured from American Radiolabeled Chemicals (St. Louis, MO). Benzene (purity 99.0%), toluene (purity 99.8%), xylene (purity 98.0%), 1,2,3,5-tetramethyl benzene (assay 80%, remainder 1,2,4,5-tetramethyl benzene), polyoxyethylene 20 oleyl ether (Brij 98), pentobarbital sodium, sodium chloride, halothane and heparin sodium were procured from Sigma–Aldrich (St. Louis, MO).

2.2. In vitro skin penetration studies

The dorsal skin was excised from hairless rats after euthanizing with an overdose of halothane anesthesia and the adhering fat and subcutaneous tissue were removed. The freshly excised skin was mounted between the donor and receptor compartments of Franz diffusion cells (Permegear Inc., Bethlehem, PA) with the stratum corneum facing the donor compartment. Each Franz cell had a diffusional surface area of 0.636 cm^2 and the maximum capacity