Effects of Prenatal Toluene Exposure on Fetal Development: A Review

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Abstract

Inhalant misuse is a growing public health concern, particularly among adolescents and females of childbearing age. Toluene is a volatile organic solvent that is used in many industries and is prevalent in consumer products. Due to the lipophilic nature of toluene, it can readily diffuse across the placenta, which lends to its potential as a teratogen when inhaled by pregnant individuals. Toluene inhalation can occur through incidental occupational exposure and intentional misuse by inhalation of vapors from toluene-containing products done to achieve intoxication. This literature review was conducted using PubMed, EbscoHOST, and Google Scholar databases to summarize the neurological and physical effects of prenatal toluene exposure on fetal development evaluated at several stages of life. The teratogenic effects of toluene exposure to fetuses have been documented in animal models, rodents, and rabbits, as well as in humans. Experiments performed on rodents have determined a wide range of physical and neurological defects due to toluene exposure. Some physical defects include growth restrictions, premature delivery, and congenital malformations. Neurological defects include but are not limited to demyelination and dysfunction of the cerebellum, cerebral cortex, and subcortical regions of the brain. These defects lead to problems with growth and neurological disorders in the form of delayed motor skill development, poor memory, and poor cognitive function. Rodent studies have also established the neuroprotective effects of phytochemicals, melatonin, and omega-3 fatty acids. Rabbits have also been used to model toxicologic endpoints that would be seen in larger animals and humans. Rabbit models revealed the importance of administering toluene in concentrations at a level that mirrored human binging to understand the potential fetal impacts. Rabbits injected with high concentrations of toluene had structural damages to several brain regions and decreased levels of several important central nervous system (CNS) factors. Toluene's neurological effects on the human brain are similar to those seen in rodents and rabbits. Our review highlights the importance of public health intervention to curb the detrimental effects of intentional and unintentional toluene exposure.

Introduction

Toluene is an organic solvent that is prevalent in many industries. It is used in gasoline, adhesives, inks, fabric dyes, and as an extraction solvent for some pharmaceutical and cosmetics as well as many other consumer products (1–4). Toluene is a clear, colorless, volatile aromatic hydrocarbon with a half-life of 3.4 hours in human blood (1, 5). One of the dangers of toluene is brought about by its chemical structure, shown in Figure 1, which makes it lipophilic. This allows it to enter the blood after inhalation and in pregnant women, toluene can cross the placenta to enter fetal blood circulation (6). Due to the ubiquity of toluene usage in making consumer products, many individuals could be exposed to the vapors during their daily work. The prevalence of toluene and ease of accessibility makes it a target for inhalant misuse.

Inhalant abuse is common among adolescents and young adults aged 18–25 as an easily accessed and relatively inexpensive means for achieving intoxication. This age group falls within prime childbearing years for women (3, 7). The percentage of high school-aged girls who reported they had "ever used inhalants" was 10%, which surpassed males at 7.9% (6, 8). With increasing exposure of females to toluene and other volatile substances through inhalant abuse while in childbearing ages, it is important to understand the potential effects on fetal neurodevelopment. The focus of this review was on the neurodevelopmental effects of toluene on fetuses whose mothers are exposed to toluene while pregnant.

Methods

Three databases were used to retrieve articles for use in this literature review: PubMed, EbscoHOST, and Google Scholar. The following key terms were used to find relevant articles: prenatal, toluene, teratogen, and neurological. No date range or journal exclusion was applied. The chemical structure was made in ChemDraw version 19.0.0.

Discussion

Toluene exposure in rats

Physical defects of toluene exposure

Several case studies using rodents, rats and mice, show that exposure to toluene through inhalation can negatively affect maternal health and fetal development (3, 9–11). These investigations have helped foster a better understanding of the toxic effects of toluene exposure alone versus its synergistic

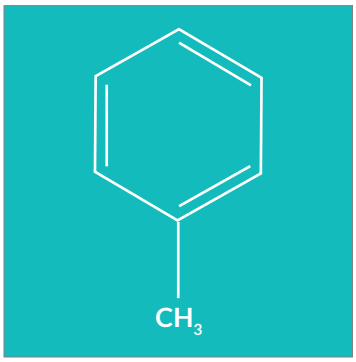

Figure 1. Toluene Chemical Structure. Molecular Formula: C6H5CH3, PubChemCID: 1140, IUPAC name: methylbenzene (5)

effects with other factors like stress (12). Toluene and stress during pregnancy lead to a reduction in dams' body weight gain (12). Additionally, their pups showed decreased appetite and physical defects such as musculoskeletal malformations including missing digits and craniofacial abnormalities (13–14). The appetite suppressing effect of toluene leading to failure to thrive over a prolonged time has been demonstrated in many rat studies (15–18). Adverse effects pertaining to affected animal fetuses include growth restriction, premature delivery, and congenital malformations (3, 7, 19–21).

Neurological and neurobehavioral effects of toluene exposure

Physical defects due to toluene exposure led to poor cognitive and motor skill development (3, 7, 19–21). Neuronal damage with resultant cognitive and learning disabilities were key findings in dam studies with toluene exposure. Toluene toxicity leads to detrimental effects in postnatal development (22–23). This can be partly explained by the observed neuronal cell death in neonatal rats (22). Neuropathological studies using methods such as TUNEL staining, DNA-laddering, and caspase-3 activity have led to a greater understanding of the histological effects of toluene (22). Findings include decreased apoptotic activity occurring in the cerebellum and hippocampus (22). Toluene embryopathy could occur due to the arrested neurogenesis process (24) and its negative impact on the somatosensory cortex (7, 24–25). Toluene causes changes in brain synaptic formation (26) leading to poor visual perception and disorders of learning processes (27–28). Toluene also leads to a reduction in the density of synapsin and densin, two novel proteins abundant in the forebrain and cerebellum. This result could explain toluene's suppressive effect on glutamatergic synapses in hippocampal neurons (24).

The Morris water maze was mainly used in rat studies as a behavioral procedure to observe spatial learning and memory skills (29). The maze was designed with four different starting points from which the time it took to find a hidden platform was measured (29). The learning process included training the rats through the maze consecutively for a few days until their performance was stable. The memory phase occurred five weeks later, when the rats were tested again for their ability to complete the maze. In each trial, there were four imaginary starting points arranged in a pseudorandom sequence; north, south, east, and west. The rats were directed into one of the starting points to complete the trial by reaching the hidden platform.

Rats prenatally exposed to toluene showed signs of impaired cognitive function based on their ability to complete the Morris water maze (29, 30). Additionally, male offspring had mid-frequency hearing loss within the range of 8–20 kHz (30). The Morris water maze was used in another investigation completed in 2017 to evaluate if prenatal toluene exposure impaired maze performance in adolescent rats (6). Rats exposed prenatally to 12,000 ppm toluene struggled to find the platform during reversal learning. An explanation for this finding is that toluene exposure damaged long-term spatial memory (6). A contradictory study, however, determined that prenatal 6,000 ppm toluene exposure for 30 minutes twice daily from gestation day 8 through gestation day 20 impaired short-term memory which was evaluated by using an object recognition test (31). Rats in this report also did not appear to have physical malformations (31).

Neuroprotective and regenerative effects of phytochemicals, melatonin, and omega-3 fatty acids

While several experiments have shown the detrimental neurological effects of toluene exposure in rats, specific studies have shed some light on possible treatments for toluene neurotoxicity. Findings from multiple studies highlight the neuroprotective and possible regenerative properties of Nigella sativa (NG), derived thymoquinone (TQ), melatonin, and omega-3 fatty acids following toluene exposure (32–37). NG is a black seed which comes from the plant family Ranunculaceae and contains fixed oils and volatile oils such as TQ. Studies have identified some healing properties of the extract of NG which can aid in bronchodilation, immune modulation, hepatoprotection, antihistamine effects, and neuroprotection (32). TQ is an active ingredient from the volatile oil in NG which has also proved to reduce cellular damage and toxicity in vital organs (32).

Specifically, there are several beneficial effects of NG and derived TQ on neurodegeneration affecting the hippocampus of rats exposed to toluene. One study demonstrated the beneficial effects of TQ and NG used rats that inhaled 3,000 ppm toluene over 12 weeks (32). The control group was not treated with NG and TQ. The treated group received TQ and NG orally through gastric intubation at a dosage of 400 mg/kg per body weight and 50 mg/kg, respectively (32). Tissue sampling showed distorted nerve cells in the untreated group versus no significant distortion in the treated group. Additionally, the control group showed destroyed endoplasmic reticulum, swollen mitochondria, and chromatin disorganization in the hippocampal neurons. Treatment with NS and TQ was able to reduce the immunoreactivity of degenerating neurons from exposure to toluene (32, 33). TQ can also help neuronal injury and leads to remarkable morphological improvement on neurodegeneration of the frontal cortex (34). Histopathological findings showed that in the TQ treated group, there were still irregular chromatin clumps and swollen mitochondria in neurons, but there was an improvement in degenerative changes in the cytoplasm and nuclei of neuronal cells affected by toluene exposure (34). The control group had an increased number of apoptotic neurons in frontal cortex tissues compared to the rats that were treated with TQ.

Another possible treatment examined the effectiveness of omega-3 fatty acids in preventing toluene-induced neurotoxicity in the prefrontal cortex. Adult male Wistar rats were divided into three groups. The first group was the control group that did not receive toluene or treatment. The second group was the untreated group which was intraperitoneally injected with a dose of 500 mg/kg per day of toluene. The third group being the treated group received the same amount of toluene as the second group and was additionally treated with a dosage of 0.4 g/kg per day of omega 3 fatty acids via intragastric gavage (35). Omega-3 fatty acids are known for their antioxidant properties and important role in improving the function of the brain. The second group, the untreated toluene-exposed rats, had shrinkage of neuron bodies, detachments in pia mater, and increased hemorrhage. In the omega-3 fatty acid treated group, overall morphologic structure improved from the detrimental

exposure of toluene, with no detachments in pia mater or bleeding. Antioxidant enzyme glutathione peroxidase (GSH-Px) was reported to be high in the omega-3 fatty acid treated group, which is a possible explanation for the prevention of toluene-induced neurotoxicity (35).

Melatonin is another treatment that has been shown to prevent neuronal damage, including dendritic impairment, from toluene exposure (36). To model these effects, rats were put under deep ether anesthesia in which their brains were dissected out and stained using the Golgi-Cox-Sholl procedure (36). Golgi-Cox staining is an essential method used for visualizing dendritic branching patterns which help to identify morphologic changes to neurons. Results showed that the rats exposed to 5,000–6,000 ppm toluene vapors had decreased dendritic size and branching. For the treated group of rats that received intraperitoneal administration of 0.5–10 mg/kg melatonin after exposure to toluene, dendritic impairment was prevented. Another finding from this group was that frontal neurons had a 33%–40% increase in dendritic size and branching compared to the untreated group (36). This same pattern was also identified for dendritic size and branching in the parietal and occipital neurons. The ability of melatonin to prevent neuronal damage, specifically dendritic impairment, due to toluene exposure was significant. Melatonin also can protect the CNS by reducing reactive gliosis (37). Reactive gliosis is a reaction of damaged astrocytes and neurons from exposure to harmful chemicals such as toluene (37–38). Collectively, data has shown that melatonin provides antioxidant protection against neurotoxic chemicals by enhancing the activity of glial cells that protect and provide support to neurons (37).

Toluene exposure in rabbits

To strengthen the predictive potential of exposing animal models to toluene prenatally, it is important to compare rodent (mouse or rat) and non-rodent (rabbit) species (39–40). Rabbits have an added advantage in that they are the smallest, least expensive animal model that still allows for measuring toxicologic endpoints that would be evaluated in larger animals or humans (40). Prenatal toxicity of toluene was studied in artificially inseminated female Himalayan rabbits which are known to be sensitive to teratogenic substances (39). Rabbits were exposed to either 0, 30, 100, or 500 ppm of toluene for 6 hours per day from days 6 to 18 post-insemination. Although differences between the toluene-exposed and control groups were not found, maternal toxicity was not observed which is a potential limitation of this study. Teratology studies on several animals, including rabbits, were reviewed to understand the relationship between embryo-fetal mortality and fetal malformations with maternal toxicity (41). Fetal malformations in rabbits were associated with maternal toxicity and those malformations were rare or absent when maternal toxicity did not occur. Additionally, there is potential that the rabbit experiment published in 1992 did not adequately represent the concentrations of toluene human fetuses experience when their mother inhales toluene recreationally (39). Women who abuse toluene can inhale up to 15,000 ppm in one binge episode (3).

In 2017 rabbits were again used as a model to determine the detrimental effects of toluene on different regions of the brain (42). A single dose of 876 mg/kg (99.9%) toluene solution was intraperitoneally administered. The specific regions of focus were the prefrontal cortex, hippocampus, hypothalamus, substantia nigra, and entorhinal cortex. A significantly increased level of tumor necrosis factor-alpha (TNF-α) was identified when comparing the toluene-exposed and control groups in each of the brain structures. Additionally, levels of several factors were significantly decreased in the toluene-exposed group versus the control group: dopamine serum levels from the substantia nigra, nerve growth factor (NGF) from hippocampal neurons, and glial fibrillary acidic protein (GFAP) from astrocytes. The brain tissue of rabbits exposed to toluene also had distinct damage that differed from a normal structure. There were areas of abscess formation, gliosis, and perivascular demyelination in the brain cortex. The nuclei of oligodendrocytes were also malformed and cells with dispersed borders were identified. Structural damage was also identified in the sequential neurons of the hippocampus for toluene-exposed rabbits.

Effects of toluene on humans

Fetal developmental effects

In humans, many effects have been documented for fetuses exposed to toluene during gestation. Toluene "abuse" by pregnant women has been documented to result in embryopathy and malformations as well as perinatal death (2). More specifically, toluene-exposed infants presented with craniofacial abnormalities and dysmorphology of the body (2, 43). Craniofacial abnormalities can include deep-set eyes, low set ears, a small face, and micrognathia (3, 44–45). The craniofacial abnormalities are thought to occur due to either abnormal differentiation and migration of neural crest cells or increased embryonic cell death (43–44). Similar to rodents and rabbits (7, 42), toluene exposure in humans can cause microcephaly, demyelination, and dysfunction of many brain areas like the cerebellum and cerebral cortex (43, 45). Preterm delivery was also related to toluene and benzene exposure during gestation (2, 43, 46). Postnatally, children whose mothers abused toluene during their gestation exhibited growth retardation and developmental delays (44–45).

Neurobehavioral effects

Many detrimental neurobehavioral effects arise as a result of toluene exposure. Toluene is known to cause neurotoxicity, which can negatively affect the liver, renal system, and nervous system (47–50). When high levels of toluene around 10,000 – 30,000 ppm were introduced into the body, consequences such as ataxia, a lack of coordination, and unconsciousness occurred (47, 49). There are many case reports that highlight toluene's destructive effects (48, 50). The CNS of 20 patients was found to have toxicity, in all cases following the misuse of toluene (48). Each of these individuals suffered indelible effects such as acute encephalopathy, personality impairment, and cerebellar ataxia as a consequence of their misuse (50). In another report, a 33-year-old man who was documented to have been exposed to toluene inhalation over 14 years while working in an aircraft-manufacturing company was shown to have cerebellar degeneration and permanent damage to his CNS (50). Both of these studies are consistent with evidence that validates that toluene affects white matter, periventricular, and subcortical regions of the brain (47, 49). These long-lasting neurological effects result in numerous deficits that include but are not

limited to memory loss, attention deficiency, lack of speed, executive functioning, and language impairments (47, 49).

Public health policies and educational programs

In Japan, pregnant women who hold occupations in house renovation and decoration are regularly exposed to harmful paints and dyes. These chemicals increased the risk of their offspring having congenital heart disease (CHD), limb defects, cleft lip/palate, and even gastrointestinal obstructions (41, 51–53). Likewise, women who work in cosmetology, such as nail technicians and hairdressers, were found to have increased birth defects, specifically CHD and oral clefts (41, 51–52). With proper handling precautions and ventilation in the workplace, risks of inhaling an amount of toluene that would be harmful can be minimized (2–3). However, organic solvents like toluene, benzene, and xylene are typically abused through inhalation. This repeated, intentional binging on high concentrations of toluene can lead to very high concentrations in the blood (2–3).

Education regarding toluene exposure, use, and toxicity can aid in the development of public health awareness (11, 49). Currently, the Occupational Safety and Health Administration (OSHA) has recommendations on how to reduce exposure. When handling this chemical, they advise individuals to follow instructions and safety precautions provided by the manufacturer (52, 54–55). They also suggest that when adequate ventilation cannot be ensured, usage of this chemical should be avoided entirely. These limits have been set by OSHA to prevent any detrimental long-term effects on the nervous system that may occur as a result of exposure. Likewise, the importance of risk assessment is taken into consideration for safety.

The German Commission for the Investigation of Health Hazards of Chemical Compounds evaluated toluene in work-related areas to assess reproductive and developmental toxicity (56). By conducting their evaluations, they were able to provide a maximum workplace concentration (MAK) value that determined the rate at which prenatal toxic effects are not to be expected, despite its use (56). The MAK value was determined as 50 ml toluene/m3 and therefore was classified as Pregnancy Risk Group C where no prenatal toxic effects were to be expected. There have also been alternative policies such as propositions implemented for consumer education. States such as California are required by proposition 65 to include warnings to their consumers, workers, and other personnel regarding significant exposure to chemicals that have the possibility of being carcinogenic, increasing the risk of birth defects, or other reproductive harm (54, 57). Toluene is one of these chemicals which must be listed on supplies containing it since significant exposure while pregnant may lead to developmental delays.

Among the few initiatives targeting toluene misuse, there is a 12-step program and outpatient dependency treatment strategy which were implemented for public education (57). These programs include diverse techniques to help individuals continue abstinence and maintain healthy long-term recovery (57). The purpose is to provide education regarding neurological damages associated with misuse and to also supply health care providers with enhanced screening measurements that are centered around reproductive health (57). More states and organizations should be required by law to implement these practices to ensure proper education is being delivered to both consumers and patients. They should also be held accountable for maintaining a safe work environment by preventing incidental inhalation of dangerous volatile chemicals. By providing reliable resources and giving recommendations, departments can implement long-term protocols that prioritize the health and safety of their workers, especially for pregnant females.

Conclusion

Misuse of volatile organic solvents like toluene is a growing public health concern specifically among adolescents and females of childbearing age. This is due to its detrimental physical and neurological effects on a fetus in utero. In addition to intentional misuse, toluene inhalation can occur through incidental occupational exposure, making it even more urgent to document toluene's teratogenic effects. Animal studies have shown negative effects from in utero exposure that extend through adolescence. In studies on both rodents and rabbits, structural damage was indicated in several brain areas such as the cerebellum, cerebral cortex, and subcortical regions. However, phytochemicals, melatonin, and omega-3 fatty acids have shown promise for protecting against and reversing the damaging neurological effects of toluene. A strategic prevention framework should be built on the following: public health education to promote awareness, policies to prevent or limit workplace exposures, and tailored interventions directed at current misusers to protect both pregnant women and their children. Longitudinal studies in humans looking at exposure in utero through adulthood will help fill the knowledge gap and aid in designing a tailored approach to decrease the prevalence of birth defects due to fetal toluene exposure.

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