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Developmental Toxicity of Prenatal Exposure to Toluene

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ABSTRACT

Organic solvents have become ubiquitous in our environment and are essential for industry. Many women of reproductive age are increasingly exposed to solvents such as toluene in occupational settings (ie, long-term, low-concentration exposures) or through inhalant abuse (eg, episodic, binge exposures to high concentrations). The risk for teratogenic outcome is much less with low to moderate occupational solvent exposure compared with the greater potential for adverse pregnancy outcomes, developmental delays, and neurobehavioral problems in children born to women exposed to high concentrations of abused organic solvents such as toluene, 1,1,1-trichloroethane, xylenes, and nitrous oxide. Yet the teratogenic effects of abuse patterns of exposure to toluene and other inhalants remain understudied. We briefly review how animal models can aid substantially in clarifying the developmental risk of exposure to solvents for adverse biobehavioral outcomes following abuse patterns of use and in the absence of associated health problems and co-drug abuse (eg, alcohol). Our studies also begin to establish the importance of dose (concentration) and critical perinatal periods of exposure to specific outcomes. The present results with our clinically relevant animal model of repeated, brief, high-concentration binge prenatal toluene exposure demonstrate the dose-dependent effect of toluene on prenatal development, early postnatal maturation, spontaneous exploration, and amphetamine-induced locomotor activity. The results imply that abuse patterns of toluene exposure may be more deleterious than typical occupational exposure on fetal development and suggest that animal models are effective in studying the mechanisms and risk factors of organic solvent teratogenicity.

KEYWORDS: toluene, inhalant abuse, pregnancy, fetal, teratogen

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INTRODUCTION

Organic solvents are a large structurally diverse group of chemicals that have become widespread in our environment because of their essential use in industry. Organic solvents are also found in many household consumer products such as paint thinners, gasoline, cleaning fluids, cosmetics, glues, inks, correction fluids, and many more. The extensive use of solvents in the general population suggests that people are frequently exposed to these potentially toxic agents. However, the extent and degree of solvent exposure vary substantially. With proper use of these products, and an ever increasing awareness and enforcement of safe practices, only low-vapor concentrations should be produced in the typical home or workplace. On the contrary, dangerously high, acute concentrations can occur in industrial accidents¹⁻⁴ and in cases of voluntary inhalation and inhalant abuse.⁵⁻⁷ The latter is the focus of this mini-review.

The organic solvents most commonly abused by inhalation⁶ are the aromatic hydrocarbons found in glues and cleaners (eg, toluene, benzene, xylenes); the aliphatic hydrocarbons found in fuels and cigarette lighters (eg, butane, propane, gasoline); and the alkyl halides found in correction fluids, degreasing agents, paint strippers, and aerosol propellants (eg, 1,1,1-trichloroethane [TCE], methylene chloride). Other inhaled substances include the aliphatic nitrites found in room air fresheners (eg, isoamyl and isobutyl nitrite); ketones and acetone found in adhesives and nail polish remover; the volatile anesthetics (eg, isoflurane, halothane), and nitrous oxide ("laughing gas") used as a propellant in food dispensers (eg, whipped cream dispensers, or "whippets").

INCIDENCE AND PREVALENCE

Inhalant abuse is the deliberate inhalation of vapors to alter one's consciousness or to become intoxicated. Inhalant abuse is a common form of drug abuse that has been documented in many regions of the world and across a diversified group of individuals. The most recent estimates of the

prevalence of solvent abuse⁸ are that 9.5% of Americans 12 years old and older (23.8 million people) reported deliberate inhalant use at least once in their lifetimes, and 0.9% (over 2.2 million people) reported using inhalants in the previous year. Also, over 1.6 million women in their prime childbearing period between 18 and 25 years old reported inhalant abuse in 2004, a 15% increase over the number of women in this age group reporting inhalant abuse in 2000.⁹ Similar increases in inhalant abuse have been reported in Canada, and among certain populations such as isolated Inuit and aboriginal communities, inhalant use (especially gasoline) is pandemic.¹⁰ Pearson et al¹¹ estimated some time ago that more than half of chronic abusers are women of reproductive age. Because such large numbers of inhalant abusers are young women in their childbearing years,^{6,12-14} there are rising concerns about the potential negative effect of inhalants on the physical and mental development of the unborn.^{15,16}

The attraction to organic solvents by mostly socioeconomically disadvantaged youth is that these solvents are readily accessible, not restricted by law (as are alcohol and tobacco), and are relatively inexpensive compared with other drugs of abuse (eg, cocaine and barbiturates).⁶ Intoxication is achieved by quickly inhaling volatile substances to deliver the highest possible concentrations through the lungs to the brain. Administration is uncomplicated and several methods of voluntary inhalation/self-administration of products containing volatile organic solvents rapidly achieve high blood concentrations. These various inhalation routes include “huffing” (breathing fumes from a solvent-soaked rag placed near the mouth and/or nose), “bagging” (breathing fumes from substances placed in a paper or plastic bag), “sniffing” or “snorting” (nasal inhalation directly from container or heated pan), and “spraying” (spraying aerosol compounds directly into the mouth or nose). Intoxication occurs usually within seconds and the effects last anywhere from 15 to 60 minutes. Initial central nervous system (CNS) dose-dependent effects are disinhibitory, progressing to inhibition, and are characterized by euphoria, excitement, a floating sensation, dizziness, slurred speech, ataxia, and a sense of heightened power.¹⁷

PRENATAL ORGANIC SOLVENT EXPOSURE

The clinical literature is limited by inherent methodological issues including low numbers of subjects, uncertain dose-dependency, retrospective or self-restricted subject selection, and/or potential recall biases, and potential confounding features such as uncertain or multiple solvents, unspecified and/or uncontrolled co-drug exposures or risk factors. Clinical and epidemiological studies of the reproductive effects of occupational exposures to organic solvents, usually toluene (with or without other co-exposures) have been incon-

sistent in identifying altered rates of spontaneous abortion or congenital malformation.¹⁵ Although the risk for fetal demise or major malformations is much less with low to moderate occupational toluene exposure, there may be behavioral sequelae. In one clinical study of self-referred pregnant women concerned about possible occupational solvent exposure, an ordinal metric was constructed to classify women by level of occupational exposure to any number or combination of solvents.¹⁸ Relatively high exposure was associated with impairments in language and “graphomotor” ability in 4- or 5-year-old children.

In contrast, clinical cases of toluene-related embryopathy and malformations have been reported after toluene abuse by pregnant women.^{11,19-23} Very high levels of maternal solvent exposure typical of abuse can lead to perinatal death,^{19,24} and there are reports that surviving neonates show evidence of morphological teratogenicity. At birth, the affected infants were typically premature and/or growth retarded and microcephalic with severe facial dysmorphology (eg, deep-set eyes, small face, low set ears, micrognathia), and spatulate fingertips and small fingernails.^{19-23,25} The term *fetal solvent syndrome* has been adopted to describe this constellation of morphological and behavioral effects, following the model of fetal alcohol spectrum disorders (FASD)^{11,19} and comparing the phenotype of toluene embryopathy to the effects of prenatal alcohol exposure.¹⁶ In general, there are parallel outcomes including facial dysmorphology, perinatal growth, and neurobehavioral delay. In particular, craniofacial features linked with the abuse of either toluene or alcohol included small palpebral fissures, a thin upper lip, and midface hypoplasia.²⁶ Other features found in both fetal toluene syndrome and fetal alcohol syndrome (FAS), but more consistently after prenatal toluene than in FAS, included micrognathia, ear anomalies, a narrow bifrontal diameter, abnormal scalp hair patterning, down-turned corners of the mouth, and a large fontanelle. Follow-up evaluations of the toluene-exposed children up to 3 years of age revealed developmental delays, language impairment, hyperactivity, cerebellar dysfunction, and postnatal growth retardation.^{11,19,21,22} features also reported after prenatal alcohol exposure.²⁷⁻³¹ This pattern of teratogenicity, which is similar to that of FAS, is prevalent in all human studies of excessive in utero exposure to toluene.³² The popularity of these organic solvents, combined with what little is known about their prenatal effects, confirms the necessity for greater focused attention on the effects of inhaled solvent abuse on pregnancy outcome.

ANIMAL MODELS OF FETAL SOLVENT EXPOSURE

While still relatively new compared with established animal models assessing prenatal alcohol exposure,³³⁻³⁵ basic/animal research has begun to elucidate the developmental toxicity

of prenatal solvent exposure, and its effect on biobehavioral maturation of rodent offspring. Animal studies of prenatal organic solvent exposure (usually toluene or TCE) have used varying routes of administration—administered, intubated, ingested, and inhaled. Many of these studies focused on growth and the gross morphological abnormalities following relatively low-level chronic exposure modeling occupational settings,³⁶⁻⁴⁰ rather than the repeated high-dose “binge” model of abuse exposure.

Noninhalation Models

Gospe and Zhou⁴¹ exposed pregnant rats to a single daily dose of toluene by gavage (650 mg/kg) from gestation day (GD) 6 through GD 19 and found age-dependent reductions in brain development. This level of exposure was thought to produce blood toluene concentrations (BTCs) equivalent to what would be seen had animals been exposed to toluene via inhalation for 3 hours at >4100 ppm. Young offspring had 15% reductions in forebrain cell numbers, which were normalized by weaning. In sharp contrast, reductions in cortical myelination did not emerge until postnatal (PN) day 21, indicating that behavioral sequelae may also not emerge until after weaning.

Inhalation Model

York and colleagues⁴² exposed female Long-Evans rats to 2100 ppm TCE for 6 h/d before mating (5 d/wk for 14 days), or during pregnancy (7 d/wk for 20 days), or both before mating and during pregnancy. Decreases in fetal body weight were observed following gestational exposure alone, while skeletal and soft tissue abnormalities were seen following exposure before and during gestation. Thiel and Chahoud⁴³ exposed pregnant Wistar rats to inhaled concentrations of toluene ranging from 100 ppm to 1200 ppm for 6 h/d from GD9 through GD21 and reported that even the highest concentration of toluene with this exposure regimen did not significantly affect behavior. There were no “clear-cut” significant differences owing to these toluene levels in early maturation or in locomotion, coordination (rotorod), or in an operant discrimination task. Another group of researchers similarly exposed Wistar rats to 1200 ppm toluene for 6 h/d from GD7 to GD18⁴⁴ or to 1800 ppm toluene for 6 h/d from GD7 to GD20.⁴⁵ These exposures were associated with lower pup birth weight and affected early maturation, as revealed in deficits in surface righting at PN3 and in auditory startle at PN13.⁴⁴ However, there were inconsistent effects on open field activity and no effects on rotorod performance or prepulse inhibition of startle.^{44,45} There were also sex-influenced effects on spatial learning in the Morris maze such that toluene increased the latencies to find the hidden platform during both acqui-

sition and reversal training, more so in females than in males,^{44,45} and as the authors in this group noted, these effects were very similar to our prior findings with prenatal alcohol exposure.^{46,47}

Since the timing of the exposure period can influence outcome, animal protocols that pattern exposure to model solvent abuse practices will be able to assess the teratogenic potential of abused inhalants with greater sensitivity and fidelity to the clinical situation than other patterns.⁴⁸ Inhalant abuse is periodic or episodic rather than continuous and most prior prenatal toluene studies used chronic, long-term exposures (ie, >6 hours per day). Solvent abusers reportedly inhale several times a week in brief bouts with some abusers reporting daily use.^{49,50} Previous animal models have varied the temporal pattern of exposure over a restricted range during pregnancy. Compared with continuous lower-dose exposures (eg, 2000 ppm for 17 hours), 2 studies have shown that briefer exposures (60 minutes) of pregnant mice to higher concentrations of TCE (7000 ppm to 8000 ppm) produced similar developmental delays and greater reductions in birth weight and behavioral effects in offspring.^{51,52} Jones and Balster⁵³ approximated an abuse pattern in pregnant mice with inhalations up to 2000 ppm of toluene limited to 3 60-minute periods per day for a 5-day period within the human equivalent of mid second trimester (GD12 to GD17). There were small, transient toluene-induced reductions in pup weight, surface righting, inversion righting, and grip strength.

In our studies,⁵⁴ we used a similar strategy with pregnant rats exposed to repeated 15-minute bouts of very high toluene concentrations (8000 ppm or 12 000 ppm) twice a day from GD8 through GD20 to mimic solvent abuse during pregnancy. Our results demonstrate that these high-concentration prenatal toluene exposure patterns significantly increase the rate of negative postnatal outcome, including decreased body weight and strength, and altered negative geotaxis behavior, and increased numbers of minor malformations, “runting,” and death, while producing little effect on gestation length, litter size, or litter sex ratios.⁵⁴ These deficits in growth were more persistent after our repeated 15-minute binge exposures to high concentrations than were seen with much longer exposures (6 hours) to lower concentrations (1800 ppm) in the few other studies with similar prenatal inhalation exposures,^{43,45,53} even though the apparent total daily exposures were fairly equivalent. Jones and Balster⁵³ exposed pregnant dams to low-level toluene inhalations (200 ppm, 400 ppm, or 2000 ppm) for 3 hours a day (3 × 60-minute exposures with 2000 ppm) limited to GD12 through GD17. This generated a crudely measured high “load” of 6000 ppm/h/d, equivalent to the similarly calculated “load” with our highest dose (2 × 15-minute exposures with 12 000 ppm). Yet with our pattern of inhalation exposure, there were no significant deficits in perinatal outcome

measured as neonatal deaths or minor malformations. While the effects on offspring body weight on PN1 were more modest and effects on negative geotaxis were absent, compared with the effects of the abuse pattern of exposure in the current study the longer exposures were associated with significant decreases in weight gain and in grip strength.⁵⁴ Another study by Thiel and Chahoud⁴³ exposed pregnant Wistar rat dams to toluene inhalations (300 ppm, 600 ppm, 1000 ppm, or 1200 ppm) for 6 h/d from GD9 to GD21. This generated a high “load” of 7200 ppm/h/d, or 120% of the daily “load” with our highest concentration. This study reported lower offspring body weight after their highest doses, only one minor indication of maturational effects, but no other measures were in common with our study. Although the biological relevance of these derived crude indices of exposure (ie, “load” as “ppm/h/d”) in this assessment is uncertain, and there are other differences among the studies (eg, total number of days exposed; strain of rat), the pattern and period of inhalation exposure appear to be very important variables worthy of further investigation. Taken together, these results are consistent with the hypothesis that binge patterns of exposure to high concentrations of toluene may pose a greater risk to fetuses than continuous exposures at lower concentrations, at least for some outcomes.

FUTURE PERSPECTIVES AND CONCLUSIONS

Current epidemiology of inhalant abuse indicates that there is a growing potential for adverse pregnancy outcomes, developmental delays, and neurobehavioral problems in children born to abusers far beyond what might be predicted from continuous lower-level occupational exposure.^{15,16,19} The clinical picture of prenatal exposure to toluene,^{11,19} or to any one of the classes of specific organic solvents and anesthetic gases that are currently and commonly abused, may be confounded by the variety and multiplicity of solvents abused,¹⁸ and by exposures to other substances.³² Despite these difficulties in clinical studies, there are indications of dose- and exposure pattern-dependent effects of solvents, which need to be elaborated.^{15,18} Animal models can be effective in clarifying the developmental risk of exposure to specific organic solvents in the absence of associated health problems and co-drug abuse (especially alcohol), as well as in establishing the importance of dose and critical periods of exposure, issues that appear to require further study.

The routes and patterns of toluene administration used in most studies with animals to date model occupational or environmental exposure and have not captured key pharmacodynamic features of inhalant abuse that can put fetuses at greater risk. Specifically, clinically relevant brief repeated high peak BTCs, compared with relatively constant lower

exposures that may produce the same areas-under-the-curve, and that occur at certain critical periods of perinatal neural maturation in the rat, are likely to cause greater teratogenic damage with life-long biobehavioral consequences. Pregnant solvent abusers are exposing themselves and their fetuses to very high concentrations, inhaling up to 15 000 ppm per binge episode, and our animal model is mimicking these patterns of periodic, acute, very high-dose prenatal toluene exposures necessary to evaluate teratogenic risk of abused toluene—or other related inhaled organic solvents. Noninhalation models can produce BTCs approximating average levels attained via chronic inhalation,⁵⁵ but the pharmacodynamics of intragastric intubation, for example, will yield peak BTCs that vary substantially from acute inhalation because of differential diffusion and absorption, susceptibility to gastric metabolism, access to storage depots, excretion, and the kinetics of repeated administration,⁵⁶ resulting in a different shape to the “dose/concentration-BTC” curve over time, and different outcomes.

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