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The inhalation effects of by-products from chlorination of heated indoor swimming pools on spinal development in pup mice

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ABSTRACT

Introduction: It has been postulated that swimming in heated indoor swimming pools in the first year of life is associated with the development of spinal deformity in children. We explored in pup mice whether exposure to certain disinfection by-products resulting from chlorination of heated pools would affect the future development of the spinal column.

Methods: Mice, from birth and for 28 consecutive days, were exposed to chemicals known to be created by disinfection by-products of indoor heated swimming pools. The study made use of a body fluid analogue and a chlorine source to recreate the conditions found in municipal pools. A cohort of 51 wild-type C57B6 mice, male and female, were divided into two groups: experimental (n = 29) and controls (n = 22). 24 mice were observed for 8 months (32 weeks), with 27 culled at 4 months (16 weeks). Serial CT scanning was used to assess the spines.

Results: Exposure to disinfection by-products resulted in an increase in the normal thoracic kyphotic spinal angle of the mice when compared with their controls at 10 weeks; experimental mice kyphosis range 35–82° versus 29–38° in controls. At 14 weeks the kyphosis of the experimental mice had reduced in size but never to that of the control group.

Conclusion: We have demonstrated the ability to influence spinal development in pup mice through environmental factors and shown that the developmental deformity became evident only after a significant latent period.

1. Introduction

Postnatal development constitutes a vulnerable period in a human's early infant life with regard to the effects of environmental hazards. Subtle effects during development, when the central nervous system (CNS) is both developing and maturing, can lead to functional deficits and increased risk of disease later in life. Barouki et al. (2012) stated that changes can be dependent on cell, tissue type and sex, and may not be apparent until after a latent period, which can last from months to years or decades. The White Paper of 2012 (Srader-Frechette, 2012) stated that children in their early postnatal developmental periods are particularly sensitive to developmental disruption by environmental-chemical exposures, with potentially adverse consequences for health later in life. An environmental assault can influence chemical marks on the DNA and the proteins that make up the genome, thus altering the genes without changing the DNA sequence (Dolinoy and Jirtle, 2008).

Normal growth of the spine can deform on the sagittal and/or coronal planes. McMaster et al. (2006) found in a case-control study a statistically significant association between the introduction of infants to heated indoor swimming pools and the subsequent development of an adolescent idiopathic scoliosis, which is a spinal deformity in the sagittal plane observed at the onset of the skeletal growth spurt in the early teenage years. A neurogenic hypothesis (McMaster, 2011) was proposed for the etiology of the deformity of adolescent idiopathic

List of abbreviations: BFA, Body fluid analogue; CNCl, Cyanogen chloride; CNS, Central nervous system; CSF, Cerebrospinal fluid; CT, Computerized tomography; DPD, N,N-diethyl-p-phenylenediamine; LPS, Lipopolysaccharide; NCl_3, Nitrogen trichloride; THM, Trihalomethane

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scoliosis which postulated that toxins from chlorine in such pools might cross the infant's blood-brain barrier and act deleteriously on the immature CNS, comprising brain and spinal cord.

The primary focus of the study was to assess possible effect of exposure to three chemical disinfection by-products (gases) found in heated indoor swimming pools: nitrogen trichloride (NCl_3), cyanogen chloride (CNCl), and chloroform.

The putative portals of entry into the blood for the chlorine-based chemicals could be dermal, oral, or respiratory. We looked at entry via the respiratory route. Entry of circulating small molecules to the brain would be via the blood-brain barrier, blood-cerebrospinal fluid (CSF) barrier, and the circumventricular organs. Karrow's review (Karrow, 2006) reported that inflammatory stressing during neonatal development, when critical windows of early-life are developing, can alter the programming of the neuroendocrine-immune axis. He states that there is some evidence to suggest that lipopolysaccharide (LPS) may be able indirectly to activate the hypothalamus-pituitary-adrenal axis by binding to LPS receptors on cells within the circumventricular organs and the blood-brain barrier.

During the first 6 months of human life CSF contains higher concentrations of proteins that are immunologically identical to proteins in plasma; this is attributed to mechanisms continued from fetal brain development rather than immaturity (Dzueguekewsja and Saunders, 1988). The clinical significance of this vulnerability might be that a large proportion of toxins which bind to plasma proteins can cross the choroid plexus (Saunders and Dziegielewska, 1998) and diffuse into the CSF, from where they can reach the individual brain structures. There is evidence of such apparent vulnerability of the blood-brain barrier which can occur in developing rodents' brains, particularly given differences in the fragility of cerebral blood vessels and abundance of transport proteins leading to increased permeability (Saunders et al., 2012).

Swimming pools in the UK safeguard public health against horizontal transmission of pathogens, usually by the use of calcium hypochlorite or sodium hypochlorite; however, these same agents may inadvertently be damaging human health. These oxidizers react with microorganisms, endogenous human excretions, and cosmetic chemicals to form a range of disinfection by-products (Kim et al., 2002). This study is concerned with three gaseous compounds found within chlorinated swimming pools: NCl_3, chloroform, and CNCl.

NCl_3 (trichloramine), considered to be the chlorine odor found in swimming pools, has been reported to be an irritant to the upper airways and eyes, with similar irritant potency to chlorine gas (Gagnaire et al., 1994). It is also found to increase hyperpermeability of lung epithelium (Bernard et al., 2006) and thus may weaken the protective nature of the lungs. Chloroform is grouped as a trihalomethane (THM) and known to be a common disinfection by-product. THMs are toxic to humans with repeated exposure and also known to be carcinogenic in rodents (Beddowes et al., 2003). The main exposure method and entry into the body is by inhalation (Aggazzotti, 1993). Finally, CNCl is present in all chlorinated swimming pools (Weaver et al., 2009) and is known to form when chlorine reacts with nitrogenous human excretion compounds such as urine (Weng et al., 2012) and is a registered chemical warfare agent.

The acceptable concentrations of CNCl, chloroform, and NCl_3 are not regulated for the swimming pool industry in the UK. Although there are work exposure limits for CNCl and chloroform, it is not standard practice for routine analysis because of impracticability. Numerous studies have been carried out on adults with regards to disinfection by-products (Gagnaire et al., 1994; Aggazzotti, 1993; Zwiener et al., 2007).

2. Materials and methods

2.1. Animals

All in-vivo experimental procedures were approved by the University of Edinburgh animal ethics committee and performed in compliance with UK Home Office regulations under the Animals (Scientific Procedures) Act 1986. 51 wild-type C57BL/6 mice, male and female, were divided into an experimental group and a control group. Animals came from multiple litters, and were randomly assigned to cage shortly after weaning at 19 days, stratified by sex. The experimental group consisted of 29 animals exposed to disinfection by-products (22 females and seven males). The control group had 22 animals (19 females and three males) that were not treated. We included substantially fewer males in our study, given that adolescent idiopathic scoliosis in humans is predominantly found in females. Animals were housed in unisex cages, with between two and five animals per cage. Control and exposed animals were housed separately. At 6 weeks siblings require separation to present reproduction.

Using an exposure chamber, pup mice together with the dam were placed into the chamber for 2 h a day from birth for 28 consecutive days. Once the gas exposure period was terminated, all animals were kept in ordinary cages (see below) and were left to develop, free from disinfection by-products. Having reached puberty 27 were culled at the age of 4 months (16 weeks). The remaining 24 animals (experimental 15, controls 9) were kept and CT scanned monthly up to the age of 7.5 months (30 weeks), which is equivalent to middle age in humans.

The animals in this study were obtained from in-house breeding colonies. The mice were maintained in polycarbonate boxes (335 cm^2 floor area) with weekly change of bedding of sterilized white Aspen shavings under conditions of 12 h of light and 12 h of darkness, with ambient temperature of 21 °C (± 2 °C) and humidity 55% (± 10%). Mice were given tap water triple filtered and an RMI standard diet of autoclaved pellets (22.4% protein, 4.2% fat, 1.15% calcium, 0.82% phosphorous, trace mineral, and vitamin fortified).

2.2. Chemistry

2.2.1. Chemicals

The concentrations used for this study were chosen by reviewing literature regarding concentrations found within pools around the world (Table 1). We also considered the maximum exposure limits set by the World Health Organization.

Table 1
Concentrations selected for study.

	Literature review average/ existing values in pools (mg/ m^3; ppmV)	Literature review average/ existing values in pools (μg/ L; ppb)	Original work exposure limits	Study concentration aim (mg/m^3)	Study concentration achieved (mg/L)
Nitrogen trichloride (MW: 120.365 g/mol)	0.50	102.0	0.5mgm^{-3} (French ref value)	0.5	0.102
Chloroform (MW: 119.36 g/mol)	0.565	115.78	9.9mgm^{-3} / 8 h	0.5	0.102
Cyanogen chloride (MW: 61.46 g/mol)	0.303	120.80	0.77mgm^{-3} / 15 min	0.2	0.199

MW = molecular weight. ppmV = parts per million per volume, measured as a gas. ppb = parts per billion, measured as a liquid.