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Full Length Article

Continuous exposure to low-frequency noise and carbon disulfide: Combined effects on hearing

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ABSTRACT

Carbon disulfide (CS_2) is used in industry; it has been shown to have neurotoxic effects, causing central and distal axonopathies.However, it is not considered cochleotoxic as it does not affect hair cells in the organ of Corti, and the only auditory effects reported in the literature were confined to the low-frequency region. No reports on the effects of combined exposure to low-frequency noise and CS_2 have been published to date. This article focuses on the effects on rat hearing of combined exposure to noise with increasing concentrations of CS_2 (0, 63,250, and 500 ppm, 6 h per day, 5 days per week, for 4 weeks). The noise used was a low-frequency noise ranging from 0.5 to 2 kHz at an intensity of 106 dB SPL. Auditory function was tested using distortion product oto-acoustic emissions, which mainly reflects the cochlear performances. Exposure to noise alone caused an auditory deficit in a frequency area ranging from 3.6 to 6 kHz. The damaged area was approximately one octave (6 kHz) above the highest frequency of the exposure noise (2.8 kHz); it was a little wider than expected based on the noise spectrum.Consequently, since maximum hearing sensitivity is located around 8 kHz in rats, low-frequency noise exposure can affect the cochlear regions detecting mid-range frequencies. Co-exposure to CS_2 (250-ppm and over) and noise increased the extent of the damaged frequency window since a significant auditory deficit was measured at 9.6 kHz in these conditions.Moreover, the significance at 9.6 kHz increased with the solvent concentrations. Histological data showed that neither hair cells nor ganglion cells were damaged by CS_2. This discrepancy between functional and histological data is discussed. Like most aromatic solvents, carbon disulfide should be considered as a key parameter in hearing conservation régulations.

1. Introduction

Noise exposure is known to cause hearing loss, and the main contributor to occupational hearing loss is exposure to high-intensity noise in the working environment. The permissible threshold limit values for occupational noise in Europe and the United States are 87 dB(A) and 90 dB(A), respectively. The types of injury incurred by the auditory receptor are numerous. For instance, high-intensity noise or impulse noises can damage the stereociliae of hair cells (Carreres Pons et al., 2017; Liberman and Dodds, 1987; Wang et al., 2002); noise can provoke hair cell loss and even collapse of Hensen cells (Campo et al., 2014; Kurabi et al., 2016; Wang et al., 2002); some authors have reported possible Reissner membrane disruptions (Wang et al., 2002); and recently, synaptopathies and swelling underneath the hair cells were linked to temporary and permanent hearing loss (Kobel et al., 2016; Liberman and Kujawa, 2017; Moser et al., 2013; Wang et al., 2002).

Hearing loss induced by low-frequency noises differs from that caused by mid- or high-frequency noises. For instance, hearing deficits caused by low-frequency noise cover a wider frequency window than those induced by mid- and high-frequency noises (Burdick, 1982). Thus, low-frequency noises first cause damage in the cochlear region where low-frequencies are discriminated (apex), then sweep down toward the mid- and high-frequency

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Acronyms

AS	Amplitude Shift of DPOAE levels at the end of exposure
CNS	Central Nervous System
CS2	Carbon Disulfide
dB SPL	Decibel Sound Pressure Level
dB(A)	Decibel weighted A
DPOAEs	Distortion Product Oto-Acoustic Emissions
IHC	Immunohistochemistry
LEX,8h	Equivalent continuous noise level calculated over 8 h
LM	Light microscopy
OHC	Outer Hair Cells
PAS	Permanent Amplitude Shift of DPOAE levels 4 weeks after exposure
PBS	Phosphate Buffer Saline
SDH	Succinate DeHydrogenase
SEM	Scanning electronic microscopy
TTCA	2-Thio-1,3-thiazolidine-4-carboxylic acid
TWA	Time Weighted Average. Average exposure within the workplace using the baseline of an 8-hour day or 40-hour week work schedule

regions (base) (Bohne and Harding, 2000). However, the nature of the damage caused by this type of noise is not well described in the literature.

Carbon disulfide (CS_2) is a volatile, inflammable solvent which is widely used in the production of viscose rayon fibers and cellophane films (Rolecki and Tarkowski, 2000). In industrial viscose production, daily exposures of around 40 ppm have been reported in the literature (Göen et al., 2014; Vanhoorne et al., 1995). These exposure levels are well above the threshold limit values (TWA) authorized in Europe (5 ppm) and the United States, where the OSHA recommends 20 ppm. Currently, based on numerous epidemiological studies, experts recommend even lower limit values, ranging between 1 and 10 ppm (Beauchamp et al., 1983; Newhook and Meek, 2002).

The most common toxic effects of CS_2 reported in the literature are neurofilamentous axonopathies (Llorens, 2013), which can affect both sensory and motor neurons (Hirata et al., 1996; Johnson et al., 1983; Takebayashi et al., 1998). Vascular complications have also been reported (Kotseva et al., 2001; Partanen et al., 1970; Sulsky et al., 2002). In rats, an abnormal accumulation of neurofilaments in the long axons of the peripheral and central nervous systems has been observed (Clerici and Fechter, 1991; Gottfried et al., 1985; Knobloch et al., 1979; Pappolla et al., 1987). Rebert and Becker, 1986, and Hirata et al., 1992, demonstrated CS_2-induced axonopathies in peripheral and central auditory fibers in rats. In occupational environments, CS_2 exposure is most frequently associated with co-exposure to noise, and significant hearing loss is often found in co-exposed workers. According to Chang et al., 2003, Morata, 1989, hearing loss is more frequent and severe in cases of co-exposure than with exposure to noise alone. Similar effects have been observed with other aromatic solvents, and experimental studies in rats clearly demonstrated synergistic effects on hearing of co-exposure to noise (Campo et al., 2013; Chen and Henderson, 2009; Pouyatos et al., 2005; Venet et al., 2015). Very few studies have been performed to date in humans (Chang et al., 2003; Morata, 1989) combining exposure to noise and CS_2, and no histological analyses have been performed in rats.

For these reasons, the main purpose of the current investigation was to analyze how co-exposure to a low-frequency noise and a range of CS_2 concentrations affected hearing in rats. The impact of the exposure scenario on hearing was assessed using distortion product oto-acoustic emissions (DPOAEs) which reflect outer hair cell (OHC) motility (Avan et al., 2001). OHC are innervated by afferent nerve fibers (10%) and efferent nerve fibers (90%) which control their mechanical activity and determine frequency discrimination (Dannhof and Bruns, 1993). Functional investigations were complemented by a morphological analysis of the cochlea. In addition, 2-thiothiazolidine-4-carboxylic acid (TTCA) was assayed in rat urine as it is known to be the best urinary metabolic indicator of CS_2 exposure in both humans (Riihimäki et al., 1992) and rats (Cox et al., 1996). Circulating CS_2 levels were also determined in blood.

The results obtained are discussed with regard to current occupational threshold limits which are supposed to regulate the risks encountered by all workers, including co-exposed workers.

2. Materials and methods

2.1. Animals

The animal facilities where experiments were performed are fully accredited by the French Ministry of Agriculture (authorization No. D 54–547-10). While conducting the research described in this article, investigators adhered to the Guide for Care and Use of Laboratory Animals promulgated by the European Parliament and of the Council (DIRECTIVE 2010/63/EU, 2010). The study, referenced as APAFIS#3950-201602051 1372481, was approved by the ethics committee at the Ministry of Education and Research. Adult female Long Evans rats (n = 117) weighing approximately 250 g

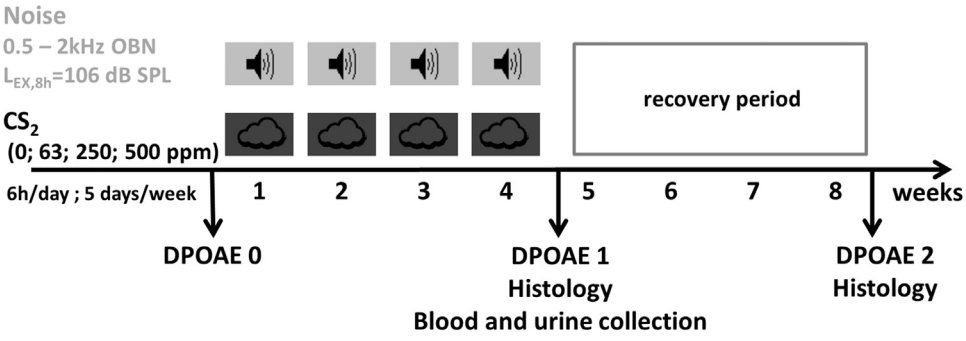

Fig. 1. Experimental protocol. Exposure to carbon disulfide (CS_2) and noise lasted 6 h/day, 5 days/week, for 4 weeks. The $L_{EX,8h}$ for the noise was 105 dB SPL and the spectrum was an octave band noise (OBN) ranging from 0.5 to 2 kHz. The CS_2 concentration was 0, 63, 250 or 500 ppm. Hearing was tested using cubic distortion product oto-acoustic emissions (DPOAEs) prior to exposure (DPOAE0), at the end of exposure (DPOAE1), and 4 weeks after exposure (DPOAE2). Blood and urine samples were collected at the end of the exposure period. Histological analyses were performed at the end of the exposure period and 4 weeks post-exposure.