Hearing loss in rats from combined exposure to carbon monoxide, toluene and impulsive noise

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INTRODUCTION

In combined exposure with ototoxic chemicals, potentiation of noise induced hearing loss (NIHL) may certainly be a hazard (Sliwinska-Kowalska et al. 2007). However, as impulsive noise has the potential to induce hearing loss even at low levels of daily noise exposure ($L_{EX,8h}$), the greatest risk for hearing loss from combined exposures seems to be from simultaneous exposure to ototoxic chemicals and impulsive noise. Toluene exposure may cause hearing loss in rats at high levels of exposure without exposure to noise, but in combination with exposure to noise synergistic interaction may potentiate the hearing loss, especially in combined exposure to impulsive noise (Lund & Kristiansen 2008). The mechanisms involved in the ototoxicity of toluene and other aromatic organic solvents have not been fully elucidated, but toluene may act to impair the auditory medial efferent system, thereby augmenting the acoustic energy absorbed by the cochlea in response to the noise exposure (Lataye et al. 2007). However, in another experiment toluene treatment did not modify the responses in the cochlea in rats with non-functional middle ear muscles, although toluene did instead inhibit the action of the middle ear reflex, possibly by their anticholinergic effect on the efferent motor neurons (Campo et al. 2007). Altogether, exposure to organic solvents appears in general to have additive rather than synergistic effects in combined exposure with noise, while asphyxiants like carbon monoxide (CO) appear capable of true synergistic effects on NIHL (Fechter 2004). CO exposure by itself does not seem to have persisting effects on the hearing of rats, but it does potentiate the effects of NIHL at exposure levels of 500 ppm and higher. The potentiation of noise by CO may not be related to a specific effect of CO on the auditory cells, but may instead reduce the cell's ability to repair the noise induced damage (Chen & Fechter 1999). The combined effect of impulsive noise and both toluene and CO may reveal the full potential for NIHL from impulse noise exposure, because the functional protective mechanisms as well as the repair processes may be hampered. Nevertheless, this combination of exposures does appear to be a rather realistic scenario in the working environment. In order to test this hypothesis, groups of rats were exposed to impulsive noise, CO and toluene. The hearing was tested before and after exposure by assessment oto-acoustic emissions over 30 frequencies between 1 and 70 kHz, and cochleograms was made on 3-4 animals in each group.

METHODS

The animal welfare committee, appointed by the Danish Ministry of Justice, has granted ethical permission for the studies. All the procedures were carried out in compliance with the EC Directive 86/609/EEC and with the Danish law regulating experiments on animals. The exposures of the rats were performed in dedicated 1200 l inhalation chambers with walls made of stainless steel and glass. The airflow

is driven by a radial fan at the outlet, giving a slight negative pressure within the chambers, eliminating possible leakage of toluene or CO to the surroundings. The air exchange rate was 12 per hour, air temperature 20 ± 2 ºC and humidity 55 ± 10 %RH. To be exposed, the rats were transferred daily from their home cages to the inhalation chambers and kept in pairs in wire mesh cages without access to food and drinking water. All groups were exposed to CO and/or toluene 8 hours a day for 10 days, but only to noise 6 hours a day, starting 2 hours after the onset of the chemical exposure. CO was fed to the air inlet of the chamber, under the control of simple flow meters (Porter). The CO concentration was measured with an infrared gas cell spectrophotometer (Foxboro MIRAN-1A) in one chamber every 5 minutes, automatically changing from chamber to chamber. The toluene and the noise exposure have been described recently (Lund & Kristiansen 2008), and only a brief description will be given in the following. Toluene (purity >99.5 % GC; CAS-No. [108-88-3]) was evaporated in the air inlet of each exposure chamber by individual HPLC-pumps feeding the toluene to the top of glass spirals, which were slightly heated by circulating water. The toluene concentration in the chambers was measured with an infrared gas cell spectrophotometer. Noise was generated by a PC with a 16-bit D/A-converter board, amplified by audio amplifiers, and delivered by dome tweeters located above each cage. The noise exposure used was a mixture of impulse and Wide band noise (WBN), with the main energy (75 %) as impulsive noise, composed of sound impulses with a peak level just above 130 dB, and the different levels of noise were generated by varying the interval between the impulses. The sound field was measured at various points at the floor level of the cages with a ½" condenser microphone (B&K4133) and a spectrum analyzer (HP35670A) by averaging a large number of samples (4096 or more). Further analyses of the noise exposures (Leq) were made on time samples of several minutes at 100 kHz sampling frequency. The impulse frequency distribution of the impulse noise was somewhat unequal due to a slightly higher energy level toward the lower frequencies, and the level varied up to ±1.5 dB between measuring points within the sound field. In order to find the lowest-observed-adverse effect level (LOAEL) between exposure to noise and CO, two groups of rats (n=12) were exposed to CO (0 ppm and 500 ppm) and either L_{eq8h} = 81 dB or L_{eq8h} = 84 dB SPL impulsive noise. Further, groups of rats (n=12) were exposed to impulsive noise (L_{eq8h} = 84 dB SPL), CO (0 ppm, 300 ppm and 500 ppm) and toluene (0 ppm, 500 ppm and 1000 ppm). The hearing was tested before and after exposure by assessment of DP-grams, i.e. the cubic distortion product (CDP) from distortion product oto-acoustic emissions (DPOAE) over 30 frequencies of f2 between 1 and 70 kHz (Lund and Kristiansen 2008). The DP-grams was made at a fixed ratio of the primaries (f2=f1x13/16=1.23) and fixed levels of stimulation (L1=L2+10 dB=60 dB SPL). Further, cochleograms (not shown) were made on 3-4 animals in each group.

RESULTS

Exposure to the impulsive noise alone at L_{eq8h} = 81 dB had little effect on hearing, while the effects were barely notably at exposure to L_{eq8h} = 84 dB SPL. However, when combined with exposure to CO, the hearing impairment at the latter noise level was statistically significant from the group without CO exposure at the 500 ppm level (see Figure 1).

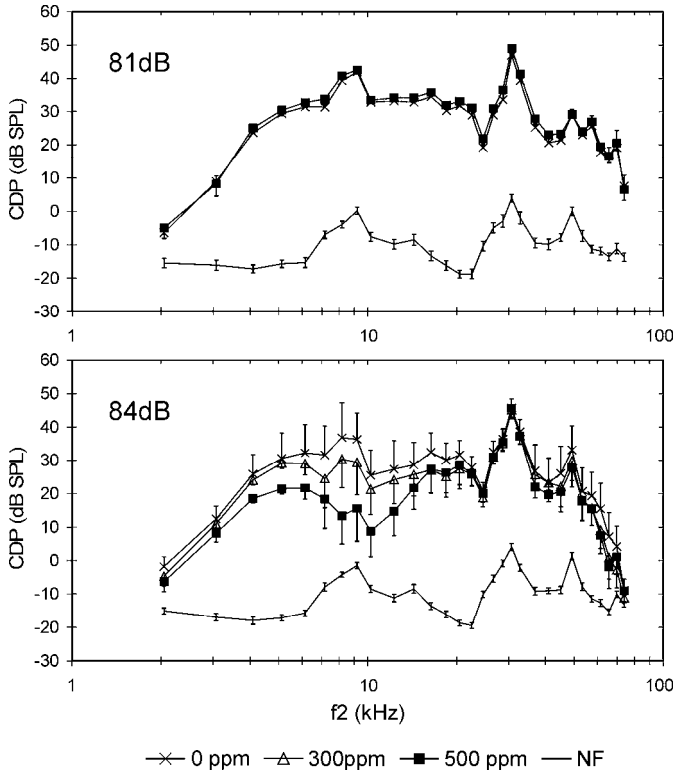

Figure 1: DP-grams (f2/f1= 1.23; L1= 60 dB and L2= 50dB SPL) from group of rats two weeks after the end of 10 days combined exposure to either 0, or 500 ppm carbon monoxide (CO) and impulsive noise exposure at $L_{eq8hours}$ = 81 dB SPL (Top Panel), and 0 ppm, 300 ppm or 500 ppm CO and impulsive noise exposure at $L_{eq8hours}$ = 84 dB SPL (Bottom Panel). Each point marks the mean with indication of 95 % CI, and NF denotes the noise floor, determined by the mean value in the frequency bins next to the CDP. No effects from combined exposure are observed at the 81 dB noise level, but clear dose-dependant effects can be seen at the 84 dB level.

The main effect of the noise exposure occurs within the 8-10 kHz frequency range, but the frequencies above 30 kHz seem also to be slightly affected. Further, addition of toluene exposure without CO did not increase the hearing impairment from the impulsive noise, but the addition of CO to the toluene exposure, did increase the hearing loss considerably. The dose-effect relationship seems to be rather complex (see Figure 2): Both CO and toluene exposure potentiated the effects of the 84 dB impulsive noise exposure, although not in fully dose-dependant manner. The toluene exposure without CO did not potentiate the effects of the impulsive noise exposure, but with addition of 300 ppm CO, there was clear potentiation at both levels of toluene exposure. Increasing the CO level to 500 ppm only exacerbated the effects of the 1000 ppm toluene exposure, but not the effects of the 500 ppm toluene exposure.

DISCUSSION

The exposure to impulsive noise at L_{eq8h} = 81 dB SPL for 10 days did not induce notably hearing impairment even in combined exposure with 500 ppm CO. This CO level has previously been shown to be the LOAEL for elevations of NIHL in combined exposure with steady state noise (Fechter 2004). In the exposure to impulsive noise at L_{eq8h} = 84 dB SPL there was an effect of the impulsive noise exposure.

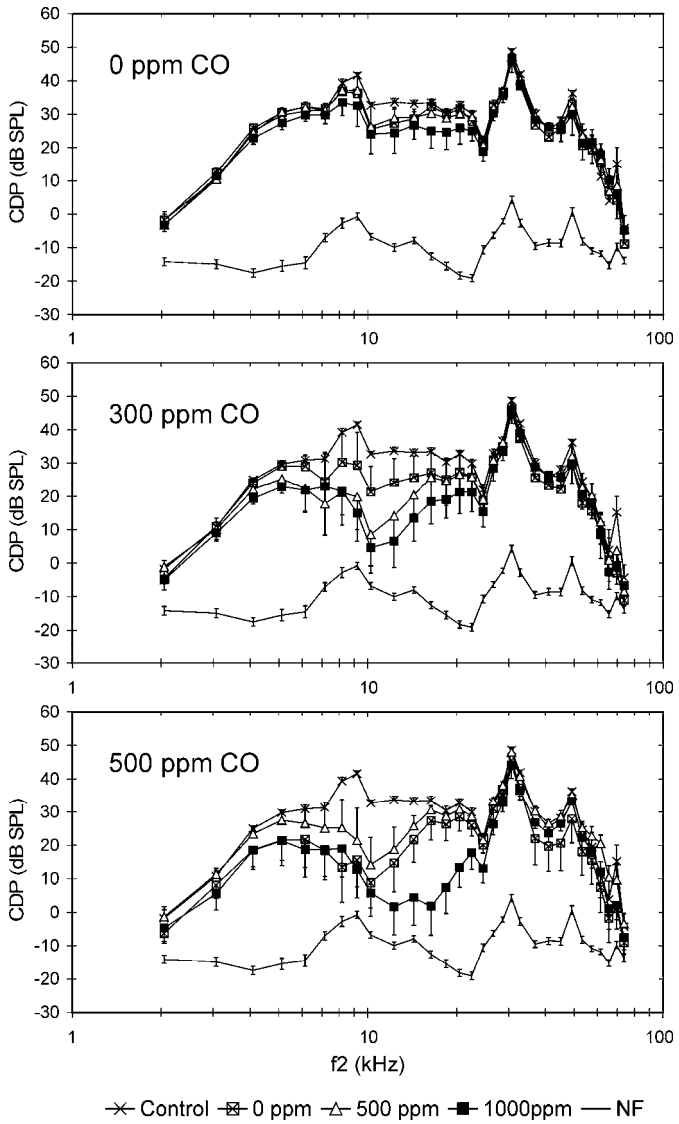

Figure 2: DP-grams (f2/f1= 1.23; L1= 60 dB and L2= 50dB SPL) from groups of rats two weeks after the end of 10 days combined exposure to impulsive noise ($L_{eq8hours}$ = 84 dB SPL), carbon monoxide (CO) in at levels of either 0 ppm (Top panel), 300 ppm (Middle panel) or 500 ppm (Bottom panel) and either 0 ppm, 500 ppm or 1000 ppm toluene. Each point marks the mean with indication of 95 % CI, and NF denotes the noise floor, determined by the mean value in the frequency bins next to the CDP. For comparison, a completely unexposed control group is also shown in each panel.

However, neither 500 ppm nor 1000 ppm toluene exposure did increase the noise induced hearing impairment, while 300 ppm as well as 500 ppm CO did seem to potentiate the effects from the impulsive noise exposure, but the effect at the 300 ppm CO was borderline, thereby confirming the mentioned 500 ppm LOAEL of CO in combined exposure with noise. However, as CO was added to the exposure to both toluene and impulsive noise, there was obviously a clear potentiation of the hearing impairment. The effects of interaction were for both chemicals synergistic at certain levels of exposure. The mechanisms of action previously described for CO (Chen & Fechter 1999) and toluene (Campo et al. 2007; Lataye et al. 2007) are consistent with data from the present study. The relative high levels of toluene necessary to induce potentiation in experimental studies with rats compared to the present day exposure in the working environment is related to the inactivity of the rats during exposure, but if the rats are forced to work during exposure, the same effects may be expected at approximately 50 % reduction in the organic solvent exposure (Lataye et al. 2005). Overall, the present study demonstrated that impulsive noise exposure at

L_{eq8h} = 84 dB SPL has little or no margin of safety, and even if the occupational exposure limits are not exceeded, concomitant exposure to ototoxic chemicals may increase the hazards of impulsive noise exposure considerably.

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