TOXICOLOGICAL SCIENCES **58,** 315–323 (2000)

Predicting Exposure Conditions that Facilitate the Potentiation of Noise-Induced Hearing Loss by Carbon Monoxide

Laurence D. Fechter,[1] Guang-Di Chen, Deepa Rao, and Jason Larabee

Center for Toxicology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73190

Received July 7, 2000; accepted September 5, 2000

Hearing loss is the most common occupational disease in the United States, with noise serving as the presumed causative agent in most instances. This investigation characterizes the exposure conditions that facilitate the potentiation of noise-induced hearing loss (NIHL) by carbon monoxide (CO). Auditory function was compared in rats exposed 4 weeks earlier to noise alone, CO alone, combined exposure, and air in the exposure chamber. This interval between exposure and auditory threshold assessment was selected to permit recovery of temporary threshold shifts. The compound action potential (CAP) threshold evoked by pure tone stimuli was used as a measure of auditory sensitivity. The no adverse effect level (NOAEL) with respect to potentiation of NIHL was found to be 300 ppm CO. Potentiation of NIHL by CO increases linearly as CO concentration increases between 500 –1500 ppm. Benchmark dose software (version 1.1B) published by the U.S. EPA National Center for Environmental Assessment was employed to determine a benchmark concentration of CO that produced either a 5-dB potentiation of NIHL or an increase in auditory threshold equivalent to 10% of the effect of noise alone. The lower bound for these benchmark concentrations were 320 and 194 ppm CO, respectively. Unlike CO dose, the relationship between noise severity and potentiation of NIHL by CO shows a nonlinear relationship. The greatest potentiation was observed at moderate noise exposures (100 dB, 2-h, octave band–limited noise, or OBN) that produce limited permanent threshold shifts. Repeated exposures to 95-dB noise for 2-h periods in combination with 1200 ppm CO also yielded potentiation of NIHL, though such effects were not observed following a single combined exposure. These results underscore the potential risk of hearing loss from combined exposure to noise and CO, and the risks associated with repeated exposure.

Key Words: **carbon monoxide; ototoxicity; chemical asphyxiants; noise; risk assessment; complex exposures; rat.**

Noise-induced hearing loss (NIHL) is the most common occupational disease in the United States (NIOSH, 1996); approximately 30 million workers are exposed to potentially hazardous noise levels in the workplace (Franks *et al.,* 1996). OSHA has established a permissible exposure level (PEL) in the Hearing Conservation Amendment (OSHA, 1981) to the standard for occupational exposure to noise designed to protect workers against NIHL. The Amendment specifies an action level of 85dB (A) with impact noise levels factored into the dose. In addition, sound levels of 90 dB (A) are permitted based upon an 8-h average exposure. As noise duration decreases from continuous 8-h exposure, a 5-dB trade-off is applied when duration of noise is halved. Thus, exposures of 90 dB (A) would be permitted for a duration of 8 h, and a level of 95 dB (A) would be permitted for a duration of 4 h. The maximum permitted level is 115 dB (A) for continuous noise. The A-weighting used for measuring sound intensity is designed to reflect the shape of the human audiogram at moderate noise levels, with its sensitivity between approximately 20 Hz and 16 kHz.

One potential risk factor for the occurrence of significant hearing loss even under conditions of relatively low noise exposure is the influence of other environmental agents present along with noise. Many chemical contaminants are ototoxic. These include organic solvents (Campo *et al.,* 1997; Crofton *et al.,* 1994; Crofton and Zhao, 1993, 1997; Fechter *et al*. 1998; Johnson *et al.,* 1988; Morata *et al.,* 1993, 1994, 1997 a, b); metals (Fechter *et al.,* 1992; Rice and Gilbert, 1992; Schwartz and Otto, 1991; Wu *et al.,* 1985); chemical asphyxiants (Chen and Fechter, 1999; Chen *et al.,* 1999; Fechter *et al.,* 1988; Fechter, 1989; Young *et al.,* 1987); and prescription drugs (c.f. Rybak, 1986). Simultaneous and even successive exposure to certain of these agents along with noise can increase greatly susceptibility to NIHL (Chen and Fechter, 1999; Chen *et al.,* 1999; Fechter *et al.,* 1988; Johnson *et al.,* 1988, 1990; Johnson, 1993, Lataye and Campo, 1997; Morata *et al,* 1993).

The risk of NIHL under noise exposure conditions that might appear to be safe is underscored by laboratory studies demonstrating the potentiation of NIHL by CO exposure in rats (Fechter *et al.,* 1988; Fechter, 1989; Young *et al.,* 1987). In those studies, broadband noise exposure that, by itself, did not produce an auditory threshold shift produced significant auditory impairment when CO was presented simultaneously with noise. CO by itself has no permanent effects on auditory

[1] To whom correspondence should be addressed at The University of Oklahoma, Health Sciences Center, College of Pharmacy, P.O. Box 26901, Oklahoma City, OK 73190. Fax: (405) 271-7477. E-mail: fechter@ouhsc.edu.

sensitivity (Fechter *et al.*, 1988; Fechter, 1989; Young *et al.*, 1987).

The research presented here evaluates the relationships between noise severity and CO concentration on potentiation of NIHL in pigmented rats. The results of three different experiments are reported. In experiment one, we investigated the effect of CO concentration on NIHL induced by 100-dB OBN over an 8-h exposure period. The objective was to determine a function relating CO concentration to potentiation of NIHL so that prediction of the CO concentration at which hearing impairment began could be made. This process of risk assessment is critical in estimating a reference concentration that can be considered safe for human exposure.

In the second experiment, the role of noise severity in identifying potentiation of NIHL was evaluated. Subjects were exposed to a single concentration of CO in order to determine how the potentiation of NIHL changes as noise exposure becomes more severe. This is important because the 5-dB trade-off between noise intensity and doubling of noise duration used by OSHA might not hold during a mixed exposure to two agents. The objective here was parallel to that addressed in the first experiment, but focused on the role of noise severity (intensity and duration) in determining the potentiating effects of CO.

Finally, in experiment three, subjects were repeatedly subjected to combined noise and CO exposure over successive days at levels of CO and noise that did not affect auditory function when presented on a single occasion. This was done in order to determine whether repeated exposure might yield adverse effects. The study of repeated exposure is important in developing a model for certain occupational exposures to noise and CO and for understanding mechanisms that might be involved in the potentiation phenomenon.

MATERIALS AND METHODS

Subjects. A total of 144 Long-Evans male pigmented rats, 2–3 months of age, obtained from Harlan Sprague Dawley were employed for all experiments. The subjects were housed with free access to food and water in their home cages. Background sound levels in the colony room were below 50 dB (A) with sound levels in octave bands centered at 2 kHz and above registering 39 dB and lower. Thus the background sound in the colony was primarily low in frequency. Temperature was maintained at 21 ± 1°C. Lights were on from 0630 to 1830. All exposures and testing were performed during the daytime.

Exposure procedure. The basic experimental design employed a minimum of four treatment groups consisting of noise exposure alone, CO exposure alone, combined exposure to noise + CO, and a control condition that entailed placement of subjects in the exposure chamber and no CO. Octave band analysis showed that noise levels in the exposure chambers when no noise was intentionally added were below 35 dB for all octave bands with center frequency of 2 kHz and higher. Auditory function was assessed 4 weeks following the exposure in order to determine whether permanent impairment of cochlear function resulted.

Subjects were assigned randomly to treatment groups. Exposures were conducted in a reverberant 40-liter glass cylinder equipped with stereo speakers for delivering sound, a Quest 1-in. microphone and sound level meter for monitoring sound, and a CO monitor (Industrial Scientific) for measuring the chamber gas concentration. The subjects were placed within small wirecloth enclosures (15 × 13 × 11 cm) within the chamber. They were conscious and free to move within the enclosures.

Noise exposure. Broadband noise was generated by a function generator (Stanford Research System, Model DS335) and bandpass filtered (Frequency Devices, 9002) to provide an OBN with center frequency of 13.6 kHz. The roll-off for the filter system was 48 dB/octave. This octave band was selected because it results in a clear preferential disruption of high-frequency auditory function while preserving lower-frequency auditory function intact (Chen and Fechter, 1999; Chen *et al.*, 1999). The preservation of normal auditory function at low frequencies provides a useful control for determining that the electrode for recording auditory threshold is positioned correctly on the cochlea and that the sensitivity of the equipment is within normal specifications.

The noise was amplified by a power amplifier (SAE 2200) and delivered to two tweeters (Vifa D25AG-05-06, 709) in the exposure chamber. The animal cages were located under the speakers with a vertical distance (to cage floor) of about 18 cm. Noise intensity was measured with a Quest sound level meter (model 215) using a linear frequency response. The noise level varied less than 2 dB within the space accessible to the subjects.

CO exposure. Air exchange rate in the exposure chamber was 8.5 l/min (approximately one air change every 5 min), which was monitored by a Top Trak 821-1-PS mass flow meter. The CO concentration in the exposure chamber reached the desired level within 30 min of exposure onset. Previous studies showed that carboxyhemoglobin levels approach steady state within 30 min of exposure onset (Chen and Fechter, 1999) and have stabilized by 90 min of exposure. Therefore, CO exposure began for the appropriate subjects 90 min prior to the onset of noise to assure equilibration of carboxyhemoglobin. Noise exposure durations were 8 h long in the initial CO dose-response study and varied between 2 and 8 h in the second experiment that investigated the interaction of noise severity with CO. A noise regimen (95 dB for 2 h) was then selected from that second study that did not produce NIHL alone or in combination with CO. This exposure was used on 5 successive days in experiment three to assess the effects of repeated exposure. The total daily exposure time to CO and air exposure time varied, therefore, between 3.5 and 9.5 h based on experimental design.

Assessment of cochlear function. Four weeks following exposure, a time interval designed to permit recovery of temporary threshold shift (Chen and Fechter, 1999), auditory thresholds were assessed in all subjects. The subjects were anaesthetized with xylazine (13 mg/kg, i.m.) and ketamine (87 mg/kg, i.m.), and normal body temperature was maintained using a DC heating unit built into the surgical table. The temperature of the cochlea was also maintained using a low-voltage high-intensity lamp. The auditory bulla was opened via a ventrolateral approach to allow the placement of a silver wire electrode onto the round window. A silver chloride reference electrode was inserted into the neck muscle. The CAP signals evoked by pure tones were amplified 1000× between 0.1 and 1.0 kHz with a Grass A.C. preamplifier (Model P15). The sound level necessary to generate a visually detectable CAP response on a digital oscilloscope (approximate response amplitude of 1μV) was identified. The CAP response was not averaged.

Pure tones for eliciting CAP were generated by a SR530 lock-in amplifier (Stanford Research Systems, Inc.). A programmable attenuator controlled the tone intensity, and the output of the attenuator was amplified by a high-voltage amplifier and then delivered to the sound transducer in the rat's external auditory meatus. Auditory thresholds were determined for tones of 2, 4, 6, 8, 12, 16, 20, 24, 30, 35, and 40 kHz using tone bursts of 10-msec duration with a rise/fall time of 1.0 msec. The repetition rate of the tone bursts was 9.7 times/sec. Sound levels at all the testing frequencies were calibrated with a $\frac{1}{2}''$ probe microphone (ACO Pacific model #4015) located near the eardrum. This microphone, in turn, was referenced to a B&K $\frac{1}{4}''$ microphone (model 4136) that has a flat frequency response up to 70 kHz. This cross referencing of the $\frac{1}{2}''$ probe microphone to the $\frac{1}{4}''$ microphone was achieved by making successive measurements of tone intensity with each microphone coupled to the sound

source and creating a computer-based look-up table for subsequent calibrations within the subject's ear. Calibrations were conducted for each subject.

Characterization of the CO dose response for potentiation of NIHL. Five groups of eight subjects each were exposed for 8 h to both noise (100 dB OBN) and to CO (300, 500, 700, 1200, and 1500 ppm). Three additional groups (n = 8) were exposed to the noise alone, to 1200 ppm CO alone, or a control exposure in the chamber without noise and CO present. The potentiation of NIHL in the frequency range between 12 and 40 kHz was evaluated by determining the difference between the mean auditory threshold among subjects exposed to noise alone and each subject that received noise + CO exposure. This frequency range was selected because the lower bound corresponded closely to the lower bound of the OBN exposure used, and because previous research (Chen and Fechter, 1999) has shown that high-frequency auditory function is impaired by combined exposure to noise + CO. The potentiation of NIHL by different CO concentrations was evaluated using benchmark dose software (version 1.1B) published by the U.S. EPA National Center for Environmental Assessment. A continuous linear model was employed to determine a benchmark concentration of CO that produced either a 5-dB potentiation of NIHL or an increase in auditory threshold equivalent to 10% of the effect of noise alone. The criterion of a 5-dB elevation in threshold was selected because it approaches the smallest reliable difference that could be readily determined using our threshold recording procedure. The alternative criterion, disruption of auditory threshold equivalent to 10% of the effect of noise alone, was chosen because it corresponds to a more familiar criterion for estimating a benchmark dose (MacPhail and Glowa, 1999).

Alteration of noise severity. Eight groups of six subjects each were employed in this study. Three groups were exposed to 1200 ppm CO in combination with the OBN for 2 h (95 and 100 dB) or for 4 h (100 dB). The additional groups received CO alone (1200 ppm for 4 h) and each of the three noise conditions alone. Results were compared to an unexposed control group. For comparison, the PEL to noise in humans is 90 dB (A) for 8 h, and the equivalent values for shorter duration exposure are 95 dB (A) and 100 dB (A) for 4 and 2 h, respectively.

Assessment of repeated CO-noise exposures. Four groups of eight subjects each were exposed to CO alone, noise alone, combined exposure, or air exposure for 5 successive days. The CO level was 1200 ppm and the noise exposure was OBN for 2 h (95 dB).

Statistical analysis. The data from each experiment were analyzed using split-plot ANOVA in which treatment served as a between-subjects variable and frequency was evaluated as a within-subjects variable. Pairwise comparisons were made between treatment groups using Scheffe's tests. Because the focus of this research is on potentiation of noise-induced hearing loss by CO exposure, these effects were evaluated using orthogonal comparisons within stepdown analyses of variance. Statistical comparisons were also undertaken to determine whether CO exposure by itself impaired auditory sensitivity compared to the air-exposed control group.

RESULTS

Figure 1 presents the CAP thresholds obtained for groups of subjects receiving OBN alone, 1200 ppm CO alone, noise + CO at each of six levels of CO, and air-exposed controls. In general, the audiograms from all groups are parallel within the lower frequency range, whereas those for noise alone and noise + CO are elevated at higher frequencies. Among control subjects, the greatest auditory sensitivity is found at 12 kHz, with a loss in sensitivity at higher and lower test frequencies. It is apparent from inspection of this figure that CO by itself does not alter auditory function 4 weeks after exposure relative to the untreated control group. Also, the effects of noise treatment alone are comparable to control subjects for sound frequencies below 10 kHz and show a distinct elevation at higher frequencies. The average shift in auditory threshold due to noise varied between approximately 20 and 40 dB (A) at frequencies between 12 and 40 kHz. Finally, as CO dose increases, potentiation of NIHL is observed to occur in a dose-dependent fashion. Potentiation is very limited at 300 ppm (Fig. 1A), becomes more apparent at 500 ppm with an increase in threshold above the noise-exposed group of approximately 5–10 dB (Fig. 1B), and is quite distinct with CO exposure of 700 ppm and higher. The potentiation observed at 1500 ppm is as great as 30 dB above the effect of noise alone. In addition, as CO concentration increases, especially at 1200 ppm and above, the auditory impairment appears to broaden to lower test frequencies.

The global analysis of variance demonstrates a significant treatment effect ($F_{8/80} = 60.55$, $p < 0.0001$) and a significant interaction between treatment and test frequency ($F_{80/630} = 14.09$, $p < 0.0001$). Therefore, stepdown analyses of variance were run to compare the effects of each CO concentration upon NIHL. Differences between subjects exposed to noise alone and noise +CO at each concentration were assessed using planned orthogonal comparisons. Although auditory thresholds among subjects receiving noise + 300 ppm CO did not differ significantly from subjects receiving noise alone ($T = 1.1424$, $p > 0.05$), all groups receiving higher CO concentrations simultaneously with noise exposure did differ significantly from the noise-only group (500 ppm $T = 2.22$, $p < 0.05$; 700 ppm $T = 2.29$, $p < 0.03$; 1000 ppm $T = 3.614$, $p < 0.0015$; 1200 ppm $T = 5.2568$, $p < 0.0001$; 1500 ppm $T = 9.309$, $p < 0.00001$).

Figure 2 provides a graphical representation of the additional loss in auditory threshold sensitivity shown by subjects exposed to noise + CO compared to the mean group threshold of those subjects receiving noise exposure alone. The threshold loss is averaged across high frequencies (12–40 kHz) as a function of CO concentration. The relationship between extent of potentiation of NIHL and the CO concentration combined with the noise exposure is estimated using linear regression generated by the benchmark dose software. Two different analyses are presented. First, a linear continuous model was run using an absolute shift in auditory function of 5 dB as the criterion for estimating the benchmark CO concentration. The second analysis estimated the benchmark CO concentration yielding a 10% increment in the effect of noise alone. The benchmark concentrations using these two methods are 388 and 236 ppm, respectively. The lower bound of the benchmark concentration with 0.95 confidence levels are 320 and 195 ppm, respectively.

In contrast to the direct linear relationship observed between potentiation of NIHL and CO concentration, increasing the total noise energy level (sound intensity and exposure duration) does not have comparable effects on the potentiation of im-

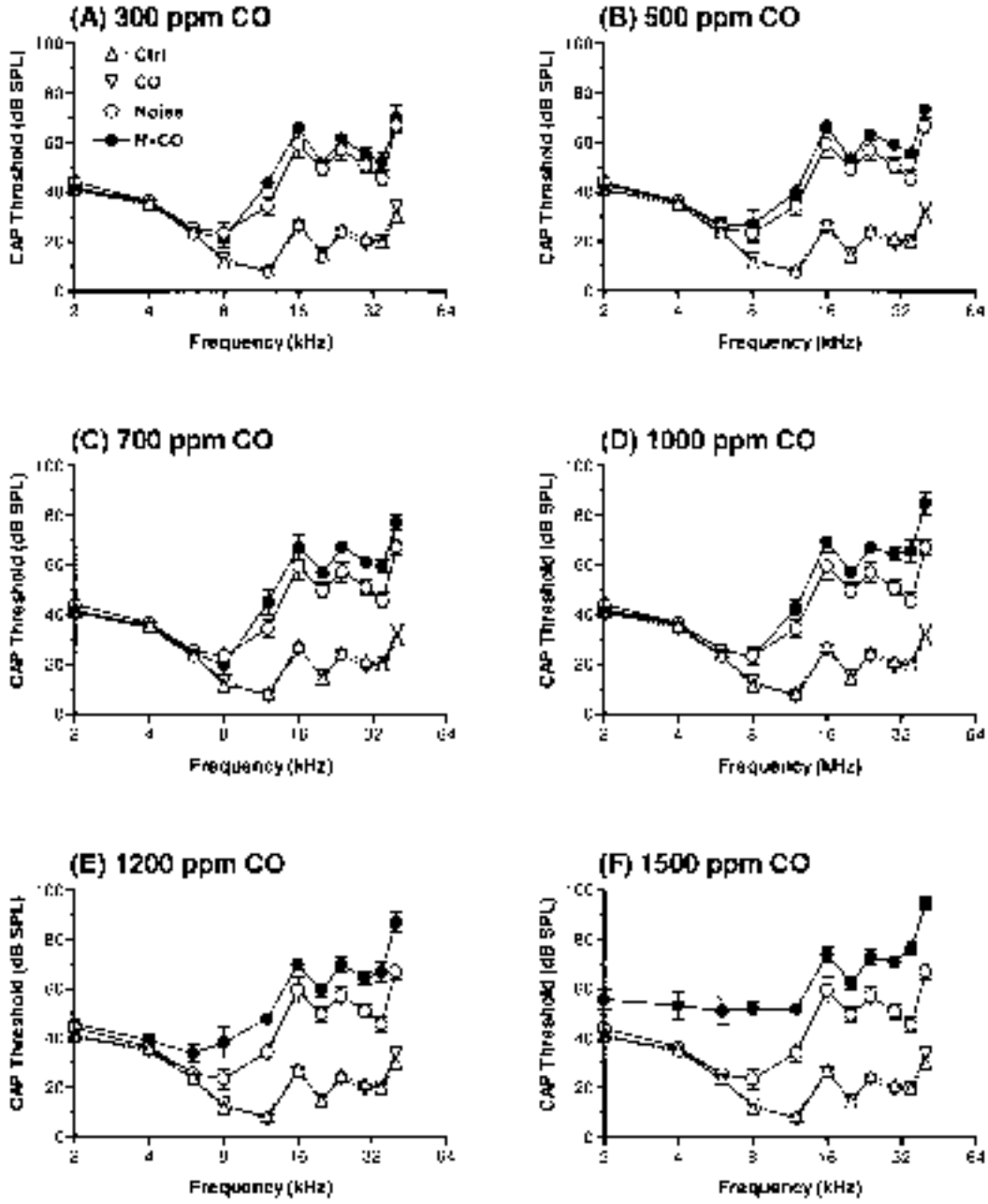

FIG. 1. Pure tone auditory thresholds for subjects exposed to a fixed noise level (100 dB, OBN, 8 h) alone or in combination with CO at concentrations of 300–1500 ppm. Auditory thresholds in subjects receiving 1200 ppm CO alone and untreated control subjects are also represented. All thresholds were recorded 4 weeks following experimental exposures. The NOAEL is 300 ppm; the LOAEL is 500 ppm. Data represent the mean ± SEM.

pairment resulting from simultaneous exposure to noise + CO (Fig. 3). For subjects that receive noise only, auditory thresholds increase linearly in direct relationship to total noise energy at frequencies of 12 kHz and above (Fig. 3). For the mildest noise exposure, impairments in auditory function do not exceed 10 dB and are typically at or below 5 dB relative to the untreated controls (Fig. 3A). For moderate noise exposure, auditory thresholds are elevated 10–20 dB above those seen in air control subjects (Fig. 3B). Finally, under the most severe noise exposure condition, auditory impairments of 30–40 dB are observed for all frequencies above 12 kHz (Fig. 3C). A linear regression analysis shows that noise severity accounted for 99% of the variability in threshold scores among subjects receiving noise treatment alone. The slope obtained for this line of best fit is significantly different from zero ($p < 0.05$).

The influence of noise severity on the combined effect of

noise + CO is also represented in Figure 3. The auditory thresholds recorded in subjects receiving the mildest combined noise + CO exposure show a minimal increase (generally 5 dB or less) in impairment beyond that observed in subjects exposed to this noise exposure alone (Fig. 3A).

At the intermediate level of noise severity (100 dB for 2 h), a clear potentiation of NIHL by CO is observed, ranging up to 30 dB at high frequencies (Fig. 3B) beyond the damaging effect of noise alone. However, even further increases in noise severity, by increasing duration of the 100-dB noise exposure from 2 to 4 h during combined exposures, do not yield any further increase in potentiation (Fig. 3C). The loss in threshold sensitivity obtained by 100 dB, 4-h OBN treatment alone obscures any elevation in NIHL resulting from the CO treat-

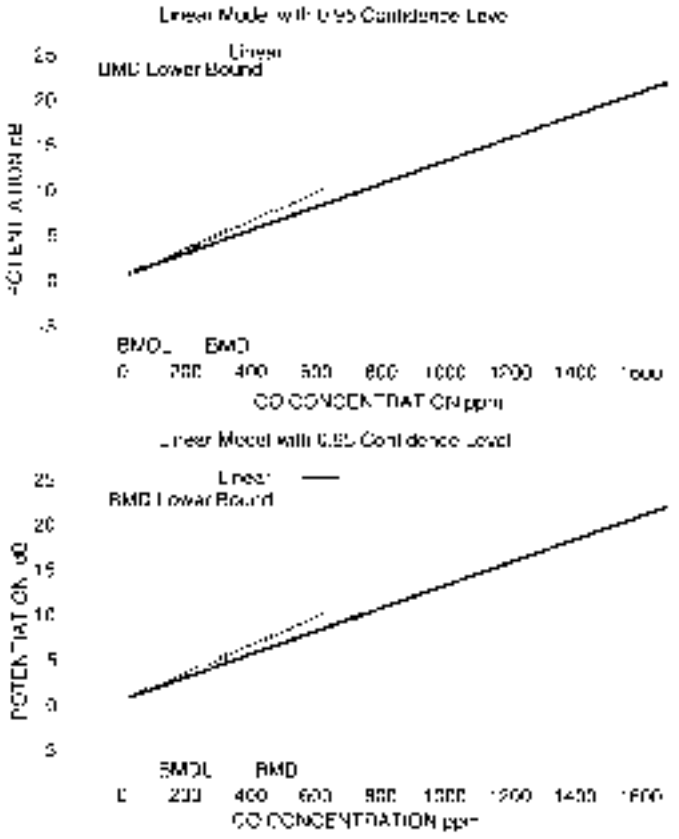

FIG. 2. Potentiation of noise-induced hearing loss measured as the average loss in CAP threshold sensitivity in excess of the loss produced by noise alone over the frequency range 12–40 kHz is shown for subjects receiving simultaneous exposure to varying concentrations of CO. Data were fit using Benchmark Dose Software version 1.1 beta published by the U.S. EPA. The upper panel shows the predicted benchmark dose that would yield an elevation in auditory threshold equivalent to 10% of the effect produced by noise alone and its lower bound. The predicted benchmark concentration is 236 ppm CO, and the lower bound for the benchmark concentration is 195 ppm. The lower panel shows the predicted benchmark dose that would yield a 5-dB elevation in auditory threshold beyond that produced by noise alone and its lower bound. The predicted benchmark concentration is 388 ppm CO, and the lower bound for the benchmark concentration is 320 ppm.

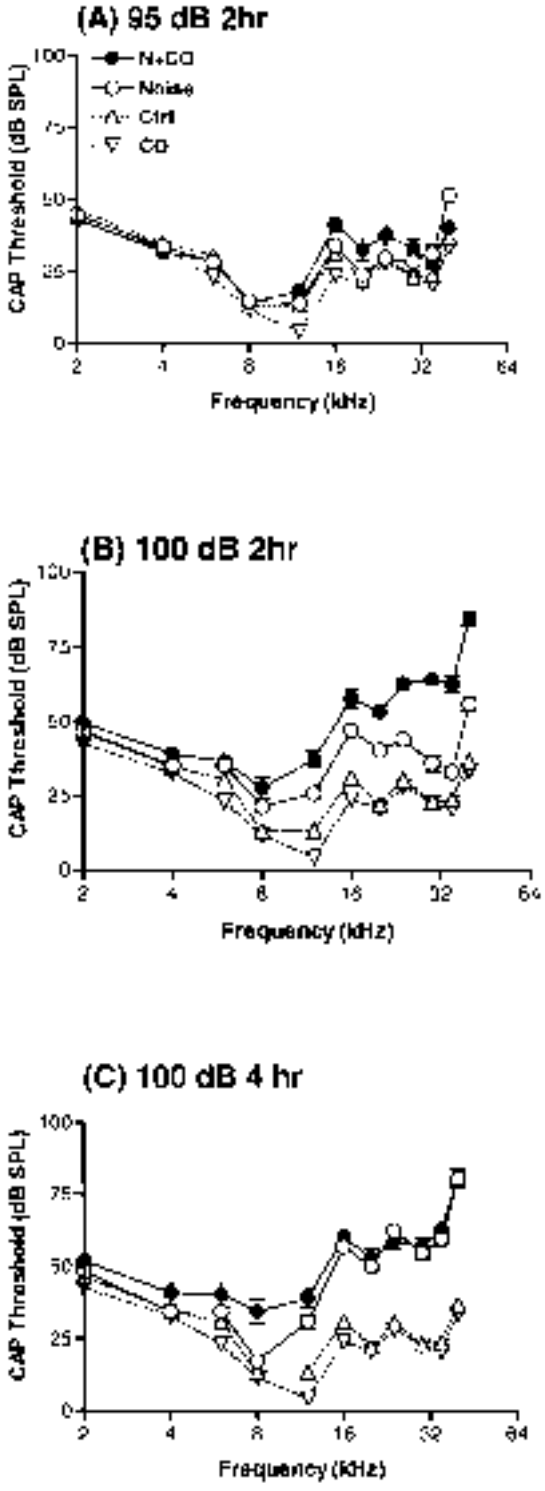

FIG. 3. Pure tone auditory thresholds for subjects exposed to a fixed CO level (1200 ppm) alone or in combination with increasing levels of noise severity ranging from 2 to 4 h in duration and 95–100 dB intensity. Auditory thresholds in subjects receiving noise alone and untreated control subjects are also represented. All thresholds were recorded 4 weeks following experimental exposures. Data represent the mean ± SEM.

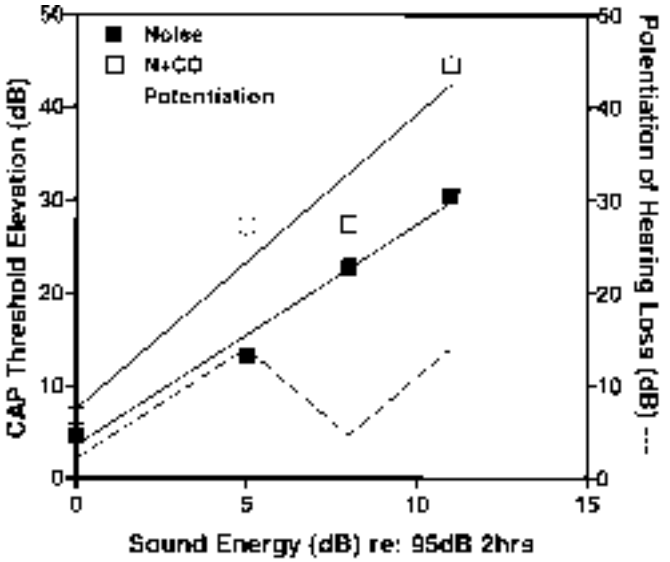

FIG. 4. The mean ± SEM loss in auditory threshold sensitivity averaged for frequencies between 12 and 40 kHz is represented for subjects exposed to noise alone or to noise + 1200 ppm CO. Sound energy is referenced to 95 dB OBN for 2 h. Data are abstracted from results presented in Figures 1E and 3. Also represented is potentiation of NIHL measured as the excess average loss in CAP threshold sensitivity due to combined exposure relative to the loss produced by each noise severity alone. Although direct linear relationships were found between noise severity by itself and threshold shift for both noise alone and noise + CO conditions, potentiation was not linearly related to noise severity.

ment. As CAP threshold loss of at least 60 dB relative to controls can be detected by this method (Chen and Fechter, 1999), it appears that the failure to find additional impairment in auditory threshold among subjects subjected to 100 dB OBN for 4 h + CO does not reflect merely insensitivity of the method for assessing cochlear function.

Analysis of variance shows a significant interaction between treatment and frequency ($F_{60,350} = 4.191$, $p < 0.0001$) as well as significant main effects of treatment ($F_{6,35} = 9.892$, $p < 0.0001$) and frequency ($F_{10,350} = 37.044$, $p < 0.0001$). Post hoc analysis of the interaction effect shows a significant difference between air control subjects and those exposed to noise levels of 100 dB for both 2 and 4 h ($p < 0.02$ and 0.0001, respectively) and between air controls and subjects receiving 100 dB noise + CO for both 2 and 4 h ($p < 0.0001$). Significant differences were also found between subjects receiving noise alone for 2 h and those receiving noise + CO for this time interval ($p < 0.01$).

Figure 4 demonstrates the relationship between noise severity and potentiation of the NIHL by 1200 ppm CO exposure averaged for frequencies between 12 and 40 kHz. Noise severity is referenced to the mildest noise exposure condition (95 dB OBN for 2 h). The data were abstracted from experiment 2 (Fig. 3) and from the 1200 ppm CO + 8-h noise condition in experiment 1 (Fig. 1E). Threshold elevation increases linearly as a function of sound energy for both the subjects receiving noise alone and combined noise + CO. Linear regression relating threshold elevation to noise alone accounted for 98% of the variance, and the slope differed significantly from zero ($p < 0.01$). A similar linear regression conducted for subjects exposed to noise +CO accounted for 93% of the variance and the slope also differed significantly from zero ($p < 0.05$). However, the potentiation of threshold elevation by combined exposure, represented by the difference between these functions, does not increase beyond that observed at 95 dB for 4 h. Thus the relationship between noise intensity and potentiation of NIHL by CO follows a non-monotonic function.

Figure 5 portrays the effect of repeated daily exposure to 1200 ppm CO and 100 dB OBN for 2 h per day on auditory thresholds. The data demonstrate elevation of auditory thresholds in subjects exposed both to noise by itself and also noise + CO. Repeated exposure to CO alone has no effect on auditory threshold. Among those subjects receiving noise exposure alone, auditory thresholds were typically 5–10 dB higher than among control subjects at all frequencies except 40 kHz. At this extreme high frequency, noise-exposed subjects showed nearly a 20-dB elevation in threshold. The auditory threshold observed in the noise +CO–treated rats, by contrast, was approximately 5 dB above the threshold seen in noise-treated rats at frequencies at or below 8 kHz. Above 8 kHz, potentiation ranged from 10 to 15 dB above noise-exposed subjects. Statistical analysis showed a significant effect of treatment ($F_{3/28} = 14.08$, $p < 0.0001$) and a significant treatment × frequency interaction ($F_{30/280} = 2.36$, $p < 0.0002$) on threshold sensitivity. Subsequent comparisons confirmed that noise and noise + CO groups differed significantly from each

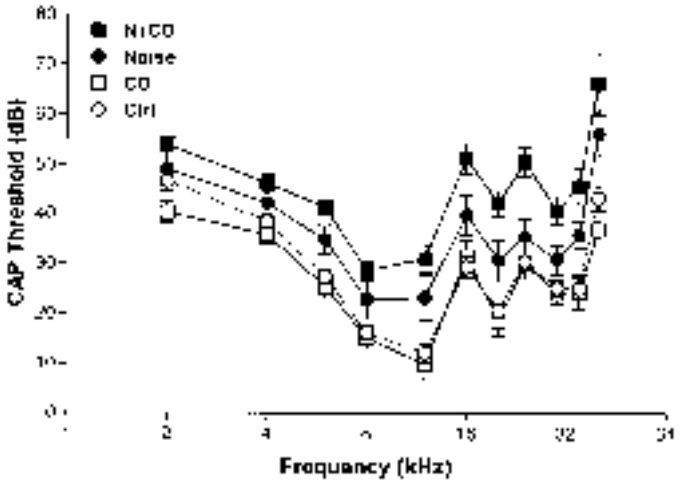

FIG. 5. Pure tone auditory thresholds for subjects exposed to a fixed noise level (95 dB, OBN, 2 h) alone or in combination with 1200 ppm CO for 5 consecutive days. Auditory thresholds in subjects receiving five daily exposures to 1200 ppm CO alone and untreated control subjects are also represented. All thresholds were recorded 4 weeks following experimental exposures. Data represent the mean ± SEM.

other ($p < 0.05$), and that both the air and CO groups varied from the noise-exposed group ($p < 0.05$).

DISCUSSION

Noise is clearly the predominant environmental hazard to hearing. Although exposure standards for noise are of primary importance in protecting against NIHL, complex exposures that include noise must be evaluated for their potential to injure the ear (NIOSH, 1996). The data presented here provide an initial understanding of the conditions under which an important chemical asphyxiant, CO, can potentiate NIHL. The data show a linear relationship between CO concentration and extent of potentiation of NIHL. Statistically significant elevations in NIHL are observed with CO exposures of 500 ppm and higher, yet benchmark concentration analyses suggest that much lower CO concentrations are able to potentiate NIHL in rats. The question of how much lower the concentration of CO need be to yield potentiation of NIHL is dependent in part upon selection of criteria for determining what the benchmark effect should be.

Agencies and scientists differ with regard to the precise manner in which risk determinations are made (e.g., Ohanian *et al.,* 1997); however, risk assessments frequently entail laboratory investigations designed to characterize a threshold dose for some toxic agent that produces the smallest recognizable adverse effect. Through the process known as "benchmarking," a function is fit to data in order to predict the concentration of a chemical having a known adverse effect (Slikker *et al.,* 1996) e.g., the elevation of auditory threshold. Determination of a benchmark dose or benchmark concentration can be achieved by a number of different methods (cf. Glowa and MacPhail, 1995; MacPhail and Glowa, 1999; Slikker and Gaylor, 1995) that take into account the background variability in the data obtained from the control group and attempt to relate this normal variability to the outcome scores obtained from toxicant-treated subjects. The criteria selected in the current study, a 5-dB elevation in threshold above the effect of noise alone and a value corresponding to 10% above the mean effect of noise alone, can be justified in terms of the biological relevance of a 5-dB elevation in auditory threshold above an already elevated threshold resulting from noise alone and the variability in auditory threshold resulting from noise alone. Obviously, other definitions might also be used. For example, Crofton and Zhao (1997) used a 15-dB threshold shift as the basis for benchmark concentration estimates for the ototoxic effects of the solvent trichloroethylene. The rationale they cite for this definition is the judgment that a threshold loss of this magnitude corresponds to a clinically significant hearing loss. This definition was rejected in part in the current work, as the focus here was on potentiation of a threshold shift that already represented a significant departure from auditory acuity of untreated control subjects. In addition, the methods adopted in the current work yielded very small standard errors, permitting the identification of a threshold shift as small as 5 dB.

The benchmark concentration must then be modified by safety factors that represent uncertainty concerning the relationship between the study sample and the human population with which we are actually concerned. There is an ongoing debate about the size of the safety factor that should be accorded on the basis of species differences, extrapolation from acute to chronic exposures, and increased sensitivity of particular groups within the population such as the elderly or very young, for example. In the current study, development of a safety factor is important in trying to extrapolate the results obtained from a small sample of rats (eight subjects per exposure condition) to a large human population and from a single acute exposure to repeated daily exposures or chronic exposures. It is fairly common to adopt a safety factor of 3 and 10 to account for each factor relevant to the toxicant and study population, although this is not a universal practice. Thus, in setting permissible human exposures based upon laboratory animal data (cf. Slikker *et al.,* 1996), the benchmark dose would be adjusted downward to provide an adequate safety factor. This reference concentration then becomes the best estimate of a safe exposure for humans. Based on our data, we predict a lower bound to the benchmark dose of CO for potentiation of NIHL of 195–320 ppm. Adjustment of this benchmark by a factor of 10 would place the reference concentration within the permissible range of human workplace exposure. Moreover, the experimental results obtained when a limited number of daily repeated exposures were employed suggest increased risk of potentiation with repeated exposures to CO and noise. This suggests the need to consider additional adjustments in developing a reference concentration for CO in the presence of noise. One potential weakness in our determination of a benchmark concentration for CO reflects the fact that the noise exposure conditions selected in the CO dose-response study are not optimal for producing potentiation by CO. If less intense noise were used (e.g., 100 dB OBN for 2 h rather than 8 h), the extent of potentiation produced by all CO concentrations might have been more apparent. Thus, the current estimated benchmark dose might be high.

The predicted benchmark concentration at which CO exposure potentiates NIHL far exceeds permissible exposure levels for CO. In the United States, the Environmental Protection Agency permits ambient exposure levels of 9 ppm averaged over 24 h and 35 ppm averaged over 1 h. For work environments, the standards proposed by ACGIH and by OSHA are 50 ppm averaged over an 8-h workday, with a peak level of 200 ppm. However, actual workplace CO levels do exceed these permissible levels, at least for short time intervals. CO is the most abundant pollutant in air and is present along with noise in many work environments. CO exposure is of particular concern among acetylene workers, steel and coke oven workers, and pulp and paper workers, among others (U.S. EPA,

1991). Exposure to noise and carbon monoxide (CO) is common among firefighters (Gold *et al.*, 1978; Reischl *et al.*, 1979; Treitman *et al.*, 1980), automobile workers (Morley *et al.*, 1999), and traffic and toll booth personnel (Burgess *et al.*, 1977). Firefighters, for example, are exposed to a very wide variety of chemical combustion products. However, CO can be considered to be a ubiquitous agent in all fires, and one that is especially likely to be an acute health hazard (Gold *et al.*, 1978; Treitman *et al.*, 1980). Gold *et al*. (1978) reported CO levels in excess of 400 ppm in roughly 20% of air samples they obtained using personal air samplers in a large number of structural fires in Boston. The geometric mean CO level was 110 ppm. Lowry *et al.* (1985) reported similar data in their studies of Dallas area fires. The 400 ppm short-term exposure limit (STEL) was exceeded in nearly 30% of fires studied (Lowry *et al.*, 1985). Treitman *et al*. (1980) reported CO levels averaging above 200 ppm and a range from 0 to nearly 5000 ppm, based on personal monitoring devices carried by firefighters in over 200 Boston structural fires. Jankovic *et al*. (1991) found CO levels from 0 to 1900 ppm, with one-third of the samples exceeding the 400 ppm STEL and roughly 10% exceeding 1500 ppm, a level immediately dangerous to life and health (IDLH). Although these measurements reflect ambient air levels within fires, the use of self-contained breathing apparatus (SCBA) greatly limits actual CO exposures of firefighters. Nevertheless, in addition to CO emanating from the active fire, firefighters can be subjected to significant CO during the knockdown phase of the fire and from exhaust from fire trucks and pumping engines where SCBA equipment may not be in routine use. Nevertheless, it can be predicted that exposure levels employed in this study will generally far exceed those experienced by working individuals.

Unlike the finding of increased potentiation of NIHL as a function of increasing CO concentration, the current data show that the potentiation of NIHL by CO does not grow with increasing severity of noise exposure. Rather, the potentiating effects of CO are most apparent with intermediate noise exposures that by themselves result in threshold shifts of approximately 20 dB. As expected, permanent threshold shifts attributable to noise alone do increase as noise exposure becomes more severe; however, there is no added effect or potentiating effect of CO when noise exposure exceeds a specific level (in this instance, OBN of 100 dB_{Lin} for 2 h). Whether this reflects a common cochlear target for noise alone and noise + CO such as the outer hair cell (Chen *et al*., 2000), or whether it reflects limitations in measuring increased auditory impairment beyond that observed in the high-noise condition will require further investigation. However, other nonlinearities have been identified with respect to potentiation of NIHL by CO as a function of the nature of the noise exposure. Recently, Chen *et al*. (1999) showed that simultaneous exposure to CO and noise disrupts the relationship between noise duty cycle (the proportion of time that an intermittent noise is turned on) and the extent of NIHL. Although rats exposed to noise alone show increasing NIHL with increasing duty cycles ranging from 40% to 67%, simultaneous exposure to 1200 ppm CO produces equivalent levels of NIHL for all duty cycles studied. Thus, it appears that the potentiating effects of CO on NIHL are most likely to occur under noise exposure conditions that would be predicted to result in very limited NIHL.

The current investigation does not fully address the possibility that potentiation of NIHL by CO can occur under noise conditions that produce no hearing loss whatsoever. In all cases where CO potentiation of NIHL was observed, the appropriate comparison group that received only noise exposure did show at least some limited NIHL. However, earlier studies that provided longer recovery periods of up to 8 weeks following exposure (e.g., Fechter *et al*., 1988; Young *et al.*, 1987) did show that CO could potentiate NIHL at noise exposure conditions that had no significant effects on auditory function. In any case, when studying occupational noise exposure, there are a considerable number of individuals who do ultimately develop a threshold shift. The current data suggest that these cohorts are at greatest risk for the potentiating effects of simultaneous CO exposure.

The current work focused upon predicting conditions under which NIHL would be increased by CO exposure and not upon the mechanism(s) by which such an effect might occur. Other research shows that combined exposure to CO and noise results in greater loss of sensory cells (in particular the outer hair cells) than does noise exposure alone (e.g., Fechter *et al.*, 1988). Studies conducted using free radical spin trap agents suggest that the potentiation of NIHL by CO may reflect, in part, free radical damage (Rao and Fechter, 2000).

ACKNOWLEDGMENTS

This research was supported in part by NIOSH (OH 03481) and NIH (ES 08082) and by a cooperative agreement through the EPSCoR Office of NSF (EPS9550478). W. Tawackoli conducted some of the experiments involving repeated exposures to noise and CO. The authors are grateful to J. Storm for help in undertaking the benchmark dose analysis and to M. McWilliams and P. Meder for their help in preparing this manuscript.

REFERENCES

Burgess, W. M. A., Diberardinis, L., and Speizer, F. E. (1977). Health effects of exposure to automobile exhaust—V. exposure of toll booth operators to automobile exhaust. *Am. Ind. Hyg. Assoc. J.* **38,** 184–191.

Campo, P., Lataye, R., Cossec, B., and Placidi V. (1997). Toluene-induced hearing loss: a mid-frequency location of the cochlear lesions. *Neurotoxicol. Teratol.* **19,** 129–140.

Chen, G. D., and Fechter, L. D. (1999). Potentiation of octave-band noise induced auditory impairment by CO. *Hear. Res.* **132,** 149–159.

Chen, G. D., McWilliams, M. L., Fechter, L. D. (1999). Intermittent noise-induced hearing loss and the influence of carbon monoxide. *Hear. Res.* **138,** 181–191.

Chen, G. D., McWilliams, M. L., and Fechter, L. D. (2000). Succinate

dehydrogenase (SDH) activity in hair cells: a correlate for permanent threshold elevations. *Hear. Res.* **145,** 91–100.

Crofton, K. M., Lassiter, T. L., and Rebert, C. S. (1994). Solvent-induced ototoxicity in rats: an atypical selective mid-frequency hearing deficit. *Hear. Res.* **80,** 25–30.

Crofton, K. M., and Zhao, X. (1993). Midfrequency hearing loss in rats following inhalation exposure to trichloroethylene: evidence from reflex modification audiometry. *Neurotoxicol. Teratol.* **15,** 413–423.

Crofton, K. M., and Zhao, X. (1997). The ototoxicity of trichloroethylene: extrapolation and relevance of high-concentration, short-duration animal exposure data. *Fundam. Appl. Toxicol.* **38,** 101–106.

Fechter, L. D. (1989). A mechanistic basis for interactions between noise and chemical exposure. *Arch. Com. Environ. Studies* **1,** 23–28.

Fechter, L. D., Clerici, W. J., Yao, L., and Hoeffding, V. (1992). Rapid disruption of cochlear function and structure by trimethyltin in the guinea pig. *Hear. Res.* **58,** 166–174.

Fechter, L. D., Liu, Y., Herr, D. W., and Crofton, K. M. (1998). Trichloroethylene ototoxicity: evidence for a cochlear origin. *Toxicol. Sci.* **42,** 28–35.

Fechter, L. D., Young, J. S., and Carlisle, L. (1988). Potentiation of noise induced threshold shifts and hair cell loss by carbon monoxide. *Hear. Res.* **34,** 39–47.

Franks, J. R., Stephenson, M. R., and Merry, C. J. (1996). Preventing occupational hearing loss–a practical guide. USDHHS, Center for Disease Control and Prevention.

Glowa, J. R., and MacPhail, R. C. (1995). Quantitative approaches to risk assessment in neurotoxicology. In *Neurotoxicology Approaches and Methods,* (L. W. Chang and W. Slikker, Eds.), pp. 777–788. Academic Press, New York.

Gold, A., Burgess, W. A., and Clougherty, E. V. (1978). Exposure of firefighters to toxic air contaminants. *Am. Ind. Hyg. Assoc. J.* **39,** 534–539.

Jankovic, J., Jones, W., Burkhart, J., and Noonan, G. (1991). Environmental study of firefighters. *Ann. Occup. Hyg.* **35(6),** 581–602.

Johnson, A. C. (1993). The ototoxic effect of toluene and the influence of noise, acetyl salicylic acid, or genotype. A study in rats and mice. *Scand. Audiol. Suppl.* **39,** 1–40.

Johnson, A.-C., Juntunen, L., Nylen, P., Borg, E., and Hoglund, G. (1988). Effect of interaction between noise and toluene on auditory function in the rat. *Acta Otolaryngol.* **105,** 56–63.

Johnson, A. C., Nylen, P., Borg, E., and Hoglund, G. (1990). Sequence of exposure to noise and toluene determine loss of auditory sensitivity in the rat. *Acta Otolaryngol.* **109,** 34–40.

Lataye, R., and Campo, P. (1997). Combined effects of a simultaneous exposure to noise and toluene on hearing function. *Neurotoxicol. Teratol.* **19(5),** 373–382.

Lowry, W. T., Juarez L., Petty C. S., and Roberts, B. (1985). Studies of toxic gas production during actual structural fires in the Dallas area. *J. Forensic Sci.* **30,** 59–72.

MacPhail, R. C., and Glowa, J. R. (1999). Quantitative risk assessment in neurotoxicology: past, present, and future. In *Neurotoxicology* (H. A. Tilson and G. J. Harry, Eds.), pp. 367–382. Taylor and Francis, New York.

Morata, T. C., Dunn, D. E., Kretschmer, L. W., LeMasters, G. K., and Keith, R. W. (1993). Effects of occupational exposure to organic solvents and noise on hearing. *Scand. J. Work Environ. Health* **19,** 245–254.

Morata, T. C., Dunn, D. E., and Sieber, W. K. (1994). Occupational exposure to noise and ototoxic organic solvents. *Arch. Environ. Health* **49,** 359–365.

Morata, T. C., Engel T., Durao A., Costa T. R., Krieg E. F., Dunn D. E., and Lozano M. A. (1997a). Hearing loss from combined exposures among petroleum refinery workers. *Scand. Audiol.* **26,** 141–149.

Morata, T. C., Fiorini, A. C., Fischer, F. M., Colacioppo, S., Wallingford, K. M., Krieg, E. F., Dunn, D. E., Gozzoli, L., Padrao, M. A., and Cesar,C. L. (1997b). Toluene induced hearing loss among rotogravure printing workers. *Scand. J. Work Environ. Health* **23,** 289–298.

Morley, J. C., Seitz, T., and Tubbs, R. (1999). Carbon monoxide and noise exposure at a monster truck and motocross show. *Appl. Occup. Environ. Hyg.* **14,** 645–655.

National Institute for Occupational Safety and Health, U.S. Department of Health and Human Services. National Occupational Research Agenda, April 1996.

Occupational Safety and Health Act of 1970. Public Law 91-596. 91st Congress, S. 2193.

Occupational Safety and Health Administration. (1981). Final Regulatory Analysis of the Hearing Conservation Amendment. Washington, DC: U.S. Department of Labor, Occupational Safety and Health Administration. 46 Fed. Reg. 4076.

Ohanian, E. V., Moore, J. A., Fowle, J. R. III, Omenn, G. S., Lewis, S. C., Gray, G. M., and North, D. W. (1997). Risk characterization: a bridge to informed decision making. *Fundam. Appl. Toxicol.* **39,** 81–88.

Rao, D. B., and Fechter, L. D. (2000). Protective effects of phenyl-N-*tert*-butylnitrone on the potentiation of noise-induced hearing loss by carbon monoxide. *Toxicol. Appl. Pharmacol.* **167,** 125–131.

Reischl, U., Bair, H. S., Jr., and Reischl, P. (1979). Fire fighter noise exposure. *Am. Ind. Hyg. Assoc. J.* **40,** 482–489.

Rice, D. C., and Gilbert, S. G. (1992). Exposure to methylmercury from birth to adulthood impairs high frequency hearing in monkeys. *Toxicol. Appl. Pharmacol.* **115,** 6–10.

Rybak, L. P. (1986). Drug ototoxicity. *Ann. Rev. Pharmacol Toxicol.* **26,** 79–99.

Schwartz, J., and Otto, D. (1991). Lead and minor hearing impairment. *Arch. Environ. Health* **46,** 300–305.

Slikker, W., Jr., Crump, K. S., Andersen M. E., and Bellinger, D. (1996). Biologically based, quantitative risk assessment of neurotoxicants. *Fundam. Appl. Toxicol.* **29,** 18–30.

Slikker, W., Jr., and Gaylor, D. W. (1995). Concepts on quantitative risk assessment of neurotoxicants. In *Neurotoxicology Approaches and Methods*. (L. W. Chang, and W. Slikker, Eds.), pp. 771–776. Academic Press, New York.

Treitman, R. D., Burgess, W. A., and Gold, A. (1980). Air contaminants encountered by firefighters. *Am. Ind. Hyg. Assoc. J.* **41,** 796–802.

U.S. EPA (1991). *Air Quality Criteria for Carbon Monoxide.* Environmental Criteria and Assessment Office, U.S. EPA, Research Triangle Park, NC.

Wu, M. F., Ison, J. R., Wecker, J. R., and Lapham, L. W. (1985). Cutaneous and auditory function in rats following methyl mercury poisoning. *Toxicol. Appl. Pharmacol.* **79,** 377–388.

Young, J. S., Upchurch, M. B., Kaufman, M. J., and Fechter, L. D. (1987). Carbon monoxide exposure potentiates high-frequency auditory threshold shifts induced by noise. *Hear. Res.* **26,** 37–43.