

Potential of Noise-Induced Hearing Loss by Low Concentrations of Hydrogen Cyanide in Rats

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Noise-induced hearing loss is the most prevalent occupational injury in the United States despite the adoption of clear permissible exposure limits and protocols for hearing conservation. This study identifies low-level chemical asphyxiant exposure as a risk factor capable of potentiating noise-induced hearing loss. Rats were exposed to 10, 30, and 50 ppm hydrogen cyanide (HCN) alone for 3.5 h ($n = 28$) or in combination with 2 h octave band noise exposure (100 dB_{im}; $n = 28$). Additional groups received noise exposure alone ($n = 16$) and no treatment other than placement in an inhalation chamber with clean air and quiet ($n = 16$). Pure tone compound action potential (CAP) thresholds were determined 4 weeks following the exposure in order to assess pure tone auditory sensitivity and permanent threshold impairment. Cochleae from an additional 13 subjects were processed for light microscopy to permit assessment of hair cell loss. The results demonstrate that the noise exposure alone impaired CAP threshold by about 10 dB, averaged between 12–40 kHz, and produced a 5% loss of outer hair cells at the base of the cochlea, but no inner hair cell loss. The combined exposure to noise and HCN caused a cyanide dose-dependent CAP threshold impairment that exceeds the noise exposure alone. This effect reached statistical significance at a HCN level of 30 ppm. Combined exposure also produced more outer hair cell loss than noise alone. HCN alone did not cause significant hearing loss or hair cell loss. A risk assessment analysis was conducted for the auditory threshold data using benchmark dose software published by the U. S. EPA (BMDS version 1.3). A continuous model showed that the data could be described by a linear function. For a benchmark response corresponding to a 5 dB increase in auditory threshold above the effect of noise alone, the lower bound on the 95% confidence interval for the benchmark dose was 9 ppm. The benchmark dose that impaired auditory threshold 10% above the effect of noise alone had a lower bound of 2 ppm. The lower bound to the HCN dose that produced a 1 SD elevation in noise-induced hearing loss was 16 ppm. These exposure levels provide a range of concentrations below to slightly above the short-term exposure limit for HCN. However, if these levels are adjusted for an 8 h time-weighted average (TWA), the resulting levels are below the permissible exposure level (PEL) for HCN.

Key Words: hydrogen cyanide; ototoxicity; noise; complex exposures; noise-induced hearing loss; potentiation; rats.

Noise-induced hearing loss is the most common occupational injury in the United States (NIOSH, 1996). Approximately 30 million workers in the United States are exposed to potentially hazardous noise levels in the workplace (Franks *et al.*, 1996) and noise is considered to be the most significant environmental contributor to acquired hearing loss. While OSHA has adopted a permissible exposure level (PEL) in the Hearing Conservation Amendment (46 Fed. Reg. 4078, 1981) to the U.S. Occupational Safety and Health Act of 1970 (PL 91-596), designed to prevent noise-induced hearing loss, the problem of noise-induced hearing loss has not abated. The OSHA PEL for nonimpact noise is based upon sound intensity averaged according to the human audiometric curve with its sensitivity between approximately 20 Hz–16 kHz. This averaging convention is referred to as an “A weighting,” and sound intensity using this convention is referred to as dB (A). The OSHA PEL for nonimpact noise is a level equivalent (LEQ) to 90 dB (A) Leq. That is, sound levels equivalent to 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 upper (peak) permissible noise limit is 115 dB (A) for continuous noise.

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. Organic solvents (Campo *et al.*, 1997, 1999; Cappaert *et al.*, 1999; Crofton, 1994; Fechter *et al.*, 1998; Johnson *et al.*, 1988; Lataye *et al.*, 2001; Loquet *et al.*, 2000; Morata *et al.*, 1993, 1994, 1997), metals (Fechter *et al.*, 1992; Schwartz and Otto, 1987), and chemical asphyxiants (Chen and Fechter, 1999; Chen *et al.*, 1999; Fechter *et al.*, 1988, 2000a,b; Young *et al.*, 1987) are all known to have ototoxic potential. Simultaneous and even successive exposure to certain of these agents along with noise can increase greatly susceptibility to noise-induced hearing loss both for humans (Morata *et al.*, 1993, 1994, 1997) and laboratory animals (Chen and Fechter, 1999; Chen *et al.*, 1999; Fechter *et al.*, 1988, 2000a,b; Johnson *et al.*, 1988, 1990; Johnson, 1993; Lataye and Campo, 1997).

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The potentiation of noise-induced hearing loss by carbon monoxide (CO) exposure is particularly well established (Chen and Fechter, 1999; Chen *et al.*, 1999; Fechter *et al.*, 1988, 2000a,b; Fechter, 1989; Young *et al.*, 1987). This fact relates to findings that the mammalian cochlea represents a highly active structure metabolically vulnerable to the effects of hypoxia and chemical asphyxiants. Indeed, disruption of blood supply (ischemia) and reduction in available oxygen levels (hypoxia) have been suggested to be fundamental mechanisms that are responsible for many forms of sudden hearing loss and drug ototoxicity (Hawkins, 1976; Lawrence, 1970; Thorne and Nuttall, 1987).

Unlike the case for hypoxic hypoxia (reduction of oxygen concentration in inspired air typically by diluting air with nitrogen), carbon monoxide, and ischemia, the effect of cyanide on auditory function has not been well defined. Van Heijst *et al.* (1994) studied 20 patients in Tanzania with sudden onset polyneuropathies correlated with elevated blood cyanide and plasma thiocyanate. Hearing loss was identified in nearly half of these cases. The source of cyanide exposure was believed to be increased dietary intake of cassava due to food shortages. Direct experimental evidence that cyanide can produce cochlear impairment is limited to two studies in which cyanide salts were perfused through the cochlea (Evans and Klinke, 1982; Konishi and Kelsey, 1968) and one study of acute impairment due to systemic KCN injection (Tawackoli *et al.*, 2001). These studies focus on acute effects of cyanide on the stria vascularis, a cochlear structure with extremely high rates of oxidative metabolism. They provide little guidance on the chronic functional effects of this contaminant on hearing.

The primary purpose of this study was to evaluate the potential for cyanide gas inhalation, at concentrations close to the human PEL, to potentiate noise-induced hearing loss. While such potentiation might be anticipated based on the commonality of cyanide and CO in disrupting oxidative processes, the marked differences in the probable cochlear mechanisms by which these two toxicants produce their acute auditory effects underscore the possibility of agent-specific actions. While the acute administration of both KCN and CO can disrupt auditory function transiently (Tawackoli *et al.*, 2001), the severity of the impairment, the pattern of effect on critical tissue beds within the cochlea, and the time scale of effect vary greatly for the two agents. KCN causes a profound reduction in the endocochlear potential indicative of dysfunction in the stria vascularis. Along with this loss of the endocochlear potential and, perhaps, as a result, a parallel drop in CAP amplitude is also detected. Thus the stria vascularis appears to be very sensitive to cyanide as it is for ischemia (Thalman *et al.*, 1975). CO, on the other hand, produces a much slower impairment of CAP amplitude and no change or nearly no change in the endocochlear potential (Tawackoli *et al.*, 2001). It appears to exert its effects principally on the inner hair cells (Fechter *et al.*, 1988; 1992).

In addition to inadvertent exposure as a combustion product,

TABLE 1
Summary of Experimental Treatments

Treatment group	Number of subjects
Physiological study	
Control	16
10 ppm HCN	10
30 ppm HCN	12
50 ppm HCN	6
Noise	16
Noise + 10 ppm HCN	10
Noise + 30 ppm HCN	12
Noise + 50 ppm HCN	6
Histological study	
Noise	4
Noise + 10 ppm HCN	3
Noise + 30 ppm HCN	3
Control	3

cyanides are also used intentionally in the extraction of low-grade ores, in electroplating, and as chemical intermediates (ATSDR, 1995). Cyanides are used in the manufacture of synthetic fibers, various plastics, dyes, pigments, and nylon. Hydrogen cyanide (HCN) is a frequent component of fire fighting environments as it is a common combustion product of polyurethane foam, acrylics, wool, and urethane, among others (Gold *et al.*, 1978; Ives *et al.*, 1972; Treitman *et al.*, 1980). However, the concentration of HCN varies widely depending upon the nature of the burning material. The OSHA PEL for HCN is 10 ppm as an 8-h time weighted average, and the short-term exposure limit (STEL) is also set at 10 ppm.

MATERIALS AND METHODS

Subjects. A total of 101 Long-Evans male pigmented rats, 2–3 months of age, obtained from Harlan Sprague Dawley (Chicago, IL), 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). A spectral analysis of this background noise level showed that sound levels in the frequency range used for threshold assessments in the rats were below 40 dB. Temperature was maintained at $21 \pm 1^\circ\text{C}$. Lights were on from 0630 to 1830 h. All exposures and testing were performed during the daytime.

Exposure procedure. Subjects were randomly assigned to 8 different treatment groups. Group size varied between 6 and 16 rats depending upon whether or not a treatment protocol was replicated as described below. The groups were exposed to noise alone, HCN alone at concentrations of 10, 30, and 50 ppm, combined exposure to noise + 10, 30, and 50 ppm HCN, and a control condition that entailed placement of subjects in the exposure chamber and no HCN. The exposures to 10 and 30 ppm HCN alone and to noise + 10 and 30 ppm HCN were subsequently replicated. Each replication also included treatment groups that received HCN alone, noise alone, and no experimental treatment. Table 1 provides information on exposure groups and subject numbers for each group. Limited histological data were obtained from rats that had received noise alone ($n = 4$), 10 and 30 ppm HCN + noise ($n = 3$), and untreated controls ($n = 3$). Physiological testing could not be conducted on these subjects due to equipment failure.

An analysis of the noise spectrum in the exposure chambers when no noise

was added intentionally were below 35 dB for all octave bands having a center frequency (geometric mean frequency) of 2 kHz and higher. An octave can be defined as having a minimum value of F and a maximum value of $2F$. The lowest frequency that was employed in audiometric testing was 2 kHz.

Subjects were assigned randomly to treatment groups. Exposures were conducted in a reverberant 40 l glass cylinder equipped with stereo speakers for delivering sound, a Quest (Oconomowoc, WI) 1" microphone and sound level meter for monitoring sound, and a HCN monitor (Industrial Scientific, Oakdale, CA) for continuous measurement of chamber gas concentration. The subjects were placed within small wire cloth 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 octave band noise with center frequency of 13.6 kHz. 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 is positioned correctly on the cochlea and that the sensitivity of the equipment is within normal specifications. The roll-off for the filter system was approximately 48 dB/octave. Acoustic measurement in the exposure chamber demonstrated similar spectral intensities between 10–16 kHz and about 10 dB lower at 20 kHz. The acoustic intensity between 5–10 kHz was decreased about 40 dB.

The noise was amplified by a power amplifier (SAE 2200) and delivered to 2 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 using a linear weighting. The sound level meter was checked weekly using a calibrated noise source of 94 dB at 1000 Hz. The noise level varied less than 2 dB within the space accessible to the subjects.

HCN exposure. All exposures were conducted in an airtight exposure chamber located within a dedicated chemical exhaust hood. The air exchange rate in the exposure chamber was 8.5 l/min (approximately 1 air change every 5 min), which was monitored by a Top Trak 821-1-PS mass flow meter. The source for HCN gas was a calibrated gas mixture that was stored within an aluminum cylinder (500 ppm HCN in nitrogen gas) fitted with all stainless steel regulators and tubing. The HCN gas was metered into a mixing chamber using a microvalve. A solenoid switch was employed to stop the flow of HCN if activated by power failure, failure of the ventilation system, and excursions of HCN levels above those desired in the chamber or detection of HCN outside of the hood. The HCN concentration in the exposure chamber reached the desired level within 30 min of exposure onset. The electrochemical HCN sensor within the chamber was calibrated on a monthly basis using calibration standards of 20 and 50 ppm HCN in nitrogen. Exhaust gas from the chamber was passed through NaOH to trap HCN prior to its release. HCN exposure began for the appropriate subjects 90 min prior to the onset of noise. Noise exposure duration was 2 h long.

Characterization of the HCN dose response for potentiation of noise-induced hearing loss. Rats were exposed to both noise (100 dB octave band noise, 2 h) and to HCN (10, 30, and 50 ppm, 3.5 h). Parallel groups were exposed to the noise alone, to 10 ppm, 30 ppm, and 50 ppm HCN alone; or they underwent a control exposure in the chamber without HCN present and no added noise.

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, im) and ketamine (87 mg/kg, im) and normal body temperature was maintained using a dc heating unit built into the surgical table. The temperature of the cochlea was maintained using a low voltage high-intensity lamp. The auditory bulla was opened via a ventro-lateral 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 generated in response to sound presentation were amplified 1000× between 0.1–1.0 kHz with a Grass (Quincy, MA) A.C. preamplifier (Model P15). The sound level necessary to generate a visually detectable CAP response on a digital oscilloscope (approximate response amplitude of 1 μ V) 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 attenuators 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/s. Sound levels at all test frequencies were calibrated with a probe microphone located near the eardrum.

Histology. The subjects used for histological study were euthanized 4 weeks after exposure by anesthetizing them prior to decapitation. Cochleae were removed immediately. Round and oval windows and the apex of the cochlea were opened to facilitate perfusion. The cochleae were perfused with SDH incubative solution (0.05 M sodium succinate, 0.05 M phosphate buffer, and 0.05% tetranitro blue tetrazolium) and immersed in the solution for 1 h (37°C). Then the cochleae were fixed with 10% formalin for at least 2 days. After fixation, the cochleae were decalcified in 7% EDTA solution (EthyleneDiamine Tetraacetic Acid) for 3 days or longer as needed. Cochlear microdissection was accomplished under a light microscope to yield surface preparations of each cochlear turn. These were oriented to permit correlation between location of hair cell loss and impairment of specific frequencies physiologically. Successive image pictures (covering 200 to 300 μ m basilar membrane) were obtained with Optimetic Image system (Edmonds, WA). Counting of hair cell loss was achieved as a function of cochlear location using Scion Image software (Bethesda, MD).

Statistical analysis. The data from each replication were analyzed separately using repeated measures ANOVA in which treatment was a between subjects variable and frequency (or cochlear locations) was evaluated as a within subjects variable. Pair-wise comparisons were made between treatment groups using Scheffe's tests. Because equivalent results were obtained between replications for groups receiving 10 and 30 ppm HCN, the data were collapsed across replication and included in an overall ANOVA along with the 50 ppm HCN exposure. Statistical differences beyond a $p = 0.05$ were considered to be significant.

The potentiation of noise-induced hearing loss by HCN was subsequently evaluated using benchmark dose software (version 1.3) published by the U.S. EPA National Center for Environmental Assessment. A continuous linear model was employed to determine a benchmark concentration of HCN that produced a specific impairment in auditory function that exceeded the effect of noise treatment alone. The model also provided an estimate of the lower bound for the 95% confidence interval for the benchmark dose.

RESULTS

Figure 1 presents CAP thresholds for rats exposed 4 weeks earlier to 0, 10, 30, and 50 ppm HCN alone for 3.5 h. CAP thresholds were equivalent among subjects receiving 0, 10, and 30 ppm. The animals exposed to 50 ppm HCN had slightly elevated thresholds (approximately 10 dB) restricted to the highest and the lowest test frequencies. However, the repeated measures ANOVA does not show a significant difference in CAP thresholds among the 4 treatment groups presented in Figure 1 ($F_{3/40} = 2.26, p = 0.0960$).

The effect of noise alone and noise + HCN on CAP threshold is shown in Figure 2. Subjects receiving 2h-noise exposure (13.6 kHz octave band noise, 100 dB_{L_{in}}) show a moderate CAP

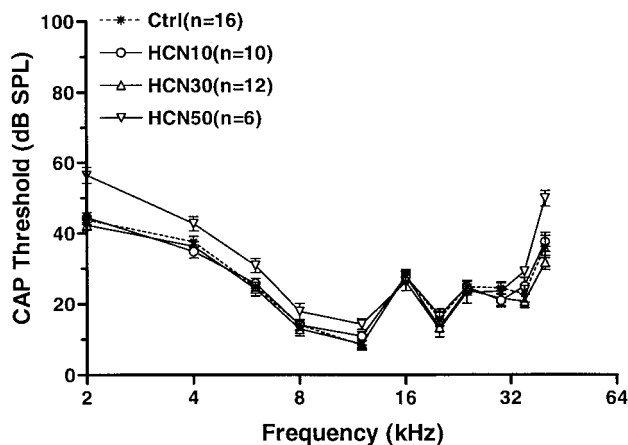


FIG. 1. CAP thresholds of control rats ($n = 16$) and those receiving 10 ($n = 10$), 30 ($n = 12$), and 50 ppm HCN exposure alone ($n = 6$) show no effect on auditory threshold. The thresholds were measured 4 weeks after the exposure. Vertical bars are SE.

threshold elevation (12 ± 1 dB averaged across frequencies of 12–40 kHz) comparing to the controls. Auditory thresholds for noise-treated subjects are normal between 2–8 kHz. When HCN is presented in combination with noise exposure, greater auditory impairment was observed. The group receiving combined exposure to noise and 10 ppm HCN had 15 ± 2 dB CAP threshold elevation averaged across frequencies of 12–40 kHz. The group receiving combined exposure to noise and 30 ppm HCN had 24 ± 2 dB threshold elevation. The animals exposed to noise + 50 ppm HCN had an average threshold impairment of 36 ± 2 between 12–40 kHz. In addition, rats receiving the highest HCN concentration + noise also showed auditory impairment of nearly 20 dB for 8 kHz tones.

The repeated measures ANOVA shows a significant difference in CAP thresholds among the 5 groups presented in Figure 2 (between treatment, $F_{4/55} = 18.34$, $p < 0.0001$; treatment-frequency interaction, $F_{10/40} = 6.26$, $p < 0.0001$). Post hoc analysis shows that the noise group is significantly different from the control group ($p = 0.001$), significantly different from the group receiving noise + 30 ppm HCN ($p < 0.005$) and the group receiving noise + 50 ppm HCN ($p < 0.0001$). The noise group is not significantly different from the group that received noise + 10 ppm HCN ($p > 0.05$).

For the risk assessment analysis, the average impairment in CAP threshold between 12–40 kHz was calculated for the groups receiving noise alone and noise + HCN relative to the untreated control group. This frequency range was predicted based upon previous studies (e.g., Fechter *et al.*, 2000a,b) and upon the nature of the noise exposure used to contain the maximal threshold shifts. In addition, parallel post hoc analyses were performed between 20–40 kHz representing the frequency range in which we observed significant deviations between the rats receiving noise alone and noise + HCN. A continuous linear model was applied to the data relating extent of threshold elevation to dose of HCN. The analysis showed

that a linear model did provide an adequate fit to the data and that equivalent BMDs and LBMDs resulted when alternative models were selected. Three specific benchmark responses were evaluated: (1) a 10% elevation in auditory impairment beyond the effect of noise alone, (2) a fixed 5 dB potentiation of noise-induced hearing loss in the frequency range most sensitive to the noise (i.e., 12–40 kHz and 20–40 kHz), and an auditory impairment that exceeded the mean effect of noise alone by 1 SD. The criterion of a 10% impairment beyond that seen in the appropriate control group is a fairly common default definition for continuous data that has been used by others undertaking risk assessment analysis (e.g., MacPhail and Glowa, 1999; Malsch *et al.*, 1994). In addition, the 10% increment in auditory impairment beyond the effect of noise alone represents a step of approximately 1 dB. 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 and it was employed in our previous analysis for noise-induced hearing loss potentiation by CO (Fechter *et al.*, 2000b). Finally, a benchmark dose corresponding to an increase in noise-induced hearing loss equivalent to 1 SD above the mean effect of noise was employed to provide a conservative estimate of a benchmark dose reliably different from the mean for noise alone.

Figure 3 shows the result of this analysis using each of the 3 benchmark responses described above for the frequency range 12–40 kHz. The benchmark concentration obtained for a 10% elevation of auditory impairment beyond the effect of noise was 3 ppm with a lower bound to the 95% confidence interval of 2 ppm (see Fig. 3A). For a benchmark response corresponding to a 5 dB elevation in threshold, the benchmark HCN concentration was estimated at 11 ppm with the lower bound of the 95% confidence interval at 9 ppm (see Fig. 3B).

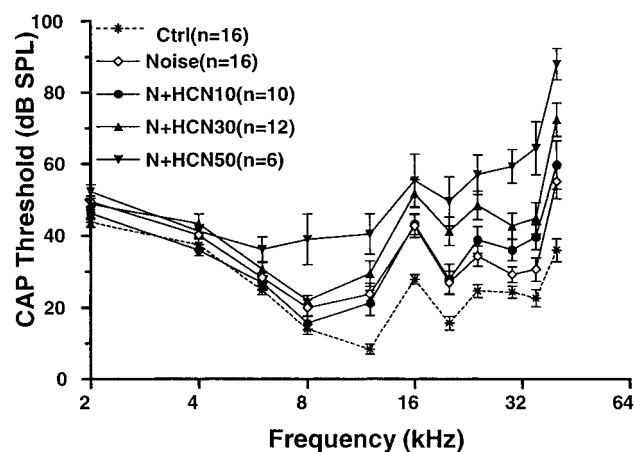


FIG. 2. CAP thresholds as functions of test frequency showing more CAP threshold loss caused by the combined exposure to noise and cyanide (filled symbols) than to noise alone (open diamonds). Octave band noise with center frequency at 13.6 kHz, $100 \text{ dB}_{\text{Lin}}$ for 2 h. HCN levels were 10, 30, and 50 ppm for 3.5 h (1.5 h prior to noise onset). Vertical bars are SE.

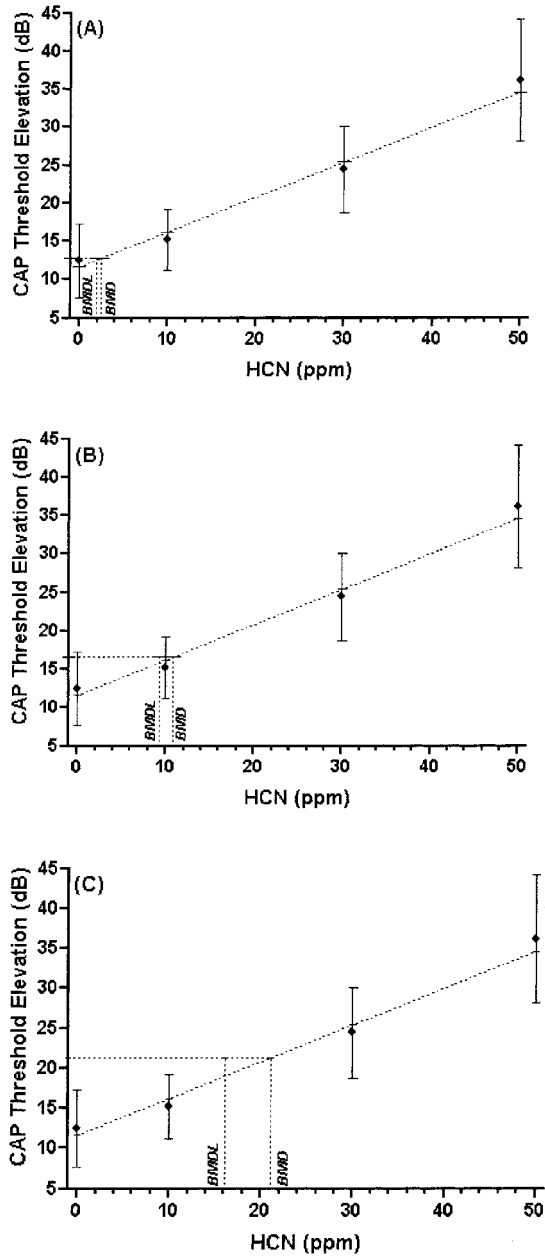


FIG. 3. CAP threshold elevation averaged across 12–40 kHz as a function of HCN concentration showing a linear HCN dose-dependency. Data were fit using Benchmark Dose Software version 1.3 published by the U.S. EPA. The benchmark dose (BMD) and the lower bound of the 95% confidence interval (BMDL) are shown for 3 different benchmark responses. Vertical bars are SE. (A) the BMD and BMDL for potentiation of noise-induced hearing loss by 10% are 3 and 2 ppm. (B) The BMD and BMDL for potentiation of noise-induced hearing loss by 5 dB are 11 and 8 ppm. (C) The BMD and BMDL for potentiation of noise-induced hearing loss by 1 SD are 21 and 16 ppm.

The comparable values for a response 1 SD above the effect of noise alone the benchmark was 21 ppm with a 16 ppm lower bound (see Fig. 3C). Restricting the range of frequencies over which auditory thresholds were calculated to 20–40 kHz generally had little effect upon the resulting benchmark dose

except when a definition of the benchmark response was elevation in average threshold by 1 SD. In this case, the benchmark dose was calculated as 11 ppm HCN with a lower bound to the 95% confidence interval of 16 ppm. The comparative benchmark doses and their lower bounds are presented in Table 2.

The limited histopathological data provide an estimate of the extent of hair cell impairment resulting from the noise alone and from noise in combination with 10 and 30 ppm HCN relative to untreated control subjects. Because the cochlea is organized tonotopically with high frequency tones encoded near the base and lower frequency tones encoded toward the apex, it is possible to relate the location of hair cell damage along the basilar membrane to tone frequency utilizing tone place maps that have been developed for different species. Cytocochleograms relating cell loss to location were organized using a place map published by Muller (1991) for the rat. Inner hair cell loss was limited to a small basal location corresponding to frequencies above 40 kHz (Fig. 4). Outer hair cell loss was observed among the experimental groups mainly at cochlear locations that correspond to frequencies higher than 20 kHz (Fig. 4A). This corresponds to the region of auditory function that was most impaired in the physiological experiments. However, there was evidence of impaired auditory function at even lower frequencies yet this is not reflected in an actual loss of outer hair cells. The combined exposure to noise and HCN (10 and 30 ppm) caused more outer hair cell loss ($6 \pm 3\%$ and $9 \pm 4\%$ averaged across locations in the most basal 45–90% of the cochlea) than the noise alone ($5 \pm 3\%$). HCN alone did not induce hair cell loss.

DISCUSSION

The present report demonstrates that HCN exposure for 3.5 h increases permanent noise-induced hearing loss in a dose

TABLE 2
Benchmark Doses and the 95% Lower Bound on the Confidence Intervals for Potentiation of Noise-Induced Hearing Loss by Coexposure to Hydrogen Cyanide Using 3 Alternative Benchmark Responses

Benchmark response	Benchmark HCN dose (ppm)	Lower bound to the 95% confidence level (ppm)
10% Elevation (1.2 dB) above noise alone		
12–40 kHz	3	2
20–40 kHz	2	1
5 dB Elevation above noise alone		
12–40 kHz	11	9
20–40 kHz	9	8
1 SD Elevation above noise alone		
12–40 kHz	21	16
20–40 kHz	11	9

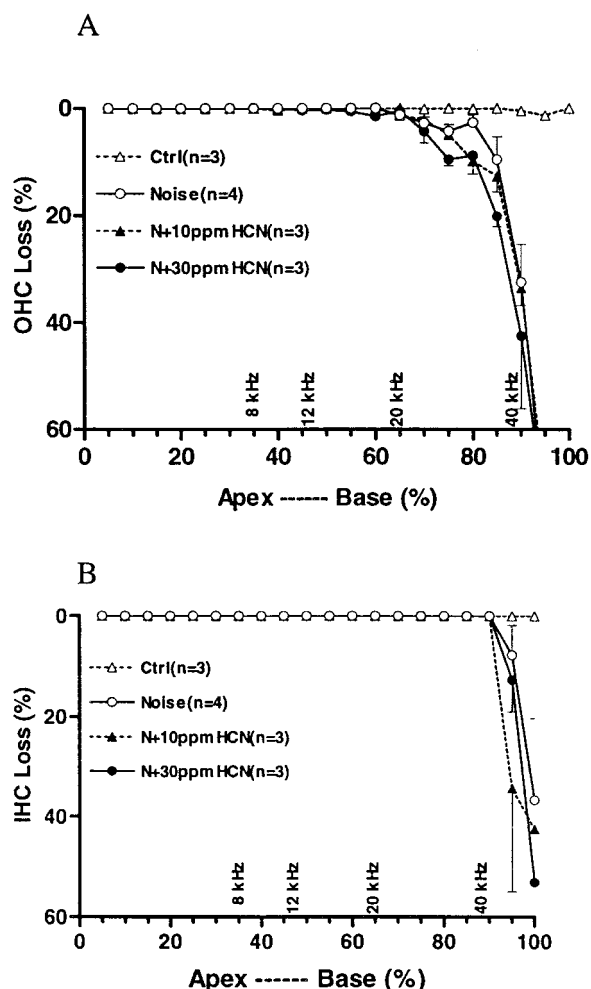


FIG. 4. Hair cell loss as function of cochlear distance from apex. (A) More outer hair cell losses caused by the combined exposure to noise and HCN (filled symbols) than by the noise alone (open circles). (B) No inner hair cell loss was observed within the region of test frequencies. Octave band noise with center frequency at 13.6 kHz, 100 dB_L for 2 h. HCN levels were 10 and 30 ppm for 3.5 h (1.5 h prior to noise onset). Vertical bars are SE.

dependent manner. Because HCN exposure has minimal effects even at exposure concentrations of 50 ppm, these data demonstrate that low concentrations of HCN can potentiate noise-induced hearing loss. These data extend similar findings reported for CO (Fechter *et al.*, 2000a,b) in showing that chemical asphyxiants can potentiate noise-induced hearing loss despite the fact that the asphyxiants by themselves do not produce a permanent shift in auditory function. In both instances increased outer hair cell loss has been observed along with physiological impairment (Fechter *et al.*, 2000a,b).

In the current study, potentiation of noise-induced hearing loss achieved statistical significance when noise was combined with 30 ppm HCN while 10 ppm HCN did not produce significant potentiation or pronounced outer hair cell loss. For the purposes of traditional risk assessment, then, both a lowest observed effect level (LOEL) and a no observed effect level

(NOEL) have been identified by this study. However, we have extended this risk assessment analysis using a benchmark dose approach to identify concentrations of HCN that produce specific adverse response based upon data obtained over a slightly broader dose range. Many different potential benchmark responses could be utilized in making this risk analysis. For example, we have previously utilized both a 10% elevation in the response above control levels (noise effect alone) and an absolute 5 dB elevation in auditory threshold above that observed in subjects exposed to noise alone. The rationales that we have used for such benchmarks (Fechter *et al.*, 2000b) are, first, that a 10% increased response above some control value has precedence in other risk assessment analyses (e.g., MacPhail and Glowa, 1999; Malsch *et al.*, 1994) and secondly, that a 5 dB elevation in auditory impairment above the effect of noise alone represents a substantial increase in stimulus intensity relative to the sensitivity of the auditory measure utilized. That is, we can be confident in our ability to measure a 5 dB elevation in auditory threshold. In the current investigation the average auditory impairment produced by noise alone was only 12 dB and so the 10% increase represents a shift of only 1 dB. Such a loss represents a statistical alteration in auditory function observable in a sample of subjects, but would not be considered to be an adverse effect in a single subject. That is, error in measurement of the CAP is greater than 1 dB. We also elected to utilize a benchmark response equivalent to 1 SD above the mean threshold for noise exposed rats. The rationale for such a benchmark has merit since noise exposure both in rats and humans produces pronounced variability in outcome between individuals. Based upon the benchmarks selected, the lower bound to the 95% confidence interval about the benchmark concentration that potentiates noise-induced hearing loss falls between 2 and 16 ppm HCN. If these values are subjected to an 8 h TWA in line with OSHA protocols, then the lower bound to the 95% confidence interval for benchmark dose would be 0.5 and 4 ppm. For comparative purposes, the current PEL for cyanide provided by OSHA is 10 ppm, based on an 8 h TWA with a STEL value also set at 10 ppm.

Other benchmark responses might also be used for risk assessment. Crofton and Zhao (1997), for example, identified a 15 dB elevation in auditory function above the level shown by untreated controls as the basis for identifying a benchmark dose of an ototoxic solvent. They reasoned that a 15 dB impairment in auditory function represented an adverse effect based upon studies of auditory function in humans. We have not selected that measure for analysis because in our study the proper control group is one that has already suffered a mild auditory impairment due to noise exposure alone. Thus, the average total auditory impairment among rats receiving both HCN and noise is on the order of 15–20 dB. Whether the benchmark dose range identified in this study has meaning for human HCN exposure depends in part on the relative sensitivity to chemical asphyxiants between rats and human. The data on HCN toxicity and particularly neurotoxicity are not suffi-

cient for making a judgment of relative sensitivity of humans and rodents (ATSDR, 1997).

Another basis for assessing the utility of a benchmark dose is by evaluation of the underlying mechanism responsible for the toxic response. What is the nature of this process at low HCN exposure levels? Here the data are somewhat limited. Acute KCN injection in anaesthetized rats yields a near immediate impairment of the stria vascularis, a tissue bed with a very high level of oxidative metabolism that is responsible for maintaining a strict ionic gradient within the cochlea essential for its polarization. The impairment of the stria vascularis is predictable given the ability of cyanide to inhibit $\text{Na}^+ - \text{K}^+$ ATPase. However, auditory function normally recovers within minutes in such rats if noise is not present along with the cyanide exposure (Tawackoli *et al.*, 2001). This time course is consistent with elimination of cyanide from blood (Tawackoli *et al.*, 2001).

There are several potential mechanisms by which HCN can potentiate permanent noise-induced hearing loss. In addition to blocking $\text{Na}^+ - \text{K}^+$ ATPase, cyanide can also inhibit mitochondrial superoxide dismutase (SOD) activity in the cochlea (Pier-son and Gray, 1982) and disrupt mitochondrial function. In the current instance, we hypothesize, that HCN + noise produce impaired auditory function by producing significant oxidative stress in the cochlea. This hypothesis is based largely upon parallel findings that have been obtained for noise-induced hearing loss-potential by CO. Rao and Fechter (2000) and Rao *et al.* (2001) showed that spin trap agents could block the potentiation of noise-induced hearing loss by CO and that spin trap adducts were formed in significant concentrations following combined exposure to CO and noise. Neither CO nor noise alone produced detectable ROS. Under at least certain conditions, noise is known to initiate ROS in the cochlea (Henderson *et al.*, 1999; Lautermann *et al.*, 1997; Seidman *et al.*, 1993; Yamane *et al.*, 1995). It is possible, then, that both inhibition of ROS sequestration and increased ROS production result from the combined exposure to HCN and to noise. In this event, it might be anticipated that HCN exposure would potentiate oxidative stress.

The limited histological data collected suggest that low dose HCN exposure + noise can increase slightly the extent of outer hair cell death relative to the noise only subjects. No inner hair cell loss was observed. The modest loss of outer hair cells is generally consistent with the comparatively mild auditory impairment observed at all but the highest tone frequencies. That outer hair cell loss corresponding to impaired cochlear function for lower tones was not observed may reflect impairment, but not cell death at more apical regions of the cochlea.

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