ELSEVIER

Hearing Research 166 (2002) 24–32

HEARING RESEARCH

www.elsevier.com/locate/heares

Susceptibility to the ototoxic properties of toluene is species specific

Rickie R. Davis [a,e,*], William J. Murphy [a], John E. Snawder [c], Cynthia A.F. Striley [c], Donald Henderson [f], Amir Khan [b], Edward F. Krieg [d]

[a] *Hearing Loss Prevention Section, Engineering and Physical Hazards Branch, National Institute for Occupational Safety and Health, 4676 Columbia Parkway, Cincinnati, OH 45226, USA*

[b] *Control Technology Section, Engineering and Physical Hazards Branch, National Institute for Occupational Safety and Health, 4676 Columbia Parkway, Cincinnati, OH 45226, USA*

[c] *Biological Monitoring Laboratory Section, Biomonitoring and Health Assessment Branch, National Institute for Occupational Safety and Health, 4676 Columbia Parkway, Cincinnati, OH 45226, USA*

[d] *Monitoring Research and Statistics Activity, Division of Applied Research and Technology, National Institute for Occupational Safety and Health, 4676 Columbia Parkway, Cincinnati, OH 45226, USA*

[e] *Department of Biological Sciences, University of Cincinnati, Cincinnati, OH, USA*

[f] *Hearing Research Laboratory, State University of New York, Buffalo, NY, USA*

Received 23 July 2001; accepted 28 November 2001

Abstract

Toluene is the most widely used industrial solvent. It has been shown to be ototoxic in mice and rats, and to increase permanent threshold shift in conjunction with exposure to noise. Chinchillas are widely used for studying noise effects on the cochlea. The present study was initiated to study toluene and noise interaction in chinchillas. Thirty-three chinchillas were exposed to a 95 dBA 500 Hz octave band noise plus 2000 ppm toluene, 8 or 12 h per day for 10 days. Auditory function was estimated using the auditory brainstem response (ABR) to tones between 500 Hz and 16 kHz. There was no effect on the ABR of toluene alone. Noise alone produced a threshold shift. There was no interaction of noise and toluene on the ear. The present study suggests that chinchillas are markedly less susceptible to the ototoxic effect of toluene than mice and rats. A working hypothesis as to the species differences was that chinchilla liver was able to detoxify the toluene. Hepatic microsomes from chinchillas, rats and humans were tested for their ability to convert toluene to the more water-soluble compound – benzyl alcohol. Chinchilla livers were found to contain more of the P450 enzymes CYP2E1 and CYP2B than rats or humans. In addition, the data show that the P450 enzymes are more active in chinchillas than in rats and humans. In conclusion, the results suggest that rats and mice are a more appropriate model for human toluene ototoxicity. However, chinchillas may provide a valuable model for investigating how ototoxic agents can be detoxified to less damaging compounds. © 2002 Elsevier Science B.V. All rights reserved.

Key words: Ototoxicity; Toluene; Hepatic microsome; CYP2E1; CYP2B; Chinchilla

1. Introduction

Toluene is a colorless liquid at room temperature with the familiar odor of 'airplane glue'. One of the most commonly used industrial solvents in the world, toluene is often abused by inhalation for its euphoric high. It is not considered carcinogenic (Dorsey and Donohue, 1994). Toluene, being a gas at body temperature, is removed quickly from the tissues of animal or human via the circulation.

Solvents have long been suspected to be ototoxic agents. Human epidemiology studies have shown a greater risk for hearing loss among workers exposed to carbon disulfide (Morata, 1989) and toluene (Morata et al., 1997) than non-exposed workers. For a review of the human literature see Franks and Morata (1996).

Animal studies in rats and mice have also shown that

* Corresponding author. Tel.: +1 (513) 533-8142; Fax: +1 (513) 533-8139.
E-mail address: rrd1@cdc.gov (R.R. Davis).

0378-5955/02/$ – see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0378-5955(02)00280-0

exposure to toluene alone leads to destruction of the outer hair cells of the organ of Corti (Sullivan et al., 1988; Johnson and Canlon, 1994; Li, 1992). These changes lead to a reduction in the auditory brainstem response (ABR). Exposure to toluene followed by exposure to noise interacted to increase the permanent threshold shift over toluene alone in rats (Johnson et al., 1990; Rebert et al., 1983). Campo et al. (1999) detected toluene in the rat organ of Corti but were not able to measure it. Lataye and Campo (1997) reported synergism between toluene and noise in the rat ear.

The experimental literature shows relatively large interspecies effects. Campo et al. (1993) exposed groups of guinea pigs to (1) 1000 ppm toluene via inhalation for 2 weeks, or (2) to an 85 dB one-third octave band noise for 8 days, or (3) to toluene plus noise, or (4) to air alone (control). They were not able to demonstrate an ototoxic effect of toluene or an additive effect of toluene with noise. In a personal communication, Brummett indicated that guinea pigs receiving chronic intraperitoneal injections of toluene did not sustain a reduction in cochlear microphonics. On the other hand, Liu and Fechter (1997) showed in vitro that toluene at 100 μM disrupts intracellular calcium levels and cell morphology in isolated outer hair cells of guinea pigs showing that the guinea pig ear is not intrinsically insensitive to the effects of toluene.

The present study was conducted to determine if chinchillas would be a valid model for toluene ototoxicity. Because its audiogram more closely aligns with the human (Fay, 1988) and the cochlea is easily harvested, the chinchilla has been used extensively for noise-induced hearing loss experiments for over 30 years.

Previous studies with rats exposed the animals to toluene and noise sequentially (Johnson et al., 1988, 1990) and reported a hearing loss that was greater than addition of the two exposures. A simultaneous exposure to noise and toluene was proposed since it more closely mimics an occupational environmental exposure and stresses the cochlea with noise while toluene is at its maximum concentration.

When little ototoxic effect was seen in the chinchilla to toluene in the first series of exposures in the present study, a positive control experiment with rats was conducted, whose results are also reported here. A third study was conducted to examine the mechanism that protected the chinchilla hearing from the ototoxic effects of toluene. The chinchilla's liver cytochrome P450 system was hypothesized to be more effective than that of the rat. The detoxifying properties of chinchilla and rat liver microsomes were compared to human liver microsomes. The results of the microsome experiments are also reported here.

2. Materials and methods

2.1. Subjects

Chinchillas were adults born in the National Institute for Occupational Safety and Health (NIOSH) colony. They were fed Purina Chinchilla Chow. Surgical, test and exposure procedures were approved by the State University of New York (SUNY)-Buffalo Animal Care and Use Committee under protocol COM-05080N and by the NIOSH Animal Care and Use Committee under protocol C73DAV. Every day, animals were weighed prior to the exposure. Each animal was examined by experienced animal staff prior to and after each exposure session to detect any exposure-related signs. All procedures were conducted under the supervision of a staff veterinarian. SUNY-Buffalo and NIOSH animal facilities were AAALAC accredited.

2.1.1. ABR test procedures

The initial set (first exposure) of 22 chinchillas were implanted at the SUNY-Buffalo Hearing Research Laboratories and pre-exposure ABRs were measured. The adult chinchillas were monauralized via left cochlear destruction and implanted with chronic electrodes in the inferior colliculus and central sulcus region (Henderson et al., 1969). The animals were tested for auditory-evoked potentials and were then transported by automobile to NIOSH Taft Laboratories in Cincinnati. The SUNY-Buffalo procedure has previously been described by Bancroft et al. (1991). Briefly, 10 ms (5 ms rise/decay time) tone bursts (0.5, 1, 2, 4, 8 and 16 kHz) with 100 ms interstimulus interval were presented in the free field. Chinchillas were awake and restrained by a collar. Potentials from the electrodes were amplified by a Grass 511 amplifier and digitized by a Loughborough DSP board. The ABR was collected and analyzed by custom software running on a PC computer.

Another 11 chinchillas (second exposure) were implanted and tested at NIOSH. Chronic electrodes were implanted as above, but subjects were not monoauralized. Upon recovery chinchillas were tested awake and restrained (Snyder and Salvi, 1994). The contralateral pinna was cleaned with an alcohol swab and a Grass® gold earlobe cup electrode with conductive gel was clipped onto the pinna and attached to the ground input. Unless otherwise noted all ABR-related equipment was from Tucker-Davis Technologies (TDT, Gainesville, FL, USA). Stimuli were generated by BioSig ABR (TDT) software. Tone bursts (5 ms rise/fall time, Blackman windowed, alternate phase) at 0.5, 1, 2, 4, 8, and 16 kHz were played to the right ear through an Etymotic Research ER-3 insert earphone. Attenuation was provided to the left ear by a second ER-3 earphone.

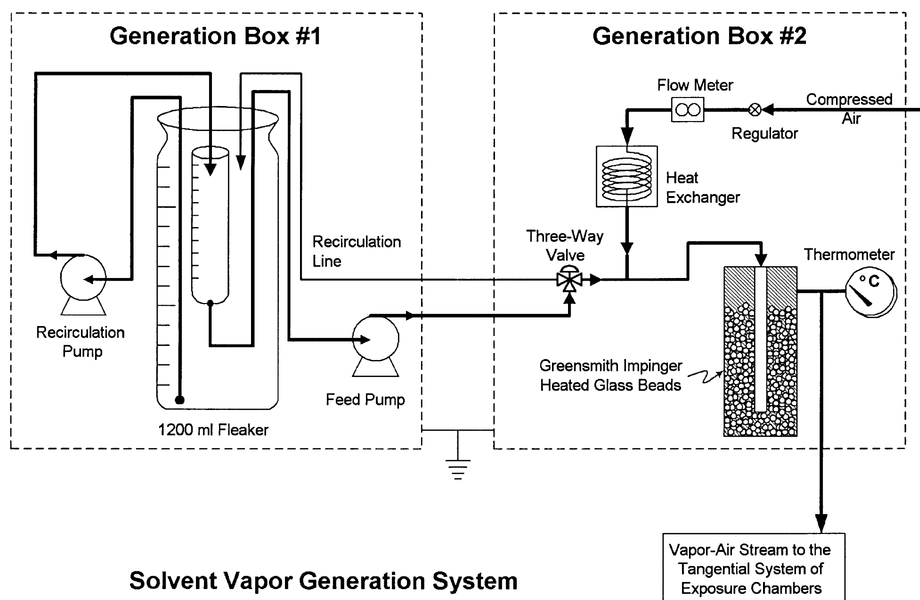

Fig. 1. Schematic diagram of the vapor generation system. The glass hypodermic syringe body provides a reservoir of toluene with a constant pressure for the feed pump. This keeps the flow of toluene constant into the chamber.

Electrophysiologic signals were amplified by a Grass P511 biological amplifier and then sampled by an analog-to-digital converter. Up to 512 responses were averaged. The time window for the response was 20 ms. Baseline pre-exposure thresholds were obtained three separate times. Post-exposure measures were made on days 1, 3, 7, 14 and 30 after the last exposure.

In addition, distortion product otoacoustic emissions (DPOEs) were measured for each chinchilla. However, the results of the DPOEs paralleled those of the ABRs and will not be presented here.

2.1.2. *Determination of exposure levels*

Pilot studies were conducted for levels of 2000–4000 ppm toluene to determine safe exposure levels. Generally chinchillas survived exposure to 2250 and 2500 ppm but died of secondary effects after a number of days in the exposure. At the highest levels (3000 and 4000 ppm) neurotoxic effects were observed: ataxia, head leaning and other vestibular signs.

2.1.3. *Procedures for toluene and noise exposures*

During exposure each animal was observed at least once every hour. Chinchillas had access to water although animals were never observed drinking. The placement within the chamber was rotated each day to remove any effects of non-homogeneous noise levels and chemical concentrations although all measurements showed noise to be ±1 dB and toluene concentrations to be ±1% within the chamber.

2.2. *Exposure facilities*

Initially the studies were carried out in two 5 m^3 inhalation chambers with toluene exposure and unexposed control animals run simultaneously (first exposure). The studies were moved to a single 0.5 m^3 chamber with 'exposed' and 'control' conditions handled sequentially (second exposure and rat exposure).

The vapor system used two pumps (Fig. 1). One pump (RP-BG25-1, Fluid Metering, Inc.) recirculated toluene (Fisher® Optima® grade toluene, stock number T291-4, assay ≥99.8%) from the glass reservoir into a 5 ml glass syringe body, which was allowed to overflow into the reservoir. This provided a constant head pressure for the feed pump. The feed pump (RP-BP25-0, Fluid Metering, Inc.) drew toluene off the glass syringe body and added toluene at a constant rate into a stream of warm, filtered air which further flowed through a warm, 500 cm^3 Green-Smith impinger (Fisher Scientific, Pittsburgh, PA, USA) filled with glass beads. The impinger and glass beads provided adequate heat transfer area and contact time between the dilution air and the toluene to ensure total vaporization. Filtered air was pushed through the glass impinger by a fan and the resulting vapor-laden air was directed into the exposure chamber. The exposure chamber was negatively pressurized with respect to the lab by an exhaust fan. The exhaust air was treated by flow through three stages of charcoal filters which scavenged the toluene. A Miran 1A infrared analyzer (Foxboro Analytical, Foxboro,

MA, USA) monitored the final exhaust air to detect any charcoal filter saturation and toluene breakthrough.

Chamber toluene concentration was constantly measured by a Miran 1A infrared analyzer located outside the chamber. The analyzer was calibrated before each daily run. Twice during the multi-day runs a chamber air sample was taken through a sampling port. As a secondary method of calibration, a heated, 1 liter glass sampling bulb was filled with chamber atmosphere and the sample was assayed in a Hewlett-Packard 5890 gas chromatograph with a flame ionization detector. All measurements indicated that we could maintain chamber toluene levels within ±1% of our target concentration.

The unexposed control animals were placed in the chambers but toluene was not injected into the airflow.

2.3. Noise

Noise levels in the chamber were monitored with a Brüel and Kjær 4165 1/2″ microphone and a Brüel and Kjær 2636 measuring amplifier. Calibrations of the noise monitoring system were performed before and after the exposure day using a Brüel and Kjær pistonphone. The background noise levels were less than 60 dBA.

A 500 Hz octave band noise stimulus was created with a 1382 General Radio random noise generator and a 3323 Krohn-Hite filter. The signal was attenuated with Wavetek 5080 manual attenuators and amplified by a Stewart PA1400 power amplifier. Two Radio Shack Optimus 1 speakers were mounted in the inhalation chamber in such a manner as to produce a diffuse sound field within the volume in which the chinchillas were placed for exposure. Homogeneity of the noise exposure spectra and energy was assessed with a Stanford Research Systems SR780 dual channel FFT analyzer prior to the exposures. The amplitude of the noise was increased until the level in the chambers reached 97.5 dB SPL A-weighted on the noise monitoring system. A-weighting was used because it filtered out the low-frequency fan vibration which was transduced by the microphone.

2.4. Exposures

Chinchillas were divided into six groups. The first four groups (first exposure) were run simultaneously, the second two groups (second exposure) were run later (see Fig. 2). All groups were exposed for 10 sequential days. Group 1 was exposed to 8 h of toluene at 2000 ppm and no noise other than background. Group 2 was exposed to 8 h of noise but no toluene. Group 3 was exposed to 8 h of noise and 8 h of toluene. Finally, a control group (group 4) was exposed to only background noise and clean air by being placed in the exposure chamber for 8 h.

Not seeing a toluene effect after the first exposure, an attempt was made to increase the toluene dose. Pilot studies showed that increasing the concentration of toluene would not be a viable option for increasing dose, so a longer exposure to toluene was initiated. The noise exposure was kept constant so results could be compared with the earlier exposures. The second set of exposures were 12 h of toluene for 10 sequential days

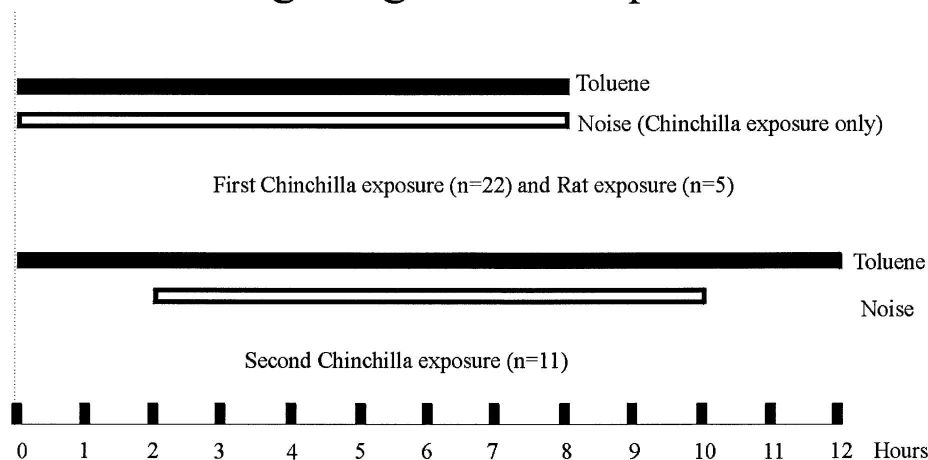

Fig. 2. Schematic diagram of the timing of the 8 (top) and 12 h (bottom) exposures. Black bar indicates the time when toluene was present in the chamber. Open bar indicates when noise was present. Group 1 received an 8 h toluene exposure with no noise. Group 2 received an 8 h noise exposure with no toluene. Group 3 was exposed to both 8 h of noise and 8 h of toluene. Group 4 was exposed to neither noise nor toluene but placed in the exposure chamber. Group 5 was exposed to 12 h of toluene and 8 h of noise. Group 6 was exposed to 12 h of toluene alone. No noise was present during the 8 h rat exposures.

(see Fig. 2). Group 5 was exposed to toluene for 12 h and noise for 8 h, with the noise beginning 2 h after the start and ending 2 h before the end of the toluene exposure. Group 6 was exposed to 12 h of toluene with only background noise present.

2.5. Rat positive control experiment

Six adult rats of the Sprague–Dawley strain were exposed to toluene at 2000 ppm for 5 days at 8 h per day by inhalation. The exposure was similar to the chinchilla exposures in the smaller chamber. Noise was limited to background noise (less than 60 dB). The pre-exposure ABR threshold for each rat served as its own control. Rats were tested before exposure and 30 days after exposure at 8, 16 and 32 kHz and with a 0.01 ms click. The ABR was evoked and averaged by an Intelligent Hearing System ABR unit (North Miami, FL, USA) controlled by a laptop computer. Rats were anesthetized with an i.m. injection of a mix of ketamine (22 mg/kg) and Rompum (1.1 mg/kg). An auditory stimulus was delivered by headphones (AKG-K340) and presented binaurally via plastic funnels. Grass® stainless steel needle electrodes were inserted subcutaneously at the vertex (active), ventrolateral to the left ear (inverting) and the dorsum (ground). A Grass 511 preamplifier amplified the biological signal 25 000–100 000× before presentation to the ABR unit for analysis and display. The signal was averaged for 512 sweeps or until a reproducible waveform could be seen. Auditory threshold was determined by the lowest stimulus intensity where at least two peaks of the ABR waveform could be visually detected. Individual hearing loss was quantified based upon the threshold shift between pre- and post-exposure ABR thresholds.

2.6. Liver microsome testing

Five toluene-naive adult chinchillas were killed with an overdose of sodium pentobarbital. After removal of cochleas for another study, livers were removed and prepared for cryogenic storage. Toluene metabolism was measured only in three of those chinchilla livers but protein content was measured in all five livers. Two pools of three toluene-naive Sprague–Dawley rat liver samples were obtained from a previous experiment. Ten frozen human liver samples were obtained from a commercial supplier (International Institute for the Advancement of Medicine, Exton, PA, USA). These samples were normal tissue–resected during transplantation. All tissues were collected, resected, rinsed in phosphate-buffered saline (PBS) and snap-frozen in liquid nitrogen, stored at −80°C until microsomes were made (same method, buffers, etc.). Microsomes were diluted in identical buffers and again snap-frozen until use. Incubations (chinchilla, rat and humans) were done at the same time, using the same buffers, chemicals, etc.

2.6.1. Chemicals

All chemicals were at least reagent grade and were obtained from Sigma (St. Louis, MO, USA) or Aldrich (Milwaukee, WI, USA) unless otherwise noted. Glucose-6-phosphate and $NADP^+$ were obtained from Boehringer-Mannhein (Indianapolis, IN, USA), and ketoconazole was purchased from Janssen Biotech (Olen, Belgium).

2.6.2. Microsome preparation and metabolism of toluene

Chinchilla, rat and human microsomes were prepared via the method of Guengerich (1989). Microsomal protein content was determined by the BCA method with bovine serum albumin as a standard. Toluene metabolism was assessed as previously reported by Nakajima et al. (1993). Briefly, 200 μg of microsomal protein was incubated for 5 min in 0.1 mM Tris buffer containing 5 mM $MgCl_2$ and 1–10 mM toluene. All reactions were initiated by the addition of an NADPH-regenerating system (in 0.1 M potassium phosphate buffer containing 14 mmol glucose-6-phosphate, 0.66 mmol $NADP^+$, and 3 U glucose-6-phosphate dehydrogenase) and were quenched after 30 min by the addition of 0.2 ml cold acetonitrile. Benzyl alcohol formed from toluene was measured by reverse-phase high-performance liquid chromatography under the following conditions: column C18, mobile phase was 70/30 water/acetonitrile (pH 3) at a flow rate of 0.2 ml/min. Under these conditions benzyl alcohol had a retention time of 21.7 min. The area under the curve of peaks was measured and the amount of benzyl alcohol formed was estimated by comparison with a standard curve of benzyl alcohol. Values were represented as nmol benzyl alcohol/min/mg protein.

2.6.3. Quantification of CYP proteins

The quantification of CYP proteins via enzyme-linked immunosorbent assay (ELISA) was conducted as previously described (Snawder and Lipscomb, 2000). Duplicate samples of microsomal protein were analyzed for CYP1A, CYP2B and CYP2E1 content. The CYP content for each CYP form was expressed as pmol CYP/mg microsomal protein.

2.6.4. Determination of individual P450 forms in chinchilla, rat and human liver samples

Contents of specific P450 forms were estimated by a direct ELISA. Briefly, 0.5 μg of microsomal protein/well was plated onto microtiter plates (carbonate–bicarbonate buffer, pH 9.0) along with microsomes containing a known quantity of the P450 form (between 1 and 1000 fmol cytochrome P450/50 μl, Gentest) of interest

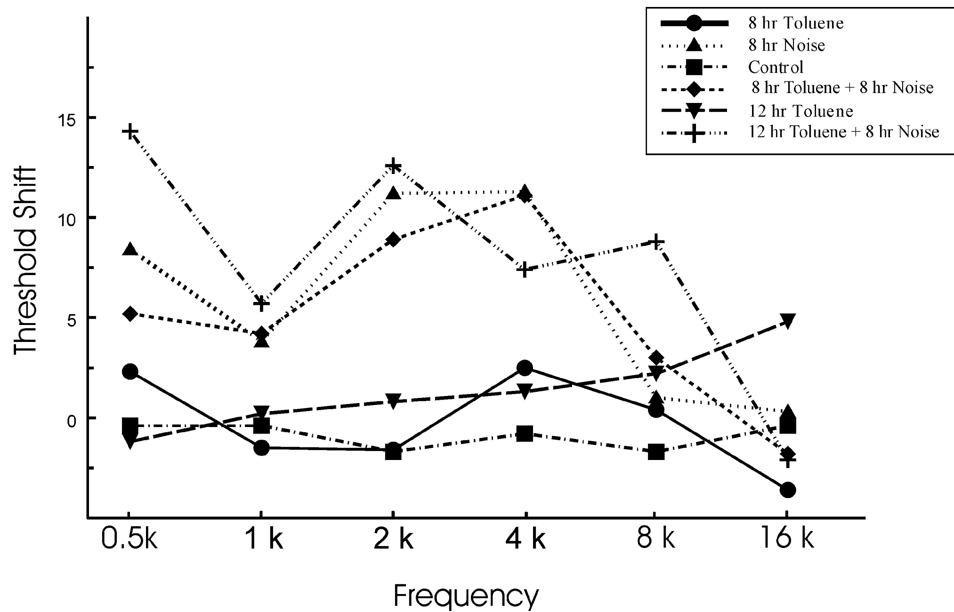

Fig. 3. Chinchilla threshold shifts 30 days post-exposure. Note the very little shift in chinchilla groups exposed only to toluene. Major shifts occurred in the chinchilla groups exposed to noise alone or in combination with toluene. There was no increased shift with noise plus toluene.

for a standard curve. Plates were incubated overnight at 4°C and the plating solution was removed the following morning. One hundred μl of 50% fetal bovine serum (FBS) in PBS was added as a blocking agent and plates were incubated for 1 h at 37°C. The blocking agent was removed and plates were washed three times (10% FBS, TBS-Tween) and were incubated at 37°C for 1 h with primary antibody (anti-CYP1A, CYP2B, CYP2C and CYP2E1, Gentest). Primary antibody was removed and plates were washed and then incubated for 1 h with 200 μl/well of anti-goat–alkaline phosphate conjugate. The secondary antibody was removed and plates were washed and 150 μl of K-Gold pre-mixed ELISA phosphatase substrate (ELISA Technologies, Lexington, KY, USA) was added to each well. After 30 min the plate was read at 405 nm. Absorbance of sample containing wells was compared to a standard curve. Values were expressed as pmol P450 form/mg protein.

2.6.5. Statistical analysis

Data were evaluated by Student's *t*-test and analysis of variance with post-hoc evaluation of differences by Student–Newman–Keuls test ($P < 0.05$). The tests were performed using SAS® (SAS Institute, Cary, NC, USA) or Sigmastat® (Jandel Scientific, San Rafael, CA, USA).

3. Results

Pre-exposure ABR thresholds in all chinchilla groups were essentially the same (data not shown). Fig. 3 shows the threshold shift results of all of the post-exposure chinchilla groups 30 days after the final exposure. Note, as expected, that the unexposed control group (squares) had about zero threshold shift across the audiogram. The striking feature about Fig. 3 is the mid-frequency division of the groups exposed to the 500 Hz octave band of noise (triangle, diamond and plus) from groups exposed to toluene alone (inverted triangle and circle). The noise exposures produced a 12 dB permanent threshold shift at 2.0 and 4.0 kHz. Analysis of

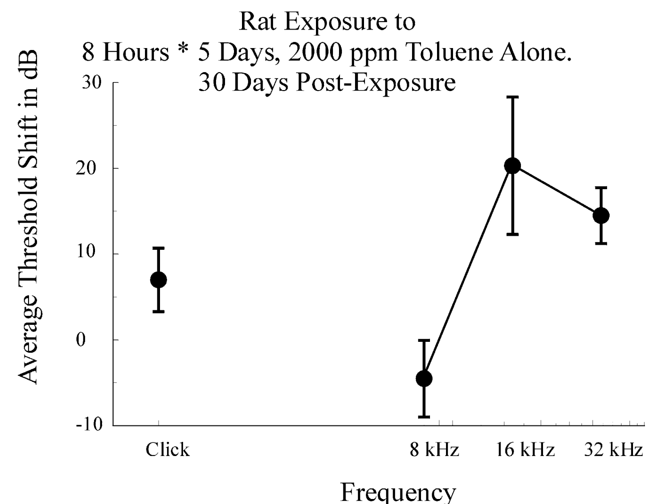

Fig. 4. Toluene-alone exposures produced large threshold shifts in rats. A 5 day, 8 h per day exposure resulted in the above threshold shifts.

variance did not detect any significant main effect due to toluene alone or an interaction of toluene with noise. The 12 h toluene exposures show a slight elevation at 16.0 kHz when exposed only to toluene, but when exposed to 12 h of toluene and 8 h of noise, the high-frequency loss disappeared and only the low-frequency noise effect was present.

Fig. 4 shows permanent threshold shift for rats exposed to 2000 ppm for only 5 days at 8 h per day. These data stand in stark contrast to the chinchilla data. The shorter rat exposures to toluene led to extensive threshold shifts of 20 and 15 dB at 16.0 and 32.0 kHz. Two conclusions can be drawn: rats were much more sensitive to the ototoxic effects of toluene than chinchillas and the exposure environment was adequate to produce ototoxic effects. The cumulative rat exposures were less than half of those of the chinchillas (5 versus 10 days) yet the threshold shifts produced were considerably larger.

The two species differ markedly in their response to toluene. Several hypotheses could explain the observed results. One hypothesis is that the two species metabolize and detoxify toluene at different rates. The most important site of detoxification in the body is the liver. To appraise the differences between the two toluene-exposed species and humans, liver microsomes were tested in vitro. Microsomes, derived from liver cells, are membrane-bound, cell-free spheres containing the P450 enzymes. The P450 enzymes convert toluene to less lipid-soluble and more water-soluble chemical forms, which are easier to eliminate via the kidney. The CYP2E1 enzyme converts toluene to benzyl alcohol (Fig. 5). The CYP1A2/1 enzymes convert toluene to

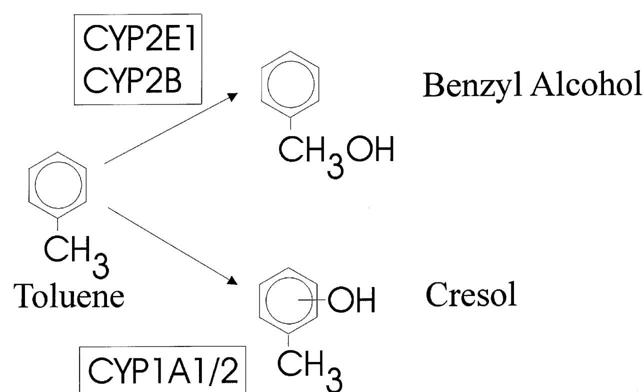

Fig. 5. Metabolism of toluene. The P450 enzymes CYP2E1 and CYP2B convert 70–90% of the toluene into benzyl alcohol. Benzyl alcohol is further transformed to benzaldehyde then benzoic acid and finally to hippuric acid, which is removed in the urine. Less than 1% of the toluene is converted to *o*- or *p*-cresol which is further conjugated with sulfate, glucuronide, glutathione or cysteine before removal in the urine (adapted from Dorsey and Donohue, 1994).

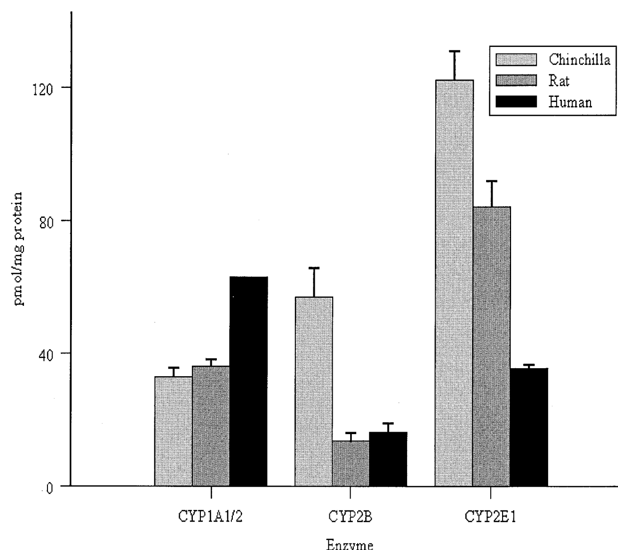

Fig. 6. Differences in P450 enzyme levels between the different species show that chinchillas have more enzyme present.

o-cresol. The CYP2B* enzymes operate at higher toluene concentrations. The ELISA analysis found that chinchillas had higher levels of the liver cytochrome enzymes CYP2E1 and CYP2B than either rats or humans. CYP1A levels were similar in chinchilla and rat while human samples show great variation across individuals (Fig. 6).

Chinchilla microsomes were found to possess the greatest metabolic activity for conversion of toluene to benzyl alcohol (Table 1). Formation of benzyl alcohol by rat microsomes was less than half that measured in chinchilla; human microsomes showed great individual variation in benzyl alcohol formation – levels varied four-fold between the sample with the highest activity compared to the sample with the lowest activity. Differences in activity for the two human samples may be due to enzyme induction by life exposure to various chemicals.

Specific P450 levels in microsome samples were correlated with toluene biotransformation. Samples containing high amounts of CYP2E1 displayed the strongest correlation with alcohol as a product of toluene metabolism ($r = 0.973$). Among other forms examined, only CYP2B displayed a consistent correlation between

Table 1
Rate of toluene conversion per mg of protein

Species	Benzyl alcohol (nmol/min/mg protein)
Chinchilla	5.90 ± 1.45*
Rat	2.12 ± 0.11
Human	1.72 ± 1.75

*$P < 0.05$. Chinchilla liver microsomes can convert toluene almost three times faster than rat or human microsomes.

protein content and benzyl alcohol formed from toluene metabolism ($r = 0.936$).

4. Discussion

Exposure to toluene in the present study produced no measurable ototoxicity in chinchillas while noise effects were detected. In contrast, rats showed a significant permanent threshold shift to toluene exposure. Negative results are always difficult to justify. The positive results of the rat experiment would argue that our procedures and exposure chambers were capable of producing toluene ototoxicity. Thus, differences between species must be considered to explain the differences in susceptibility.

Unlike aminoglycoside antibiotics or other classical ototoxins, solvent effects seem to be species specific. In particular, rat and mouse ears appear to be extremely sensitive to solvents while chinchillas and guinea pigs do not. This difference in susceptibility seems to be explained by apparent differences in liver enzyme activity between chinchillas and guinea pigs and between rats and mice. Human livers appear to have greater variability in the ability to biotransform toluene than any of the experimental animals but appear to be less able to metabolize toluene than chinchillas. Such variability in biotransformation may explain the differences seen in human populations between people more susceptible and less susceptible to toluene ototoxicity. Based on these data one would speculate that humans suffering from liver damage, e.g. cirrhosis, would be much more sensitive to the ototoxic effects of toluene. Humans receiving drugs which inhibit liver enzymes would be expected to be more susceptible to toluene ototoxicity. On the other hand, individuals who are able to build P450 enzyme levels without compromising the liver may be less susceptible to the ototoxic effect of toluene.

Metabolic variation among humans has been investigated as a risk-modifying factor for CYP2E1 substrates including toluene, chloroform and trichloroethylene (Allen et al., 1996; Pierce et al., 1996; Snawder and Lipscomb, 2000). Often, human individual variance in xenobiotic metabolism mediates differences in susceptibility to injury (Nebert and Roe, 2001). Depending on the cytochrome P450 form, metabolic activity can be influenced by genetics, lifestyle, overall health of the individual and environmental factors (de la Maza et al., 2000; Roe et al., 1999).

The hypothesis that the liver modulates ototoxicity could be further tested by utilizing liver enzyme inhibitors in chinchillas and liver enzyme inducers in rats to demonstrate a change in susceptibility to toluene ototoxicity. If the liver function of chinchillas could be chronically impaired, exposure to toluene may produce an ototoxic effect. Induction of enzymes may produce protection in rats.

These results indicate that chinchillas are not an ideal species to study direct toluene effects on the ear. However, they may be an interesting model for examining the more complex environment in which ototoxins operate. Clearly, the ear is not the only target for these agents.

Acknowledgements

Our thanks to Dr. Derek Dunn, former Director of the Division of Behavioral and Biomedical Science, for his support during the early part of this project. Dr. Thais Morata (Visiting Scientist), Jeff McLarin (Physical Tech), Dawn Ramsey (Chemist) and Dr. Alex Teass (Chemist) provided helpful support. The ILS, Inc., animal crew kept the animals safe and healthy. Yun-Hua Shen, at SUNY-Buffalo, provided chinchilla ABR and DPOE testing during the experiment. Thanks also to Mike Cheever and Dr. Lawrence Erway from the University of Cincinnati for help in ABR testing of the rats. Early reports of these data were presented as posters at the Association for Research in Otolaryngology (Davis et al., 1996; Davis and Snawder, 2000).

References

Allen, B.C., Covington, T.R., Clewell, H.J., 1996. Investigation of the impact of pharmacokinetic variability and uncertainty on risks predicted with a pharmacokinetic model for chloroform. Toxicology 111, 289–303.

Bancroft, B.R., Boettcher, F.A., Salvi, R.J., Wu, J., 1991. Effects of noise and salicylate on auditory evoked-response thresholds in the chinchilla. Hear. Res. 54, 20–28.

Campo, P., Lataye, R., Bonnet, P., 1993. No interaction between noise and toluene on cochlea in the guinea pig. Acta Acust. 1, 35–42.

Campo, P., Loquet, G., Blachère, V., Roure, M., 1999. Toluene and styrene intoxication route in the rat cochlea. Neurotoxicol. Teratol. 21, 427–434.

Davis, R.R., Snawder, J.E., 2000. Does liver control susceptibility to toluene ototoxicity? The 21st Midwinter Meeting of the Association for Research in Otolaryngology. Abstract 411.

Davis, R.R., Murphy, W.J., Zheng, X.Y., Henderson, D., Morata, T., Khan, A., 1996. Effects of toluene and noise on the chinchilla auditory system. 19th Midwinter Research Meeting of the Association for Research in Otolaryngology. Abstract 124.

de la Maza, M.P., Hirsch, S., Petermann, M., Suazo, M., Ugarte, G., Bunout, D., 2000. Changes in microsomal activity in alcoholism and obesity. Alcohol Clin. Exp. Res. 24, 605–610.

Dorsey, A.S., Donohue, J.M., 1994. Toxicological Profile for Toluene. US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, TP-93/14.

Fay, R.R., 1988. Hearing in Vertebrates: A Psychophysics Databook. Hill-Fay Associates, Winnetka, IL.

Franks, J.R., Morata, T.C., 1996. Ototoxic effects of chemicals alone

or in concert with noise: a review of human studies. In: Axelsson, A., Borchgrevink, H.M., Hamernik, R.P., Hellström, P.A., Henderson, D., Salvi, R. (Eds.), Scientific Basis of Noise-Induced Hearing Loss. Thieme, New York, pp. 437–446.

Guengerich, F.P., 1989. Principles and Methods of Toxicology, 2nd edn. Raven Press, New York, pp. 777–814.

Henderson, D., Onishi, S., Eldredge, D.H., Davis, H., 1969. A comparison of chinchilla evoked response and behavioral response thresholds. Percept. Psychophys. 5, 41–45.

Johnson, A.C., Canlon, B., 1994. Progressive hair cell loss induced by toluene exposure. Hear. Res. 75, 201–208.

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

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

Li, H-S., 1992. Genetic influences on susceptibility of the auditory system to aging and environmental factors. Scand. Audiol. Suppl. 36, 1–39.

Liu, Y., Fechter, L.D., 1997. Toluene disrupts outer hair cell morphology and intracellular calcium homeostasis in cochlear cells of guinea pigs. Toxicol. Appl. Pharmacol. 142, 270–277.

Morata, T.C., 1989. Study of the effects of simultaneous exposure to noise and carbon disulfide on workers' hearing. Scand. Audiol. 18, 53–58.

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., Cesar, C.L., 1997. Toluene-induced hearing loss among rotogravure printing workers. Scand. J. Work Environ. Health 23, 289–298.

Nakajima, T., Wang, R.-S., Elovarra, E., Park, S.S., Gelboin, H.V., Vainio, H., 1993. Cytochrome P450-related differences between rats and mice in the metabolism of benzene, toluene and trichloroethylene in liver microsomes. Biochem. Pharmacol. 45, 1079–1085.

Nebert, D.W., Roe, A.L., 2001. Ethnic and genetic differences in metabolism genes and risk of toxicity and cancer. Sci. Total Environ. 274, 93–102.

Pierce, C.H., Dills, R.L., Morgan, M.S., Nothstein, G.L., Shen, D.D., Kalman, D.A., 1996. Interindividual differences in 2H8-toluene toxicokinetics assessed by semiempirical physiologically based model. Toxicol. Appl. Pharmacol. 139, 49–61.

Rebert, C.S., Sorenson, S.S., Howd, R.A., Pryor, G.T., 1983. Toluene-induced hearing loss in rats evidenced by the brainstem auditory-evoked response. Neurobehav. Toxicol. Teratol. 5, 59–62.

Roe, A.L., Howard, G., Blouin, R., Snawder, J.E., 1999. Characterization of cytochrome P450 and glutathione *S*-transferase activity and expression in male and female ob/ob mice. Int. J. Obes. Relat. Metab. Disord. 23, 48–53.

Snawder, J.E., Lipscomb, J.C., 2000. Interindividual variance of cytochrome P450 forms in human hepatic microsomes: correlation of individual forms with xenobiotic metabolism and implications in risk assessment. Regul. Toxicol. Pharmacol. 32, 200–209.

Snyder, D.L., Salvi, R.J., 1994. A novel chinchilla restraint device. Lab. Anim. 23, 42–44.

Sullivan, M.J., Rarey, K.E., Conolly, R.B., 1988. Ototoxicity of toluene in rats. Neurotoxicol. Teratol. 10, 525–530.