Acute Toluene Exposure and Rat Visual Function in Proportion to Momentary Brain Concentration

William K. Boyes,*1 Mark Berccegay,* Quentin Todd Krantz,† Elaina M. Kenyon,† Ambuja S. Bale,* Timothy J. Shafer,* Philip J. Bushnell,* and Vernon A. Benignus‡

*Neurotoxicology Division, †Experimental Toxicology Division, and ‡Human Studies Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina

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Acute exposure to toluene was assessed in two experiments to determine the relationship between brain toluene concentration and changes in neurophysiological function. The concentration of toluene in brain tissue at the time of assessment was estimated using a physiologically based pharmacokinetic model. Brain neurophysiological function was measured using pattern-elicited visual evoked potentials (VEP) recorded from electrodes located over visual cortex of adult male Long–Evans rats. In the first experiment, VEPs were recorded before and during exposure to control air or toluene at 1000 ppm for 4 h, 2000 ppm for 2 h, 3000 ppm for 1.3 h, or 4000 ppm for 1 h. In the second experiment, VEPs were recorded during and after exposure to clean air or 3000 or 4000 ppm toluene. In both experiments, the response amplitude of the major spectral component of the VEP (F2 at twice the stimulus rate in steady-state responses) was reduced by toluene. A logistic function was fit to baseline-adjusted F2 amplitudes from the first experiment that described a significant relationship between brain toluene concentration and VEP amplitude deficits. In the second experiment, 3000 ppm caused equivalent VEP deficits during or after exposure as a function of estimated brain concentration, but 4000 ppm showed a rapid partial adaptation to the acute effects of toluene after exposure. In general, however, the neurophysiological deficits caused by acute toluene exposure could be described by estimates of the momentary concentration of toluene in the brain at the time of VEP evaluation.

Key Words: neurotoxicity; PBPK model; volatile organic compound; organic solvent; visual evoked potential.

Understanding the relationships between exposure parameters, target tissue dose, and toxic outcome can improve quantitative risk assessments in a variety of different circumstances (Andersen, 1995). For example, acute exposure guideline levels (AEGLs) are a set of values intended to describe the risks of acute exposures to hazardous substances that are established for three levels of severity and five different durations of exposure, including 10 min, 30 min, and 1, 4, and 8 h (National Research Council, 2001). Because data are rarely available for the set of exposure durations of interest, the setting of AEGL standards typically involves extrapolations or adjustments across exposure durations. Because momentary concentration of trichloroethylene (TCE) in brain was predictive of acute neurological impairment following inhalation exposure, it was possible to estimate air exposure concentrations that would provide the same internal dose, and presumably the same risk, over several different exposure durations (Boyes et al., 2005b; Bruckner et al., 2004; Simmons et al., 2005). It is of interest to determine similar relationships for additional common volatile organic compounds so that quantitative estimates of risk under a variety of exposure conditions may be derived.

Toluene is an aromatic volatile organic compound that is one of the most commonly used substances in industry and commerce. Toluene is a constituent of gasoline, and is present in numerous other consumer products including glues, cleaning products and oil-based paints. Toluene was included on a list of 188 chemicals and chemical classes designated as Hazardous Air Pollutants by the U.S. Congress in the 1990 Amendment to the Clean Air Act. Under this statute, the U.S. Environmental Protection Agency (USEPA) is charged with evaluating the risks of acute and chronic exposure to the residual emissions from industrial sources releasing hazardous air pollutants after those sources have been controlled using the maximum available control technology (USEPA, 1999). Toluene is the most commonly emitted hazardous air pollutant, accounting for 18% of all air toxic emissions, and is a consideration in the residual emissions from approximately 40 industrial source categories, more than any other substance. Toluene was detected in the majority of blood samples from the third National Health and Nutrition Examination Survey, a cross-sectional study of U.S. citizens (Ashley et al., 1994). Toluene was found above the methodological detection limits in 45–75% of serial blood samples taken from U.S. children (Sexton et al., 2005). In addition, toluene is a main component of some inhalants of abuse and is behaviorally reinforcing (Bowen et al.,
Because of the large volume of use and ubiquitous exposure, it is important to develop an accurate understanding of the risks of exposure to toluene.

Acute exposure to toluene impairs neurological functions, including general disruption of sensory, cognitive, and motor function, similar to the effects of acute exposure to other volatile organic compounds (Arlien-Søborg, 1992). Exposure initially may produce euphoria, followed with continued or increasing levels of exposure by central nervous system depression. Reports of impaired visual function are prominent among the neurological deficits caused by exposure to toluene. Among these deficits are impaired perception of color, impaired visual acuity, impaired performance of neurobehavioral tasks with visual–motor or visual–spatial components, impaired flicker fusion frequency, and alterations of visual evoked potentials (VEPs) (USEPA, 2005). The latter, VEPs, are a set of electrophysiological procedures in which electrocortical potentials are recorded from electrodes placed above visual cortex in response to stimulation of the eyes with flashed or patterned visual stimuli. VEPs reflect the functional integrity of the primary visual afferent pathway and visual cortex, and are among other sensory evoked potentials that have been used widely to evaluate actions of neurotoxic substances (Herr and Boyes, 1995). Chronic exposure to toluene, either through toluene abuse (Kiyokawa et al., 1999) or occupational exposure (Vrca et al., 1995), alters VEPs in humans. Changes in VEPs were among the set of adverse outcomes that together were used by the USEPA to define a point of departure in the establishment of a reference concentration value for toluene (USEPA, 2005).

Acute behavioral alterations have been related to brain toluene concentrations (Benignus et al., 1998; Bruckner and Peterson, 1981; Kishi et al., 1988; Miyagawa et al., 1984). In one case, however, repeated exposure to toluene in high level concentration spikes caused behavioral impairments that were not directly related to peak brain toluene concentrations (van Asperen et al., 2003). Acute exposure to toluene also alters electrophysiological sensory-evoked potentials of rats, including suppression of flash or pattern-elicited VEPs (Dyer et al., 1988; Rebert et al., 1989). These electrophysiological studies, however, did not relate the magnitude of changes observed to a measure of internal dose of toluene.

Electrophysiological approaches offer advantages in linking temporal changes in internal dose to changes in neurological function, because they can be acquired in a matter of a few minutes or less, compared to many minutes required for behavioral assessments. One set of measures that has proved useful in this regard is steady-state pattern-elicited VEPs recorded from visual cortex of rats watching a changing visual pattern, in which the predominant response component occurs at double the temporal frequency of visual pattern modulation (F2). The amplitude of the F2 response was reduced by acute exposure to another volatile organic compound, TCE, in a manner that was dependent on the concurrent momentary concentration of TCE in the brain, as estimated by a physiologically based pharmacokinetic (PBPK) model (Boyes et al., 2003, 2005a).

The current study sought to evaluate the effects of acute toluene exposure on rat VEPs, and to relate those changes to brain toluene concentration at the moment of VEP assessment. The potential mechanisms of action through which these effects occur are explored in another manuscript (Bale et al., 2007).

MATERIALS AND METHODS

The experimental methods were similar in many respects to those described in detail elsewhere (Boyes et al., 2003, 2005a) and therefore are described here more briefly.

Test Compound

Spectrophotometric grade toluene (99.5% pure) (CAS # 108-88-3) was obtained from Aldrich Chemical Co., St Louis, MO.

Test Animals

The experimental subjects were male Long–Evans rats (LE) (Crl:LEBR) obtained from Charles River Laboratories (Raleigh, NC) at approximately 60 days of age. Animals were housed individually in polycarbonate cages with kiln-dried pine shaving bedding (Northeastern Products, Warrensburg, New York), and had ad libitum access to tap water and rat chow (LabDiet PMI #5001, Richmond, Indiana). The ambient temperature and relative humidity of the animal colony were 22 ± 2°C and 50 ± 10%, respectively, with a 12:12-h light:dark cycle (lights on at 6:00 A.M.). The illumination during the light cycle ranged from approximately 190 lux (bottom shelf of the animal rack) to 390 lux (top shelf of the animal rack). All aspects of the care and treatment of laboratory animals were approved by the Institutional Laboratory Animal Care and Use Committee of the National Health and Environmental Effects Research Laboratory, and were in compliance with applicable federal guidelines for laboratory animal experimentation.

Surgery

Prior to beginning the experiment, each rat was equipped with chronic indwelling cranial electrodes. To do so, rats were anesthetized (sodium pentobarbital; 50 mg/kg i.p.), placed in a standard stereotaxic device, and prepared for cranial surgery. Recording electrodes, constructed from stainless steel screws (00-90 #5001, Richmond, Indiana). The ambient temperature and relative humidity of the animal colony were 22 ± 2°C and 50 ± 10%, respectively, with a 12:12-h light:dark cycle (lights on at 6:00 A.M.). The illumination during the light cycle ranged from approximately 190 lux (bottom shelf of the animal rack) to 390 lux (top shelf of the animal rack). All aspects of the care and treatment of laboratory animals were approved by the Institutional Laboratory Animal Care and Use Committee of the National Health and Environmental Effects Research Laboratory, and were in compliance with applicable federal guidelines for laboratory animal experimentation.

Infalation Exposure

Toluene vapors were generated using a J-tube inhalation system (McGee et al., 1994) and delivered into a (10 × 10 × 17 cm) head-only exposure chamber (Boyes et al., 2003). The head-only exposure apparatus was constructed of stainless steel with the exception of a glass front plate to allow the rat to view the video monitor displaying visual stimuli outside of the chamber, and a glass side plate allowing observation of the experimental subject. Rats were equipped with a rectal thermometer probe, restrained in a plastic cone with the eyes, nose, and ears exposed, and placed into the exposure chamber. A gas-tight latex seal was established around the upper torso to isolate the atmosphere of the head-only exposure chamber. Electrophysiological...
signals were recorded from a flexible cable entering the head-only exposure chamber through a gas-tight port and connected to the electrode headset.

The air concentrations of toluene in the head-only exposure chamber were monitored continuously online by a real-time infrared spectrophotometer (Miran, 1A, Foxboro, Co., East Bridgewater, MA), which provided continuous output on a strip chart recorder. The strip chart was monitored by the operator throughout each experiment, and the experiment was stopped if there was any noticeable deviation from the expected concentration. In order to calculate actual concentrations presented, an average of 3–8 points was taken from the strip chart, depending on the length of the exposure period. The actual concentrations were then computed relative to a standard calibration curve, and mean and SD values were calculated for each rat. Group mean concentrations for each dose group were calculated from the individual rat mean values.

**Visual Stimuli**

Visual stimuli were presented on a video monitor located outside the glass face of the exposure chamber approximately 15 cm from the eye. The stimuli were generated with a computer-based system described elsewhere (Boyes et al., 2003; Hamm et al., 2000). The stimulus generation system was calibrated to achieve a linear relationship between video input signal voltage and screen luminance. The entire assembly was covered in a black cloth so that the primary source of light for the rat was the video screen.

The parameters of the visual stimuli were identical to those used previously to study TCE (Boyes et al., 2003, 2005a). Specifically, the stimulus pattern was a vertical grating with a sinusoidal spatial luminance profile and a spatial frequency of 0.16 cycles per degree visual angle. The stimulus modulated between contrast of 0 (i.e., a uniform nonpatterned screen) and peak contrast of 60%, with a temporal sinusoid of approximately 4.55 Hz. These values of spatial frequency and temporal modulation rate were selected because they are near the peak of the pigmented rat spatial and temporal response functions, and give an unambiguous steady-state response. In addition, pattern VEP amplitudes recorded using these stimulus parameters have been sensitive to and give an unambiguous steady-state response. In addition, pattern VEP near the peak of the pigmented rat spatial and temporal response functions, spatial frequency and temporal modulation rate were selected because they are well defined (multiple consistent experimental results with low variability) or relatively noninfluential (e.g., organ volumes other than fat). Sensitivity analyses were conducted to evaluate the potential for individual parameters to influence the model output by manipulating the values of specific model parameters while holding other parameters steady. This analysis revealed that the most influential parameters for these experimental conditions were cardiac output, alveolar ventilation and fat volume. For this reason, fat volume was estimated using regression equations developed specifically for the LE rat (Simmons et al., 2002) and activity-based estimates were used for cardiac output and alveolar ventilation (Kenyon et al., in press). Baseline values were obtained from Simmons et al. (2002) for cardiac output, and increases above baseline for cardiac output were benchmarked against increased heart rate measured in LE rats implanted with telemers. Values for alveolar ventilation rate during toluene exposure were optimized under various conditions of toluene exposure (actively performing tasks and resting). Resting values were used to derive estimates for this study; since these rats were not actively performing a task such as lever pressing during the exposures. This parameterization of the model allowed successful prediction of other literature data not used for model development or parameter estimation. After elimination of one data set from the literature which had lower concentrations than the others, the model showed a correlation coefficient of 1.01, which was not significantly different from 1, between the predicted and combined observed data across three different experiments. The PBPK model was used to estimate brain toluene concentrations at the times of VEP recordings based on the mean body weights of each group as tested.

**Evoked Potential Recording**

Electrophysiological potentials were recorded using a system described in Hamm et al. (2000), amplified (10K), bandpass filtered (0.1–1000 Hz), and sampled at a rate of 1.2 kHz in 5-s epochs. Each VEP was constructed from the average of 20 5-s epochs. Each averaged waveform was submitted to a spectral analysis. The spectrum was calculated using a mixed-radix Fast Fourier Transform routine (Bergland and Dolan, 1979), and then calculating the real magnitude spectrum from the complex spectrum. The spectral amplitude was measured at the rate of the visual pattern modulation (F1) and twice that rate (F2). In addition to recording evoked potentials to the 60% contrast visual stimulus, averaged waveforms were also recorded to a nonmodulated mean luminance screen (0% contrast), paired to each signal session, to indicate the recording noise levels.

**Pharmacokinetic Modeling of Toluene Exposures**

The PBPK model used to simulate the exposures conditions of this study was developed and calibrated for LE rats of the age and weight range used in this study (Kenyon et al., in press). Briefly, the compartments in the model were lung, slowly and rapidly perfused tissue groups, fat, liver, GI tract, and brain. Tissue transport was blood-flow limited and metabolism occurred in the liver. Initial parameters for organ volumes and blood-flow rates were obtained from the literature (Brown et al., 1997) or were specific for the LE rat (Simmons et al., 2002). Partition coefficients were obtained from Thrall et al. (2002) and metabolic rate constants from DeJongh and Blauhofer (1996). A combination of literature review and sensitivity analysis indicated that certain parameters (e.g., partitions and metabolic rate parameters) were either well defined (multiple consistent experimental results with low variability) or relatively noninfluential (e.g., organ volumes other than fat). Sensitivity analyses were conducted to evaluate the potential for individual parameters to influence the model output by manipulating the values of specific model parameters while holding other parameters steady. This analysis revealed that the most influential parameters for these experimental conditions were cardiac output, alveolar ventilation and fat volume. For this reason, fat volume was estimated using regression equations developed specifically for the LE rat (Simmons et al., 2002) and activity-based estimates were used for cardiac output and alveolar ventilation (Kenyon et al., in press). Baseline values were obtained from Simmons et al. (2002) for cardiac output, and increases above baseline for cardiac output were benchmarked against increased heart rate measured in LE rats implanted with telemers. Values for alveolar ventilation rate during toluene exposure were optimized under various conditions of toluene exposure (actively performing tasks and resting). Resting values were used to derive estimates for this study; since these rats were not actively performing a task such as lever pressing during the exposures. This parameterization of the model allowed successful prediction of other literature data not used for model development or parameter estimation. After elimination of one data set from the literature which had lower concentrations than the others, the model showed a correlation coefficient of 1.01, which was not significantly different from 1, between the predicted and combined observed data across three different experiments. The PBPK model was used to estimate brain toluene concentrations at the times of VEP recordings based on the mean body weights of each group as tested.

**Experimental Design**

Two experiments were conducted. In Experiment 1, five independent groups of rats were exposed to either 0, 1000, 2000, 3000, or 4000 ppm toluene ($n = 9$ or 10 per dose group). Each dose group was run in a block of approximately 1-week duration in order to best maintain consistent and calibrated air toluene concentrations in the exposure chamber. Each rat experienced a single exposure session during which multiple VEPs were recorded. VEPs were recorded both before and during inhalation exposure. Recording times after onset of exposure included 1, 1.3, 2, or 4 h for 0 and 1000 ppm; 1, 1.3, and 2 h for 2000 ppm; 1.3 h for 3000 ppm; and 1 h for 4000 ppm. Data from the 4-h time point were omitted due to reduced waveform amplitudes in the control group relative to baseline. F2 amplitudes of the control group did not change significantly from baseline at time points of less than 4 h.

In Experiment 2, three independent groups of rats were exposed to toluene in concentrations of 0, 3000, or 4000 ppm ($n = 8–10$ per dose group). Each rat was exposed only once, and VEPs were recorded repeatedly both during and after termination of exposure. The duration of inhalation exposure was 1.3 h for 3000 ppm, and 1 h for 4000 ppm. The recording times were selected using the PBPK model to give estimates of the brain toluene concentration ranging from 0 to 150 mg/l at the time of VEP testing. For 3000 ppm, the testing times during exposure included 0.17, 0.33, 0.53, 0.83, 1.23, and 1.3 h, and after termination of exposure included 1.37, 1.53, 1.83, and 2.3 h (time 0 is defined as onset of exposure). Similarly, for 4000 ppm the testing times during exposure included 0.1, 0.2, 0.33, and 0.47 h, and after termination of exposure included 0.67, 1.07, 1.17, 1.37, 1.67, and 2.17 h.

**Statistical Analysis and Curve Fitting of Experiment 1 Data**

**Correcting for measured noise.** The raw F2 amplitude data from Experiment 1 were transformed to adjust for the measured noise by subtracting the noise amplitude from the F2 amplitude for each trial.

**Transforming F2 amplitude to effect magnitude.** The goal was to develop a quantitative model relating the magnitude of change in F2 amplitudes to the estimated concurrent concentration of toluene in the brain. To facilitate such comparison, the F2 amplitudes were transformed so that they ranged in value from 0.0 to 1.0 in which the value of 0.0 was interpreted as no effect and the value of 1.0 was given the interpretation of maximum effect. Logistic curves require values of the dependent variable to range from 0.0 to 1.0. The 0–1 effect scale expresses the magnitude of effect independent of the raw data scale and thus permits comparison of results across experiments and across
dependent variables. In this experiment a baseline value (just before exposure) was provided for each subject and under each toluene concentration. The transform was designed so that no effect (i.e., equal F2 amplitudes in toluene and under baseline conditions) yielded a value of zero, and the maximum possible effect (zero amplitude) yielded a value of 1.0. The transformed F2 is given as

\[ E = \frac{(F_{2n} - F_2)}{F_{2n}} \]  

in which \( E \) is the transformed F2 effect magnitude, \( F_2 \) is a particular experimental value of F2, and \( F_{2n} \) is the appropriate preexposure baseline for F2.

The data from the group of unexposed subjects were analyzed first to determine if there was an effect of the amount of time spent in the experiment. For this analysis, the \( E \) data from the unexposed group were tested for significant differences among the five measurement times by a repeated-measures ANOVA. If the ANOVA was statistically significant, time effects would be fitted and subtracted from the data in the exposed groups. Therefore, the exposure data would reflect only the effects of brain toluene concentration.

\( E \) data were analyzed in the exposed groups by fitting a function to estimate the effect magnitude of the toluene exposure. This is identical to the analysis of variance where the independent variables are considered continuous (random) variables.

Because a repeated-measures experimental design was used, the VEP amplitude values obtained repeatedly from individual rats were not independent, and a mixed-model statistical analysis was required. For this reason, the results were analyzed with a repeated-measure, mixed-model ANOVA. A similar nonlinear mixed model was used to fit the logistic curve, using the function shown in Equation 2

\[ E = \frac{1}{1 + \exp(\beta_1 + \beta_2 [\ln(Tol)])} \]  

in which \( E \) is effect magnitude, \( \exp \) is the base of natural logarithms, \( \beta_1 \) and \( \beta_2 \) are empirical parameters, \( \ln \) is the natural logarithm and [Tol] is the brain toluene concentration. This function has been shown to fit empirical dose-effect data well, and its upper and lower asymptotes are 1 and 0. The SAS (Statistical Analysis System) Proc NLMIXED (SAS, Cary, NC) statistical routine was used to perform the fits, and provide statistical tests and confidence limits. The brain toluene concentrations were estimated from the exposure conditions by a PBPK model (Kenyon et al., in press). Note that the data from Experiment 2 were not subject to this transformation, since baseline data were not collected prior to exposure. The raw F2 amplitude data from Experiment 2 were subjected to SAS Proc NLMIXED to evaluate dose effects and differences during and after exposure as a function of estimated brain concentration.

RESULTS

**Experiment 1**

The animals appeared to remain in good health and be generally alert during the VEP test sessions. No motor impairment or sedation was noted upon termination of toluene exposure and removing animals from the restraint device. The concentration of toluene (mean ± SD) measured in the head-only chamber during the experiment was 989 ± 27, 2042 ± 35, 3013 ± 38, and 4067 ± 38, for nominal concentrations of 1000, 2000, 3000, and 4000 ppm, respectively. The body weights of the rats (mean ± SEM in g) prior to the onset of inhalation exposure on the day of testing were 440 ± 8, 425 ± 9, 417 ± 13, 410 ± 8, and 402 ± 7, for groups of rats exposed to 0, 1000, 2000, 3000, or 4000 ppm, respectively. The slight differences in the group mean body weights of the different dose groups reflect slight differences in age at the onset of testing of each exposure group. The body temperatures (mean ± SEM in °C) of the rats during their final test session were 37.5 ± 0.3, 37.8 ± 0.2, 37.0 ± 0.3, 36.8 ± 0.2, or 37.3 ± 0.2 for rats exposed to 0, 1000, 2000, 3000, or 4000 ppm, respectively.

Exposure to toluene reduced the amplitudes of VEPs, and induced a loss of the F2 profile of the recorded waveforms. Group mean VEP waveforms are presented for rats treated with control air or toluene in Figure 1, and their spectra are presented in Figure 2. Amplitude reductions caused by toluene were evident principally in the F2 component. The amplitude of F1 was not significantly changed by toluene exposure (data not shown). The mean F2 amplitudes of the groups exposed to control air or toluene are presented in Figure 3 as a function of time after onset of exposure.

The transformed effect magnitude data (\( E \)) from the unexposed group were tested to determine if there was an effect of time spent in the experiment. With the omission of the 4-h time points, there was no significant effect of testing time (\( F = 1.237, df = (4, 33), p = 0.315 \)).

Figure 4 is a plot of the function fitted to the \( E \) data from Equation 2 with ± 95% confidence limits. The plotted points with standard error bars are the univariate means of each exposure group with a given inhaled toluene concentration and duration, and thus a specific concentration of toluene in the brain as predicted by the PBPK model. A statistically significant fit was obtained between estimated brain toluene concentration and the magnitude of effect on VEP F2 amplitude.

**Experiment 2**

The average concentrations (mean ± SD in ppm) in the head-only exposure chamber during toluene exposure sessions were 2974 ± 100, and 4033 ± 93 for nominal concentrations of 3000 and 4000 ppm, respectively. The body weights (mean ± SEM in g) of the rats prior to exposure in Experiment 2 were 434 ± 10, 477 ± 10, and 468 ± 14, for rats exposed to 0, 3000, or 4000 ppm toluene, respectively.

The VEP F2 amplitudes were reduced in rats treated with either 3000 or 4000 ppm toluene (Fig. 5). The F2 deficit appeared both during exposure and after the exposure was terminated. At 3000 ppm, the magnitude of amplitude deficit was approximately equal during or after exposure based on the estimated concurrent brain concentration (Fig. 5 top, right panel). At 4000 ppm, however, there appeared to be a difference in the amount of amplitude reduction during and after exposure (Fig. 5 bottom, right panel). Statistical analysis of these data showed a significant effect of estimated brain toluene concentration (\( F(1, 8) = 8.70, p < 0.0184 \)), and a significant difference in F2 amplitude as a function of estimated brain level during and after treatment (\( F(1, 79) = 28.85, p < 0.0001 \)). The amplitude of F2 appeared to recover faster after exposure was terminated than was expected on the basis of estimated tissue concentration alone.
FIG. 1. Experiment 1. Group mean VEP waveforms before and during exposure to clean air (0 ppm) or toluene. Only the first 1 s of each 5 s recording epoch is displayed. The waveforms of the control rats were reduced in amplitude at 240 min, but retained a clear 2F response profile visible as 10 peaks in each second of the recording epoch. Rats exposed to toluene showed lower response amplitudes as well as a loss of the 2F response profile.
FIG. 2. Experiment 1. Spectral analysis plots of the waveforms in Figure 1. The amplitude of the frequency double response component (F2) was reduced by toluene exposure.
DISCUSSION

Acute inhalation of toluene vapors reduced the F2 amplitude of pattern-elicited VEPs recorded from pigmented rats during and after exposure. The amount of amplitude deficit was predictable from the estimated brain toluene concentration at the moment of assessment during exposure; estimated concentrations predicted the deficit after termination of exposure to 3000 ppm toluene but not after 4000 ppm toluene.

Data from the first experiment were fit to a logistic function that showed the proportional change in VEP amplitude as a function of estimated brain toluene concentration. Functions such as this enable quantitative linkage of dosimetric models with pharmacodynamic information into comprehensive exposure–dose–response models. These models have applications in risk assessment, such as predicting potential risks of exposure conditions of varying concentration and duration (Boyes et al., 2005b; Simmons et al., 2005), and also provide a framework for incorporating and synthesizing additional information for integrated risk assessments (Andersen, 1995).

In the second experiment, VEPs were recorded both during and after exposure. At 3000 ppm there was little difference in the amount of amplitude reduction during or after exposure when expressed as a function of estimated concurrent brain concentration. This result agrees with the previous observation that amount of F2 amplitude reduction caused by TCE inhalation was similar during and after exposure when expressed as a function of momentary brain concentration (Boyes et al., 2005a). The case observed here for toluene was somewhat different in that the F2 amplitude appeared to recover faster than expected following the 4000 ppm exposure. One possible explanation for this observation is that the PBPK model may be overestimating the persistence of toluene in the brain after termination of exposure, and that high-dose levels are cleared faster than the model predicts. This would lead to less toluene in the brain than we have estimated, and therefore faster recovery; however, the model does not appear to overpredict at 3000 ppm.

The ability of the PBPK model used here to estimate rat brain toluene concentrations was evaluated using both newly generated data, for which the model was optimized, and also preexisting data from the scientific literature (Kenyon et al., in press). The fact that the model predicted both newly generated and literature data on brain toluene concentrations well justifies use of the model here to estimate brain toluene concentrations, and makes it unlikely that the apparent partial recovery after 4000 ppm exposure was due to misestimating the toluene brain concentrations after termination of exposure.

A second possibility is that high-dose exposure to toluene invokes adaptive responses in the nervous system, such as changes in receptor sensitivity or receptor number, in order to mitigate the effects of the high concentrations of toluene. The observed difference in response to 4000 ppm toluene during and after exposure suggests that it may induce acute functional tolerance, a phenomenon well-documented for ethanol and some barbiturates (Erwin et al., 2000). This form of tolerance is characterized by greater dysfunction (usually motor) in animals at a given blood ethanol concentration when the ethanol concentration in the blood is rising, compared to when it is falling after an acute oral dose. It has not been reported previously for inhaled solvents. Tolerance to ethanol can also

![FIG. 3. Experiment 1. F2 amplitude group means ± SEM for rats treated with control air or toluene as a function of time after onset of exposure.](http://toxsci.oxfordjournals.org/)

![FIG. 4. Experiment 1. The function fitted to variable E from Equation 2 (E = 1/1 + exp(β1 + β2 (ln[Tol]))) with ± 95% confidence limits is plotted as a function of estimated brain concentration. The parameters of the function fit were β1 = 5.87 (t = 3.28, df = 41, p < 0.0021) and β2 = -1.48 (t = -3.53, df = 41, p < 0.0010). Estimated brain concentration is plotted using two alternate units of measure including mg/l (lower axis) and mM (upper axis). The plotted points with standard error bars are the means of each exposure group at each inhaled toluene concentration and duration.](http://toxsci.oxfordjournals.org/)
be induced over longer time frames by appropriate manipulations including practice of motor and cognitive skills during intoxication. Longer-term tolerance has been observed following 5–10 days of repeated daily exposures to toluene (Oshiro et al., in press) or TCE (Bushnell and Oshiro, 2000; Oshiro et al., 2001). Thus, the differential effect of toluene during and after inhalation exposure observed here, and the long-term tolerance previously described for toluene and TCE may reflect different aspects of an adaptive response to solvent intoxication.

Investigators have previously described behavioral changes caused by acute toluene exposure in relationship to brain toluene concentrations. Miyagawa et al. (1984) observed an inverted U-shaped dose–response relationship on the response rate of rats performing a variable interval schedule-controlled operant task. Response rates increased when the brain toluene concentrations were less than 200 μg/g, but response rates decreased when brain toluene concentrations were between 200 and 350 μg/g. van Asperen et al. (2003), however, found that repeated high-dose spikes producing observed peak brain concentrations in rats up to 248 mg/l did not predict well the level of behavioral impairment observed across different exposure scenarios. Benignus et al. (1998) performed a meta-analysis of four published studies in which shock avoidance behavior was measured in rats inhaling toluene. These studies were combined by using a PBPK model to estimate the concentration of toluene in the blood from the experimental conditions (air concentration, duration, test time) reported in each study. In this meta-analysis, the response rate in toluene-exposed rats showed an inverted U-shaped dose-effect curve with an ED10 for the increasing response rate portion of about 13 mg/l blood, a peak of about 85 mg/l blood, and an ED10 for the falling limb of the about 152 mg/l. In contrast, the proportion of successful avoidance responses in these studies showed a monotonic reduction with an ED10 value of about 73 mg/l blood. The ED10 value derived here from Experiment 1 was approximately 12.6 mg/l brain, which was lower than or generally comparable to those previously reported, given that the brain concentration of toluene in rats can be expected to

FIG. 5. Experiment 2. VEP F2 amplitudes (mean ± SEM) are presented as a function of time on the two left panels, with the onset of toluene exposure occurring at 0 min. The same VEP F2 amplitude data are presented as a function of the estimated concentration of toluene in brain at the moment of VEP recording in the two right panels. The two upper panels reflect 3000 ppm toluene exposure and the two lower panels reflect 4000 ppm toluene exposure. A single control group was run and the data from this group are repeated in the 3000 and 4000 ppm panels. In the two right hand panels, the estimated brain concentration is plotted as mg/l on the lower axis, and as mM on the upper axis of each panel. The symbol legends presented in the right side panels are appropriate for the corresponding left side panels as well.
be approximately twice the concurrent blood concentrations (Kenyon et al., in press).

The effects of toluene inhalation on pattern-elicited VEPs were qualitatively similar to those of TCE. Inhalation of both compounds caused a reduction of F2 amplitude that could be related to brain concentration of the parent compound at the time of VEP assessment. On a tissue dose basis the ED10 for TCE reduction of F2 amplitude was approximately 4.5 mg/l of TCE in brain tissue (Boyes et al., 2005a), or approximately one third of that of toluene. When calculated on a molar basis, the ED10 for toluene (molecular weight [MW] = 78) would be approximately 0.15 mM and for TCE (MW = 130) approximately 0.035 mM, meaning that TCE is approximately 4.5 times more potent than toluene.

Exposure to toluene, like TCE (Boyes et al., 2003, 2005a), caused a selective and reversible reduction of the amplitude of the F2 response component without significantly changing F1 amplitude. The visual system may be considered to be comprised of several parallel subsystems based on the functional response properties of visual neurons. There are several different approaches to distinguishing components of the visual system, one of which involves distinguishing “linear” from “nonlinear” response components (Regan, 1989). For linear elements the response output is a linear summation of visual inputs, including temporal response patterns that occur primarily at the temporal frequency of visual stimulation (F1). In contrast, nonlinear components respond at rates that are not reflective of a linear summation of the visual inputs and, in particular, tend to respond at twice the stimulus temporal modulation frequency (F2). Nonlinear components are more reflective of changes in visual patterns than to the patterns per se (Lennie and Perry, 1981). These properties make the nonlinear visual response elements ideal motion detectors. Therefore, the selective reduction of F2 amplitude caused by exposure to toluene or TCE may be interpreted as a reduction in responsiveness of the nonlinear visual system, reflecting perhaps impaired perception of visual movement. Alternatively, since the F2 component is the major response mode of rats to visual contrast modulation, an F2 amplitude reduction may also reflect a more general deficit of visual contrast perception.

The effects reported here are relevant to predicting the risks of acute single exposure episodes. It is likely that momentary brain toluene concentration will not be sufficient to predict persistent outcomes that may follow chronic exposures, and other dose metrics must be evaluated in order to develop predictive exposure–dose–response models for chronic toluene exposure.

There is increasing interest in the development of quantitative predictive models for application to risk assessment (e.g. Andersen, 1995; Simmons et al., 2005). In this paper, a quantitative relationship was developed linking estimated brain concentration of toluene to changes in visual physiology. Other studies show similar relationships between internal dose and behavior (Bushnell et al., 2007). The incorporation of mechanistic information into such a model could further improve the ability of the model to make accurate quantitative predictions, and to be applied in a variety of contexts such as predictions across species, or development of predictive screening assays that do not require whole animal experimentation. When tested in vitro, toluene and other volatile organic compounds alter the function of several ligand or voltage-gated nerve membrane ion channels including the nicotinic acetylcholine, n-methyl-D-aspartate, gamma-amino-butyric acid a-type and glycine receptors, and voltage-sensitive calcium channels (reviewed by Bushnell et al., 2005). To enable incorporation of mechanistic information into a quantitative model, we have begun to evaluate potential role of selected ion channels in generation of pattern-elicited VEPs, and influence of those ion channels in mediating the expression of toluene acute toxicity in vivo (Bale et al., 2007).

In summary, acute exposure to toluene caused a reversible reduction of VEP amplitude that was related to the estimated momentary concentration of toluene in the brain. Estimated brain toluene concentration was linked to VEP deficits through a predictive logistic function. After termination of exposure to 3000 ppm, recovery of VEP function followed the estimated reduction of brain concentration. After exposure to 4000 ppm, however, VEP recovery was somewhat more rapid than expected on the basis of estimated brain concentration alone. For practical purposes such as estimating the risks of exposure to acute toluene episodes, predicting momentary brain concentrations will likely provide a reasonable assessment of the amount of functional impairment to be expected.

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