Acute Perchloroethylene Exposure Alters Rat Visual-Evoked Potentials in Relation to Brain Concentrations

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These experiments sought to establish a dose-effect relationship between the concentration of perchloroethylene (PCE) in brain tissue and concurrent changes in visual function. A physiologically based pharmacokinetic (PBPK) model was implemented to predict concentrations of PCE in the brains of adult Long-Evans rats following inhalation exposure. The model was evaluated for performance against tissue concentrations from exposed rats (n = 40) and data from the published scientific literature. Visual function was assessed using steady-state pattern-elicited visual-evoked potentials (VEPs) recorded from rats during exposure to air or PCE in two experiments (total n = 84) with concentrations of PCE ranging from 250 to 4000 ppm. VEP waveforms were submitted to a spectral analysis in which the major response component, F2, occurring at twice the visual stimulation rate, was reduced in amplitude by PCE exposure. The F2 amplitudes were transformed to an effect-magnitude scale ranging from 0 (no effect) to 1 (maximum possible effect), and a logistical function was fit to the transformed values as a function of estimated concurrent brain PCE concentrations. The resultant function described a dose-response relationship between brain PCE concentration and changes in visual function with an ED10 value of approximately 0.684 mg/l and an ED50 value of approximately 46.5 mg/l. The results confirmed that visual function was disrupted by acute exposure to PCE, and the PBPK model and logistic model together could be used to make quantitative estimates of the magnitude of deficit to be expected for any given inhalation exposure scenario.

Key Words: neurotoxicity; PBPK model; volatile organic compound; organic solvent; visual-evoked potential.
of apartments and day-care workers in buildings with colocated dry cleaners had reduced visual contrast sensitivity (Schreiber et al., 2002). With regard to acute exposures, Altmann et al. (1990) reported that human volunteers breathing 50-ppm PCE vapors for 4 h showed increased latencies of pattern VEPs over the course of the exposure period and a trend for reduced thresholds for perception of visual contrast. Thus, although acute and chronic exposure to PCE may change many aspects of neurological function, reports of visual system changes are common.

It was shown previously that acute exposure to other volatile organic compounds including trichloroethylene (TCE) and toluene altered the visual function of rats as measured by pattern-elicited steady-state VEPs recorded from electrodes over visual cortex while the animals watched changing visual patterns (Boyes et al., 2003, 2005, 2007). Pattern-elicited VEPs take on a “steady-state” form when the stimulus rate is above approximately 4–5 Hz, and the waveform resembles a continuous sinusoid (Boyes and Dyer, 1983). When sinusoidal steady-state VEP waveforms from pigmented rats are analyzed using fast Fourier transforms (FFTs), the predominant response component occurs at a harmonic frequency of twice the stimulus rate, or F2 (Boyes, 1994). The amplitude of the F2 component was reduced by exposure to either TCE or toluene.

The concentration of TCE or toluene in the brain was estimated using physiologically based pharmacokinetic (PBPK) models that were previously calibrated against measured tissue concentrations of these substances (Kenyon et al., 2008; Simmons et al., 2002). The amount of VEP F2 amplitude reduction was predictable by the estimated momentary concentration of either TCE or toluene in the brain at the time of VEP recording (Boyes et al., 2005, 2007). The momentary tissue concentration was also related to the magnitude of deficits in behavioral signal detection tasks in rats performing during inhalation exposure (Boyes et al., 2000; Bushnell et al., 2007). Rats acutely exposed to PCE showed decreased accuracy and increased latency of signal detection performance similar to that caused by TCE or toluene (Oshiro et al., 2008a), and those deficits were better predicted by momentary brain PCE concentration than by other dose measures (Oshiro et al., 2008b). The relationship between tissue concentrations and impairments of visual or behavioral functions is valuable for a number of applications including extrapolation of hazard estimates across exposure durations (Boyes et al., 2005; Bruckner et al., 2004; Simmons et al., 2005) or across species (Benignus et al., 1998, 2007; Bushnell et al., 2005).

For these reasons, visual function was assessed during acute inhalation of PCE and the relationship between momentary concentration of PCE in the brain and changes in visual function was determined. In the experiments reported here, pattern-elicited steady-state VEPs were evaluated in pigmented rats during acute inhalation of PCE vapors. In addition, a PBPK model was implemented and evaluated against measured PCE concentrations in the brain and blood of exposed rats. The PBPK model was then used to estimate concentrations of PCE in the brain under the exposure conditions and times of VEP recordings. Finally, the VEP F2 amplitude and estimated brain PCE concentration data were fit mathematically with a logistic function to describe the dose-effect relationships. It was hypothesized that exposure to PCE would reduce VEP F2 amplitudes, and that the amount of amplitude reduction would be accurately predictable from the concentration of PCE in the brain at the time of VEP assessment.

METHODS

Test compound. HPLC grade PCE (1,1,2,2-tetrachloroethylene) (99.9+ % pure) (CAS # 127-18-4) was obtained from Sigma-Aldrich Chemical Co. (St Louis, MO).

Test animals. Male Long-Evans (LE) rats (Crl:(LE)BR) were obtained from Charles River Laboratories (n = 40 from Portage, MI, for PK studies; n = 93 from Raleigh, NC, for VEP studies). The rats were individually housed in polycarbonate cages with kiln-dried pine shaving bedding (Northeastern Products, Warrensburg, New York), and had ad libitum access to tap water. The animal colony was controlled to an ambient temperature of 22 ± 2°C and relative humidity 50 ± 10%, with a 12:12-h light:dark cycle (lights on at 6:00 AM). 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 PCE exposures occurred in the light phase of the cycle. The animal housing facility was fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care according to National Institutes of Health guidelines. Animal research protocols were reviewed and approved by the National Health and Environmental Effects Research Laboratory Institutional Animal Care and Use Committee which ensured conformance with the 1996 NRC “Guide for the Care and Use of Laboratory Animals,” the Animal Welfare Act, and Public Health Service Policy on the Humane Care and Use of Laboratory Animals.

Pharmacokinetic studies of PCE. Forty male Long-Evans rats (n = 4 per group; 5 time points × 2 concentrations × 4 animals in each group) were housed individually and maintained at 350 ± 10 g body weight by scheduled home cage feeding (Ali et al., 1992) of rat chow (Ralston Purina, St Louis, MO). Tap water was available ad libitum in the home cage. Rats were 3.5 months old at the time of tissue collection.

PCE vapor was generated in four inhalation chambers and monitored as previously described (Bushnell, 1997; Oshiro et al., 2008a). Inlet air was cleansed with carbon and high efficiency particulate air filters and conditioned to 22 ± 2°C and 50 ± 10% relative humidity. Rates of air flow through the exposure chambers were approximately 20 l/min. The rise and fall times (τ34) of PCE vapor concentrations in the test chambers were approximately 6 min. Each rat was exposed individually to PCE at vapor concentrations of 500 or 1500 ppm for either 20 or 60 min; air controls were not used under the assumption that no PCE would be present in the tissues of animals not exposed to PCE. The order of exposure and sacrifice was balanced across exposure conditions. Each rat was introduced to the chamber in air at time zero and PCE exposures started approximately 5 min later. One group was killed immediately after 20-min exposure to PCE and a second group after 60 min. Other groups were exposed to PCE for 60 min and were then left in the chambers after PCE delivery was stopped for 30, 60, or 300 min, to assess clearance of PCE from the animals. Each rat was quickly removed from the chamber at the appropriate collection time, killed immediately by cervical dislocation, and decapitated within 1 min thereafter for tissue collection. PCE concentrations were assayed using trunck blood and whole brain homogenates by gas chromatography using a headspace sampling method as described previously (Bushnell et al., 2007).

Pharmacokinetic modeling of PCE exposures. A PBPK model was developed and calibrated for Long-Evans rats of the age and weight range used in this study and used to simulate the experimental exposures conditions. The
model structure and physiological parameters were the same as those in a previously published model for toluene (Kenyon et al., 2008). The model was implemented in AcslXtreme 2.3.0.14 (XCellon, The Aegis Technologies Group, Austin, TX). Sensitivity analysis was performed using the central difference method with sensitivity coefficients normalized to both response variables (e.g., venous blood and brain concentrations, CV, and CBR) and parameters (e.g., alveolar ventilation rate, QPC, etc.). The responses evaluated were those for which data were available (CV and CBR). Parameters selected for evaluation were those which previously have been demonstrated to be influential or of interest for other volatile chemicals, that is, cardiac output (QCC), alveolar ventilation rate (QPC), blood-air partition coefficient (PB), brain-air partition coefficient (PBR), fat volume (VFC), fat blood flow (QFC), and metabolism parameters (Vmax and Km). Simulation conditions for sensitivity analysis were selected to span the range of pharmacokinetic data available for PCE in Long-Evans rats described in this paper and exposure concentrations used in the VEP studies, that is, 250, 500, 1500, and 4000 ppm for one hour of exposure followed by 6 h of no exposure. Model parameters were categorized as having low, medium, or high influence based on the criteria of Clewell et al. (1994).

The model included compartments representing the lung, slowly and rapidly perfused tissues, fat, liver, GI tract, and brain (Table 1). Tissue transport was blood-flow limited and metabolism occurred in the liver. Parameters for organ volumes and blood-flow rates were obtained from the literature (Brown et al., 1997) or were derived specifically for Long-Evans rats (Simmons et al., 2002). Chemical-specific parameters are provided in Table 2. This model structure and parameterization enabled simulations of the exposure scenarios, the results of which were compared with concentrations of PCE measured in the blood and brain of Long-Evans rats during and following the VEP exposures. The model also adequately predicted pharmacokinetic data from the literature collected for PCE in Long-Evans rats described in this paper and exposure concentrations used in the VEP studies.

### TABLE 1

<table>
<thead>
<tr>
<th>Parameter, units</th>
<th>Symbol</th>
<th>Value</th>
<th>Footnote</th>
</tr>
</thead>
<tbody>
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<td>a</td>
</tr>
<tr>
<td>Cardiac output, l/h-kg$^{0.75}$</td>
<td>QCC</td>
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<td>b</td>
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<td>QPC</td>
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<tr>
<td></td>
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<tr>
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<td>QRC</td>
<td>1 – ΣQc</td>
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<tr>
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<tr>
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<td>VRC</td>
<td>1 – ΣVc</td>
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</table>

a Body weight is an experiment-specific measurement.

b Experiment-specific based on type of acclimation and activity level of rat. These rats were “normal” in the sense of having a regular diurnal cycle of being active and feeding at night and studied during daylight hours (Kenyon et al., 2008).

c Values are from two main sources (Brown et al., 1997; Simmons et al., 2002).

d Lung receives all of cardiac output.

e Values that are LE rat specific were calculated from regression equations (Simmons et al., 2002) for a 0.35 kg rat. Other values are from a standard reference (Brown et al., 1997). All calculated values were checked against reported ranges of reference values (Brown et al., 1997).

f Experiment-specific based on body weight of rat using regression equation from Simmons et al. (2002). An ear tag was assigned to each rat prior to beginning the experiment. Prior to electrode implantation surgery, each rat was anesthetized with sodium pentobarbital (50 mg/kg i.p.) and placed in a standard stereotaxic device. The electrodes were constructed from stainless steel screws (00–90 $\frac{1}{16}$) soldered to nichrome wires. Electrodes were implanted epidurally in the following locations: 1 mm anterior to lambda and 3 mm lateral right of midline for ground and reference electrodes, respectively. The electrode wires were attached to a plug and the entire assembly was encased in dental acrylic. Details of this preparation have been described previously (Boyce et al., 2003).

**Inhalation exposure for VEP experiments.** PCE vapors were generated using a J-tube inhalation system (McGee et al., 1994) and delivered into a (10 x 10 x 17 cm) head-only exposure chamber (Boyce et al., 2003). The head-only chamber 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 subject. Each rat was fitted with a rectal thermometer probe and restrained in a plastic cone with the eyes, nose, and ears exposed. The rat was then positioned into the exposure chamber, and a latex seal was formed around the upper torso to isolate the atmosphere of the head-only exposure chamber. The rat’s eyes were approximately 15 cm from the video monitor presenting visual stimuli, located directly in front of the rat outside the glass face of the inhalation chamber. The exposure chamber and video monitor were covered with a black cloth so that the primary source of light for the rat was the video screen. Electrophysiological signals were recorded using a flexible cable connected to the electrode headset and exiting the head-only exposure chamber through a gas-tight port.

**Surgery for VEP experiments.** Adult rats were obtained at approximately 60 days of age, individually housed with ad lib access to tap water and rat chow (LabDiet PMI #5001, Richmond, IN), and allowed to adapt to the animal colony for about one week. Chronic indwelling electrodes were then surgically implanted into the skull overlying the visual cortex approximately one week prior to beginning the experiment. Prior to electrode implantation surgery, each rat was anesthetized with sodium pentobarbital (50 mg/kg i.p.) and placed in a standard stereotaxic device. The electrodes were constructed from stainless steel screws (00–90 x 1/16) soldered to nichrome wires. Electrodes were implanted epidurally in the following locations: 1 mm anterior to lambda and 4 mm left of midline overlying the left primary visual cortex; and 2 mm anterior to bregma and 2 mm lateral left and right of midline for ground and reference electrodes, respectively. The electrode wires were attached to a plug and the entire assembly was encased in dental acrylic. Details of this preparation have been described previously (Boyes et al., 2003).

**Visual stimuli for VEP experiments.** Visual stimuli were generated using a computer-based system described elsewhere (Boyce et al., 2003; Hamm et al., 2000) and presented on the video monitor located outside the glass face of the exposure chamber. The video system was calibrated to achieve a linear relationship between video input signal voltage and screen luminance over a range that included the stimulus values used. The parameters of the visual stimuli were identical to those used previously to study TCE and toluene (Boyce et al., 2003, 2005, 2007). The stimulus was a green vertical grating with a sinusoidal spatial luminance profile and a spatial frequency of 0.16 cycles per
The VEP testing times after the onset of exposure included 0, 0.1, 0.33, 0.8, and 1.3 h for 250 ppm PCE; 0, 0.1, 0.2, 0.32, 0.56, 0.8, 1.12, 1.5, and 1.92 h for 500 ppm PCE; and 0, 0.08, 0.1, 0.25, 0.44, 0.55, 0.88, and 1.3 h for 1000 ppm PCE. Rats from the control group (0 ppm) were tested at the same times as the 500 ppm group. The exposure to PCE was terminated after the final VEP test.

Transforming F2 amplitude to effect magnitude. The F2 amplitude data from the two experiments were combined for curve fitting in order to provide more robust statistical fits. First, the raw F2 amplitude data were transformed to adjust for the measured noise levels by subtracting the noise amplitude from the F2 amplitude for each corresponding trial. The noise-adjusted F2 amplitude values were then transformed to an effect-magnitude scale using Equation 1:

\[ E = \frac{F_2 - F_2_{\text{B}}}{F_2_{\text{B}}}, \]

where \( E \) is the transformed F2 amplitude, \( F_2 \) is a particular experimental value of \( F_2 \), and \( F_2_{\text{B}} \) is the appropriate pre-exposure baseline for \( F_2 \). The effect-magnitude scale ranges from 0 to 1, with 0 representing no effect of treatment (i.e., the baseline F2 amplitude value before exposure) and 1 representing the maximum possible effect of treatment, which in the case of VEP amplitudes would be 0 μV. In this case, the effect scale is equivalent to a simple proportional decrease of F2 amplitude from baseline.

Statistical analysis and curve fitting of VEP data. Logistic dose-response functions were fit to the transformed F2 amplitude effect-magnitude data with respect to estimated concurrent concentration of PCE in the brain. Logistic functions were selected because they permit comparison across experiments and across dependent variables, the range of the functions is from zero to one, and because they were used previously to fit similar dose-response curves (Benignus et al., 1998, 2005a,b, 2007; Boyes et al., 2005, 2007). The goal of fitting these functions was to develop a quantitative model relating the estimated concentration of PCE in the brain to the magnitude of change in F2 amplitude and eventually to other outcome measures as well (Benignus et al., 2007). No alternative quantitative models such as the Hill equation, which is simply a simple proportional decrease of F2 amplitude from baseline.

The experimental design involved within-subjects repeated measures, and therefore the multiple VEP amplitude values obtained from each rat were not statistically independent. For this reason, the results were analyzed with a repeated-measure, mixed-model ANOVA. A nonlinear mixed model was used to fit the logistic curve, using the function shown in Equation 2,

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

Evaluation of the PBPK Model

The PBPK model was evaluated by comparing predictions of the model to measured values of blood and brain PCE concentrations during and following PCE exposures to either 500 or 1500 ppm PCE (Fig. 1). Brain concentration data were expressed as mg PCE per liter of tissue, which is equivalent to mg PCE per kg of tissue assuming tissue density equal to that of water. In addition, the model predictions were compared with data available from the previously published scientific

### TABLE 2

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</tr>
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<td>PN</td>
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<td>35.0</td>
<td>(1)</td>
</tr>
<tr>
<td>Liver:air</td>
<td>PL</td>
<td>None</td>
<td>35.0</td>
<td>(1)</td>
</tr>
<tr>
<td>Brain:air</td>
<td>PBR</td>
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<tr>
<td>Fat:air</td>
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<td>None</td>
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<td>(1)</td>
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<td>Gut:air</td>
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<td>SPTG:air</td>
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<td>None</td>
<td>25.0</td>
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</table>

Note. (1) Partition coefficients used are from Mahle et al. (2007). Lung:air and gut:air assumed to have same partition coefficient as liver. Kidney and muscle were used as surrogates for rapidly perfused tissue:air partition coefficient and slowly perfused tissue:air partition coefficient, respectively. Brain:air partition coefficient was optimized based on data from Long-Evans rats and is within one SD of reported literature values. (2) Metabolism parameters are from Dallas et al. (1994).
literature (Dallas et al., 1994). These data were not used for development of the current model or parameter estimation. In this latter case, the model simulated concentrations of PCE in arterial blood following 2-h inhalation of either 50 or 500 ppm PCE (Fig. 2). The results showed that the predictions of the PBPK model fell within the range of the measured tissue concentration in most cases, with the exception of under-prediction of tissue concentrations during the late stages of elimination after termination of exposure (Fig. 2). Sensitivity analysis was conducted on this model and its implications for parameter selection and data interpretation are discussed in the Appendix.

**VEP Experiment 1**

The concentrations of perchloroethylene measured in the exposure chamber during the experiment were 1000 \( \pm 7.4 \), 1993 \( \pm 8.3 \), 3018 \( \pm 6.9 \), and 4016 \( \pm 19 \) (mean \( \pm \) SE), for nominal concentrations of 1000, 2000, 3000, and 4000 ppm, respectively.

The animals appeared to remain in good health and were generally alert during VEP testing. Successful experiments could not be completed on 4 of the 50 rats tested due to the exposure concentration being out of bounds \((n = 1)\); or because of poor electrophysiological signal quality \((n = 3)\), resulting in final sample sizes of \( n = 9 \) (0 ppm), \( n = 9 \) (1000 ppm), \( n = 9 \) (2000 ppm), \( n = 9 \) (3000 ppm), and \( n = 10 \) (4000 ppm). The VEP waveforms from control animals showed a steady-state response profile consisting of a sinusoidal waveform with a primary response frequency at double the frequency of pattern stimulation \((F_2;\) Fig. 3). Spectral analysis of the VEP waveforms showed corresponding amplitude peak at \( F_2 \), consistent with prior experience recording steady-state pattern VEP waveforms from pigmented rats (Boyes et al., 2003, 2005, 2007). The VEP waveform shape and the amplitude of \( F_2 \) in the spectral analysis remained relatively stable over the course of the experiment for rats breathing only clean air.

Treatment with 1000, 2000, 3000, or 4000 ppm PCE caused a change in the shape of VEP waveforms and a reduction in VEP \( F_2 \) amplitude (Fig. 3). At 60 min after onset of exposure there was a clear dose-related reduction in \( F_2 \) amplitude. All concentrations of inhaled PCE reduced the \( F_2 \) amplitude.

**VEP Experiment 2**

In the second experiment, the measured concentrations of PCE in the exposure chamber were 249 \( \pm 1.1 \), 488 \( \pm 2.9 \), and 1053 \( \pm 9.6 \) ppm (mean \( \pm \) SE) for nominal concentrations of 250, 500, and 1000 ppm, respectively.

Experimental data were successfully collected from \( n = 10 \) (0 ppm), \( n = 9 \) (250 ppm), \( n = 9 \) (500 ppm), and \( n = 10 \) (1000 ppm) rats. Animals were eliminated from the experiment due to exposure concentrations being out of bounds \((n = 4)\) or opened electrodes \((n = 1)\). Group average VEP waveforms representing all the animals of the control group in Experiment 2 are

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**FIG. 1.** Data (symbols) and simulations (lines) for PCE concentration (mg/l) in blood and brain of Long-Evans rats during and following exposure to 500 ppm (A) or 1500 ppm (B) PCE for one hour.

**FIG. 2.** Model predictions and experimental data for PCE concentration (mg/l) in arterial blood (CA) during and following exposure to 50 or 500 ppm PCE for 2 h. Data were digitized from graphs in Dallas et al. (1994).
shown from representative sample times across the course of the experiment in Figure 4, along with corresponding spectral analyses of these group average waveforms. The overall shape of the VEP waveforms and the spectral F2 amplitudes were fairly stable across the course of the recording period in animals breathing clean air. For animals treated with 500 ppm PCE, group-mean VEP waveforms from representative sample times are presented in Figure 5, along with the corresponding spectral analyses of those waveforms. At each time point after PCE was introduced into the chamber, there was a discernable alteration in the VEP waveforms and a reduction of F2 amplitude. These changes became more pronounced over time.

FIG. 3. Experiment 1. Group average steady-state VEP waveforms (left side) elicited by a pattern on/off modulation at 4.5 Hz from the groups breathing only clean air (0 ppm) or 1000, 2000, 3000, or 4000 ppm PCE for 60 min. The time axis of the VEP waveforms was expanded to show only the first 1 s of the 5-s epoch. On the right side are the frequency spectra of the corresponding waveforms to the left. The spectra show a small peak at approximately 4.5 Hz, the frequency of stimulation, and a large peak at approximately 9 Hz, twice the frequency of stimulation (F2). F2 amplitude was reduced by exposure to PCE.
but there remained a clear F2 amplitude response profile throughout the session.

**Dose Metric**

The F2 amplitude data from both experiments were combined and plotted in Figure 6 in four ways: as a function of (1) the time of recording; (2) the $C \times t$ product of the air concentration on the exposure duration; (3) the concentration of PCE in the brain at the time of VEP recording as estimated with the PBPK model; and (4) the area under the curve (AUC) of estimated brain PCE concentration spanning from onset exposure until the time of recording. The F2 amplitudes of control rats were relatively stable over time. The reduction in F2 amplitude was apparent following PCE treatment in all the
plots. No dose metric appeared to be substantially better than the others in describing the effects. In general, the dose-effect curves showed a rapid onset but a flat slope after the stage of initial deficits. Mean F2 amplitudes did not go below about 5–6 μV, even after exposure to relatively high concentrations of PCE.

Dose-Effect Function

Previously, VEP F2 amplitudes were shown to be a function of momentary brain concentrations of TCE or toluene, and momentary concentrations were a substantially better predictor of impairments than were other measures of dose (Boyes et al., 2003, 2005, 2007). In order to provide a consistent comparison to the effects of toluene and TCE, the combined data from the two PCE experiments were modeled against momentary brain PCE concentrations. The group-mean F2 amplitudes from each time point of the two VEP experiments were combined into a single data set. After transformation according to Equation 1, a single dose-effect function was calculated according to

![Group Average Waveforms](image1.png)

**FIG. 5.** Experiment 2. Group average VEP waveforms from rats treated with 500 ppm PCE beginning after the baseline recording at time 0. The time frame on the x-axis was expanded to show only the first 1 s of the 5-s epoch. The experimental time points presented are the same as those for the control group in Figure 4. Treatment with 500-ppm PCE disrupted the shape of VEP waveforms. Right column: Spectral analysis of the group average waveforms presented to the left from rats breathing vapors of PCE at 500 ppm. Exposure to 500 ppm PCE caused a reduction of F2 amplitude as a function of time after exposure.
Equation 2. The results are plotted in Figure 7. The parameters of the logistic function and the statistical goodness of fit values were: $$\beta_1 = 1.871\ (t = 5.17, \text{df} = 64, p < 0.0001);$$ and $$\beta_2 = -0.4977\ (t = -5.95, \text{df} = 64, p < 0.0001).$$ The covariance matrix for the two beta parameters was:

<table>
<thead>
<tr>
<th></th>
<th>$\beta_1$</th>
<th>$\beta_2$</th>
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<tr>
<td>$\beta_1$</td>
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<tr>
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</table>

The dose-effect function showed a rapid slope up to a brain concentration value of approximately 50 mg/l, above which it rose more slowly. From the dose-effect curve, estimated values of the effective dose (ED) causing a 10 and 50% reduction of F2 amplitude were: $\text{ED}_{10} = 0.684\ \text{mg/l}$ and $\text{ED}_{50} = 46.5\ \text{mg/l}$, respectively. Note that although the $\text{ED}_{10}$ value lies below the range of the observed data from PCE exposures, the value of zero on the effect scale is based on the mean data from pre-exposure baseline recordings. Therefore, the $\text{ED}_{10}$ value represents an interpolation between data points rather than an extrapolation beyond the range of the observations. The $\text{ED}_{50}$ value lies within the range of observed data from PCE-exposed animals. The smallest mean reduction in F2 amplitude was 0.19 which occurred at a brain PCE concentration of 16.4 mg/l. The lowest observed brain PCE concentration was 7.8 mg/l, which produced an F2 reduction of 0.51. The lowest points for F2 amplitude reduction and brain PCE concentration do not coincide because of scatter in the data.

**DISCUSSION**

Inhalation of PCE vapors reduced the amplitude of pattern-elicited VEPs recorded from pigmented rats. Other volatile organic compounds tested in this paradigm, TCE and toluene, have also reduced VEP F2 amplitude in a manner dependent on the momentary concentration of the compound in the brain at the time of VEP testing (Boyes et al., 2003, 2005, 2007). Unlike the other solvents tested, however, it appeared that momentary brain concentration of PCE was not appreciably better than $C \times t$ or AUC in describing F2 amplitude reductions. This may be due to the slower clearance and longer tissue half life of PCE, yielding more stable brain concentrations over time (Dallas et al., 1994), than toluene or TCE. When tissue concentrations are stable over time, dose measures of peak concentration, AUC and $C \times t$ become correlated, and their predictive power is redundant. However, the current study occurred during acute exposures when brain concentrations should have been increasing. Alternatively, because F2 amplitudes showed a rapid initial deficit, but little additional reduction with increasing amounts of exposure, the dynamic range among of F2 amplitude values may not have been sufficient to distinguish among the different dose metrics. Regardless of this problem, momentary concentration of PCE was, as for other solvents tested, a reasonable dose metric for describing the magnitude of F2 amplitude reduction across a range of concentrations and durations of exposure.

Like VEP F2 amplitude, the accuracy and speed of operant signal detection performance have been linked to the momentary concentration of volatile organic compounds in the brain (Bushnell et al., 2007). The shape of the PCE dose-effect function for accuracy of performance resembled that observed here in that it had a rapid initial descent but progressed little with increasing dose, despite a comparable range of dose levels (Oshiro et al., 2008a). In a further analysis of the same behavioral data (Oshiro et al., 2008a), however, the momentary brain PCE concentration was a noticeably better predictor of behavioral performance deficits than other dose metrics (Oshiro et al., 2008b).

The momentary concentration of TCE, toluene or PCE in the brain has been generally predictive of the magnitude of impairments caused by acute solvent exposures in behavioral and neurophysiological measures (Benignus et al., 2007; Bushnell et al., 2005). The $\text{ED}_{10}$ value estimated for PCE to reduce VEP F2 was approximately 0.685 mg/l brain tissue. This is substantially lower than the $\text{ED}_{10}$ estimates for either TCE (4.5 mg/l) or toluene (12.6 mg/l) (Boyes et al., 2005, 2007). In contrast, the $\text{ED}_{50}$ value for TCE (~20 mg/l) was somewhat lower than those for PCE or toluene, which were similar to each other (about 50 mg/l). These values reflect a rapid amplitude reduction after the onset of exposure, followed by a flatter, shallower slope at longer durations and higher internal concentrations. Although PCE had a rapid onset, it caused less amplitude reduction at higher doses than either TCE or toluene. Both of the other solvents reduced F2 amplitudes near the recording noise levels of 1–2 $\mu$V, but the F2 amplitudes of rats breathing PCE vapors remained at 5–6 $\mu$V, despite high concentrations and durations of exposure. This might suggest that more than one target site in the visual system is responsible for the F2 amplitude reductions, and that PCE had high sensitivity for one causing the initial changes, but low efficacy at another causing the inability to further reduce F2 amplitude to noise levels.

The molecular sites of action of volatile organic compounds have been reviewed recently (Bowen et al., 2006; Bushnell et al., 2005). Numerous ligand-gated and voltage-gated nerve membrane ion channels have been discovered to be sensitive to these substances. In this regard, it is noteworthy that PCE was more sensitive than toluene in disrupting nicotinic acetylcholine receptors in vitro (Bale et al., 2005a), and a nicotinic antagonist, mecamylamine, was similar in action to toluene or PCE and reduced F2 amplitude in vivo (Bale et al., 2005b). PCE was more sensitive than toluene or TCE in disrupting the function of voltage-sensitive calcium channels in vitro (Shafer et al., 2005), but no in vivo assessment of the effects of solvents on this pathway has occurred. The NMDA receptor also appears to be involved in vivo in the reduction of VEP F2 amplitude by toluene because administration of the NMDA antagonist, MK801, prior to toluene exposure blocked the F2
amplitude decreases caused by toluene (Bale et al., 2007). Raines et al. (2004) showed that the potency of organic compounds, including volatile anesthetics, to inhibit NMDA receptors was related to the capacity for electrostatic cation-π interactions, but PCE was not among the compounds evaluated. It is possible that the differences observed between the actions of PCE and other organic solvents may reflect sensitivity differences at various neuronal ion channels, but not all of these solvents have been tested for comparative sensitivity against isolated nerve membrane ion channels in the same assay systems. At this time, it is not possible to correlate the in vivo F2 amplitude reductions with the relative actions of these chemicals at numerous potential nerve membrane target sites.

The scientific literature contains several reports suggesting that acute or chronic exposure to PCE alters the function of the visual system, although certainly other neurological systems may be impaired as well. One report involved a single case (Onofrj et al., 1998), whereas others have evaluated populations exposed occupationally (Cavalleri et al., 1994; Gobba et al., 1998) or residentially (Altmann, et al., 1995; Schreiber et al., 2002). These studies have been important in identifying adverse effects of PCE, often at low exposure levels, but they also were limited by factors including small sample sizes, limited exposure information, and in some cases potential confounding through mismatched control populations. In such cases, it is difficult to attribute unequivocally the change in visual function to PCE exposures. Other studies have involved performance of complex behavioral tasks that may have utilized a visual stimulus component, such as choice reaction time, digit-symbol, or others, in which case it is unclear that the deficits involved visual dysfunction as opposed to other behavioral or cognitive systems (Echeverria et al., 1995; Seeber, 1989). Exposure to other organic solvents or solvent mixtures is also associated with a reduction of visual contrast sensitivity (Boeckelmann and Pfister, 2003; Broadwell et al., 1995; Campagna et al., 1995; Castillo et al., 2001; Donoghue et al., 1995; Frenette et al., 1991). In one experimental study,
Altmann et al. (1990) showed that latencies of pattern VEPs in human volunteers breathing 50-ppm PCE vapors increased over a 4-h period. The current studies report in an animal model that the amplitude of pattern-elicited steady-state VEPs was reduced by acute exposure to PCE.

The implications of a reduction in the amplitude of VEP F2 for visual function have been discussed previously (Boyes et al., 2005, 2007). Basically, visual neurons that respond at twice the temporal stimulus rate are thought to comprise a “nonlinear” response component of the visual system, because the response output of these individual cells is not a simple linear summation of their spatial luminance input (Enroth-Cugell and Robson, 1966; Hochstein and Shapley, 1976; Kaplan and Shapley, 1982; Lennie and Perry, 1981). The frequency-doubling feature of these neurons is a function of their responding to both stimulus onset and offset modulations, as opposed to responding at a constant pace to the sustained presence of a patterned stimulus. Frequency-doubling (nonlinear) neurons have properties that hypothetically would make them good motion detectors. It is tempting, therefore, to speculate that exposure to PCE or other organic solvent vapors that reduced F2 amplitude would make the organism less sensitive to the perception of moving visual stimuli. Note, however, that in both cases the cells are dependent on the ability to encode changes in visual contrast, that is, the differences between light and dark components of the patterned visual world, and it is possible that the initial encoding of visual contrast in the retina is the common factor in rats and humans disrupted by exposure to PCE and other volatile organic compounds.

FIG. 7. VEP F2 amplitude, expressed as effect magnitude (E) according to Equation 1, and as a function of brain PCE concentration at the moment of VEP testing as estimated using the PBPK model. Dose-response curves were fit to the transformed VEP F2 amplitude data according to Equation 2. Plotted data are mean ± SEM. The dashed lines represent the 95% confidence limits of the dose-effect function.

PERCHLOROETHYLENE AND VISUAL FUNCTION

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REFERENCES


buildings with colocated dry cleaners (Schreiber et al., 2002), used a stationary visual stimulus card to evaluate contrast sensitivity. The perception of stationary visual patterns and color in humans is thought to be mediated through cells innervating the parvocellular layer of the lateral geniculate nucleus (LGN), whereas motion detection is thought to involve primarily cells innervating the magnocellular layer of the LGN (Livingstone and Hubel, 1987, 1988). Rats do not have a structure in the visual pathway equivalent to the primate parvocellular pathway, and their visual system consists of cells more similar to those of the primate magnocellular system. Thus, the cells involved in perception of stationary contrast in PCE-exposed humans may differ from those generating the rat VEPs evaluated here. Note, however, that in both cases the cells are dependent on the ability to encode changes in visual contrast, that is, the differences between light and dark components of the patterned visual world, and it is possible that the initial encoding of visual contrast in the retina is the common factor in rats and humans disrupted by exposure to PCE and other volatile organic compounds.


Oshiro, W. M., Krantz, Q. T., Kenyon, E. M., and Bushnell, P. J. (2008b). Acute behavioral effects of inhaled perchloroethylene (PCE) in rats are directly related to its concentration in the brain and blood. Abstract presented at the annual meeting of the Society of Toxicology, Seattle WA.


APPENDIX

Sensitivity Analysis for the PCE PBPK Model

Sensitivity coefficients (SCs) for PCE in venous blood (CV) and brain (CBR) are shown in Tables A1 and A2, respectively, for four different concentrations spanning the range of concentrations used in the rat VEP studies. SCs for the 1-h time point are shown for the sake of brevity and because these were generally representative of the relative magnitude of the SCs over time, except where noted. It is noteworthy that the metabolic rate parameters (VmaxC and Km) were not influential for either tissue over the range of exposure concentrations and times evaluated. The metabolic rate parameters did not approach moderate influence until exposure concentrations were around 1 ppm PCE (data not shown). It is of interest that there was some variation in estimated metabolic rate parameters in the literature (e.g., Reitz et al., 1996). The metabolic rate parameters estimated by Dallas et al. (1994) were selected because they were generated in the same strain of rat (i.e., Sprague-Dawley) as were most partition coefficients used in the model (i.e., Mahle et al., 2007). However, because of the concentration range of interest for this study, the relative value of metabolic rate parameters was not critical.

Sensitivity analysis also demonstrated that blood:air (PB) and brain:air (PBRC) partition coefficients were highly influential for CV and CBR, respectively. Interestingly, in the literature there was a fair degree of variation in PB reported for PCE; for example, Thrall et al. (2002) and Gargas et al. (1989) report values of 22.6 ± 3.3 and 18.9 ± 1.1 for male F344 rats, respectively, whereas Mahle et al. (2007) and Dallas et al. (1994) report values of 13.6 ± 6.4 and 19.8, respectively, for male Sprague-Dawley rats. In our experience, the Sprague-Dawley rats were more similar to Long-Evans, and thus parameters from Sprague-Dawley rats were given preference in the absence of Long-Evans strain-specific parameters. Given that Mahle et al. (2007) used a well-validated method (vial equilibration) for experimental determination of partition coefficients, whereas Dallas et al. (1994) used a less common method (area method), we chose to use the partition coefficients from Mahle et al. (2007). The observed variation reported in partition coefficients for PCE was a function of both rat strain and age (Mahle et al., 2007), illustrating the importance of parameter selection in relation to experimental conditions and goals of the modeling exercise.

As expected, alveolar ventilation rate (QPC) was a highly influential parameter for both CV and CBR. Previous work with toluene (Kenyon et al., 2008) suggested that a resting value for QPC was most appropriate for rats during the VEP testing conditions. The model tended to underpredict blood and brain concentrations at times greater than 2-h postexposure (Fig. 2). This may be a function of changes in activity level once rats have been removed from exposure chambers and returned to their home cage, although data do not exist to allow evaluation of this hypothesis.

<table>
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<th>250 ppm</th>
<th>500 ppm</th>
<th>1500 ppm</th>
<th>4000 ppm</th>
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