Chapter 5
Brain and Behavioral Changes in Rodent Models

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Abstract Trichloroethylene (TCE) exposure results in central nervous system (CNS) effects in experimental animals that can result from acute, subchronic, or chronic exposure. This chapter focuses on the behavioral and neurological changes that have been observed, to date, in rodent models (primarily rat and mice). Some of the neurological changes such as hearing impairment (ototoxicity) and nerve degeneration are classified as persistent and/or non-reversible. Other impairments such as vision and motor movement have been classified as primarily reversible or non-permanent neurological changes. The neurological changes that are observed include nerve conduction changes, sensory effects, cognitive deficits, changes in psychomotor function, and changes in mood and sleep behaviors. In addition, general pathological and neurotransmitter changes in the brain are discussed and may be relevant to the observed behavioral changes.

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Keywords Neurotoxicity • Rodents • Trichloroethylene • Brain • CNS depressant

5.1 TCE Exposure to Rodents and the Nervous System

Several studies with TCE exposure in rodents have been conducted to better understand the neurological effects that have been observed in humans. Neurotoxicological effects have been studied in humans and, briefly, the major neurological impairments are changes in nerve function, some observations of

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K.M. Gilbert, S.J. Blossom (eds.), *Trichloroethylene: Toxicity and Health Risks*, Molecular and Integrative Toxicology, DOI 10.1007/978-1-4471-6311-4_5,

hearing and vision impairment, decreased cognitive function and impaired vestibular function. Many studies found that humans exposed to TCE present abnormalities in trigeminal nerve function (Feldman et al. 1988, 1992; Kilburn and Warshaw 1993; Kilburn 2002; Ruijten et al. 1991). Auditory impairments in humans, with children under 9 years of age being particularly susceptible, were reported in three studies that were conducted on the population in the TCE Subregistry from the National Exposure Registry (NER) developed by the Agency for Toxic Substances Disease Registry (ATSDR) (Burg and Gist 1995, 1999; ATSDR 2003). Human studies have also reported visual impairment resulting from TCE exposure including color discrimination (Kilburn 2002), contrast sensitivity (Reif et al. 2003) and visual depth perception (Vernon and Ruff 1969). TCE exposure on psychomotor response in humans have been studied primarily as the impact on reaction times (RT), and generally it has been found the RT is increased with exposure (Kilburn 2002; Kilburn and Warshaw 1993; Reif et al. 2003; Kilburn and Thornton 1996). However, there are significant limitations in the human neurotoxicity studies with TCE. Including lack of exposure information and duration, inability to determine effect levels.

To circumvent to the inherent limitations of studies involving TCE-mediated neurotoxicity in humans, rodent models allow investigators to better characterize the potential neurological targets that may be associated with TCE exposure. For most of the neurological effects associated with TCE, there has been a high degree of concordance between the rodent models and the observed effects in humans. In vivo studies in rodents and in vitro models have demonstrated that TCE produces functional and physiological neurological changes. Documenting changes in brain pathology and neurotransmitter signaling in rodents exposed to TCE may help verify that the observed behavior changes are due to TCE exposure. Overall, these effects collectively indicate that TCE has CNS depressant-like effects at low level exposures and causes anesthetic-like effects at high exposures.

5.2 Persistent Neurotoxic Effects of TCE

5.2.1 Auditory Effects

Perhaps the most carefully assessed persistent and/or permanent neurotoxic effect of adult TCE exposure in laboratory animals is a loss in auditory sensitivity that occurs preferentially for mid-frequency sound. The ability of TCE to permanently disrupt auditory function and produce abnormalities in inner ear histopathology has been demonstrated in several rodent studies using a variety of test methods. At least four independent research groups have replicated the finding that subacute TCE inhalation exposures in the range of 2,000–4,000 ppm produce a preferential impairment of mid-frequency hearing in several strains of rats (see Table 5.1 for study details and findings). The effects have been well-characterized using a broad range

Table 5.1 Summary of animal auditory function studies – inhalation exposure

Reference	Species/strain/sex/number	Dose level/exposure duration	Effects
Rebert et al. (1991)	Rat, Long Evans, male, 10/group Rat, F344, male, 4–5/group	Long Evans: 0, 1,600, 3,200 ppm; 12 h/day, 12 weeks F344: 0, 2,000, 3,200 ppm; 12 h/day, 3 weeks	Brainstem auditory evoked responses (BAERs) were measured. Significant decreases in BAER amplitude and an increase in latency of appearance of the initial peak (P1). For Long Evans rats, exposures to 3,200 ppm resulted in significant decreases in BAER amplitude, whereas for the F344 rats the same effect was observed at 2,000 ppm TCE
Rebert et al. (1993)	Rat, Long Evans, male, 9/group	0, 2,500, 3,000, 3,500 ppm; 8 h/day, 5 days	BAERs were measured 1–2 weeks post-exposure to assess auditory function. Significant decreases in BAERs were noted with TCE exposures of 3,000 ppm and higher
Rebert et al. (1995)	Rat, Long Evans, male, 9/group	0, 2,800 ppm; 8 h/day, 5 days	BAER measured 2–14 days post-exposure at a 16 kHz tone. Hearing loss ranged from 55 to 85 dB in rats exposed to 2,800 ppm TCE
Crofton et al. (1994)	Rat, Long Evans, male, 7–8/group	0, 3,500 ppm TCE; 8 h/day, 5 days	BAER measured and auditory thresholds determined 5–8 weeks post-exposure. Selective impairment of auditory function for mid-frequency tones (8 and 16 kHz) in rats exposed to 3,500 ppm TCE
Crofton and Zhao (1997), Boyes et al. (2000)	Rat, Long Evans, male, 9–12/group Rat, Long Evans, male, 8–10/group Rat, Long Evans, male, 8–10/group Rat, Long Evans, male, 8–10/group	0, 4,000, 6,000, 8,000 ppm; 6 h 0, 1,600, 2,400, 3,200 ppm; 6 h/day, 5 days 0, 800, 1,600, 2,400, 3,200 ppm; 6 h/day, 5 days/week, 4 weeks 0, 800, 1,600, 2,400, 3,200 ppm; 6 h/day, 5 days/week, 13 weeks	Auditory thresholds as measured by BAERs for the 16 kHz tone increased with TCE exposure. The effect levels were dependent on exposure duration 6 h exposure – 8,000 ppm 5 day exposure – 3,200 ppm 4 week exposure – 3,200 ppm 13 week exposure – 2,400 ppm No significant changes in BAERs were observed at lower exposures for the individual exposure durations

(continued)

Table 5.1 (continued)

Reference	Species/strain/sex/number	Dose level/exposure duration	Effects
Fechter et al. (1998)	Rat, Long Evans, male, 12/group	0, 4,000 ppm; 6 h/day, 5 days	Cochlear function measured 5–7 weeks after exposure. Loss of spiral ganglion cells noted. Auditory function was significantly decreased as measured by compound action potentials in the animal exposed to 4,000 ppm
Jaspers et al. (1993)	Rat, Wistar derived WAG-Rii/MBL, male, 12/group	0, 1,500, 3,000 ppm; 18 h/day, 5 days/week, 3 weeks	Auditory function assessed repeatedly 1–5 weeks post-exposure for 5, 20, and 35 kHz tones; No effect at 5 or 35 kHz; Decreased auditory sensitivity at 20 kHz in animals exposed to 1,500 ppm and higher
Muijser et al. (2000)	Rat, Wistar derived WAG-Rii/MBL, male, 8	0, 3,000 ppm	Auditory sensitivity decreased with TCE exposure at 4, 8, 16, and 20 kHz tones for the animals exposed to 3,000 ppm
Albee et al. (2006)	Rat, Fischer 344, male and female, 10/sex/group	0, 250, 800, 2,500 ppm	Mild frequency specific hearing deficits; Focal loss of hair cells and cochlear lesions in only the animals exposed to 2,500 ppm
Yamamura et al. (1983)	Guinea Pig, albino Hartley, male, 7–10/group	0, 6,000, 12,000, 17,000 ppm; 4 h/day, 5 days	No change in auditory sensitivity at any exposure level as measured by cochlear action potentials and microphonics at any TCE exposure levels

of well validated test methods including brainstem auditory evoked response (BAER) (Rebert et al. 1993, 1995; Albee et al. 2006), a behavioral method known as reflex modification audiometry (Jaspers et al. 1993; Muijser et al. 2000; Crofton et al. 1994; Crofton and Zhao 1997; Boyes et al. 2000), and electrophysiological assessment of cochlear potentials (compound action potential threshold, compound action potential growth functions, and cochlear microphonic isopotential curves) (Fechter et al. 1998). In one study (Fechter et al. 1998), multiple methods were used to document qualitatively similar auditory impairments within the same subjects through collaborative investigations among laboratories. In addition, there are limited histopathological data to support a loss of sensory receptor cells and neuronal cells in the cochlea that are consistent with the functional impairment and document an irreversible effect (Albee et al. 2006; Fechter et al. 1998). Collectively, TCE has been shown to produce ototoxicity in rodents at mid-frequency tones (4–24 kHz) and no observed changes in auditory function were observed at either the low (<4 kHz) or high (>24 kHz) frequency tones. Additionally, deficits in auditory effects were found to persist for at least 7 weeks after the cessation of TCE exposure (Rebert et al. 1991; Jaspers et al. 1993; Crofton and Zhao 1997; Fechter et al. 1998; Boyes et al. 2000). For example, Jaspers et al. (1993) exposed Wistar rats to 1,500 and 3,000 ppm TCE for 18 h/day, 5 days/week, for 3 weeks. Selective hearing loss at the 20 kHz tone in the rats exposed to 3,000 ppm TCE, but not in the 1,500 ppm TCE group, was reported for up to 6 weeks after cessation of exposure. Similarly, in male Long-Evans hooded rats, animals had increased hearing thresholds at a 16 kHz tone for up to 5 weeks following a acute, short-term, or subchronic exposures (up to 13 weeks) (Crofton and Zhao 1997).

Decreased amplitude and latency were noted in the BAERs (Rebert et al. 1991, 1993, 1995) suggesting that TCE exposure affects central auditory processes. Decrements in auditory function following reflex modification audiometry (Jaspers et al. 1993; Crofton et al. 1994; Crofton and Zhao 1997; Muijser et al. 2000) combined with changes observed in cochlear histopathology (Fechter et al. 1998; Albee et al. 2006) suggest that ototoxicity is occurring at the level of the cochlea and/or brainstem.

5.2.1.1 Reflex Modification

Reflex modification was used in several studies to evaluate the auditory function in TCE-exposed animals (Jaspers et al. 1993; Muijser et al. 2000; Fechter et al. 1998; Crofton and Zhao 1993; Crofton et al. 1994; Crofton and Zhao 1997; Boyes et al. 2000; Yamamura et al. 1983). In these studies, tones of different kHz were presented at increasing decibels (sound intensity). The decibel level at which the rodent became startled (e.g. acoustic startle response) was recorded. A decrease in auditory function is noted when an increased decibel level is needed to elicit a startle response. These studies collectively demonstrate significant decreases in auditory function at mid- frequency tones (8–20 kHz tones) for TCE exposures greater than 1,500 ppm after acute, short-term, and chronic durations. Only one study in guinea

pigs (Yamamura et al. 1983) did not demonstrate impairment in auditory function from TCE exposures as high as 17,000 ppm for 4 h/day over 5 days. However, auditory testing was not performed in an audiometric sound attenuating chamber and extraneous noise could have influenced the outcome, and the guinea pig has been reported to be far less sensitive than the rat to the effects of ototoxic aromatic hydrocarbons such as toluene.

5.2.1.2 Brainstem Auditory Evoked Responses (BAERs)

Brainstem auditory-evoked potentials (BAERs) were also measured in several studies (Rebert et al. 1991, 1993, 1995; Albee et al. 2006) following TCE exposures ranging from 3 to 13 weeks. BAERs were generally measured by presenting tones to the rodents and measuring the responses using an electrode that was placed over the brainstem area. Rebert et al. (1991) measured BAERs in male Long Evans rats (n = 10) and F344 rats (n = 4 – 5) following stimulation with 4, 8, and 16 kHz sounds. The Long-Evans rats were exposed to 0, 1,600, or 3,200 ppm TCE, 12 h/day for 12 weeks and the F344 rats were exposed to 0, 2,000, or 3,200 ppm TCE, 12 h/day for 3 weeks. BAER amplitudes were significantly decreased at all frequencies for F344 rats exposed to 2,000 and 3,000 ppm TCE and for Long Evans rats exposed to 3,200 ppm TCE. In subsequent studies Rebert et al. (1993, 1995) again demonstrated TCE significantly decreases BAER amplitudes and also significantly increases the latency of appearance. Similar results were obtained by Albee et al. (2006) for male and female F344 rats exposed to TCE for 13 weeks.

5.2.1.3 Pathology Changes in the Auditory System

Notable pathology changes were also reported in a few auditory studies. Histological data from cochleas in Long-Evans rats exposed to 4,000 ppm TCE indicated that there was a loss in spiral ganglion cells (Fechter et al. 1998). Similarly, there was an observed loss in hair cells in the upper basal turn of the cochlea in F344 rats exposed to 2,500-ppm TCE (Albee et al. 2006).

5.2.2 Neuronal Degeneration and Neuronal Impairment

There is evidence of persistent and/ or permanent neurological effects from TCE exposure on neuronal degeneration. The available studies primarily demonstrate selective neuronal injury following TCE administration.

5.2.2.1 Dopaminergic Neurons

In two separate animal studies, subchronic administration of TCE has resulted in a decrease of dopaminergic (DA) cells in both rats and mice.

Gash et al. (2008) assessed the effects of subchronic TCE administration on dopaminergic neurons in the central nervous system. Fischer 344 male rats were given 1,000 mg/kg TCE in olive oil by gavage, 5 days/week for 6 weeks. Degenerative changes in DA containing neurons in the substantia nigra were reported as indexed by a 45 % decrease in the number of tyrosine hydroxylase positive cells. Additionally, there was a decrease in the ratio of 3,4-dihydroxyphenylacetic acid (DOPAC), a metabolite of DA, to DA levels in the striatum. This shift in ratio, on the order of 35 %, was significant by Students t test, suggesting a decrease in release and utilization of this neurotransmitter. While it is possible that long-term adaptation might occur with regard to release rates for DA, the loss of DA cells in the substantia nigra is viewed as a permanent adverse effect. The exposure level used in this study was limited to one high dose and more confidence in the outcome will depend upon replication and development of a dose-response relationship. If the results are replicated, they might be important in understanding mechanisms by which TCE produces neurotoxicity in the central nervous system. The functional significance of such cellular loss has not yet been determined through behavioral testing.

Guehl et al. (1999) also reported persistent effects of TCE exposure on DA neurons. In this study, OF1 male mice (n = 10) were injected ip daily for 5 days/week for 4 weeks with TCE (400 mg/kg/day). Following a 7 day period when the subjects did not receive TCE, the mice were euthanized and tyrosine hydroxylase immunoreactivity was used to measure neuronal death in the substantia nigra pars compacta. Treated mice presented significant dopaminergic neuronal death (50 %) in comparison with control mice based upon total cell counts conducted by an examiner blinded as to treatment group in six samples per subject.

5.2.2.2 Gamma-Aminobutyric Acid (GABA) and Glutamatergic Neurons

Disruption of GABAergic and glutamatergic neurons by environmental agents can represent serious impairment as GABA serves as a key inhibitory neurotransmitter while glutamate is equally important as an excitatory neurotoxicant. Moreover, elevations in glutamatergic release have been identified as an important process by which more general neurotoxicity can occur through a process identified as excitotoxicity. Consequently, GABA and glutamatergic neurons represent potentially important targets of TCE neurotoxicity.

Briving et al. (1986) exposed Mongolian gerbils to 50 and 150 ppm TCE continuously for 12 months via inhalation and reported changes in amino acids levels in the hippocampus and cerebellar vermis, and on high affinity uptake of GABA and glutamate in those same structures. An elevation of glutamine in the hippocampus of approximately 20 % at 150 ppm was reported, but no other reliable changes in amino acids in either of these two structures. With regard to high affinity uptake of glutamate and GABA, there were no differences in the hippocampal uptake between control and treated gerbils although in the cerebellar vermis there was a dose related elevation in the high affinity uptake for both of these neurotransmitters. Glutamate uptake was increased about 50 % at 50 ppm and 100 % at 150 ppm. The corresponding increases for GABA were 69 and 74 %. It is unclear if this finding in cerebellar vermis is also present in other brain tissues and should be studied further.

Shih et al. (2001) provided indirect evidence in male Mf1 mice that TCE exposure by injection might alter GABAergic function. The mice were injected ip with 250, 500, 1,000 and 2,000 mg/kg TCE in corn oil and the effect of these treatments on susceptibility to seizure induced by a variety of drugs was observed. Shih et al. reported that doses of TCE as low as 250 mg/kg reduced signs of seizure induced by picrotoxin, bicuculline and pentylenetetrazol, all GABAergic antagonist drugs. TCE treatment had a more limited effect on seizure threshold induced by non-GABAergic convulsant drugs such as strychnine (glycine receptor antagonist), 4-aminopyridine (alcohol dehydrogenase inhibitor) and N-methyl-d-aspartate (glutamatergic agonist) than was observed with the GABAergic antagonists. While these data suggest the possibility that TCE could act at least acutely on GABAergic neurons, there are no direct measurements of such an effect.

5.2.3 Demyelination Following TCE Exposure

Because of its anaesthetic properties and lipophilicity, it is hypothesized that TCE may disrupt the lipid-rich sheaths that cover many central and peripheral nerves. This issue has also been studied both in specific cranial nerves known to be targets of TCE neurotoxicity (namely the trigeminal nerve) and in the central nervous system including the cerebral cortex, hippocampus and cerebellum in particular. For peripheral and cranial nerves, there are limited nerve conduction velocity studies that are relevant as a functional measure. For central pathways, the most common outcomes studied include histological endpoints and lipid profiles.

A significant difficulty in assessing these studies concerns the permanence or persistence of effect. There is a very large literature unrelated to TCE which demonstrates the potential for repair of the myelin sheath, and at least partial if not full recovery of function. In the studies reviewed, where nerve myelin markers were assessed, it was not possible to determine if the effects were transient or persistent.

5.2.3.1 Trigeminal Nerve Demyelination

Rodent studies that have examined nerve function focused on the trigeminal nerve, or the fifth cranial nerve, that mediates facial sensations and motor functions including chewing and biting. Rodent studies have focused on impaired trigeminal nerve function because there are several human studies that have associated TCE exposure to decreased functionality in this nerve. Recent findings have also suggested that dichloroacetylene (DCA), an ex vivo TCE degradation product, may also be responsible for the impairment of trigeminal nerve function. The overall published information from the rodent studies suggest that the breakdown product of TCE, DCA, may be responsible for the trigeminal nerve effects.

Morphological Changes

Rodent studies have found that exposure to TCE results in morphological changes following a 3 day or a 10 week oral dose of 2,500 mg/kg-day (Barret et al. 1991, 1992). Examination of the pathology of the nerve revealed that TCE exposed animals had thinner trigeminal nerve fibers. Specifically, the thickness of the myelin sheath was significantly decreased. Also, it was observed that the internodal length was decreased. Effects were also evaluated with DCA (17 mg/kg) and it was found that the morphological changes were more severe than with TCE alone. TCE-dosed animals only exhibited changes in the smaller Class A fibers where internode length increased marginally (<2 %) and fiber diameter increased by 6 %. Conversely, DCA-treated rats exhibited significant and more robust decreases in internode length and fiber diameter in both fiber classes A (decreased 8 %) and B (decreased 4 %). Although the changes were noted, the administered doses in these studies were significantly higher than an incidental human exposure to TCE. Thus, DCA, a degradation product of TCE, was found to produce more severe morphological changes, but in animals exposed to TCE only the smaller nerve fibers (class A) were impacted.

Functionality

In order to verify the observed morphological changes in the trigeminal nerve, evaluations of trigeminal nerve functionality were conducted in rodents following inhalation exposures to TCE. Rats were exposed to various concentrations (250–2,500 ppm) of TCE for 13 weeks, and the trigeminal sensory evoked potentials (TSEPs) were measured (Albee et al. 2006). Stimulation of the trigeminal nerve was accomplished by sending electrical signals at the vibrissae pads (whiskers of the rat), and the evoked potentials were measured with electrodes placed over the somatosensory cortex where responses from the trigeminal nerve would traverse. TSEPs were not changed with TCE exposure, but when rodents were exposed to DCA, there were significant disruptions in the TSEP (Albee et al. 1997, 2006).

Kulig (1987) also measured peripheral (caudal nerve) nerve conduction time in male Wistar rats and failed to show an effect of TCE with exposures as high as 1,500 ppm for 16 h/day, 5 days/week for 18 weeks.

5.2.3.2 Demyelination in Central Nervous System

There are two studies (Isaacson and Taylor 1989; Isaacson et al. 1990) that document selective hippocampal histopathology when Sprague Dawley rats are exposed to TCE within a developmental model. Both of these studies employed oral TCE administration via the drinking water.

Isaacson and Taylor (1989) examined the development of the hippocampus in neonatal rats that were exposed in utero and in the preweaning period to TCE via their dam. TCE was added to the drinking water of the dam, and daily maternal doses were estimated based upon water intake of the dam as being 4 and 8.1 mg/day. Based upon body weight norms for 70 day old female Sprague Dawley rats, which would predict body weights of about 250 g at that age, such a dose might approach 16–32 mg/kg/day initially during pregnancy. Even if these assumptions hold true, it was not possible to determine how much TCE was received by the pups although the authors did provide an estimate of fetal exposure expressed as μg/ml of TCE, trichloroethanol, and trichloroacetic acid. The authors reported a 40 % decline in myelinated fibers in the CA1 region of the hippocampus of the weanling rats where the dams were exposed to a daily dose of 4.0 or 8.1 mg/day TCE. Since there was no effect of TCE treatment on myelination in several other brain regions including the internal capsule, optic tract or fornix, this effect appeared to be restricted to the CA1 region of the hippocampus at the tested exposures.

In a second publication by that group (Isaacson et al. 1990), weanling rats were exposed to TCE via their drinking water at doses of 5.5 mg/day for 4 weeks or 5.5 mg/day for 4 weeks, followed by a 2 week period with no TCE, and then a final 2 weeks of exposure to 8.5 mg/day TCE. Spatial learning was studied using the Morris water maze and hippocampal myelination was examined histologically starting 1 day post exposure. The authors reported that the subjects receiving a total of 6 weeks exposure to TCE showed *better* performance in the Morris swim test ($p < .05$) than did controls while the 4 week exposed subjects performed at the same level as did controls. Despite this apparent improvement in performance, histological examination of the hippocampus demonstrated the hippocampal myelin was significantly reduced in the TCE exposed groups, while normal myelin patterns were found in the internal capsule, optic tract and fornix. The authors did not evaluate the signs of gross toxicity in treated animals such as growth rate which might have influenced hippocampal development.

Ohta et al. (2001) administered 300 or 1,000 mg/kg TCE, i.p., to male ddY mice. Twenty-four hours after TCE administration, the mice were sacrificed and hippocampal sections were prepared from the excised brains and long term potentiation was measured in the slices. A dose related reduction in the population spike was observed following a tetanic stimulation relative to the size of the population spike elicited in the TCE mice prior to tetany. The spike amplitude was reduced 14 % in the 300 mg/kg TCE group and 26 % in the 1,000 mg/kg group. Precisely how such a shift in excitability of hippocampal CA1 neurons relates to altered hippocampal function is not certain, but it does demonstrate that injection with 300 mg/kg TCE can have lingering consequences on the hippocampus at least 24 h following ip administration.

A critical area for future study is the potential that TCE might have to produce demyelination in the central nervous system. It is realistic to imagine that an anaesthetic and lipophilic agent such as TCE might interact with lipid membranes and produce alterations, for example, in membrane fluidity at least at anaesthetic levels. However, from the available data it appears that chronic lower doses of TCE (50 and

150 ppm for 12 months, 320 ppm for 90 days, 510 ppm 8 h/day for 5 months) might alter fatty acid metabolism in the brains of Sprague Dawley rats and Mongolian gerbils (Kyrklund et al. 1983, 1986, 2002; Kyrklund and Haglid 1990; Kyrklund 1992). High doses were not included in these studies. Because the lower doses produced only sporadic significant effects and those tended to be of small magnitude (5–10 %) it is not certain that they are truly observing events with biological significance, or whether they are observing random effects. It could be hypothesized that the alterations in fatty acid metabolism could be an underlying mechanism for demyelination. However, it is not apparent that one brain region is more vulnerable to the effects of TCE than is another region. Significant changes in levels of cholesterol, neutral and acidic phospholipids or total lipid phospholipids were reported throughout the brain regions that have been measured and suggested a shift in lipid profiles between treated and untreated subjects.

5.3 Nonpersistent Neurotoxic Effects of TCE

5.3.1 Vestibular Function

The effect of TCE on vestibular function in animals has been evaluated by either (1) promoting nystagmus (vestibular system dysfunction) and comparing the level of effort required to achieve nystagmus in the presence and absence of TCE, or (2) using an elevated beam apparatus and measuring the balance of subjects exposed to TCE. Overall, it was found that exposure to TCE disrupts vestibular function. Impairment of vestibular function in male and female pigmented rats was observed after an acute inhalation exposure to TCE (2,700–7,200 ppm; Niklasson et al. 1993). An increased ability to promote nystagmus (fast and uncontrollable movement of the eyes related to vestibular function) was observed with acute TCE exposure. Complete recovery of the vestibular function in rats was reported within minutes of terminating a direct arterial infusion of TCE (Tham et al. 1979, 1984).

5.3.2 Visual Effects

Changes in visual function have been demonstrated in rodent studies during acute (Boyes et al. 2003, 2005) and subchronic exposure (Rebert et al. 1991; Blain et al. 1994) to TCE. In these studies, the effect of TCE on visual evoked responses to patterns (Boyes et al. 2003, 2005; Rebert et al. 1991) or a flash stimulus (Rebert et al. 1991; Blain et al. 1994) were evaluated. Overall, the studies demonstrated that exposure to TCE results in significant changes in the visual evoked response, which is reversible once TCE exposure is stopped. All of the rodent studies evaluated central visual function by measuring changes in evoked potential response

following a visual stimulus that was presented to the animal. Two acute exposure inhalation studies (Boyes et al. 2003, 2005) exposed Long Evans rats to TCE based on a concentration × time schedule (Haber's law), and reported decreases in visual evoked potential amplitude and indicated decreased visual function. Additionally, Boyes et al. (2003, 2005) found that brain TCE concentration was best correlated with changes in visual function as measured by evoked potentials under acute exposure conditions. Two subchronic exposure studies (Rebert et al. 1991; Blain et al. 1994) demonstrated visual function changes as measured by pattern reversal evoked potentials (Rebert et al. 1991) or electroretinograms/oscillatory potentials (Blain et al. 1994). In one of these studies changes in ERGs and oscillatory potentials were noted following a 12-week exposure at 350 ppm (LOAEL) in rabbits (Blain et al. 1994). In the second study rats exposed to 3,200-ppm TCE for 12 weeks showed decreases in pattern reversal evoked potentials but no effect was noted in the 1,600-ppm exposure group (Rebert et al. 1991). Both subchronic studies examined visual function following an exposure-free period of either 2 weeks (Rebert et al. 1991) or 6 weeks (Blain et al. 1994) and found that visual function returned to pre-exposure levels, thus demonstrating that the TCE effects were reversible.

5.3.3 Cognitive Function

Many rodent studies have demonstrated significant differences in performance of learning tasks such as the speed to complete the task following TCE exposure. Impairment in operant-conditioning cognitive tasks has been reported following TCE exposure in both rats and mice (Kulig (1987); Umezu et al. 1997; Bushnell and Oshiro 2000). Wistar rats exposed to 250–4,000 ppm TCE and higher showed a significant decrease both in the total number of lever presses and in avoidance responses compared with controls. The rats did not recover their pre-exposure performance until about 2 h after exposure (Kulig (1987)). Likewise, a depressed rate of operant responding in male ICR strain mice intraperitoneally injected with 1,000 mg/kg TCE was observed in a conditioned avoidance task. Increased responding during the signal avoidance period at lower doses (250 and 500 mg/kg) suggested an impairment in ability to inhibit responding or failure to recognize the signal.

Rats trained in an operant visual signal detection task and then exposed to TCE (2,000 or 2,400 ppm, inhalation, 70 min daily for 9 days) had significant decrements in the accuracy of signal detection and response time (Bushnell and Oshiro 2000). In a follow-up, repeated exposure study with the operant visual signal detection task rats were inhalationally exposed to TCE (0, 1,600, 2,400) for 6 h/day for 20 days (Oshiro et al. 2004). No significant differences were observed among the exposure groups with respect to acquisition of the visual discrimination response or in the

reaction times. Therefore, it was suggested that TCE exposure with this cognitive task does not result in persistent effects since repeated inhalational exposure to TCE (Oshiro et al. 2004) did not result in the same impairments that was observed in the acute exposure study (Bushnell and Oshiro 2000).

Although cognitive impairments were noted in some studies, other studies indicated no change, or even improvement, in cognitive tasks with continuous TCE exposure. No decrements in cognitive function as measured by the radial arm maze were observed in Mongolian gerbils exposed continuously by inhalation to 320 ppm TCE for 9 months (Kjellstrand et al. 1980). Improved performance, despite a loss in hippocampal myelination, was noted in a Morris swim test for weanling rats orally dosed with 5.5 mg/day for 4 weeks followed by 2 weeks of no exposure and an additional 2 weeks of 8.5 mg/day (Isaacson et al. 1990). Overall, cognitive function is impaired by TCE exposure primarily in tests that measure working memory (e.g., avoidance, operant responding, visual signal detection task). For spatial learning and memory tasks, such as the radial arm maze and the Morris swim test, TCE exposure in animals does not result in impaired performance.

5.3.4 Psychomotor Effects

Several animal studies have demonstrated that TCE exposure produces changes in psychomotor function. At high doses (≥2,000 mg/kg) TCE causes mice to lose their righting reflex when the compound is injected intraperitoneally (Shih et al. 2001; Umezu et al. 1997). At lower exposures (inhalation and oral), TCE produces alterations in neurobehavioral measures including locomotor activity, gait, operant responding, and reactivity. However, these effects may also be due to anesthetic properties associated with TCE exposure.

5.3.4.1 Loss of Righting Reflex

Impaired righting reflexes have been primarily tested in mice. Acute intraperitoneal (i.p.) injections of TCE in male ICR mice resulted in a dose-dependent disrupted righting reflex at doses of 2,000 mg/kg and higher (Umezu et al. 1997). Similarly, impaired righting reflexes at exposure doses of 5,000 mg/kg (i.p.) in male Mf1 mice were observed (Shih et al. 2001). When mice were pretreated with a CYP2E1 inhibitor, dimethyl sulfoxide or disulfiram, the TCE-induced loss of the righting reflex was delayed in a dose related manner. In contrast, the alcohol dehydrogenase inhibitor, 4-metylpyradine, did not delay the loss of the righting reflex following TCE (5,000 mg/kg) treatment. These data suggest that the anesthetic properties of TCE involve its oxidation via CYP2E1 to an active metabolite.

5.3.4.2 Activity, Sensory-Motor and Neuromuscular Function

TCE exposure in animals has resulted in changes in sensory-motor and neuromuscular function primarily following acute or short-term exposure (Kishi et al. 1993; Moser et al. 1995, 2003). Male Wistar rats inhalationally exposed to 250–4,000 ppm TCE for 4 h showed a significant decrease both in the total number of lever presses and in avoidance responses at 140 min of exposure compared with controls (Kishi et al. 1993). In adult female Fischer 344 rats acute and short-term (14 day) administration of TCE resulted in decreased performance in the neuromuscular and sensorimotor function tests which were conducted as part of a functional observational battery (general battery of behavioral observations and tests; Moser et al. 1995). Acute exposure to TCE produced the most significant effects in motor activity (activity domain), gait (neuromuscular domain), and click response (sensorimotor domain). In the 14-day study, only the activity domain (rearing) and neuromuscular domain (forelimb grip strength) were significantly different from control animals. In a separate 10-day study, TCE administration significantly reduced motor activity, tail pinch responsiveness, reactivity to handling, hind limb grip strength and body weight (Moser et al. 2003).

Although significant changes in neuromuscular and sensorimotor function have been observed following a shorter term exposure to TCE, longer term exposures (13–18 weeks) have not been able to establish an association between TCE exposure and impairment in this neurological domain. Male and female Fischer 344 rats inhalationally exposed to TCE (250–2,500 ppm) for 13 weeks did not have any impairments in the neuromuscular and sensorimotor tests conducted as part of the functional observational battery (Albee et al. 2006). No treatment related differences in grip strength or landing foot splay were demonstrated in this study. Similarly, in an 18 week exposure study with TCE (500–1,500 ppm), no changes in spontaneous activity, grip strength, or coordinated hind limb movement were reported (Kulig (1987)). Measurements were made every 3 weeks during the exposure period and occurred between 45 and 180 min following the previous TCE inhalation exposure. Therefore, it appears that acute or short-term exposures to TCE result in neuromuscular and sensorimotor function deficits and there may be some development of tolerance in longer term exposures since these neurological functions were not impaired for the longer exposure durations even though the exposure concentrations were comparable.

5.3.4.3 Locomotor Activity

The observed effects of TCE on locomotor activity in rodents are inconsistent. Several studies showed that TCE exposure can decrease locomotor activity in mice and rats (Wolff and Siegmund 1978; Moser et al. 1995, 2003). Reduced locomotor activity was reported in including AB mice (n = 18) treated acutely 182 mg/kg TCE, i.p. at one of four time points during a 24-h day (Wolff and Siegmund 1978) and in female Fischer 344 rats (n = 8 – 10) gavaged with TCE over an acute

(LOAEL = 5,000 mg/kg TCE) or subacute period (LOAEL = 500 but no effect at 5,000 mg/kg) (Moser et al. 1995, 2003). Rats were also reported to have an increased response latency (potentially an indication of decreased locomotor activity) to a two choice visual discrimination following 1,000- and 1,500-ppm TCE exposures for 18 weeks. However, no significant changes in grip strength, hindlimb movement, or any other motor activity measurements were noted (Kulig (1987)).

There are also a few studies (Fredriksson et al. 1993; Waseem et al. 2001) generally conducted using lower exposure doses that failed to demonstrate impairment of motor activity or ability following TCE exposure. Male Wistar rats dosed with TCE (350, 700, and 1,400 ppm) in drinking water for 90 days or exposed to 500, 1,000, and 1,500 ppm for 16 h/day, 5 days/week, for 18 weeks did not have any changes in locomotor activity (Waseem et al. 2001). No changes in locomotor activity were observed for 17-day-old male NMRI mice that were dosed postnatally with 50 or 290 mg/kg/day from Day 10 to 16 (Fredriksson et al. 1993). However, rearing activity was significantly decreased in the NMRI mice at Day 60.

5.3.4.4 Mood Effects and Sleep Disorders

Evaluating mood changes in rodents is difficult, but some investigators have reported in rats that exposure to TCE results in increased handling reactivity. The increased handling reactivity or hostility in rats was reported in male and female Fischer 344 rats following either an oral gavage of TCE (Moser et al. 2003) or an inhalation exposure (Albee et al. 2006).

Sleep disturbances with TCE exposure have been demonstrated in male Wistar rats exposed to 50–300 ppm TCE inhalation for 8 h/day, 5 days/week, for 6 weeks. Electroencephalographic (EEG) responses were measured and used to determine the number of awake (wakefulness hours) and sleep hours. TCE exposure significantly decreased amount of time spent in wakefulness (W) during the exposure period (Arito et al. 1994). The sleep changes in rodents are a highly sensitive effect in comparison to other observed neurological changes.

5.4 Summary

Exposure to TCE results in several neurological effects in rodent models. The most studied TCE-mediated neurotoxicological effect in the rodents was hearing impairment. The major findings are that hearing loss occurs at mid frequency tones (8–20 kHz), at inhalation exposures starting at 2,000 ppm. These effects persist as measured by loss in spiral ganglion and focal hair cells in the cochlea in addition to the lack of recovery in the hearing function tests. Neuronal degeneration and neuronal impairment was also reported in rodent models and these findings may be extended to numerous human reports of TCE exposure and nerve (specifically trigeminal nerve) impairment. Changes in vestibular function, decrements in visual, cognitive,

and psychomotor effects were also observed in the rodent models and consistent with human findings. Sleeping disorders were also observed with TCE exposure in rodents, but have not been studied, to date, in humans and may represent a neurological hazard for humans. Overall, the rodent models help to strengthen the association between TCE exposure and neurotoxicity in humans. In addition, further behavioral studies with these rodent models may help to uncover more neurological changes and mechanistic events that might be occurring in humans with TCE exposure.

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