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# The Neurobehavioral Toxicity of Trichloroethylene

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ANNAU, Z. *The neurobehavioral toxicity of trichloroethylene.* NEUROBEHAV. TOXICOL. TERATOL. 3(4) 417–424, 1981.—Trichloroethylene is an industrial solvent used primarily in degreasing operations with some use as an anesthetic agent as well. The primary route of exposure is inhalation and the central nervous system effects consist of headache, nausea, sleepiness, burning of the eyes. Human cases of intoxication have been associated with trigeminal neuropathy, however this is probably caused by a breakdown product dichloroacetylene. Fatal exposures may be the result of cardiac failure. Chronic exposure in industrial settings may cause alterations in a variety of behavioral parameters such as reduced memory and intellectual functioning. Experimental human exposures reveal fatigue and sleepiness effects and possible alterations in reaction time, but no deterioration in performance on manual dexterity tasks up to 300 ppm exposures. Animal experiments using acute exposures generally fail to reveal behavioral effects at concentrations below 1000 ppm, with a range of 75–2000 ppm. Cessation of exposure results in rapid behavioral recovery with no residual behavioral deficits. Exposure of dogs to 3000 ppm chronically results in severe cerebellar pathology, with no trigeminal nerve damage. No neurochemical effects of exposure have been documented. The neurobehavioral literature on the toxic effects of trichloroethylene is fragmented and poorly documented suggesting that more and better quality work is needed to understand the potential toxicity of this compound.

Trichloroethylene Neurobehavioral toxicity Human Animal Pathology Behavior Toxicology

TRICHLOROETHYLENE was synthesized in Germany by Fisher in 1964. It is used as an industrial solvent, as a household cleaner and solvent and as an inhalation analgesic and anesthetic. The US production has been declining but in 1977 it was still estimated to be about $116.6\times10^6$ kg with another $9.7\times10^6$ kg imported. Its most popular use has been as an industrial degreasing agent although it is also used in the dry cleaning industry and as a component of adhesives and lubricants. As an anesthetic it is used mainly in short operative procedures as in dentistry, obstetrics etc. [36]. The toxicity of trichloroethylene, (TCE) has been reviewed by several authors [1,51], and this review therefore will restrict itself to the neurobehavioral effects of this compound.

Following ingestion, it disappears rapidly from the blood and can be found in body fats, adrenals and ovaries with smaller proportions in other organs. It is mostly exhaled through the lungs as TCE, but metabolism does occur and the urinary metabolites are trichloroethanol, glucuromide and trichloroacetic acid [51]. An interesting interaction with ethanol known as degreaser's flush has been observed in exposed workers. Consumption of alcohol following exposure to TCE, will produce red blotches on the face and upper parts of the body. In this case TCE levels in the blood can be 2½ times higher than without the alcohol, suggesting lower rates of metabolism, since the TCE excreted through the respiratory route remains unchanged.

A recent NIOSH estimate suggests that 3.5 million persons are occupationally exposed to TCE and that 100,000 persons are exposed on a full-time basis and that 67% of these are working under conditions where control measures are indequate or lacking [36]. Most of these workers are exposed as a result of degreasing operations, and it is thought that the majority are men, although there may be many others working in close proximity to the degreasing operation who are also exposed. The present US occupational standards for TCE are set at 100 ppm while those of the Soviet Union are set a 2 ppm as an 8 hour time weighted average [36].

## HUMAN OCCUPATIONAL EXPOSURES

Accidental overexposure resulting in fatalities has been reported fairly frequently in the literature. In five fatal cases of TCE exposure the victims complained of nausea, dizziness and abdominal pains in some cases accompanied by vomiting. The victims subsequently died suddenly without further warning of deterioration. Post mortem examinations revealed no organ abnormalities except congestion of the viscera. It was concluded that four of the victims died of cardiac fibrillation and one of hepatorenal failure [26].

A survey of 80 Swiss workers who had been employed on the average of 3.5 years in 10 different factories at air concentrations between 1 and 335 ppm of TCE showed a number of "psychic" changes as a result of their exposure histories [17]. Nineteen percent of them complained of reduced memory capability, 20% of reduced intellectual processes, 13% of perseveration of ideas, 13% of emotional lability and 17% of paucity of associations. Unfortunately, no unexposed control group of workers with similar socioeconomic background were examined so that the incidence of these com-

plaints and symptoms in the general worker population was not established. The incidence of symptoms was correlated with three factors, TCE concentration in the air, urine trichloroacetic acid and length of time in years of exposure to TCE. Statistical analyses showed that the incidence of "vegetative" and "neurological" disorders were significantly higher in workers exposed to the higher (mean of 85 ppm) than in the groups exposed to lower concentrations. Similarly groups with means of 67 and 180 mg/l of trichloracetic acid excretion had higher incidence of symptoms than groups with 20 mg/l. Finally, the symptoms were also significantly related to the length of time the workers had been exposed. These results were also verified in a subsequent Rumanian study of 70 workers [31], and by others in England [45].

At higher exposure concentrations unconsciousness can result rapidly, especially if the individual had been exposed to TCE prior to the high level exposure. Two workers overcome by TCE vapors were found unconscious inside a tank where the concentration of 3000 ppm TCE was found [32]. Both of these men had worked in the tank before taking a rest outside and had become unconscious almost immediately upon re-entering the tank. In one of these workers urinary TCAA had risen to 187 mg/l 48 hours after the accident.

More chronic exposures to relatively high levels of TCE produce swelling of the eyes, face and hands, excessive sleepiness and loss of appetite [33]. This type of exposure can also lead to addiction and there are several cases of fatal addictions recorded in the occupational literature [22]. In a typical instance a worker had been found unconscious in a vat in 1948. His addictive habit continued until 1957 despite repeated warnings from supervisors. His death occurred as in the previous cases without warning while working in a room that did not conatin TCE. No abnormal pathology was found except in the lung which showed some hemorrhages. There are numerous other reports of glue sniffing addictions using TCE in the younger population [1,19].

The lack of pathological correlates reported in many clinical observations are not consistent with some reports where severe neuropathologies are seen following similar exposure histories. In one such report, four men, one of whom died, became ill from fumes inside a tank they were cleaning [7]. One worker had to be hospitalized and showed severe signs of trigeminal nerve dysfunction including loss of taste sensation over the anterior tongue. Two and a half years later this person had still complained of numbness of the upper lip, gum and palate. Histological section of the brain of the fatal case showed striking alterations in the brainstem, in the fifth nerve nuclei, spinal tracts and nerve roots. Extensive myelin and axon degenerations were seen. The principal sensory and spinal tract nuclei of the fifth nerve showed severe nerve cell loss and this was also seen in the motor nuclei of the fifth nerve. Somewhat similar though less severe damage was seen in the sixth and seventh nerve nuclei. Less severe damage was seen in ascending sections although the red nucleus, substantia nigra and mammillary bodies all showed some damage. In the cortex, Sommer's sector in the hippocampus sustained some damage due to TCE. The authors concluded that the pathological features correlated well with the clinical symptoms experienced by the victims. No estimate of the TCE concentration inside the tanks where the workers were exposed was available in this case.

In an extensive study of a single case, the extent of cranial nerve damage was described for a worker exposed to warm fumes directly for 5 minutes and indirectly through a gas mask for 1.5 hours [11]. Three days after exposure there was restriction of the visual field, complete facial anesthesia as well as a significant increase in the latency of motor unit potentials. There was also a slowing of conduction of sensory fibers in the ulnar nerve. Partial although not total recovery occurred during the next 18 months. A similar case has been reported by others [34].

In summary, these case histories while somewhat poorly documented in terms of concentration effects nevertheless show that exposure to high concentrations of TCE can lead to neuropathy primarily of the trigeminal nerve that can also extend to more peripheral nerves. As will be seen later, this pathology may not be due to TCE, but to one of its degradation products. At lower concentrations, and chronic exposures more subjective and less clearcut effects emerge, suggesting more generalized CNS damage. Unfortunately no objective measures such as motor conduction or neuropathological sections at these lower concentrations are available to determine the extent of damage to more central structures.

## HUMAN EXPERIMENTAL EXPOSURES

In an attempt to develop tests of TCE toxicity in the field, 12 human volunteers were exposed to 1000 ppm TCE for two hours and then tested for optokinetic nystagmus [30]. The subjects viewed a hemicylindrical screen on which black bands traversed the visual field at an accelerating rate from a distance of 1 meter. When the eyes could no longer follow the traversing bands at a certain speed, the optokinetic fusion limit was reached and recorded. These measures were taken both before and after exposure to TCE. In addition, two subjects were given alcohol at 0.7 g/kg body weight. After alcohol there was a marked lowering of the fusion limit; however, after TCE exposure some subjects exhibited either no change or only slight lowering of the limit. Subjects that showed a change after TCE exposure, however, showed the effect even two hours after exposure. The authors speculated that the reason TCE showed less effect than alcohol was that the blood levels of TCE as idicated by venous blood samples were considerably below the alcohol blood levels. Since the exposure levels to TCE in this study were considerably above those found in occupational settings, the nystagmus test did not appear to hold promise as an industrial screen for solvent toxicity.

Another experimental approach to investigate the effects of TCE exposure used a variety of psychological tests in a single subject exposed to 100, 200, 300 and 500 ppm of TCE [48]. In order to mask the odor of TCE lavender oil was added to both the air and the TCE exposures. Despite this precaution which masked the odor of TCE well, the subject was aware of the presence of the higher TCE concentrations because of a slight eye and throat irritation. The tasks used were the Crawford Small Parts Dexterity Test which consisted of screwing small screws into a board with a screwdriver and placing metal pins into a plate with tweezers. A visual illusion, the Necker cube was used to detect visual disturbances, in this case the number of reversals of the cube. A card sorting task and a card sorting task compounded with an arithmetic task was also used. Finally a complex matching task of moving dials with a one second response time completed the tasks. The subject reported feeling somnolent and "whoolly-headed" during the 500 ppm exposures. No statistical analyses of the data were attempted and the greatest effect observed was a 40% decrease

in the number of reversals seen on the Necker cube at 500 ppm with lesser effect at 200 and 300 ppm. The manual dexterity tests showed no effect of TCE. Thus while this study incorporated some desirable controls such as masking the TCE odor, the use of a single subject precludes any conclusions regarding the effects of TCE on performance. A subsequent study [50] used a series of perceptual motor-tasks on eight male subjects exposed to 0, 100, 300, and 1000 ppm TCE in a random order. The subjects wore nose clips and inhaled through the mouth, and some attempt was made to mask the odor of TCE as well. Each subject was exposed for two hours and was given the tests three times, once immediately at the beginning of exposure, once 50 min. into the session and finally at 1 hr. 40 min. into the session. Results again showed that for the most part TCE had no effect on most tests. Only at 1000 ppm was there a significant effect on the Howard-Dolman depth perception test and then only after 25 min. of exposure. All other tests such as the Muller-Lyer visual illusion, a hand steadiness test and the Purdue Pegboard test either showed no effect or in the last case an improvement, probably due to practice effects.

Using the rationale that many workers consume drugs while on the job or at home, the same authors repeated their study by combining the TCE exposure with three commonly used CNS depressants [12]. These drugs were thonzylamine hydrochloride, (Anahist) at 50 mg, meprobamate (Equanil) at 800 mg and alcohol at 35 ml/70 kg body weight, in combination with 0, 300, or 1000 ppm TCE. The results showed that only the combination of TCE with alcohol affected behavior. The depth perception test and particularly the hand steadiness test were profoundly affected by the combined exposures. The interesting aspect of this study is that it showed no interaction between the narcotic effects of TCE and the effects of the two drugs administered to the subjects, thus indicating that CNS depressants do not necessarily interact when taken simultaneously.

A similar study using a combined alcohol-TCE exposure failed to find any significant effects of the combined exposures [52]. The TCE was administered at 200 ppm and alcohol at 1 ml/kg body weight. The behavioral tasks consisted of a binary choice task where the subject had to respond to a directional cue by pressing a switch either with his right or left hand. A second task was a pursuit rotor task. The authors described impaired performance on the pursuit rotor task following the combined exposure, but this was not verified statistically. While both of these studies used alcohol in combination with TCE, the latter study used a lower TCE concentration, (200 ppm) than the lower cencentration in the former, (300 ppm). In the former study there were two TCE concentrations used with the higher being 1000 ppm, and the more significant effects occurred in this condition in combination with alcohol.

A major problem emerges in the interpretation of the results of these studies. Improvement in the performance of a task as a result of practice was not adequately controlled. This "learning" effect may have played a more significant role in altering behavior during the course of the studies than the toxic agents and thus the results could be confounded by this uncontrolled variable.

In a study designed to measure the urinary excretion and alveolar concentrations of TCE in volunteers exposed for 7 hours to 200 ppm several subjective observations were recorded as well as the Romberg balance tests which consists of the person balancing on one foot with his eyes closed and with both hands at this side [46]. After two hours of exposure 80% of the subjects were unable to detect the odor of TCE and the effect became more pronounced during the week of exposure such that they were unable to detect the odor in less time each day. A feeling of fatigue and sleepiness was felt and 50% of the subjects reported that it took greater mental effort to perform the Romberg test. The results of this study while not meant to definitively assess behavioral effects, neverless confirmed many of the clinical findings of fatigue and lethargy during exposure.

An experiment in which volunteers were exposed for two 4 hr. exposures of 110 ppm TCE, each exposure interrupted by a 1½ hour rest period, indicated significant alterations in performance on a test of tachistoscopic perception, immediate memory, and complex reaction time (both in response latency and regularity) [40]. The initial experiments were performed with student volunteers, and then repeated with workers exposed to TCE at their job. According to the authors the latter test confirmed the earlier findings with the students.

The unexpected effects using such low concentrations of TCE prompted Stewart and coworkers to repeat the study in its entirety with a lower TCE concentration added (50 ppm), and two more behavioral tests, [47]. This study reported in great detail failed to confirm any of the findings reported by the Italian group [40] at 110 ppm TCE. No apparent reasons can be found for this discrepancy although Stewart *et al.* suggested that the differences may have arisen because (1) inappropriate statistical treatment of the data; (2) inadequate control of TCE concentrations; or (3) inadequate control of the subjects who could have consumed alcohol during their 1½ hour break and thus increased considerably their TCE load. Since the findings at such low levels of TCE exposure are so much out of line with the mainstream of the literature one must either wait for better presentation of the data or a replication from another laboratory. Several subsequent attempts to obtain behavioral effects at concentrations between 100 and 300 ppm have failed [10, 27, 35].

A factor that has not been explored adequately, is the duration of exposure in a manner more analogous to the workplace. Several reports suggest that low exposure levels may have subtle effects that have yet to be fully explored.

Electrophysiological events in the brain have been reported to show alterations during TCE exposure. Volunteers exposed to 50, 100 and 200 ppm TCE showed changes in the second positive peak (P2) of the auditory evoked potential after 3½ hours of 50 ppm of TCE, and alterations in the visual evoked potential after 7½ hours of exposure to 100 ppm. This latter effect was more frequently observed in females than males suggesting that gender specific neurotoxicology should be explored more throughly [47, 54]

In yet another suggestive study factory workers were monitored with EEG telemetering devices during one week while exposed to TCE [28]. The duration of alpha episodes and the amplitude of alpha waves increased significantly from control to TCE exposed episodes. This increase was also seen from the first workday to the rest of the week, and correlated well with TCE and TCA loads. Since no control subjects were used, it is impossible to assess the contribution of job tedium to this alpha effect.

A final study, however, seems to show this work week effect using choice reaction time measures in workers exposed to more or less than 100 ppm TCE on the job [18]. With measures taken before, during, and after the last work shift, control subjects not exposed to TCE showed an improvement in reaction times as a function of time. Workers

exposed to less than 100 ppm showed no change and workers exposed to more than 100 ppm (with a high of 418 ppm) showed an impairment of reaction time during the periods measured. These effects were statistically significant. The results obtained in this study would have been strengthened considerably if blood TCE levels had been obtained and correlated with the behavioral measures.

This survey of the human experimental literature suggests that a great deal of work must be done at the low concentrations of TCE in order to determine safe limits of exposure. The last studies in particular suggest that longer exposure durations are likely to yield important insight into the possible toxicity of this compound as well as the nature of this toxicity with regard to the central nervous system. Innovative studies such as telemetered EEG and on the job testing should be increased in order to evaluate the population that is actually exposed every day, rather than college students who are exposed for a few hours and are unlikely to show subtle neurotoxicity. It is also of importance to determine whether the observed changes, i.e., alterations in reaction time, are due to the pharmacologic or anesthetic effects of TCE or whether these are truly toxic effects associated with pathology.

## ANIMAL EXPERIMENTAL STUDIES

A variety of experimental models have been used in the study of TCE neurotoxicity. The first animal model derives from the Russian literature using conditioned alimentary reflexes in rats exposed to 75 ppm TCE [24]. The animals were initially trained on a conditioned discrimination, one tone signalling food, the other weaker and different frequency tone being used as the nonreinforced differential stimulus. After the animals had been trained and reached an asymptote in the latency of the correct response (75% correct responses), they were exposed to TCE. The effect of the exposure (7½ hr per day for 48–112 weeks) was to significantly reduce response latencies while at the same time the number of correct responses decreased from 75% to approximately 25%. Individual animal rather than group data were presented. The animals were described as restlessly running around the cage between stimuli, although this behavior was not seen in the exposure cage.

This type of excitability was also seen in a study where three rats were trained to climb a rope to obtain a reinforcement [15]. Animals were exposed to either 200 ppm or 800 ppm for three hours from 4 to 11 weeks and tested immediately after the daily exposures outside the exposure chamber. Results indicated no effect of TCE on rope climbing time or ability to respond to conditioned stimuli (a tone and a light), that signalled the availability of the reinforcer. There was, however, a statistically significant increase in the number of spontaneous climbs following TCE exposure reminiscent of the increased activation in the previous report.

In a more sophisticated study from the same laboratory groups of six rats were trained on a conditioned avoidance task [4]. The effect of TCE was recorded both during learning, performance and extinction. The animals were exposed either during acquisition or extinction of the response to an average concentration of 400 ppm. Because the chambers were not airtight the initial concentration during the 3–4 hour session was 800 ppm but decreased over the session to 400 ppm. There was no effect of TCE on acquisition, but there was a significant slowing down of the rate of extinction. The exposure also increased the latency of escape from shock during escape trials, suggesting a narcotic effect.

Using yet another task, rats were trained to swim a 4 meter water alley with and without a 27 g load attached to their tail [16]. The animals were exposed to 400 and 800 ppm of TCE for six hours and then tested in the swimming task immediately. The higher TCE concentration increased swimming time but only with the load. In addition, it increased the animals' fatigue as measured by increased swimming times during the sixth to tenth swimming trials following exposure. Activity measures in other groups of rats showed that only 1600 ppm exposure to TCE for five hours resulted in decrements in motor activity.

In order to measure the effects of chronic exposures to TCE, the same authors exposed two groups of ten male rats to 400 ppm, 8 hours a day for 44 weeks. The animals were tested on the aforementioned swimming task twice a week, on a modified Hebb-Williams maze using a water tank, on a conditioned avoidance task 100 trials a day and finally on a Dashiell-type exploratory maze [5]. The results confirmed some of their earlier findings in that TCE exposure significantly decreased swimming speed but only when the animal was loaded down with the extra weight. There was no effect on the rate of learning of the avoidance task between groups. The rate of exploratory behavior in the maze was significantly increased by the TCE exposure in that the animals shifted more of their activity from the outside to the inside of the maze. There were no differences between the groups in the Hebb-Williams maze.

An examination of this series of experiments from the same laboratory provides a confusing picture of the effects of low level exposure to TCE in that most behavioral measures are unaffected even at 800 ppm. Some of the measures such as motor activity and extinction rates are not examined in enough detail to provide an understanding of the mechanisms involved in the toxicity of this compound. The swimming test which showed a significant effect in both sets of experiments may have been a good animal model of the "fatigue" reported by many workers exposed to TCE and probably should have been followed up with more complete evaluation of this aspect of TCE exposure.

A task somewhat reminiscent of the rope climbing test described above was used to measure the effect of 200, 560, 1568, and 4380 ppm of TCE following four hour exposures [13]. The animals were trained to avoid a signalled electric shock by climbing a pole. The measure of the effect of the TCE was a decrease in shock avoidance and the number of escapes, i.e., responses to the shock. No dose-related effects occurred. Animals in all groups showed a decline in the number of avoidances by the tenth day of exposure. Animals in the 4380 ppm group, however, stopped responding altogether on the first day of exposure, but improved on the second day and were not different from the other groups by the tenth day. When these authors studied the effect of TCE exposure at the above concentrations on learning the same response, they obtained better separation of the groups with the groups exposed to 200 ppm learning faster than controls, (perhaps due to increased activity levels) while the group exposed to 4380 ppm being severely retarded and not improving after the third day. Several of the animals exposed to 4380 ppm died after the tenth exposure and had massive lung and intestinal hemorrhages.

A more interesting study from the same laboratory using only 125 ppm TCE, used discrete shock avoidance in rats [14]. The animals were trained to press a lever to avoid un-

signalled shock during the fourth hour in the exposure chamber. The number of shocks received per hour prior to exposure was compared to the number received during the exposure that lasted four hours daily for 25 days. There was a very significant increase in the number of shocks received by the rats during the exposure period. Not all rats were affected by TCE according to the authors, and rats that seemed to be affected on one day were consistently affected. While these data show low concentration effects of TCE on behavior they do not agree with findings from the same laboratory showing rapid adaptation to TCE exposures.

A novel approach to the behavioral studies was offered in a recent paper using ethological techniques [44]. Pairs of rats were exposed to 100, 200, 500, and 1000 ppm for six to seven hours per day for periods varying from 1 to 12 weeks. Immediately after removal from the exposure chamber the animals were observed for social behaviors by trained observers. In addition, a thirst exploration test was also used in this experiment where thirsty rats (18 hr deprived) were placed in an unfamiliar cage and the time to find water as well as exploratory movements were recorded. The results showed that all TCE exposures reduced social activity. The difference between the low and high concentrations was the time it took for the TCE to have an effect. At high doses activity was reduced earlier. In the thirst test a somewhat different picture emerged in that all exposed animals began drinking sooner than nonexposed rats, an effect the authors interpreted as loss of inhibitory control. These results suggest that the behavioral effects may be situation specific and may have important implications for human exposures.

In a test of memory following TCE exposure, Mongolian gerbils were exposed continuously for nine months to 320 ppm. Using an 8 and a 16 arm radial maze the authors found no effect of TCE on this task [25]. When subsequent to this exposure both the TCE and air control animals were challenged with 1,1,1-trichloroethane at 2300 ppm for six hours and then retested, the previously TCE exposed animals performed better than the air controls. The authors find this a puzzling effect though it is somewhat reminiscent of the state dependent psychopharmacology literature and may shed some light on the stimulus properties of these solvents.

Two studies using brief exposures to high TCE concentrations showed that some behavioral models may be more sensitive than others to TCE. In one of these reports the electrical self-stimulation of the brain was used in rats implanted with lateral hypothalamic electrodes as the behavioral baseline for 30 min daily exposures to either 2500 or 3000 ppm TCE [2]. The higher concentration of TCE depressed self-stimulation rates on three consecutive days of exposure significantly and progressively during the 30 min., reaching 15% of control rates at the end of exposure. When exposure was terminated, the animals recovered within 24 hours to control rates. At the lower concentration of TCE much less disruption of rates was seen reaching 54% of control by the end of the 30 min. on the first day. These animals demonstrated a tolerance effect on the two subsequent days and were only depressed in self-stimulation rates by about 30%. Exposing the same animals after 24 hr of dehydration showed a more rapid development of tolerance at the low concentration with even an elevation of rates above control during the first 15 min of exposure. The results indicated that the physiological state of the organism may be an important determinant of the effects of organic solvents.

A recent study from Japan used 30 min. exposures at 2600, 5000, and 8000 ppm for 80 days and evaluated the effects on retention, extinction and relearning of a FR-30 food reinforced schedule; acquisition of a DRL schedule and spontaneous activity and emotionality in rats. The only effect the author reported is a slight deficit in learning [21].

It is clear from an examination of the animal behavior literature that the data are confusing and inconsistent. This may be due to the different techniques used and the lack of statistically adequate experimental design, (many of the early studies are based on too few animals) as well as failure to precisely measure TCE concentrations. More sensitive assays have to be developed and chronic low level exposures should be used instead of the high levels reported in many studies. The work factor so prominent in human exposures should be incorporated into animal studies since fatigue may play a role in increasing the hazards of the workplace. There is also a general failure in the behavioral studies to include neuropathological evaluation of the animals following termination of exposures, thus leaving the question of the narcotic vs. toxic effects of TCE unresolved.

## NEUROPATHOLOGICAL STUDIES

The two papers devoted to this topic use different species as well as different routes of administration. In the first report, dogs were exposed to concentrations varying from 500 to 3000 ppm daily from 2 to 8 hours 5 days weekly. At the highest exposure the animals showed severe symptoms of intoxication consisting of tremor of the head, gnashing of the teeth, frothing at the mouth, ataxic movements followed by generalized rigidity, and bicycling movements of the extremities. The pathologic changes were most striking in the cerebellum where Purkinje cells were swollen, translucent and devoid of architectural components [3]. In the cerebral cortex scattered and often severe nerve cell changes were present with the most extensive changes consisting of marked shrinkage and hyperchromatic staining. Pyknotic neurons often seen in large groups, mostly in the temporal and occipital regions, were surrounded by intact elements. The myelin in the cortex was not altered. In the white matter, again most often in the temporal and occipital regions, there was extensive swelling and vacuolizations of large groups of sheaths. The brainstem, the spinal cord and the cranial nerves showed no damage.

In the second study, rabbits were treated acutely or chronically with deep intramuscular injections twice or three times a week from 41 to 247 days in the chronic phase and for 53 days in the acute phase. The dose varied from 2 ml per injections to 3 ml for a body weight of approximately 2000 g. In the acute case neurons in the telencephalic cortex, basal ganglia, and brainstem nuclei showed an eosinophilic homogenization of the cytoplasm with slight shrinkage of the cell body and hyperchromasia of the nucleus. A number of Purkinje cells also showed these changes with some loss of these cells also occurring. All experimental animals showed multiple infiltrates of lymphocyte-like cells in the pons, cerebellum and telencephalon. In the chronic treatment these changes were more pronounced. In the cerebellum both Purkinje cells and their associated basket cells disappeared. In neither exposure protocol did the authors observe alterations in the optic or trigeminal nerves [6].

Under some environmental conditions TCE can decompose and form several degradation products that are considerably more toxic [36]. In the presence of strong alkali or hot metals, dichloroacetylene can be formed and it is this chemical that is thought to lead to the trigeminal syndrome re-

ported in the occupational literature. Two studies in which rabbits and mice were exposed to dichloroacetylene at concentrations ranging from 19 to 300 ppm demonstated the extensive lesions in the cranial nerves as well as the damage in the lungs and livers seen in workers exposed to high concentrations of TCE [37,38]

The results of these studies combined with the lack of effects seen in the previous studies where pure TCE was used suggest that TCE exposure alone does not have the neuropathological effects attributed to it by occupational physicians. It must be kept in mind of course that during occupational exposures the worker is not exposed to TCE alone, but to a variety of degradation products as well, and that the monitoring of these may be as important the monitoring of TCE itself.

## BIOCHEMICAL STUDIES

Adult male rats were exposed to 200 ppm of TCE for 4 days, 6 hr per day. Animals were sacrificed at different times within this protocol. Brain RNA content was reduced after four days of exposure. The brain RNA and glutathione content increased after two hours of exposure and then gradually declined. Behavioral changes also observed in this study indicated increased activity in a number of measures 1 hr after exposures but not at 17 hr after exposure [41,49]

A different approach was used in a study of TCE exposure of Mongolian gerbils exposed to 320 ppm of TCE for 8 weeks [20]. The polypeptide pattern of all cortical areas, the cerebellar hemispheres and the brainstem showed a significant decrease in one of the major polypeptides with a m.w. of 50.000–52.000 after the 8 week exposure. The levels of S 100 protein increased continually during the exposure in the visual cortex, the hippocampus, the cerebellar posterior hemispheres and the brainstem. In the frontal and sensory cortex an initial increase in this protein after 2–4 weeks was followed by a decline to control levels.

The results of these studies are preliminary and no clear understanding of the nature of these changes is offered. What is apparent is that different brain areas react differently to solvent exposure, a result also substantiated by the pathological findings.

## NEUROPHYSIOLOGICAL STUDIES

In the isolated rat phrenic nerve diaphragm preparation, anesthetic gases including TCE caused miniature end plate potentials to disappear completely before depression of muscle contraction, with no changes in the resting membrane potential. End plate potentials were also depressed, but with TCE it was difficult to observe these because transmission failure occurred suddenly with complete disappearance of the EPP. TCE also greatly prolonged the refractory period [23].

A more thorough evaluation of neurophysiologic effects of TCE was published recently using the giant squid axon [43]. TCE decreased the resting membrane potential in a concentration dependent fashion. The depolarization was attributed to a decrease in resting postassium permeability. Both peak transient and steady state conductance increases were suppressed by TCE and the curve relating the steady state conductance to the membrane was shifted in the depolarizing direction. The reversal potential for the peak transient current was shifted in the direction of hyperpolarization mostly due to a decrease in selectivity of the peak transient channel and partly due to an accumulation of sodium ions inside. The steady state sodium inactivation curve was shifted by TCE in the direction of hyperpolarization. The authors suggest that the accumulation of sodium ions inside the nerve would be much more pronounced in small nerve fibers in the brain than in the giant squid axon and that together with the observed decrease in selectivity of peak transient channels would play a significant role in general anesthesia.

In an attempt to study the tole of TCE in central transmission, the isolated slice of guinea pig dentate gyrus was used [39]. Stimulating electrodes were placed in the perforant path and recording electrodes in the granule cells. Synaptic transmission was depressed by TCE administered in the gas phase with the perfusing gases, between the perforant path and the granule cells at concentrations below those required to maintain anesthesia in intact animals. The population of excitatory post-synaptic potentials (epsps) and massed discharge of granule cells (population spykes) were depressed at concentrations lower than those depressing compound action potentials of the perforant path. Frequency potentiation of the evoked epsp was not impaired by TCE. The authors concluded that TCE depressed synaptic transmission by reducing the amount of transmitter release or by decreasing the sensitivity of the post synaptic membrane to transmitter substance or by both of these mechanisms.

## TERATOGENIC STUDIES

In order to evaluate the toxicity of TCE it was dissolved in olive oil and injected into chicken eggs on day 2, 3, and 6 of incubation, at 5, 25, 50, and 100 $\mu$mol/egg. On the second day of incubation 50, 25, and 5 $\mu$mol produced subcutaneous edema, stunted lower extremities and reduced number of digits, external viscera, ectopia of cordis and crooked toes. A dose of 25 $\mu$mol on the third day cause profound edematogenic effects on the whole body. The dose of 100 $\mu$mol was embryotoxic [9].

Two studies evaluating the teratogenic potential of TCE at 300 ppm or 1800 ppm in rats and mice found no effects. Both studies reported some weight reductions in the offspring but no changes in motor activity or any other adnormalities [8,41].

Thus while the first study using chicken eggs reported some teratogenic effects, without comparative tissue level determinations of TCE it is impossible to make adequate comparisons between these three studies. Species differences in sensitivity to TCE may also be important in teratogenic studies and great caution must be exercised in generalizing from avian to mammalian species.

A report that cannot be strictly classified as a teratogenic study used newborn rats exposed with their mothers to 0.7, 0.2 and 0.9 ppm of TCE for 1.5 months [29]. The authors report a significant decline in growth rate, hypotension and a change in cholinesterase activity of whole blood in the pups exposed to the two higher concentrations. All indices returned to normal following cessation of exposure.

These results are totally inexplicable in view of the literature arising from all other laboratories. At the same time, it is the only report dealing with postnatal exposures and therefore until further replication, the report must stand as a warning of the potential hazards of TCE to the developing organism.

## CONCLUSIONS

This survey of the neurobehavioral toxicity of TCE leads

one to the conclusion that much is yet to be learned regarding the human health hazards of this agent. It seems clear that with exposure to high concentrations humans can suffer neuropathy particularly of the cranial nerves as well as abnormal behavioral reactions although it is unclear whether these effects are due to TCE or degradation products or a combination of the two. The effects of long term exposure to low levels are not clear, and without better animal and human experimental models, including monitoring workers for long periods of time no clear guidelines can be suggested. The two human studies on telemetered EEG and choice reaction times suggest, however, that exposures to concentrations of 100 ppm alter neurobehavioral measures during the work shift. Even though it is not clear whether these alterations are due to narcotic or pathological effects of TCE exposure, they suggest that the current exposure standards may be too high. Altered EEG and performance may lead to increased accidents in the workplace and thus indirectly increase the health risk to the worker.

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