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Acute Neurotoxicity and Assessment of a Biomarker of Effect (GFAP) In Male Sprague-Dawley Rats After a Single Exposure to 1,2-Dichloroethane

Mario Glynn Hollomon

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THESIS

BY

MARIO GLYNN HOLLOMON

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THESIS

Presented in Partial Fulfillment of the Requirements for

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By

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1997

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Chairman, Thesis Committee

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ABSTRACT

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Professor Chander S. Mehta, Advisor

DCE is a clear synthetic liquid. Its most common use today is to make vinyl chloride and several substances that dissolve grease, glue, and dirt. The purpose of this study was to investigate the effects of DCE on the central nervous system by measuring GFAP and GSH brain levels and SMA. Brain regions studied were CTX, BS, CRB and HP. 49-day male Sprague-Dawley rats were given a single dose of DCE and sacrificed 7 and 14 days later to measure GFAP levels. Dose groups for the 7-day study were 5ml/kg saline and 0.7ml/kg DCE. Dose groups for the 14-day study were 5ml/kg saline, 0.35ml/kg [L-dose] and 0.7ml/kg [H-dose] DCE. SMA for the same

dose groups as used for the 14-day study was measured on days 1,2,7 and 14 using digiscan computerized cages. Brain GSH levels were measured 2 hours following exposure to 5ml/kg saline, 0.35ml/kg and 0.50ml/kg DCE. The saline group served as the control group in all cases. In the 7-day study, GFAP levels were significantly ($p<0.05$) increased in the HP as compared to the control group. In the 14-day study, GFAP levels were significantly ($p<0.05$) increased in the BS and HP for both L-dose and H-dose compared to the control group. SMA was significantly ($p<0.05$) lower on day 1 for both L-dose and H-dose compared to the control group. Brain GSH levels were significantly ($p<0.05$) decreased in the CTX and HP for both L-dose and H-dose. The results show a region specific affect on rat brain GFAP and GSH levels following a single exposure to DCE as well as altered SMA.

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LIST OF SYMBOLS

ATSDR	Agency for Toxic Substances and Disease Registry
BCA	bicinchoninic acid
BSA	bovine serum albumin
BS	brainstem
C	Celsius
cm	centimeter
CNS	central nervous system
CRB	cerebellum
CTX	cortex
DCE	1,2-Dichloroethane
DTNB	5,5'-dithiobis-(2-nitrobenzoic acid)
EDTA	disodium ethylenediaminetetraacetate dihydrate
ELISA	enzyme linked immunosorbent assay
g	gram
GFAP	gial fibrillary acidic protein

GSH	glutathione
GSSG	glutathione disulfide
HP	hippocampus
IgG	Immunoglobulin G
LD_{50}	lethal dose which kills 50% of population
mM	millimolar
M	Molar
mg/kg	milligrams per kilogram
ml	milliliter
ml/kg	milliliters per kilogram
MPP+	pyridinium ion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
mRNA	messenger riboneucleic acid
$P<0.05$	significance
PBS	phosphate buffered solution
ppm	parts per million
SDS	sodium dodecyl sulfate
SMA	spontaneous motor activity

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CHAPTER 1

INTRODUCTION

DCE is a clear synthetic liquid, commonly used to make vinyl chloride and several substances that dissolve grease, glue, and dirt. In the past, DCE was extensively used as an insect fumigant for stored grains, a soil fumigant in fruit orchards, a textile cleaning agent and as an extractant in certain food processes (IARC V. 20, 1979; NIOSH 27, 1978). DCE was also added to leaded gasoline to act as a lead scavenger. A major portion (84%) of U.S. production of DCE is converted to vinyl chloride (Daniel et al., 1994). There are 11 domestic manufactures of DCE; production occurs at 16 sites located predominantly in Texas, Kentucky, and Louisiana. In 1990, DCE ranked 15th among the top 50 largest volume chemicals with a total U.S. production of 13.8 billion pounds (USITC 1991; Chem. Engr. News, 1991). The 11 domestic manufacturers of DCE have a combined annual production capacity of nearly 24 billion tons. The threshold level value (TLV) for DCE is 1 part per million (ppm) for an 8-hour time weighted average (TWA). The short time exposure level is 2 ppm. Drinking water is not allowed to exceed 0.005 mg/L. The LD_{50} for rats via oral

exposure is reported to be 700-770 mg/kg (Gosselin, et al., 1984; Budavari, 1989). There is little data available for the neurotoxicity of DCE via oral exposure.

Neuronal tissue consists of nerve cells, or neurons, and supporting cells called glial cells. Several types of glial cells exist within the nervous system. Astrocytes, also called astroglial cells, constitute the most numerous cellular population of the CNS (Pope, 1978). Astrocytes are involved in a number of vital homeostatic mechanisms including maintenance of the blood-brain barrier, extracellular buffering, microregulation of cerebral blood flow, production of growth factors, intercellular communication and metabolism of glutamate and ammonium (Halliday, et al., 1996; Patel and Gray, 1993; Murphy et al.,1990; Murphy, 1993). In the adult nervous system, astrocytes provide structural support to surrounding cells, regulate the ionic and neurotransmitter levels in the extracellular fluid, and secrete pleiotropic growth factors. In this manner astrocytes likely influence the physiological properties of adjacent neurons (McCall, et al., 1996). Astrocytes are more abundant in the white matter of the CNS than the gray matter of the CNS. There is evidence that molecular and functional differences exist among astrocytes in different regions of the CNS gray matter (Denis-Donini, et al., 1984).

Astrocytes have also been shown to mediate effects that are detrimental to nervous system development and maintenance. Examples include formation of glial scars which have been suggested to be detrimental to axon regeneration (Anders and Hurlock, 1996), conversion of protoxins, such as MPTP, to potent neurotoxins, MPP+ (Langston, 1985), and accumulation and release of excitotoxic amino acids (Albrecht, et al., 1991).

GFAP is the major cytoskeletal protein of astrocytes and belongs to the intermediate filament superfamily and is found predominantly in the CNS (Mecocci, et al., 1995; McCall, et al., 1996). Intermediate filaments are directly involved in the behavior of astrocyte cytoarchitecture during the postnatal proliferation, the transition to the differentiated stage, aging and astrogliosis (Eng, 1980). The gene encoding for GFAP in the human brain is located on chromosome 17. Through the technique of immunolabelling the precise location of GFAP within the astrocyte has been identified as being the centriolar region in immature astrocytes (Kalnis, et al., 1985) and the cell body and processes of mature astrocytes (Tardy, et al., 1993). It is suggested by Trady et al. (1993) that GFAP may have a role in the early mitotic process due to its localization in the centriolar region. Chui and Goldman (1984) have observed a half life of ~ 8 days for GFAP in cultured astrocytes. The documented half life of spinal cord

GFAP in vivo is ~ 9 weeks (DeArmond, et al., 1986). With maturation, GFAP becomes more stable as witnessed by the observation that with maturation the GFAP protein expressed is substantially higher than the same temporal GFAP mRNA levels (Tardy, et al., 1990). During the neonatal stage, GFAP protein expression and GFAP mRNA levels are similar. During the neonatal stage another role of GFAP may be to maintain cell integrity and participate in morphological changes that occur during the transition from proliferation to differentiation (Tardy, et al., 1993).

Hyperplasia and hypertrophy of astrocytes are commonly termed "astrogliosis" and "reactive gliosis." Accordingly, with both hyperplasia and hypertrophy of astrocytes there is an increase in GFAP levels. Hyperplasia is an abnormal or unusual increase in the elements composing a part, whereas hypertrophy is an increase in bulk without increase in the number of constituent elements. Hyperplasia of astrocytes is believed to be associated with traumatic injury of the CNS. Hypertrophy of astrocytes results from toxic injury to the CNS and GFAP levels usually return to base levels after recovery or cessation of exposure to toxicant. There are several factors that may influence an increase in GFAP levels. These include neurotransmitters (Pollenz and McCarthy, 1986;

Le Prince, et al., 1990), hormones (Morrison, et al., 1985; Clos, 1986), and age (Yoshida, et al., 1996).

Biomarkers are chemical or morphological changes that occur in response to physical or chemical insult. Biomarkers of the CNS are used to determine exposure, susceptibility and risk. Certain characteristics of the CNS biomarkers are essential to validate their usefulness. Desirable features of the CNS biomarkers include responsive to diverse types of injury to any brain area, high sensitivity (low incidence of false negatives), high specificity to the neurotoxic condition (low incidence of false positives), and simple to evaluate and quantitate (O'Callaghan, et al., 1995). Assays are available for quantitating GFAP levels in the nervous tissue which enables the use of GFAP as a biomarker for the CNS damage (O'Callaghan, 1991; Brock and O'Callaghan, 1987).

Glutathione (gamma-glutamylcysteinylglycine; reduced glutathione, GSH) is a tripeptide found ubiquitously in plant and animal tissue, which functions to protect tissues from the toxic effects of many exogenous and endogenous substances (Trenga, et al., 1991). GSH participates in phase II metabolism of xenobiotics and endogenous compounds. In the past, glutathione was called non-protein sulfhydryl. With the discovery of other non-protein sulfhydryl

compounds came a need to distinguish the non-protein sulfhydryl compounds from one another. GSH is the name given for the predominant non-protein sulfhydryl present in biological systems (Ravindranath, et al., 1989). Other non-protein sulfhydryls include cysteine and glutathione disulfide (GSSG). GSH is the reduced form of the non-protein sulfhydryl and gluthatione disulfide is the oxidized form of the non-protein sulfhydryl. GSH also has a role in protecting the cell from lipid peroxidation. Glutathione peroxidase reacts with both organic and inorganic hydroperoxides to produce GSSG and water. GSSH is converted to the reduced form of glutathione by glutathione reductase. Glutathione S-transferase catalyzes the condensation of GSH and electrophilic xenobiotics (Dolphin, et al., 1989).

CHAPTER 2

LITERATURE REVIEW

DCE Toxicokinetics

The toxicokinetics of DCE is affected by several factors. Age, sex, genetic disposition, diet, route of exposure, concentration of exposure and vehicle type (e.g. water, corn oil or neat) are important factors when considering the toxicokinetics of any chemical. DCE is well absorbed through the lungs following inhalation exposure, the gastrointestinal tract following oral exposure, and the skin following dermal exposure in humans (Spreafico, et al., 1980). Studies conducted with experimental animals have showed that peak blood levels were achieved 1-2 hours after 6-hour inhalation exposure to 150 ppm of DCE in rats (Reitz, et al., 1982). Peak blood levels were achieved as soon as 30 minutes following oral exposure to doses of 25, 50 and 150 mg/kg DCE in corn oil (Spreafico, et al., 1980). There is evidence that shows absorption is more complete and occurs quicker following oral exposure to DCE in water compared to DCE administered orally in corn oil (Withey, et al., 1983). Blood levels reached 25ug/ml after 30 minutes following dermal exposure to 2 ml of

DCE in male rats (Morgan, et al., 1991). DCE is widely distributed within the body. Following inhalation and oral exposure to DCE adipose tissue accumulates the largest portion of absorbed dose. DCE has been observed in breast milk of nursing mothers following dermal exposure to DCE (Urusova, 1953). The biotransformation of DCE has been studied extensively in rats and mice both *in vivo* and *in vitro* (for review see ATSDR Toxicological Profile for DCE 1993). Metabolism of DCE is catalyzed by the mixed function oxidase enzymes and the resulting metabolites undergo conjugation with GSH. The enzymes involved in the metabolism of DCE have been showed to become saturated at 25 mg/kg/day (gavage) and 150 ppm (inhalation) (D'Souza, et al., 1988). Enzymatic metabolites of DCE are 2-chloroacetaldehyde, 2-chloroethanol and 2-chloroacetic acid. Glutathione-DCE conjugate metabolites include S-(2 chloroethyl)-glutathione, S-(2 hydroxyethyl)-glutathione, S-carboxymethyl glutathione, S-carboxymethyl-L-cysteinylglycine, S-carboxymethyl-L-cysteine, N-acetyl-S-carboxymethyl-L-cysteine and thioglycolic acid (Diag. 1). It is expected that at low concentrations (<25mg/kg/day [gavage] or <125ppm [inhalation]) mixed function oxidase is predominantly responsible for DCE metabolism, while GSH predominates at higher concentrations due to saturation levels.

There is little data available regarding the neurotoxicity of DCE via oral exposure. After an exhaustive literature search on the neurotoxic effects of DCE, very little information was available. The most comprehensive collection of data on the toxic effects of DCE is 1993 Toxicological Profile for DCE which was prepared by the Agency for Toxic Substances and Disease Registry (ATSDR). In the ATSDR profile no animal studies investigating neurological effects following a single exposure to DCE were located. An extensive search for neurological data from 1993 forward was carried out with no studies being located. Much of the available neurotoxicity data for DCE are from actual human case studies and from studies investigating the effects of DCE on other organs which briefly address CNS affects.

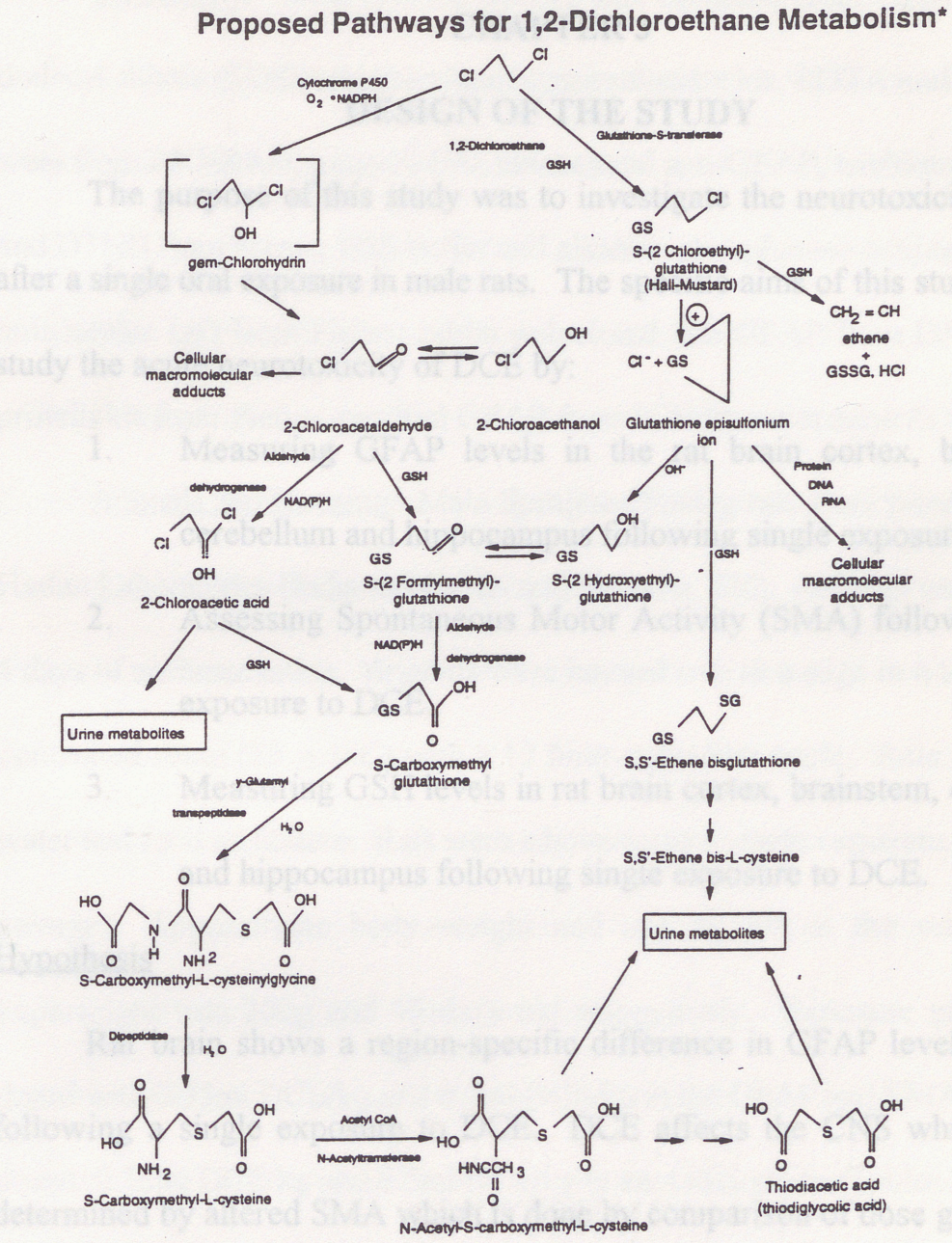
Case studies involving accidental and intentional ingestion of DCE are well documented by Yodaiken and Babcock (1973), Garrison and Leadingham (1954), Lockhead and Close (1951), and Hueper and Smith (1935). The neurotoxic effects observed in these cases studies prior to death included severe headache, dizziness and lethargic behavior. Post-mortem observations in these case studies revealed myelin degeneration, hyperemia and hemorrhage of the brain and brain edema. In a study conducted by NTP in 1991, no necrotic lesions were found in the brains of rats exposed to 480 mg/kg/day of DCE. All

of the animals died three days into the study and it was proposed that there was not sufficient time for necrosis to develop. In a multiple exposure DCE study, clinical signs in rats exposed to ≥240 mg/kg/day by gavage for ≤13 weeks included tremors, salivation, emaciation, abnormal posture, ruffled fur, and dyspnea. Upon microscopic examination, mild necrotic lesions were observed in the cerebellum of rats dosed with 240 or 300 mg/kg/day (NTP, 1991). Daniel et al. (1994) observed significant increases in brain weight in male Sprague Dawley rats following exposure to 150 mg/kg/day for 90 days.

DCE Mechanism of Action

The mechanism of action for DCE-induced toxicity is not known. Metabolites that are derived from mixed function oxidase action on DCE and DCE conjugation with GSH are believed to be responsible for the observed toxicity of DCE. These reactive metabolites are believed to be capable of binding covalently with cellular macromolecules, thus altering their function (Jean and Reed, 1989). There is also evidence that DCE causes lipid peroxidation *in vitro* (Tse, et al., 1990). As mixed function oxidase enzymes become saturated there is increased chance of DCE conjugation with GSH, thus causing increased number of GSH-DCE metabolites which are believed to be the primary reactive metabolite species responsible for DCE-induced toxicity.

Diagram 1



*Derived from NTP 1991

CHAPTER 3

DESIGN OF THE STUDY

The purpose of this study was to investigate the neurotoxicity of DCE after a single oral exposure in male rats. The specific aims of this study were to study the acute neurotoxicity of DCE by:

1. Measuring GFAP levels in the rat brain cortex, brain stem, cerebellum and hippocampus following single exposure to DCE.
2. Assessing Spontaneous Motor Activity (SMA) following single exposure to DCE.
3. Measuring GSH levels in rat brain cortex, brainstem, cerebellum and hippocampus following single exposure to DCE.

Hypothesis

Rat brain shows a region-specific difference in GFAP level alteration following a single exposure to DCE. DCE affects the CNS which can be determined by altered SMA which is done by comparison of dose group SMA to control group SMA. Rat brain GSH levels are decreased following single oral exposure to DCE.

Study Design

Chemicals. DCE (99.9% purity) was obtained from Aldrich; sodium dodecyl sulfate (SDS), alkaline phosphatase substrate kit, EDTA and Tris buffer were from BIO-RAD; triton X-100, monoclonal anti-GFAP, trichloroacetic acid and DTNB from Sigma; PBS buffer and alkaline phosphatase conjugated rabbit anti-mouse IgG from Fisher; rabbit polyclonal anti-GFAP from DAKO; BCA protein kit from Pierce; purified GFAP from ICN Pharmaceuticals Inc.

Animals and Housing. Male Sprague-Dawley rats were purchased from Harlan Laboratories (Indianapolis, IN and Houston, TX). Animals were allowed 4 days of acclimatization. Animals were housed two to a cage in a temperature controlled room (25 $\pm$ 1° C) with a 12 hour light/dark cycle. Rats were given water and food ad libitum. Rats were administered a single exposure to DCE by gavage. Approximate body weight and age of rats at the start of each experiment was 200g and 49 days old respectively. Exposure groups were dosed with 0.35ml DCE/kg and 0.7ml DCE/kg in the GFAP and SMA study and dosed 0.35ml DCE/kg and 0.5ml DCE/kg in the GSH study (Tables 1,2 and 3).

GFAP assay. Animals were sacrificed by decapitation 7 days and 14 days following single exposure to DCE and the brain excised from the skull. Brain samples were preserved with 90-95 °C 1% SDS at 1ml 1%SDS/100mg brain

sample. At time of GFAP analysis, the samples were diluted to a concentration between 125x and 500x.

GFAP was quantified using the sandwich ELISA method as described by O'Callaghan (1991) which was a modification of the "dot-immunobinding" procedure described by Jahn et al. (1984) with modifications by Van de Berg et al. (1996). A 96-well microtiter plate was coated with rabbit polyclonal anti-GFAP (1.0μg/100μl/well) and incubated for one hour at 37° C. After incubation the plate was washed four times with PBS pH 7.4 (250μl/well/time) and then blocked with BLOTTO (100μl/well) for one hour at room temperature. Following blocking with BLOTTO the plate was incubated with brain samples and GFAP standards made up in 0.5% Triton X-100 PBS (100μl/well) for one hour at room temperature. Two wells are used for each brain sample and standard. The plate was then washed four times with 0.5% Triton X-100 PBS (250μl/well/time). Following the washing, the plate was coated with monoclonal anti-GFAP (mouse) at concentration 1:400, made up in 0.5% Triton X-100 BLOTTO and incubated for one hour at room temperature. Next, the plate was washed four times with 0.5% Triton X-100 PBS (250μl/well/time). The plate was then coated with alkaline phosphatase conjugated rabbit anti-mouse IgG at concentration 1:3000 made up in 0.5% Triton X-100 BLOTTO

(100μl/well) and incubated for 30 minutes at room temperature and washed four times with 0.5% Triton X-100 PBS (250μl/well/time). Alkaline phosphatase substrate was added to the plate (100μl/well) to initiate a color reaction. After incubation of one hour at 37° C, the color reaction was stopped by adding 0.4M NaOH to the plate (100μl/well) and read using a THERMO max Microplate Reader at 405 nm at 37° C.

Preparation of GFAP standard curve. The GFAP standard curve was prepared using standard GFAP and 0.5% Triton X-100 PBS to prepare ten points: 299.7μl/0.3μl, 299.25μl/0.75μl, 298.5μl/1.5μl, 297μl/3.0μl, 285μl/15μl, 270μl/30μl, 225μl/75μl, 150μl/150μl and stock standard GFAP. Stock standard GFAP was prepared by adding 990 μl 0.5% Triton X-100 PBS to 10 μl pure standard GFAP. The units were μl 0.5% Triton X-100 PBS/μl stock standard GFAP.

Total protein assay. Total protein was measured by the method of Smith et al. (1985). The brain samples were diluted with 1%SDS to a concentration of 10x. 10 μl of brain sample and 10 μl of protein standard were pipetted into each well. Two wells were used for each brain sample and each protein standard. 200 μl BCA reagents were added to each well. The plate was then

incubated at 37° C for 30 minutes and read using a THERMO max Miroplate Reader at 562nm at 37° C.

Preparation of total protein standard curve. The total protein standard curve was prepared using BSA and SDS to prepare nine points: Blank, 1.0μg/10μl, 2.0μg/10μl, 2.5μg/10μl, 4.0μg/10μl, 5.0μg/10μl, 7.5μg/10μl, 8.0μg/10ul, 10μg/10μl. The units are μg BSA/μl SDS.

Measurement of Spontaneous Motor Activity (SMA). SMA data was collected using a Digiscan Analyzer and cages. Since rats are nocturnal animals having highest activity during the night, the SMA tests were conducted during the dark phase of the room light and dark cycle. The tests consisted of four 15 minute measurements of SMA, which amounted to a total of 60 minutes of SMA. The SMA tests included measurements of horizontal, vertical, and stereotypic activities of rats.

Glutathione Assay. Animals were sacrificed by decapitation two hours following oral exposure to 0.35 and 0.5ml DCE/kg and the brain excised from the skull. Brain samples were homoginized with 10 volumes of 0.02M EDTA (pH4.7) and immedately placed on ice. 300μL of homogenate and 30μL of 25% trichloroacetic acid were added to a 1.5mL micro test tube and placed in ice bath to precipitate the protein. Following precipitation of protein, the micro test tube

was centrifuged 5 min at 6000 g. One hundred microliters of each sample were placed in each well of a 96-well microplate. All samples in a 96-well microplate were read at wavelength 412nm at room temperature. Extinction coefficient, $13.6mM^{-1}cm^{-1}$, was used to calculate the quantity of GSH present.

Statistical Analysis. Experimental results are expressed as the mean ± standard error of the mean for 4-6 rats for each experimental group. The results were evaluated for significant differences between dosage groups by one-way ANOVA using the Student-Newman-Keuls test for multiple comparison. Differences in means of $p<0.05$ were considered significant.

CHAPTER 4

RESULTS AND DISCUSSION

Glial Fibrilliary Acidic Protein (GFAP).

The 7-day study. The cortex and brainstem (Fig. 1) showed increases in GFAP level following exposure although not significant compared to control group. The CRB showed a significant ($p<0.05$) decrease in GFAP level whereas the HP showed a significant ($p<0.05$) increase in GFAP level compared to the control group (Fig. 1).

The 14-day study. The CTX brain region showed a significant ($p<0.05$) increase in GFAP level for 0.7ml/kg DCE group following exposure (Fig. 2). The 0.35ml/kg DCE group showed an increase in GFAP level although not significant (Fig. 2). Both dose groups showed a significant ($p<0.05$) increase in GFAP level following exposure in the BS (Fig. 2). There was an increase in GFAP level in the CRB for both test groups, although the increase did not reach significant difference (Fig. 2). Both dose groups showed significant ($p<0.05$) increase in GFAP level following exposure in the HP compared to the control (Fig. 2).

SMA. Horizontal, vertical and stereotypic activity were measured at days 1, 2, 7 and 14 followng a single exposure to DCE. On day 1, both 0.35ml DCE/kg and 0.7ml DCE/kg exposed rats showed a significant ($p<0.05$) decrease in horizontal activity (Fig. 3). On day 2, only the 0.7ml DCE/kg group showed a significant ($p<0.05$) decrease in horizontal activity (Fig. 3). On days 7 and 14 there were no significant differences between the three groups for horizontal activity (Fig. 3). On day 1, both 0.35ml DCE/kg and 0.7ml DCE/kg exposed rats showed a significant ($p<0.05$) decrease in vertical activity (Fig. 4). On day 1 following exposure to DCE, rats exhibited zero vertical activity. On day 2, 0.7ml DCE/kg exposed rats showed a significantly ($p<0.05$) lower vertical activity than the control group (Fig. 4). On days 7 and 14 there were no significant differences between the three groups for vertical activity (Fig. 4). Both 0.35ml DCE/kg and 0.7ml DCE/kg had significantly ($p<0.05$) lower stereotypic activity than the control group on day 1 following DCE exposure (Fig 5). On post exposure days 2, 7 and 14 there were no significant ($p<0.05$) differences in sterotypic activity between control, 0.35ml DCE/kg and 0.7ml DCE/kg (Fig. 5).

Glutathione. As depicted in Fig. 6, GSH levels in the rat brain were reduced in the CTX, BS, CRB, and HP following exposure to 0.35ml DCE/kg

and 0.5ml DCE/kg. However, levels were significantly ($p<0.05$) lower than their respective control in the CTX and HP regions for both dose groups (Fig.6).

Home Cage. Within 30 minutes following exposure, animals exhibited signs of the CNS depression. Rats exposed to 0.7ml DCE/kg exhibited no response to click or touch, piloerection and lacrimation in home cage. Piloerection in the H-Dose group was present up to day three for 3 of 5 animals and 1 of 5 still expressed piloerection on post exposure day five. L-Dose animals had home cage behavior that was similar to that of H-Dose yet not as severe and recovery to home cage behavior similar to that of control rats was noticed 2-3 days following exposure.

Body Weight. Body weight for animals was measured at day 0, (i.e. before administration of any dose) and at 3, 7, 10, 14 post exposure days following a single exposure to 5ml saline/kg, 0.35ml DCE/kg or 0.7ml DCE/kg (Table 4). The body weight for the 0.7ml DCE/kg group was significantly ($p<0.05$) lowered on post exposure days 3, 7 and 10 compared to the respective control groups (Fig. 7).

TABLE 1 7-day GFAP Study

Treatment Group	Number of Animals
Saline (control) 5ml/kg	4
0.70ml/kg DCE	5

TABLE 2 14-day GFAP Study

Treatment Group	Number of Animals
Saline (control) 5ml/kg	5
0.35ml/kg DCE	5
0.70ml/kg DCE	4

TABLE 3 GSH Brain Study

Treatment Group	Number of Animals
Saline (control) 5ml/kg	4
0.35ml/kg DCE	4
0.50ml/kg DCE	4

TABLE 4 14-day Study Average Rat Body Weights

Treatment Group	Average body weight (gm) n= 5-6				
	Day 0	Day 3	Day 7	Day 10	Day 14
Saline (control) 5ml/kg	210.7	222.5	255.7	266.5	299.0
0.35ml DCE/kg	219.8	222.3	247.5	260.7	291.3
0.70ml DCE/kg	210.0	187.0	225.0	237.4	277.0

Figure 1. 7-day 1,2-dichloroethane Neurotoxicity Study

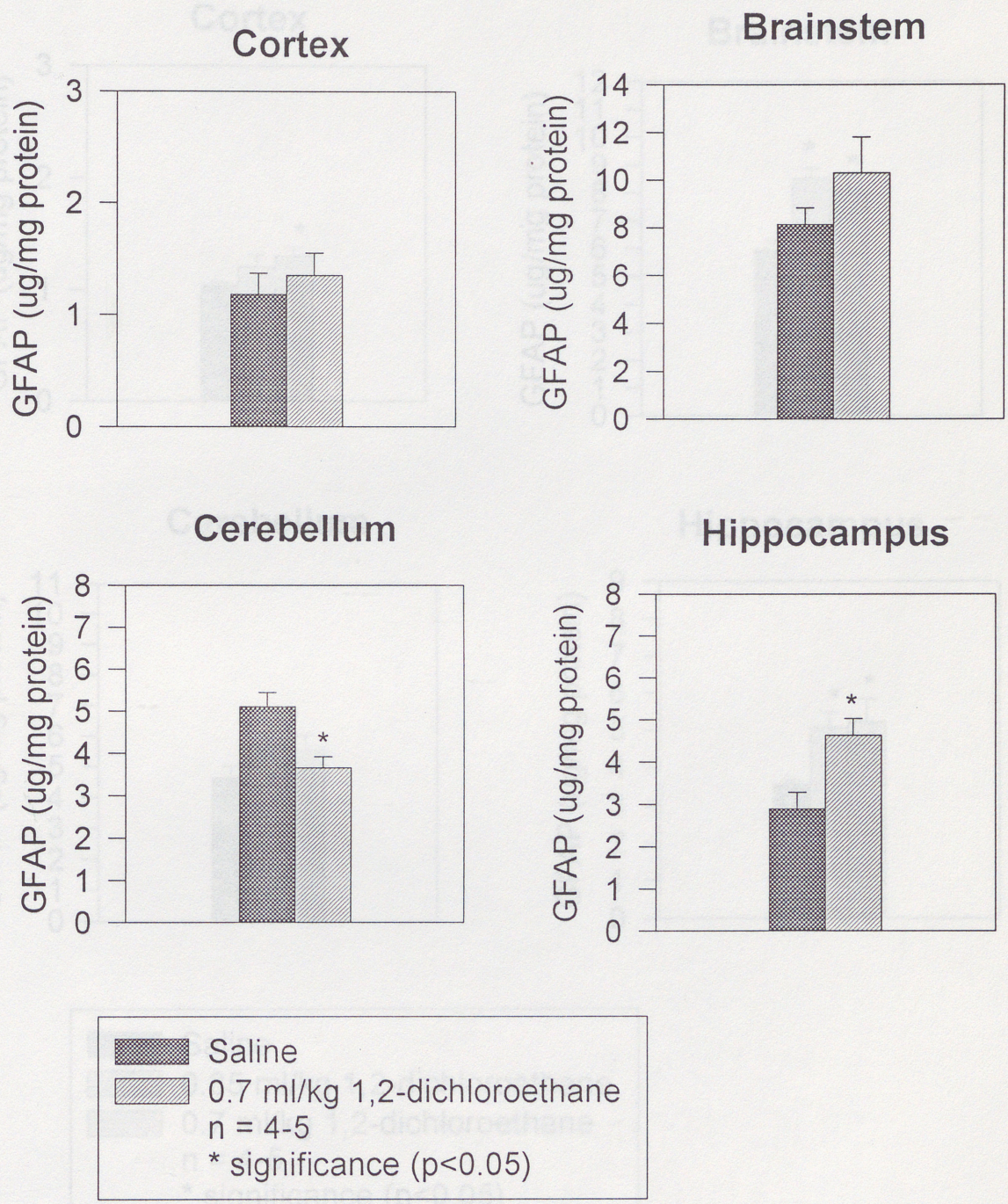


Figure 2. 14-day 1,2-dichloroethane Neurotoxicity Study

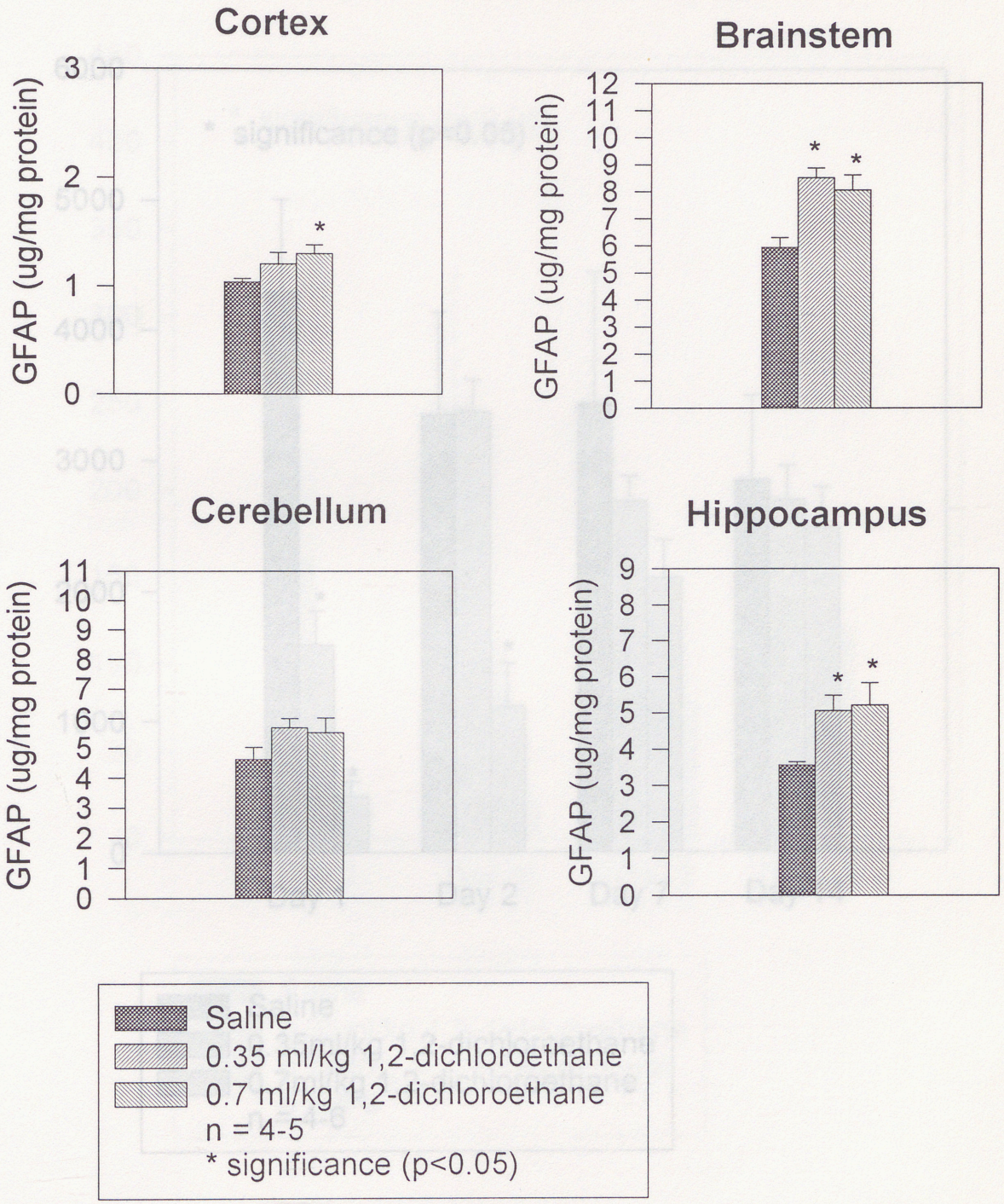


Figure 3 Horizontal Activity

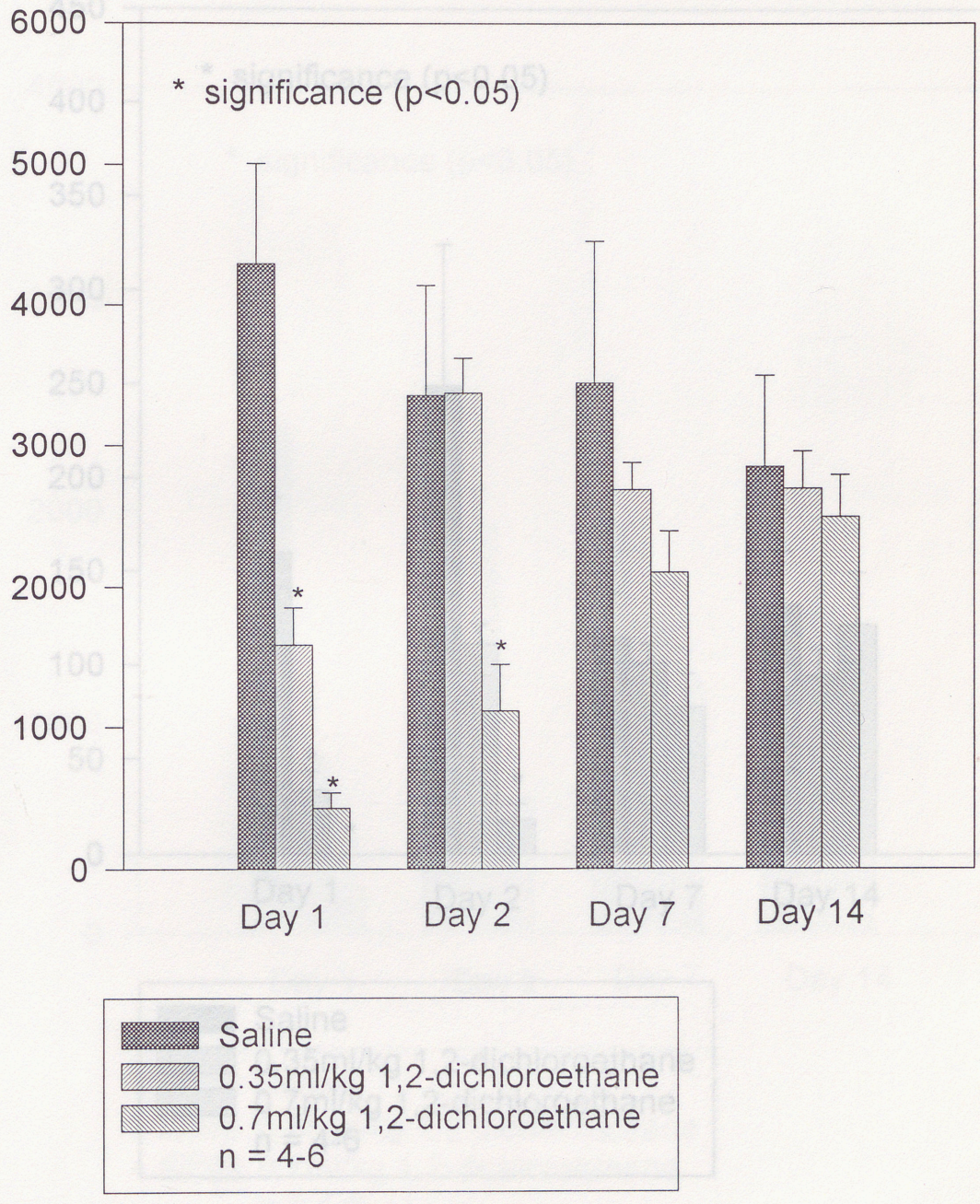


Figure 4. Vertical Activity

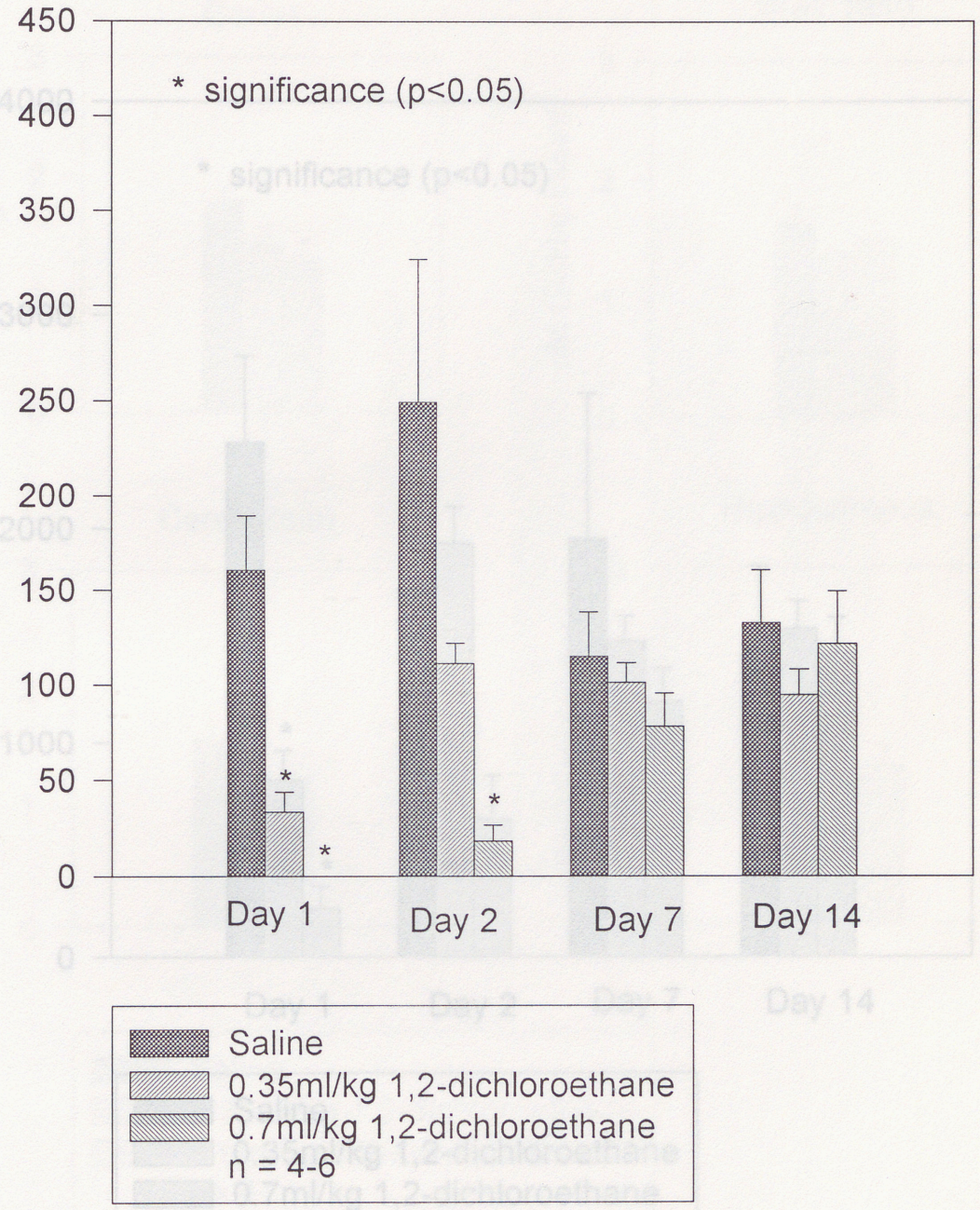


Figure 5. Stereotypic Activity

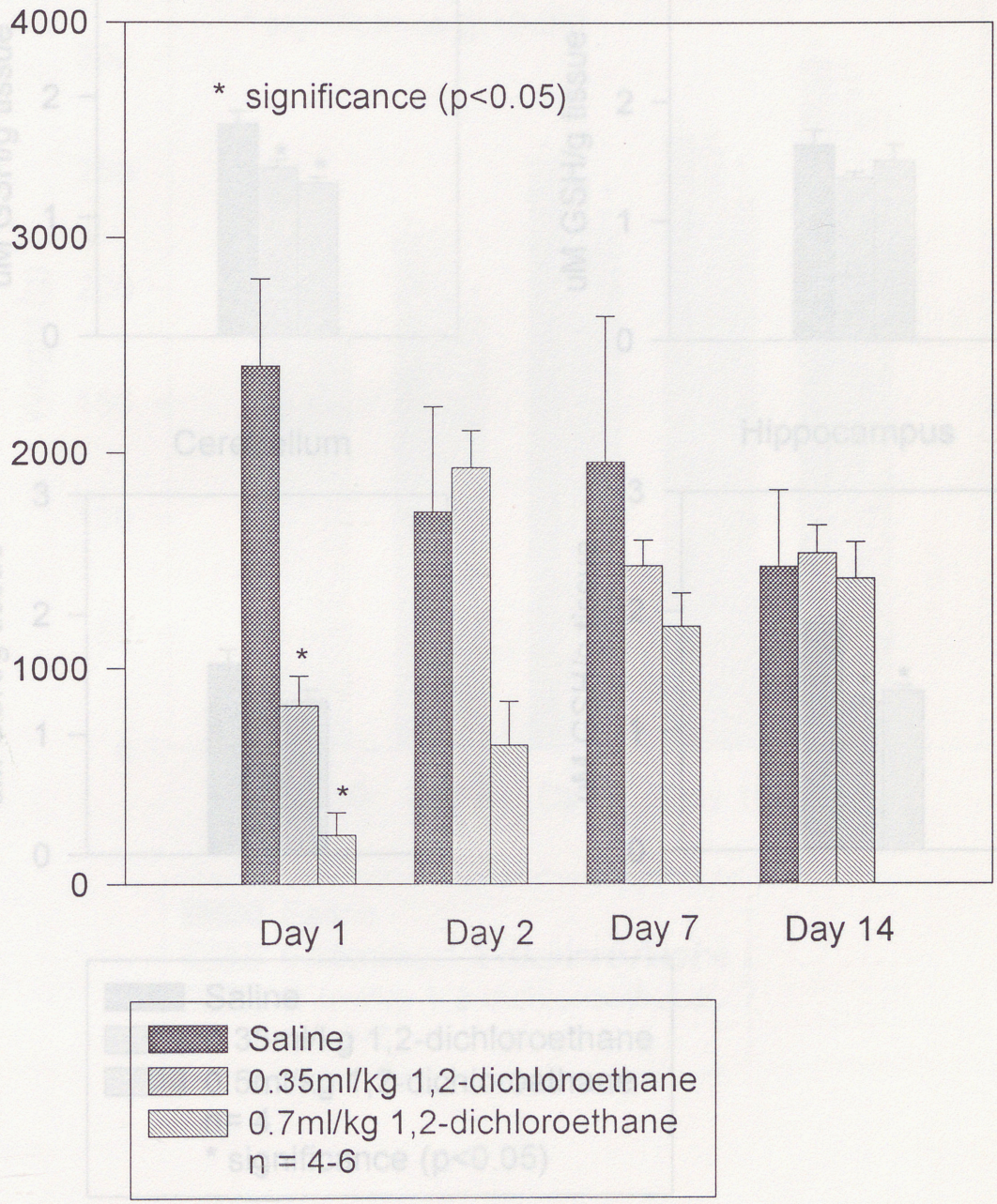


Figure 6. GSH Levels Two Hours After Exposure

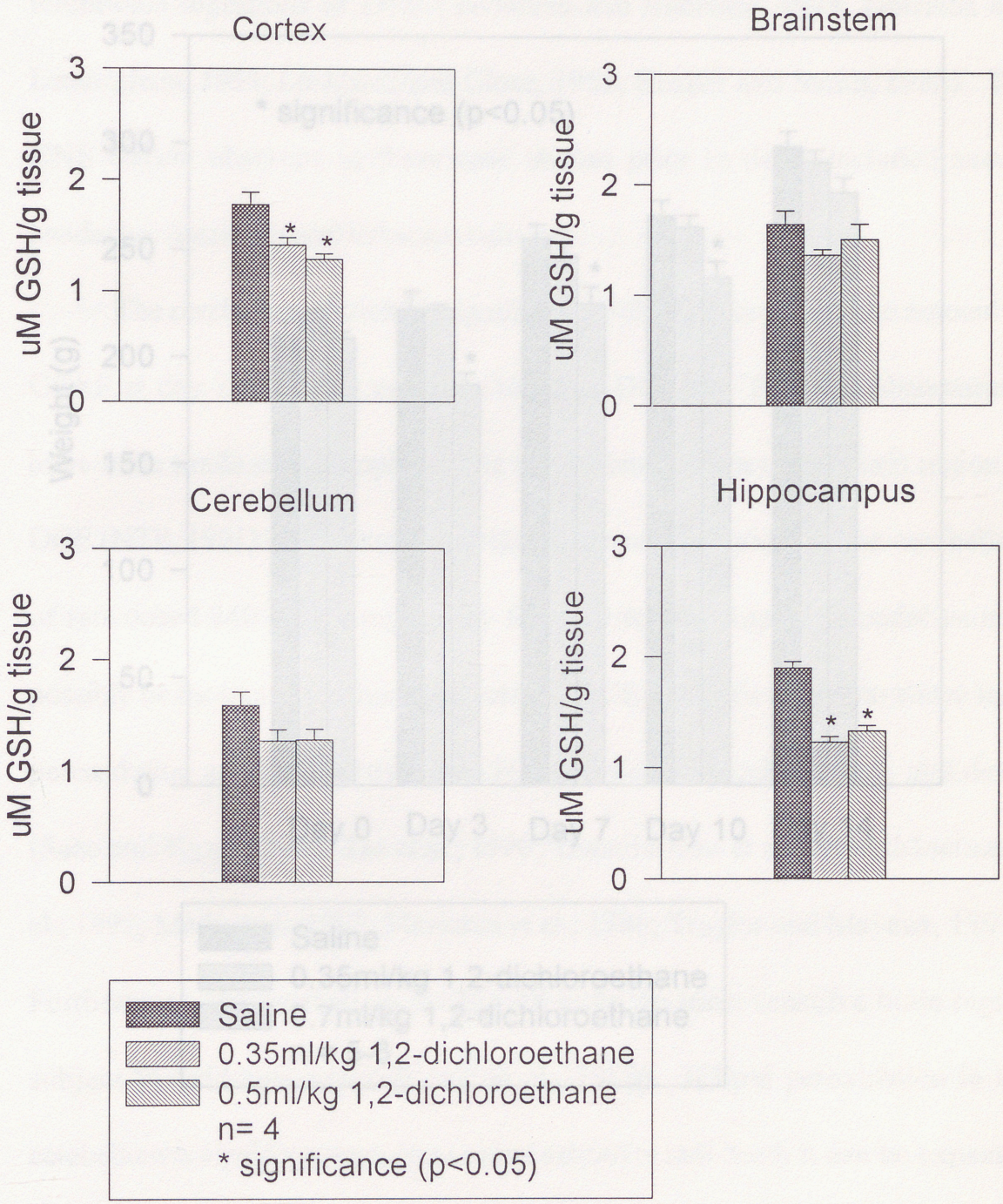
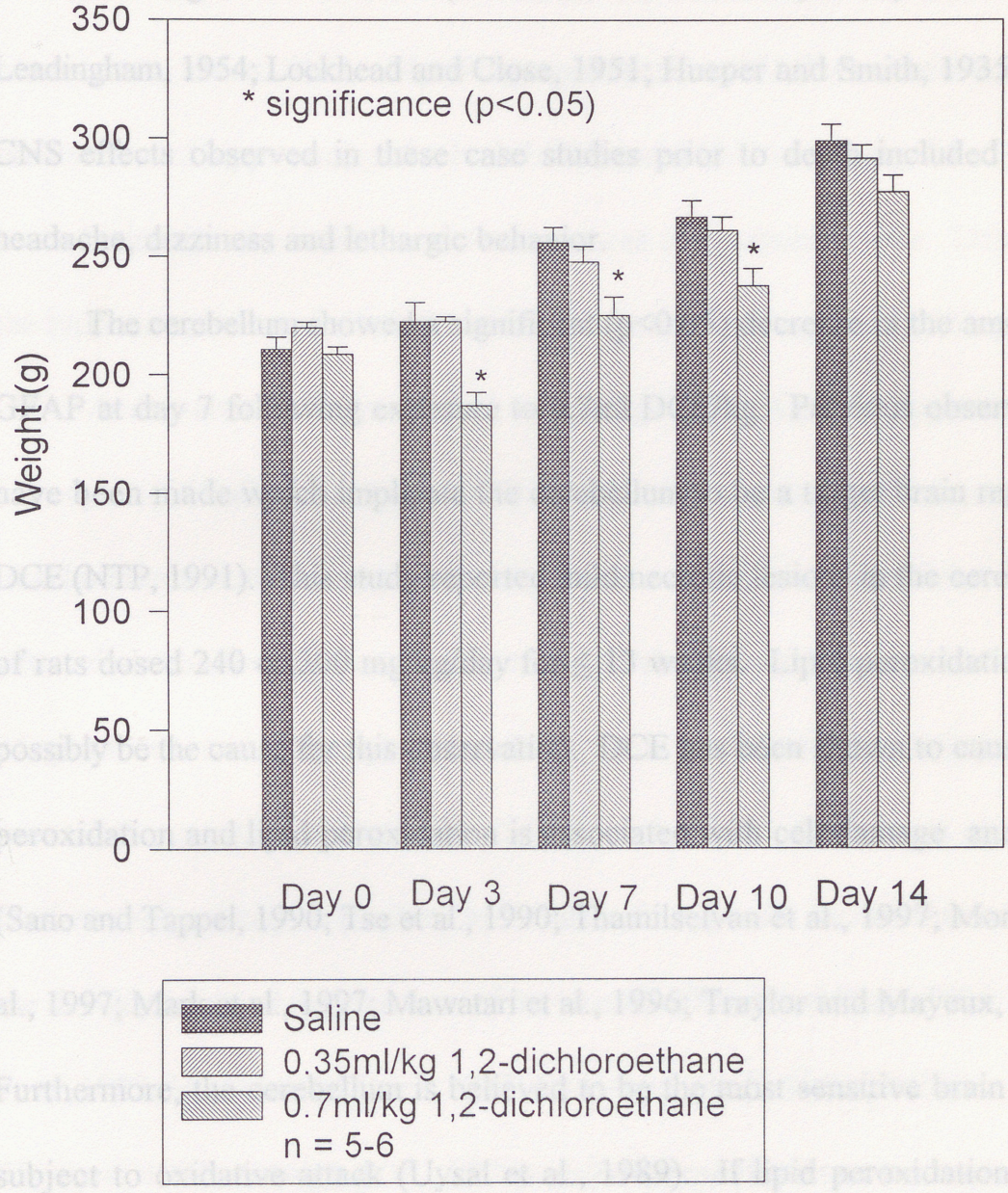


Figure 7 Group Average Weight



Discussion. The result of this study indicate that 1,2-dichloroethane has the CNS toxic effects similar to those reported following accidental or intentional ingestions of DCE (Yodaiken and Babcock, 1973; Garrison and Leadingham, 1954; Lockhead and Close, 1951; Hueper and Smith, 1935). The CNS effects observed in these case studies prior to death included severe headache, dizziness and lethargic behavior.

The cerebellum showed a significant ($p<0.05$) decrease in the amount of GFAP at day 7 following exposure to 0.7ml DCE/kg. Previous observations have been made which implicate the cerebellum to be a target brain region of DCE (NTP, 1991). This study reported mild necrotic lesions in the cerebellum of rats dosed 240 or 300 mg/kg/day for $\leq$ 13 weeks. Lipid peroxidation may possibly be the cause for this observation. DCE has been shown to cause lipid peroxidation and lipid peroxidation is associated with cell damage and death (Sano and Tappel, 1990; Tse et al., 1990; Thamilselvan et al., 1997; Montine et al., 1997; Mark et al., 1997; Mawatari et al., 1996; Traylor and Mayeux, 1997). Furthermore, the cerebellum is believed to be the most sensitive brain region subject to oxidative attack (Uysal et al., 1989). If lipid peroxidation in the cerebellum is significant enough to cause astrocyte cell death it can be expected that with a decrease in astrocytes there is a decrease in GFAP.

Nonetheless, the results from this study indicate that both an increase as well as a decrease in GFAP is a biomarker of CNS damage. With respect to the CRB, this can be inferred by the incidence of alteration in GFAP levels with concomitant altered SMA. Significant increases in GFAP was observed in the brainstem and hippocampus for both L-Dose and H-Dose on day 14. Damage to the brainstem may result in unconsciousness and possibly coma. Damage to the hippocampus causes deficits in the short-term memory and learning.

A universal cellular reaction to damage of the CNS is hypertrophy of astrocytes (O'Callaghan et al., 1995). Astrocytes are involved in a number of vital homeostatic mechanisms including maintenance of the blood-brain barrier, extracellular buffering, microregulation of cerebral blood flow and metabolism of glutamate and ammonium and have been implicated in participating in intercellular communication in the CNS (Murphy, 1993; Halliday et al., 1996). Furthermore, astrocytes produce and release four growth factors that influence the development and maintenance of nerve cells (Patel and Gray, 1993; Gnahn et al.,1983; Hayashi and Patel, 1987; Sweetnam et al., 1991; Hofmann, 1988; Thoenen, 1991; Azmitia et al., 1990). All of these functions are important to normal CNS function. A change in GFAP levels is believed to be an indication of an alteration in the astrocyte. This alteration in astrocytes could potentially

affect their normal function thus interfering with the normal CNS function. An increase in GFAP is indicative of an increase in astrocyte with consequential formation of an astroglial scar. Astroglial scars can potentially impede the regeneration of nerves (Anders and Hurlock, 1996; Faissner and Steindler, 1995). In this study, significant increases in GFAP levels compared to the control group were observed at day 7 in the hippocampus which was administered 0.7ml DCE/kg. Significant ($p<0.05$) increases in GFAP levels compared to the control group were also observed in the brainstem and hippocampus for both dose groups at day 14. With the increase in GFAP levels observed in these brain regions it is likely that glial scars were formed which have been shown to be detrimental to the CNS recovery (Anders and Hurlock, 1996). With increase in age there is an increase in GFAP levels. Therefore, it is important to use young animals when studying the effects of chemicals on GFAP levels.

Spontaneous motor activity is a commonly used behavioral test to observe the CNS following exposure to chemical insult (Mehta et. al., 1997; Lapin, 1996). The results here show 1,2-dichloroethane to cause significant ($p<0.05$) alterations in SMA as compared to control groups. GFAP levels in the brainstem were significantly ($p<0.05$) increased for both dose groups at day 14.

The cerebellum which coordinates voluntary movement also had altered GFAP levels compared to the control group. This alteration in GFAP level which is indicative of damage to the cerebellum may also have contributed to the altered Spontaneous motor activity that was recorded. Vertical activity which is more of an exploratory activity requires more CNS intergradation. Therefore damage to regions other than the cerebellum may also affect spontaneous motor activity.

GSH levels in the rat brain is reported as 1.6 - 2.6 uM per g wet tissue (Trenga et al., 1991; Akerboom and Sies, 1981; Uysal et al., 1989; Ravindranath et al., 1989). The results of this study showed a decrease in GSH levels as compared to the control group following 1,2-dichloroethane exposure. Depletion of GSH levels can potentially increase the accumulation of cytotoxic superoxide radicals which promote lipid peroxidation (Uysal et al., 1989); which would cause direct damage to the cell membranes. GSH peroxidase is responsible for the detoxification of peroxides and the amount of GSH peroxidase is limited by the amount of GSH present (Ravindranath et al., 1989). Furthermore, 1,2-dichloroethane has been shown to inhibit glutathione S-transferase in erythrocytes (Ansari et al., 1987). If this is the case in the brain, then the inhibition would decrease the amount of 1,2-dichloroethane conjugated with GSH thus increasing the levels of 1,2-dichloroethane available for MFO

metabolism. 2-chloroacetaldehyde, a metabolite of MFO metabolism, can form cellular macromolecular adducts (Fabricant and Chamlers 1980), which may alter normal function. The results of this study showed a decrease in GSH level in the rat brain following 1,2-dichloroethane exposure which agrees with the metabolic pathway (Diag. 1). The results of this study show the cortex and hippocampus to be most sensitive to GSH alteration following exposure.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

DCE is a neurotoxicant that affects GFAP and GSH levels in the brain, alters SMA and has a negative effect on body weight. The brainstem and hippocampus are more sensitive to GFAP alteration than the CTX and CRB. Whereas, the CTX and hippocampus are more sensitive to rat brain GSH alteration compared to the CRB and brainstem. The results of this study proved the hypothesis true. DCE showed region-specific difference of affect on rat brain GFAP and GSH levels and also altered normal SMA.

A study investigating GFAP levels on day 7 following exposure to L-Dose to compare to the results obtained from exposure to H-Dose on day 7 would be useful in explaining the observed decrease in GFAP level in the CRB. The amount of 1,2-dichloroethane used for H-Dose may have resulted in substantial amounts of 1,2-dichloroethane in the brain which may have overwhelmed antioxidant mechanisms. However, at lower doses, eg. L-Dose, this may not be the case.

REFERENCES

Agency for Toxic substances and Disease Registry (ATSDR). 1993. Toxicological Profile for DCE (Update). TP-93/06.

Akerboom, T.P.M., and H. Sies. 1981. Assay of glutathione, glutathione disulfide, and glutathione mixed disulfides in biological samples. Methods in Enzymology. 77: 373-382.

Albrecht, J., Simmons, M., Dutton, G.R., and Noreberg, M.D. 1991. Aluminum chloride stimulates the release of endogenous glutamate, taurine and adenosine from cultured rat cortical astrocytes. Neurosci. Lett. 127: 105.

Anders, J.J., and Hurlock, J.A. 1996. Transplanted glial scar impedes olfactory bulb reinnervation. Exp. Neurol. 142(1): 144-150.

Ansari, G.A.S., Singh, S.V., Gan, J.C., and Awasthi, Y.C. 1987. Human erythrocyte glutathione S-transferase: A possible marker of chemical exposure. Toxicol. Lett. 37: 57-62 .

Azmitia, E.C., Dolan, K., and Whitaker-Azmitia, P.M. 1990. S-100B but not NGF, EGF, insulin or calmodulin is a CNS serotonergic growth factor. Brain Res. 516: 354-356.

Brock, T.O., and O'Callaghan, J.P. 1987. Quantitative changes in the synaptic vesicle proteins, synapsin I and p38, and the astrocyte specific protein, GFAP, are associated with chemidal-induced injury to the rat central nervous system. J. Neurosci. 7: 931-942.

Budavari, S. 1989. Ethylene dichloride. In: ed. S. Budavari. The Merck Index, An encyclopedia of chemicals, drugs, and biologicals. Merck & Co., Inc. New Jersey.

Chem. and Engr. News. 1991. Production by the U.S. chemical industry. Chem. and Engr. News. 69(25): 30-31, 33

Chui, F.C., and Goldman, J.E. 1984. Synthesis and turnover of cytoskeletal proteins in cultered astrocytes. J. Neurochem. 42: 166-174.

Clos, J. 1986. Developing astroglia in abnormal thyroid states. Astrocytes. 3: 167-182.

Daniel, F.B., Robinson, M., Olson, G.R., York, R.G., and Condie, L.W. 1994. Ten and ninety-day toxicity studies of DCE in Sprague-Dawley rats. Drug Chem. Toxicol. 17(4): 463-477

De Armond, Lu Yuan-Ling, L., Kretzchmar, H.A., and Eng, L.F. 1986. Turnover of glial filaments in mouse spinal cord. J. Neurochem. 47: 1749-1753.

Denis-Donini, S., Glowinski, J., and Prochiantz, A. 1984. Glial heterogeneity may define the three-dimensional shape of mouse mesencephalic dopaminergic neurons. Nature. 307: 641-643.

Dolphin, D., Avramovic, O., and Poulson, R. 1989. Glutathione: Chemical, Biochemical, and Medical Aspects (Part B). John Wiley & Sons, Inc., Toronto, Canada.

D'Souza R.W., Francis, W.R., and Andersen, M.E. 1988. Physiological model for tissue glutathione depletion and increased resynthesis after ethylene dichloride exposure. J. Pharm. Exp. Therap. 245: 563-568.

Eng, L.F. 1980. The glial fibrillary acidic proteinn (GFAP). In: eds. R.A. Bradhaw and D.M. Schneider. Proteins of the nervous system. Raven Press, New York.

Fabricant, J.D. and Chalmers, Jr., J.H. 1980. Evidence of the mutagenicity of ethylene dichloride and structurally related compounds. In: Ames B.N., Infante P., Reitz R., eds. Ethylene dichloride: A potential health risk?

Banbury report No. 5. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.

Faissner, A., and Steindler, D.A. 1995. Boundaries and inhibitory molecules in developing neural tissues. Glial. 4: 233-254

Garrison, S.C., and Leadingham, R.S. 1954. A fatal case of ethylene dichloride poisoning in an occupational therapy department of a neuropsychiatric hospital. Am. J. Phys. Med. 33: 230-237.

Gnahn, H., Hefti, F., Heumann, R., Schwab, M.E., and Thoenen, H. 1983. NGF-mediated increase of choline acetyltransferase (ChAT) in the neonatal rat forebrain; evidence for a physiological role of NGF in the brain? Dev. Brain Res. 9: 45-51.

Gosselin, R.E., Smith, R.P., and Hodge, H.C. 1984. Ethylene dichloride In: eds. R. E. Gosselin, R.P. Smith and H.C. Hodge. Clinical Toxicology of Commerical Products. Williams & Wilkins. Los Angeles.

Halliday, G.M., Cullen, K.M., Kril, J.J., Harding, A.J., and Harasty, J. 1996. Glial fibrillary acidic protein (GFAP) immunohistochemistry in human cortex: a quantitative study using different antisera. Neurosci. Lett. 209: 29-32.

Hayashi, M., and Patel, A.J. 1987. An interaction between thyroid hormone and nerve growth factor in the regulation of choline acetyltransferase activity in neuronal cultures, derived from the septal-diagonal band region of the embryonic rat brain. Dev. Brain Res. 36: 109-115.

Hofmann, H.D. 1988. Ciliary neuronotrophic factor stimulates choline acetyltransferase activity in cultured chicken retina neurons. J. Neurochem. 51: 109-113.

Hueper, W.C., and Smith, C. 1935. Fatal ethylene dichloride poisoning. Am. J. Med. Sci. 189: 778-784.

International Agency for Research on Cancer. 1979. IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans. Some halogenated hydrocarbons. IARC. Vol. 20. pp 609. Lyon, France.

Jahn, R., Schiebler, W., and Greengard, P. 1984. A quantitative dot immunobinding assay for protein using nitrocellulose membrane filters. Proc. Natl. Acad. Sci. USA 81: 1684-1687

Jean, P.A., and Reed, D.J. 1989. In vitro dipeptide, nucleoside, and glutathione alkylation by S-(2-chloroethyl) glutathione and S-(2-chloroethyl)-L-cysteine. Chem. Res. Toxicol. 2: 455-460.

Kalnins, V.I., Subrahuranyan, L., and Fedoroff, S. 1985. Assembly of glial intermediate filament protein is initiated in the centriolar region. Brain Res. 345: 322-326.

Langston, J.W. 1985. MPTP and Parkinson's disease. Trends Neurosci. 8: 79-83.

Lapin, I.P. 1996. Antagonism by CPP (+/-)-3-(2-carboxypiperazine-4-yl)-propyl-1-phosphonic acid, of beta-phenylethylamine (PEA)-induced hypermotility in mice of different strains. Pharmacol. Biochem. Behav. 55(2): 175-8.

Le Prince, G., Copin, M.C., Hardin, H., Belin, M.F., Bouilloux, J.P., and Tardy, M., 1990. Neuronglia interactions: Effect of serotonin on the astroglial expression of GFAP and of its encoding message. Dev. Brain Res. 51: 295-298.

Lockhead, H.B., and Close, H.P. 1951. Ethylene dichloride plastic cement: A case of fatal poisoning. J. Am. Med. Assoc. 146: 1323-1324.

Mark, R.J., Pang, Z., Geddes, J.W., Uchida, K., and Mattson, M.P. 1997. Amyloid beta-peptide impairs glucose transport in hippocampal and cortical neurons: involvement of membrane lipid peroxidation. J. Neurosci. 17(3): 1046-1054.

Mawatari, K., Yasui, Y. Sugitani, K., Takadera, T., and Kato, S. 1996. Reactive oxygen species involved in the glutamate toxicity of glioma cells via antiporter system. Neuroscience Lett. 73(1): 201-208.

McCall, M.A., Gregg, R.G., Behringer, R.R., Brenner, M., Delaney, C.L., Galbreath, E.J., Zhang, C.L., Pearch R.A., Chiu, S.Y., and Messing, A. 1996. Targeted deletion in astrocyte intermediate filament (GFAP) alters neuronal physiology. Proc. Natl. Acad. Sci. USA. 93: 6361-6366.

Mecocci, P., Parnetti, L., Romano, G., Scarelli, A., Chionne, F., Cecchetti, R., Polidori, M.C., Palumbo, B., Cherubini, A., and Senin, U. 1995. Serum anti-GFAP and anti-S100 autoantibodies in brain aging, Alzheimer's disease and vascular dementia. J. Neuroimmunol. 57: 165-170.

Mehta, C.S, Sun, P.N., Zikarge, A., Mumtaz, M., and Kuhn, O. 1997. Acute neurotoxicity of trichloroethylene in male and female rats: A two week study after a single oral exposure. Res. Comm. Pharm. Toxicology. 1(4): 225-235.

Montine, K.S., Olson, S.J., Amarnath, V., Whetsell, W.O., Jr., Graham, D.G., and Montine, T.J. 1997. Immunohistochemical detection of 4-hyroxy-2-noneal adducts in Alzheimer's disease is with inheritance of APOE4. Am. J. Pathol. 150(2): 437-43.

Morgan D.L., Cooper S.W., Carlock D.L., Sykora, J.J., Sutton, B., Mattie, D.R., and Mcdougal, J.N. 1991. Dermal absorption of neat and aqueous volatile organic chemicals in the Fischer 344 rats. Environ. Res. 55: 51-63.

Morrison, R.S., De Vellis, J., Lee, Y.L., Bradshaw, R.A., and Eng, L.F. 1985. Hormone and growth factors induce the synthesis of glial fibrillary acidic protein in rat brain astrocytes. J. Neurosci. Res. 14: 167-176.

Murphy, S. 1993. Astrocytes: Pharmacology and Function. Academic Press. San Diego, California.

Murphy, S., Minor, R.L., Welk, G., and Harrison, D.G. 1990. Evidence for an astrocyte-derived vasorelaxing factor with properties similar to nitric oxide. J. Neurochem. 55: 349-351.

National Institute for Occupational Safety and Health. 1978. NIOSH Current intelligence bulletin 27: Chloroethanes: review of toxicity. DHEW (NIOSH) Publication. No. 78-181. Cincinnati, OH: Department of Health, Education, and Welfare.

NTP. 1991. Toxicity studies of DCE (ethylene dichloride) (CAS No. 107-06-2) in F344/N rats, Sprague Dawley rats, Osborne-Mendel rats and B6C3F1 mice (drinking water and gavage studies). Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Institute of Health, National Toxicology Program. NIH Publication. No. 91-3123.

O'Callaghan, J.P. 1991. Quantification of glial fibrillary acidic protein: comparison of slot-immunobinding assays with a novel sandwich ELISA. Neurotox. Terat. 13: 275-281.

O'Callaghan, J.P., Jensen, K.F., and Miller, D.B. 1995. Quantitative aspects of drug and toxicant-induced astrogliosis. Neurochem. Int. 26(2): 115-124.

Patel, A.J., and Gray, C.W. 1993. Neurotrophic factors produced by astrocytes involved in the regulation of cholinergic neurons in the central nervous system. In: S. Fedoroff, B.H.J. Juurlink and R. Doucette, eds. Biology and pathology of astrocyte-neuron interactions. Plenum Press, New York.

Pollenz, R.S., and McCarthy, K.D. 1986. Analysis of cyclic AMP-dependent protein phosphorylation and cell morphology in cultured astroglia. J. of Neurochem. 47: 9-17.

Pope, A. 1978. Neuroglia: quantitative aspects. In: E. Schoffeniels, G. Franck, D.B. Tower, eds. Dynamic properties of glial cells. Pergamon Press, Oxford.

Ravindranath, V., Shivakumar, B.R., and Anandatheerthavarada, H.K. 1989. Low glutathione levels in brain regions of aged rats. Neuroscience Lett. 101: 187-190.

Reitz R.H., Fox T.R., Ramsey J.C., Quast, J.F., Langvardt, P.W., and Watanabe, P.G. 1982. Pharmacokinetics and macromolecular interactions of ethylene dichloride in rats after inhalation or gavage. Toxicol. Appl. Pharmacol. 62: 190-204.

Sano M., and Tappel A.L. 1990. Halogenated hydrocarbon and hydroperoxide induced lipid peroxidation in rat tissue slices. J. Agric. Food Chem. 38: 437-441.

Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J., and Klenk, D.C. 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. 150: 76-85.

Spreafico F., Zuccato E., Marcucci F., Sironi, M., Paglialunga, S., Madonna, M., and Mussini, E. 1980. Pharmacokinetics of ethylene dichloride in rats treated by different routes and its long-term inhalatory toxicity. In: Ames B.N., Infante P., Reitz R., eds. Ethylene dichloride: A potential health risk? Banbury report No. 5. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.

Sweetnam, P.M., Sanon,H.R., White, L.A., Brass, B.J., Jaye, M., and Whittemore, S.R. 1991. Differential effects of acidic and basic fibroblast growth factors on spinal cord cholinergic, GABAergic, and glutamatergic neurons. J. Neurochem. 57: 237-249.

Tardy, M., Fages, C., LePrince, G., Rolland, B., and Nunez, J. 1990. Regulation of the glial fibrillary acidic protein (GFAP) and of its encoding mRNA in the developing brain and in cultured astrocytes. In: eds. J. Lauder, A. Privat, E. Giacobini, P. Timaris and A. Vernadakis. Molecular aspects of development and aging of the nervous system. Plenum Press, New York.

Tardy, M., Le Prince, S., Babajko, S., Riol, H., Fages, C., and Rolland, B. 1993. GFAP gene expression in normal and reactive astrocytes. In: eds. S. Fedoroff, B.H.J. Juurlink and R. Doucette. Biology and pathology of astrocyte-neuron interactions. Plenum Press, New York.

Thamilselvan, S., Hackett, R.L., and Khan, S.R. 1997. Lipid peroxidation in ethylene glycol induced hyperoxaluria and calcium oxalate nephrolithiasis. J. Urol. 157(3): 1059-1063.

Thoenen, H. 1991. The changing scene of neurotrophic factors. Trends Neurosci. 14: 165-170.

Traylor, L.A., and Mayeux, P.R. 1997. Nitric oxide generation mediates lipid -induced oxidant injury in renal proximal tubules. Arch. Biochem. Biophys. 338(2): 129-135.

Trenga, C.A., Kunkel, D.D., Eaton, D.L., and Costa, L.G. 1991. Effect of styrene oxide on rat brain glutathione. Neuro. Toxicology. 12: 165-178.

Tse S.Y.H., Mak, I.T., Weglicki W.B., et al. 1990. Chlorinated aliphatic hydrocarbons promote lipid peroxidation in vascular cells. J. Toxicol. Environ. Health. 31: 217-226.

Urusova T.P. 1953. [The possible presence of dichloroethane in human milk with exposure in industrial conditions.] Gig Sanit. 18: 36-37 (Russian).

USITC. 1991. Synthetic organic chemicals. United States production and sales 1990. Washington, D.C.: U.S. International Trade Commission. USITC publication number 2470: 15-18.

Uysal M., Kutalp G., Ozdemirler G., and Aykac G. 1989. Ethanol-induced changes in lipid peroxidation and glutathione content in rat brain. Drug and Alcohol Dependence. 23: 227-230.

Van Den Berg K.J., Lammers J.H.C.M., Hoogendijk E.M.G., and Kulig B.M. 1996. Changes in region brain GFAP levels and behavioral functioning

following subchronic lead acetate exposure in adult rats. Neurotoxicology 17(3-4): 725-734.

White, R.D., Daughtrey, W.C., and Wells, M.S. 1995. Health effects of inhaled tertiary amyl methyl ether and ethyl tertiary butyl ether. Toxicol. Lett. 82-83: 719-724.

Withey, J.R., Collins, B.T., and Collins, P.G. 1983. Effect of vehicle on the pharmacokinetics and uptake of four halogenated hydrocarbons from the gastrointestinal tract of the rat. J. Applied Toxicol. 3(5): 249-253.

Yodaiken, R.E., and Babcock, J.R. 1973. DCE poisoning. Arch. Environ. Health. 26: 281-284.

Yoshida T., Goldsmith, S.K., Morgan, T.E., Stone, D.J., and Finch C.E. 1996. Transcription supports age-related increase of GFAP gene expression in the male rat brain. Neurosci. Lett. 215(2): 107-110.

ACUTE NEUROTOXICITY AND ASSESSMENT OF A BIOMARKER OF EFFECT (GFAP) IN MALE SPRAGUE-DAWLEY RATS AFTER A SINGLE EXPOSURE TO 1,2-DICHLOROETHANE

ABSTRACT

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DCE is a clear synthetic liquid. Its most common use today is to make vinyl chloride and several substances that dissolve grease, glue, and dirt. The purpose of this study was to investigate the effects of DCE on the central nervous system by measuring GFAP and GSH brain levels and SMA. Brain regions studied were CTX, BS, CRB and HP. 49-day male Sprague-Dawley rats were given a single dose of DCE and sacrificed 7 and 14 days later to measure GFAP levels. Dose groups for the 7-day study were 5ml/kg saline and 0.7ml/kg DCE. Dose groups for the 14-day study were 5ml/kg saline, 0.35ml/kg [L-dose] and 0.7ml/kg [H-dose] DCE. SMA for the same

dose groups as used for the 14-day study was measured on days 1,2,7 and 14 using digiscan computerized cages. Brain GSH levels were measured 2 hours following exposure to 5ml/kg saline, 0.35ml/kg and 0.50ml/kg DCE. The saline group served as the control group in all cases. In the 7-day study, GFAP levels were significantly ($p<0.05$) increased in the HP as compared to the control group. In the 14-day study, GFAP levels were significantly ($p<0.05$) increased in the BS and HP for both L-dose and H-dose compared to the control group. SMA was significantly ($p<0.05$) lower on day 1 for both L-dose and H-dose compared to the control group. Brain GSH levels were significantly ($p<0.05$) decreased in the CTX and HP for both L-dose and H-dose. The results show a region specific affect on rat brain GFAP and GSH levels following a single exposure to DCE as well as altered SMA.

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