

AN EXPLORATORY STUDY OF THE SYSTEMIC EFFECTS OF
LEAD, TRICHLOROETHYLENE, AND A MIXTURE OF LEAD
AND TRICHLOROETHYLENE PROVIDED CONCURRENTLY
BY ORAL GAVAGE TO MALE RATS

By

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Thesis submitted to the Faculty of Virginia Polytechnic Institute & State University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Veterinary Medical Sciences

APPROVED

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December 1998
Blacksburg, Virginia

Keywords: Neurotoxicity, Trichloroethylene, Lead, Lead Carbonate, FOB

ABSTRACT

AN EXPLORATORY STUDY OF THE SYSTEMIC EFFECTS OF LEAD,
TRICHLOROETHYLENE, AND A MIXTURE OF LEAD AND TRICHLOROETHYLENE
PROVIDED CONCURRENTLY BY ORAL GAVAGE TO MALE RATS

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Committee Chairman, Dr. Marion Ehrich, Veterinary Medical Sciences

Forty rats, in groups of ten, were orally dosed with corn oil, corn oil and 2,000 mg/kg trichloroethylene (TCE), corn oil and 2,000 mg/kg lead carbonate, or a mixture of 2,000 mg/kg each TCE and lead carbonate, in an effort to determine whether or not dual administration of both TCE and lead would have an additive effect on neurotoxicity and overall health as indicated by behavioral and physiologic measurements and tissue pathology. A functional observational test battery (FOB) was performed before, during, and after dose administration to assess dose-related changes. The FOB testing assessed behavioral and physiologic measurements such as gait, open field activity, posture, grip strength, and handling reactivity. Pathological examination included assessing dosing related changes in the testis, spleen, heart, liver, kidney-adrenals, and brain.

Results indicated that each compound was toxic individually, and that the combination of the two neurotoxicants provided conflicting indications of both reduced and additive toxicity. The toxicity of lead carbonate caused the vast majority of toxic consequences in the study. A reduction in body weight and an increased resistance to cage removal were the only statistically significant changes observed in the FOB that were due to concurrent administration of lead and TCE. Organ-to-body weight and organ-to-brain weight calculations showed evidence of a statistical difference between the lead and lead/TCE dosed animals for liver, kidney-adrenals, and body weight. The significance of these changes is not fully understood.

DEDICATION

This work is dedicated to my two sons, Daniel and Benjamin - I hope their young eyes never lose their sparkle, and to their mother Terri for being there for them always.

ACKNOWLEDGMENTS

This work would not have been possible without the help and support of my friends and family. Many thanks are owed to my advisor, Marion Ehrich, for her understanding of the unique circumstances necessary for allowing me the freedom necessary to help me complete this activity while working a full-time job, undergoing several life changes, and parenting two young boys. I also want to thank my friend Chrystal who pushed me into making the completion of this thesis my New Year's resolution, and my friends Vicki and Mike who constantly questioned my progress.

A definite thank you is due to my committee members, Dr. Don Cherry, Dr. Marion Ehrich, Dr. David Moore, and Dr. John Robertson for their time and guidance. I owe John Robertson a special debt of gratitude due to the great deal of time and effort he spent performing the necessary pathological examination of the numerous tissue samples generated at the study's conclusion.

Another thank you is due to the people who assisted with the project, including Dave, Diane, Julie and Elizabeth at the Laboratory of Animal Resources, and Kristel, Dana, Linda, and Mike from the Veterinary School. These individuals cared for the animals, assisted with tissue harvesting, and generally helped to make the project go as smoothly as it did.

Without all of these people, none of this would have possible. Thank you all!

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ABBREVIATIONS

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INTRODUCTION

The purpose of this research project was to determine whether two common environmental toxicants, trichloroethylene (TCE) and lead, have increased toxicity when given together orally as co-exposure is possible from these common environmental contaminants. The project attempts to be as realistic as possible, simulating real world exposure of man and animal alike via ingestion of water contaminated with both of these compounds. The project hypothesis was that dual administration of the two neurotoxicants, TCE and lead, would have an additive effect. Endpoints for evaluation were tissue pathology, physiologic measurements, and behavioral changes.

This project has numerous merits. To the author's knowledge, no research has been performed on these two compounds given exclusively through concurrent oral gavage. Therefore, any information gleaned from this research will add new, and hopefully useful, knowledge to our existing understanding of the study compounds. Also, the possibility of simultaneous human exposure to these two common compounds is fairly high, both occupationally and to the general population. Approximately 71% of the sites listed on the National Priorities List (Superfund sites) have lead contamination, while 60% of all National Priorities List sites have TCE contamination. The actual percentage of contaminated sites may actually be higher as not all sites have been evaluated for lead and TCE contamination (Toxicological Profile for Lead, 1993; Toxicological Profile for Trichloroethylene, 1995). Of course, National Priorities List sites are a very small fraction of the contaminated sites in the United States, but they do give a good indication that a multitude of similar, non-Superfund, sites exist throughout the United States and the remainder of the world. The ground water beneath these sites is often contaminated.

Superfund sites aside, TCE is one of the most frequently found contaminants of both raw and treated water in the U.S., while lead is the most ubiquitous toxic metal with innumerable sources of being present in our environment (Kumar et al., 1992; Amdur et al., 1991).

LITERATURE SURVEY

Much information was available regarding the toxicity of TCE and lead. However, no information could be found for these two compounds in an exclusive mixture. Information on these two compounds individually may be found under the Trichloroethylene and Lead headings below.

TRICHLOROETHYLENE

TCE is a man-made compound that is a nonflammable, colorless liquid with a sweet odor and sweet burning taste. It has approximately fifty synonyms, three of the more common being Trichloroethene, Triclene and Vitran (Dow Chemical, 2/7/96). Trichloroethylene's (CAS 79-01-6) molecular formula is C_2HCl_3 . It has a specific gravity of 1.46 at 25 degrees Celsius which makes it heavier than water, a vapor density of 4.53 which makes it heavier than air, and a molecular weight of 131.38. TCE's dermal LD_{50} is approximately 10,000 mg/kg in rabbits. LD_{50} values for oral and inhalation exposure in rats are 4,920 mg/kg and 12,500 ppm for 4 hours, respectively. TCE is incompatible with certain metals such as aluminum and magnesium powders, potassium, sodium and zinc powder, strong bases and oxidizers, and heat. Decomposition products include dichloroacetylene, hydrogen chloride, chlorine, and phosgene. Its bioconcentration potential is low. Its potential for mobility in soil is high. TCE is moderately toxic to aquatic organisms on an acute basis with a LC_{50} between 1 and 10 mg/l in most sensitive species. (Dow Chemical, 2/7/96) TCE is a suspected carcinogen with experimental carcinogenic, teratogenic and tumorigenic evidence, and is also a severe eye and skin irritant (Lewis RJ, 1991; Sax NI, 1997).

TRICHLOROETHYLENE USE, PAST AND PRESENT

TCE has historically been found in many household products including typewriter correction fluid, paint remover, adhesives, and spot cleaners. TCE was also used as a surgical anesthetic for many years, (Toxicological Profile for Trichloroethylene, 1995). Use of TCE and other chlorinated solvents is in decline due to increased environmental and safety regulation. The two primary uses for TCE today include utilization as a degreaser of metal parts in general industry and as a chemical intermediate in the chemical manufacturing industry (Conversation with John East, 1997, Southchem Corporation, Bedford VA). As of December 2, 1997, stringent environmental air emission limitations are required for all degreasing units utilizing TCE and certain other halogenated solvents (US EPA, 1994). Use of TCE as an industrial degreaser is declining as a result of this new regulatory burden.

REGULATORY STATUS

EPA

The United States Environmental Protection Agency (US EPA) considers waste materials containing TCE to be hazardous wastes. Virgin product that is to be discarded as well as spill residues of unused TCE are listed as hazardous wastes and carry a Resource Conservation and Recovery Act (RCRA) listing code of U228. All listed hazardous wastes, U228 included, are strictly regulated for disposal purposes regardless of their actual TCE concentration. Used TCE and contaminated residuals may carry a RCRA characteristic waste code of D040 and/or a hazardous waste listing of F001, F002, and F003 depending on the commercial mixture's TCE concentration and the manner in which the material was used. Wastes of used TCE may carry the D040 characteristic code if their TCE level exceeds 0.5 mg/l on a toxicity characteristic leachate procedure (TCLP). Non-listed TCE contaminated materials are not considered characteristic hazardous wastes for disposal purposes if their TCLP level, a test

which involves a twenty-fold dilution, is below the 0.5 ppm level. Wastes contaminated with TCE carry the F codes if they are used for degreasing and/or if the TCE content of the parent product was 10% or more by volume, (US EPA, 1981). Prior to the passage of the RCRA in 1976 and the Hazardous and Solid Waste Amendments in 1984, TCE was used and often disposed of in a manner that allowed it to enter the soil. Dumping of used TCE on the ground was commonplace.

The EPA is currently reviewing TCE's carcinogenicity status and has issued a drinking water maximum contaminant level (MCL) limit of 5 parts per billion TCE in drinking water (effective January 9, 1989). This MCL applies to public drinking water systems and other water systems that serve the same 25 or more persons for at least six months during the year (Toxicological Profile for Trichloroethylene, 1995; US EPA, 1989).

OSHA

The Occupational Safety and Health Administration's (OSHA) current permissible exposure level (PEL) for TCE is 100 parts per million. That is, OSHA allows industrial employees to be exposed to 100 parts of TCE per million parts of air as a time weighted average assuming an 8 hour work day, 40 hours per week work schedule. (OSHA, Subpart Z) The current PEL is considered transitional and is up from the 50 ppm PEL in force during much of the 1980's. Raising of the PEL was not the result of new toxicological evidence, but was the direct result of a court decision that directed OSHA to abandon the then-current limits for a large number of compounds. OSHA expects to promulgate new exposure limits for most of the remanded chemical PEL's, including TCE, within the next several years as they deem appropriate on a risk and use based hierarchy (Conversation with Paul Saunier, 1996, Virginia Department of Labor and Industry, Roanoke, VA).

ENVIRONMENTAL FATE OF TCE

Fate of Atmospheric TCE

Toxic Chemical Release Inventory data, as reported by select industries, reveal that 49 million pounds of TCE was released into the air in 1992 (TRI92, 1994). Actual air releases should be appreciably higher since only a select number of industries are required to report their air emissions. Ninety-one percent of reported emissions in 1983 were from degreasing operations. Other sources of TCE released into the atmosphere include volatilization from ground water treatment systems, TCE manufacture, solvent evaporation losses from adhesives, paints, and coatings, and decomposition of tetrachloroethylene (US EPA, 1985).

Singh et al. (1982) reported that the dominant transformation process for TCE in the atmosphere is reaction with photochemically produced hydroxyl radicals while Class and Ballschmiter (1986) state the half-life of TCE in air is between 3 and 7 days. Degradation products include phosgene, dichloroacetyl chloride, and formyl chloride (Atkinson, 1985; Gay et al., 1976; Kirchner et al., 1990). Though the half-life is relatively short, TCE is persistent in the environment due to its constant release and its role as an intermediate of tetrachloroethylene degradation. TCE has been detected in a number of rainwater samples as it has been shown that scavenging by rainwater occurs rapidly (Jung et al., 1992). However, the majority of the TCE deposited in surface water will revolatilize quickly.

Fate of TCE in the Aquasphere

Toxic Release Inventory data reported in TRI88 (1990) estimated that 13,800 pounds of TCE was released into water from manufacturing and processing facilities required to report their releases. The level reported in TRI92 (1994) was 8,153 pounds of releases into water. TCE released into the aquatic environment

does not degrade quickly. Half-life estimates of TCE in the aquatic environment range from 10.7 months to greater than one million years (Dilling et al., 1975; Toxicological Profile for Trichloroethylene, 1995).

Microbiological degradation, as used with increased frequency to degrade halogenated solvents in ground water, is capable of degrading TCE in a matter of days to weeks under ideal conditions. Microbial degradation breakdown products of TCE in ground water may include dichloroethylene and vinyl chloride (Smith and Dragun, 1984). Since neither biodegradation nor hydrolysis occurs rapidly, most TCE in surface water in contact with the air can be expected to volatilize into the atmosphere. However, due to its density and low solubility with water, any TCE not in contact with the air may have little chance to volatilize. Also, being a dense non-aqueous liquid, TCE may find its way into pockets where it may slowly solubilize into the water or seep into the underlying aquifer where it may persist for many decades (Conversation with Jeff Peffer, 1997, Peffer Geotechnical Corporation, Lewisberry PA).

Fate of TCE in Soil and Rock

Toxic Release Inventory data reported 21,190 and 20,726 pounds of TCE were released onto the land in 1988 and 1992, respectively, from manufacturing and processing facilities in the U.S. required to report such releases (TRI88, 1990; TRI92, 1994). The majority of TCE released to the soil can be expected to volatilize into the atmosphere. Aerobic and anaerobic degradation of the TCE may take place by naturally occurring organisms. Again, microbial degradation may lead to the formation of vinyl chloride, a known carcinogen, as a breakdown product. TCE contamination in underlying soils is generally persistent, as the delicate microbiological balance necessary to degrade TCE is not generally seen in nature. TCE disposed of onto the ground, and, being a dense product, may

find its way through fissures in the underlying rock and ultimately into the ground water (Toxicological Profile for Trichloroethylene, 1995).

TRICHLOROETHYLENE EXPOSURE RISK

The US EPA estimates that over 400,000 industrial workers are exposed to TCE on a full-time basis. TCE is a common water contaminant due to past environmental contamination as well as continued deposition of TCE into the water due to industrial processes. Therefore, there is also a potential for the general population to be exposed on a routine basis as well. Mean levels in a recent study found TCE levels in ground water to have a mean value of 7 parts per billion (Toxicological Profile for Trichloroethylene, 1995). As previously stated, approximately 60% of the existing Superfund sites have known TCE contamination. Numerous non-Superfund sites throughout the country and the world also are known to be contaminated with TCE. Even on a local level, conversations with the Virginia Department of Environmental Quality (DEQ) bear out the fact that TCE contamination is common. Numerous sites in Virginia have removed TCE contamination, are currently investigating potential contamination, or are in the process of remediating TCE contaminated ground water. Due to regulatory standards, many if not most, areas of TCE contamination will most likely never be discovered (Toxicological Profile for Trichloroethylene, 1995).

TCE exposure may occur by absorption through the skin, eyes, eardrum and mucous membranes following ingestion and inhalation. Inhalation is the most prevalent route of exposure in industrial employees, while ingestion of contaminated water and food is the most common route of exposure for the human population as a whole. Industrial inhalation exposures are normally higher than ingestion exposures of contaminated food and water as roughly half the dose inhaled actually enters the bloodstream (Toxicological Profile for Trichloroethylene, 1995).

EFFECTS OF TRICHLOROETHYLENE EXPOSURE

TCE's primary target organs are the liver, heart, and central nervous system. Toxicity to these organs is not dependent on whether exposure occurred via inhalation or ingestion. Much of the TCE absorbed into the body via ingestion or inhalation will later escape through the lungs via exhaled air. The remainder of the TCE will be processed by the liver, with most of the byproducts discharged into the urine within a day. A small quantity of TCE and its metabolites will be stored in the body fat, and may build up if excessive exposure continues (Pfaffenbeger et al., 1980).

Humans metabolize between 40 and 75% of the retained dose (Toxicological Profile for Trichloroethylene, 1995), with no indication of a saturation threshold for TCE metabolism. Nomiya and Nomiya (1977) and Ikeda (1977) suggest that at inhalation doses up to 315 ppm the TCE in the blood stream is removed by the liver in a single pass. Mathematical calculations predict that saturation would occur at 2000 ppm (Feingold and Holaday, 1977). TCE is metabolized by the body via a number of metabolic pathways. The foremost pathway for metabolism utilizes liver cytochrome P-450, though glutathione conjugation, and extrahepatic metabolism occur (Bruckner et al., 1989). Excretion of the metabolites from these processes occurs primarily through the urine. Some unmetabolized TCE is excreted in the urine, though the majority is removed in the lungs during exhalation. Primary urinary metabolites are trichloroethanol, trichloroethanol glucuronide, TCA, oxalic acid, and N-(hydroxyacetyl)-aminoethanol. Some TCE is eliminated in the bile (Monster et al., 1979; Toxicological Profile for Trichloroethylene, 1995).

TCE exposure may affect the central nervous system. Effects include headache, vertigo, fatigue, short-term memory loss, decreased word associations, central nervous system depression and anesthesia. Nervous system depression or

anesthesia are normally short-lived if exposure is removed or reduced. Death can occur through central nervous system depression or heart failure due to ventricular fibrillation. Damage to the cranial nerve has been reported for both acute and chronic exposure, with reports of short and long term facial numbness as a result (Toxicological Profile for Trichloroethylene, 1995; Dow Chemical, 1996; Sax NI, 1997). Many effects of TCE have been attributed to demyelination resulting in membrane disruption (Feldman 1970; Feldman et al., 1992). Residual neuropathy from TCE exposure may also cause facial discomfort and jaw weakness which may last for several months (Buxton and Hayward, 1967; Feldman, 1970).

The primary hepatic effect of TCE exposure is liver enlargement due to cellular hypertrophy. Renal enlargement is also associated with acute exposure. Renal toxicity is due to altered biochemistry, not abnormal histology, in acute to intermediate exposure. The metabolism of TCE to dichloroacetic acid (DCA) is important to renal toxicity in rodents but this pathway seems to be less common in man (Miller and Guengerrich, 1983; Steinberg and DeSesso, 1993). High doses of TCE may saturate the P-450 monooxidase pathway of rodents, causing a switch to glutathione conjugation. The glutathione pathway may ultimately produce a metabolic product that is a renal carcinogen. There is no data for similar saturation in humans (Toxicological Profile for Trichloroethylene, 1995).

Cytochrome P-450 metabolism of TCE produces chloral initially, which is ultimately converted to trichloroethanol and eliminated via the urine. For inhalation exposure, this pathway leads to lung toxicity in some rodent species due to the limited ability of the lung to reduce chloral to trichloroethanol (Toxicological Profile for Trichloroethylene, 1995).

Hearing loss is reported to have occurred in laboratory animals upon repeated exposure to 2500 ppm or higher TCE concentration; however, it is not known whether or not this is relevant to human exposure. Positive carcinogenic response occurred in mice given large doses of TCE though it is thought that low doses in man should pose little or no carcinogenic response. Metabolism saturation occurs at lower exposures in rats than in mice (Dekant et al., 1986; Prout et al., 1985; Toxicological Profile for Trichloroethylene, 1995).

LEAD

Lead (Pb) is a naturally occurring bluish-gray metal that tarnishes upon exposure to air. It constitutes approximately 0.002% of the earth's crust to a 16 kilometer depth. Lead is very soft and easily formed, cut, melted, and cast. It is one of the metals known to the ancient world, and is the most common heavy metal. Pure water and weak organic acids in the presence of oxygen attack lead (Merck, 1989). Lead dust is flammable or explosive when exposed to heat or flame. When heated to decomposition it emits highly toxic fumes of lead (Lewis RJ, 1991).

Lead carbonate (PbCO_3), one of the many lead compounds used in industry and the lead compound utilized in this study, is a non-flammable, odorless white powder with a molecular weight of 775.1 and a specific gravity of 6.8. This material is often referred to as "white lead" (Halstab, 1994).

Lead Use, Past and Present

Lead is a naturally occurring metal that has been known and used for centuries, primarily due to its ease of use. It has a low melting point and great malleability. Past uses are numerous, including use as a pigment in paint, as an additive to gasoline, and a component of pipes and solder. While the quantity of lead found in gasoline, piping, solder, and paint has been greatly reduced during the last few

decades, lead continues to be used in many products. It persists within the environment due to past uses. Included among present uses are ammunition, fishing sinkers, hobby supplies such as pottery glazes, lead-acid batteries, and electronic components. The most important use of lead at this time is in the manufacture of lead-acid batteries (Toxicological Profile for Lead, 1993).

Lead Exposure Risk

The numerous sources of lead in our environment would make it difficult, if not impossible, for a person to avoid exposure to this compound. Ingestion and inhalation are the two most common exposure pathways for lead. Of these, inhalation of volatilized lead is the most hazardous as the lungs will absorb the majority of it, while most ingested lead will pass through the body. Flaking lead paint in older homes and soil contamination pose major health hazards to children, with exposures of up to 200 ug per day possible (Kumar et al., 1992). Old paint may be 5 - 40% lead. The burning of leaded gasoline is the single largest source of lead in the atmosphere and has been since the 1920's. This means much of this lead is still biologically available in surface dusts and soils (Toxicological Profile for Lead, 1993).

The FDA's Total Diet Food Studies conducted between 1982 and 1988 quantified the lead levels associated with a normal American diet. Interestingly, this study found that daily lead intakes dropped by approximately 50% during a period from 1980 -1984 (Gunderson, 1988) and continued to drop through 1990 for all age groups and sexes (FDA 1992; Bolger et al., 1991). Average daily intake was found to be approximately 56 ug/day during the early portion of the study and found to range from 5 to 11 ug/day near the end of the study.

Approximately 70% of the National Priorities List (Superfund) sites in the United States have lead contamination of the soil or ground water. These contaminated

sites, as well as many other non-Superfund contaminated sites, may pose a risk to neighboring communities due to the possibility of lead leaching into and contaminating the ground water, or from the liberation of airborne dust containing high levels of lead during remediation (Toxicological Profile for Lead, 1993). On a smaller scale, particles of lead paint may be liberated during building renovation and destruction. OSHA is currently implementing a requirement that contractors become certified to perform lead abatement, much akin to the current stringent certification that must be obtained prior to becoming licensed to encapsulate or remove asbestos. The certification process is intended to ensure that lead contaminated dusts and debris generated from building renovation or destruction do not contaminate the local community and environment (OSHA lead standard).

Gasoline, paint, ceramic products, solder and other household leaded products have greatly reduced lead values as compared to 10 to 20 years ago. However, the use of lead in ammunition and roofing has increased in recent years. Exposure may come from nearby hazardous waste sites, foods such as grains, fruits, vegetables, meats, soft drinks, and wine, paint chips and dust. Drinking acidic water can cause the lead in lead pipes, solder, and brass faucets to leach; smoking cigarettes, and working with stained glass also cause lead exposure. Occupational exposure is generally through inhalation of lead particles. Between 0.5 and 1.5 million workers are exposed to lead in the workplace. Families of these workers may be exposed to lead brought home by these people on their work clothes (Toxicological Profile for Lead, 1993).

Exposure of the general population is most likely to occur through ingestion of contaminated food and water and by the inhalation of leaded particles and dust. A study of inner Minneapolis found lead soil levels to be sixty times greater than in rural Minnesota. 95% of the inner city lead values are believed to have been

from the long-term use of leaded gasoline. Thus dust in the inner cities is expected to carry with it a greater risk of lead toxicity, both due to the lead from the paint of older structures and the fallout of lead from the combustion of leaded gasoline (Toxicological Profile for Lead, 1993).

The quantity of lead absorbed by the body after exposure by ingestion is age dependent. Only about 6% of the lead ingested by an adult will be absorbed by their bodies if their stomachs are full. Of this amount, 99% will be eliminated in the urine and bile while the remaining portion will become assimilated into the body tissues. In contrast, 50% of the lead ingested by a child may be absorbed under the same conditions and only 32% will be excreted in the waste. Children are, therefore, much more susceptible to the effects of lead exposure via ingestion. The greater propensity of absorption in children is further aggravated by the fact that their developing nervous system is more susceptible to damage by lead versus the fully developed, and somewhat lead resistant, adult nervous system (Toxicological Profile for Lead, 1993).

Lead's primary impact is toxicity to the nervous system, both peripheral and central. Effects include generalized neuropathy and encephalopathy that can be manifested by dullness, irritability, poor attention span, headache, muscular tremor, loss of memory, and hallucinations. These symptoms may quickly worsen to include delirium, convulsions, paralysis, coma, and death (Kumar et al., 1987). Other effects of exposure include reduced IQ and growth in children, a variety of gastrointestinal symptoms, muscular weakness in fingers, wrists, and ankles, damage to the brain, kidneys, and male reproductive system, and abortion. Effects are the same regardless of the mode of exposure. Certain lead compounds may be carcinogens but lead itself has not been proven to cause cancer (Toxicological Profile for Lead, 1993). Human encephalopathy is known to occur when blood lead level rise between 50 and 300 ug/dl. Subjective signs

of acute neurotoxicity may be seen when blood lead values are between 40 and 120 ug/dl (Toxicological Profile for Lead, 1993).

Regulatory Status Of Lead

Lead is regulated by many federal laws and is a priority water pollutant and a hazardous air pollutant. The US EPA, FDA, and other agencies regulate the quantity of lead that may be present in food items, water, fuel, and paint. The US EPA regulates materials intended for discard as a hazardous waste if they contain more than 5 ppm of leachable lead as determined by a toxicity characteristic leachate procedure (TCLP) test. Materials that leach more than 5 parts per million of lead are designated a characteristic hazardous waste with a hazardous waste code of D008. The MCL for lead in drinking water is 50 ug/ml. This MCL applies to public and other drinking water systems that serve the same 25 or more people for at least six months during the year (Toxicological Profile for Lead, 1993; US EPA, 1981; US EPA, 1989).

OSHA regulates the quantity of lead the industrial worker may be exposed to during his or her workday. Presently, OSHA enforces a permissible exposure level of 50 ug of lead per cubic meter of air (50 ug/m^3). Beyond this level, employees must be offered personal protective equipment. The employer must also attempt to improve employee protection through engineering and administrative controls. OSHA requires a medical monitoring program to be instituted at workplaces that exceed the 30 ug/m^3 action level. The PEL and action level are in force irrespective of personal protective equipment used (e.g., respirator) or actual exposure. The OSHA Lead Standard requires that affected employees have their blood lead levels checked periodically. It also requires that such employees shower and don fresh clothing prior to leaving the workplace each day. The removal of all exterior contamination in this fashion greatly

reduces the likelihood of secondary exposure from occurring to themselves and their families at home (OSHA Lead Standard; EPA, 1996).

Biological Fate of Lead

Studies show that approximately 70% of inhaled lead is absorbed by the body within 10 hours (EPA, 1986). Oral intake of lead occurs through the consumption of lead -containing food and beverages and from swallowing lead deposited in the upper respiratory tract. Children also ingest lead via normal mouthing activities and pica. Absorption occurs primarily in the gastrointestinal tract. Absorption is about 50% in children as compared to 8 % for adults (Toxicological Profile for Lead, 1993).

Once absorbed, lead is distributed into three compartments in the body, blood, soft tissue, and bone (Rabinowitz et al., 1976). Inorganic lead in the body is not known to be metabolized or biotransformed. Dietary lead not absorbed by the body is excreted via the feces. Blood lead that is absorbed but not retained is excreted by the kidneys or through the bile into the GI tract (US EPA, 1986).

Environmental Fate of Lead

Fate of Atmospheric Lead

Lead found in the atmosphere is primarily in particulate form. Lead particulate is removed from the air by wet or dry deposition. Forty to seventy percent of atmospheric lead is removed by wet fallout. Much of the recent lead contamination present in the air are oxides of lead. Older material found on surfaces are much more likely to contain lead carbonate species. Lead deposited in the soil may remain there for many years, only to be re-entrained back into the atmosphere, thus making it more available as an inhaled toxicant (Toxicological Profile for Lead, 1993).

Air is the initial recipient for much of the lead released into the environment. There has been a 64% decline in national lead emissions since 1985. This decline is primarily due to the increased use of unleaded gasoline (Toxicological Profile for Lead, 1993). The US EPA now allows up to 0.05 g lead/gallon of unleaded gasoline (US EPA, 1982). Lead emissions in 1989 were estimated to be 7.2×10^3 metric tons (US EPA, 1991).

Fate of Lead in the Aquasphere

Lead tends to form compounds of low solubility in water. The amount present in the aquatic environment depends on the pH and the dissolved salt content of the water. These relatively insoluble lead compounds may stay in the water column as suspended solids or may be present in suspended living and non-living organic matter. The ratio of lead in suspended solids to lead in dissolved form has been found to vary between 4:1 in rural streams to 27:1 in urban streams (Getz et al., 1977; Toxicological Profile for Lead, 1993).

Fate of Lead in Soil

Lead levels found in most soils are due to atmospheric deposition or from lead-based paint from housing deterioration or abatement. Most lead introduced to the soil is strongly retained there with little entering surface or ground waters, although tetraethyl and tetramethyl lead may form water-soluble compounds. The downward movement of lead from soil to ground water by leaching is very slow except under highly acidic conditions. Atmospheric lead may also enter the soil as lead sulfate. Many plants assimilate lead into their structure and fruits. This lead is returned when the plants die and decay (NSF, 1977; Toxicological Profile for Lead, 1993).

Domestic ore production and ammunition use produce great quantities of lead contaminated soil. In 1988, it was estimated that 28 million pounds of lead were released on-site to land and 28.1 million pounds were transferred off-site rather than released directly to the environment (Toxicological Profile for Lead, 1993).

EXPERIMENTAL DESIGN AND METHODS

Preliminary Study

A range finding study, utilizing 24 rats, was used to determine suitable doses for the two compounds and to provide practice in dosing and Functional Observational Battery (FOB) administration for the investigator. Rats were dosed at 500, 1,000, and 2,000 milligrams per kilogram (mg/kg) with lead carbonate, and/or 2,000, 3,000, and 4,000 milligrams per kilogram of TCE for twenty-three days. Indices of effect were physiologic and behavioral responses as found during pre and post functional observational test batteries. The data from this pre-study was not statistically analyzed due to a variety of factors; therefore, the findings of the pre-study are purely subjective. Likewise, no pathological examination was performed on the animals. The data was, instead, used as an aid in dosage selection for the definitive study.

Preliminary Study Results

Rats dosed at 500 and 1,000 mg/kg lead carbonate exhibited little notable effect, although rats dosed at 2,000 mg/kg showed decreased exploratory behavior (investigating an open field) at day 4, reduced muscle tone, (determined by qualitatively assessing muscle firmness), at day 9, an exaggerated noise response by day 10, greatly reduced food intake by day 13 and drooping eyelids as well as extreme lethargy by day 20. Blood lead levels on the one test animal checked was 470 ug/dl, a level sufficient to cause notable lead poisoning effects and encephalopathy in many species.

Using the FOB, rats dosed with TCE exhibited marked behavioral and gait changes shortly after each dosing due to the anesthetic and intoxicating effects of the compound, but no definite cumulative effect was noted. Gait changes

included lack of coordination and stumbling. Mortality was seen at all dosages, most likely due to inexperience with gavage technique on the part of the investigator. No definitive cumulative toxic effect was seen in the TCE dosed animals.

Two animals that had been dosed with lead carbonate at 2,000 mg/kg for ten days were also dosed with 2,000 mg/kg TCE for thirteen additional days. Notable effects were seen after five days of dual administration, including rear limb locomotion difficulty and "tiptoe" walking which progressed to dragging of the rear limbs by day 22. The study was terminated at day 23.

From the preliminary results, dosages of 2,000 mg/kg were chosen for both lead and TCE during the definitive thesis project. Also due to the preliminary study, TCE dosing was delayed in the thesis project until after an initial lead-dosing period to prevent undue mortality and/or morbidity.

EXPERIMENTAL DESIGN AND METHODS FOR THESIS PROJECT

Study Groups

Forty animals were divided into four groups of ten animals each. Group A received corn oil only. Group B received corn oil daily for the first nine days and trichloroethylene at 2,000 milligrams per kilogram for the remainder of the study. Group C received lead carbonate exclusively at 2,000 milligrams per kilogram of body weight daily for the entire study period. Group D received lead carbonate at 2,000 milligrams per kilogram daily for the first nine days of the study, and lead carbonate and TCE, both at 2,000 milligrams per kilogram, concurrently each day for the remainder of the study period.

Test Subjects and Husbandry

Male Sprague Dawley rats (Rattus norvegicus) purchased from Harlan Sprague-Dawley (Indianapolis, Indiana) were utilized in the study. Animals were born on 3/12/97 and were shipped at 225 to 249 grams. Rats were received on 5/14/97 and after a quarantine period were deemed healthy by a Laboratory Animal Veterinarian prior to use in the research project. Animal weights ranged from 293 to 330 grams, with the mean weight equaling 313.65 grams, with a standard deviation of 10.29 grams, at the onset of the study on 5/27/97.

Animal Holding Environment

All animals were housed at Virginia Tech's Laboratory of Animal Resources (LAR) under controlled conditions. Temperature was maintained at 22 degrees Celsius plus or minus 2 degrees, with relative humidity kept between 30 and 70 percent. Air was 100% fresh air with 10 to 15 air changes per hour. All animals were housed two to a cage in transparent polycarbonate cages covered with stainless steel wire tops.

Feed

Animals were fed Tekland 7001 4% (fat) Mouse/Rat Diet throughout the study. This feed contains a minimum crude protein of 24%, a minimum fat content of 4%, and maximum crude fiber of 5%. Feed was readily available ad libitum throughout much of each day except during a 5⁺ hour fasting period prior to each dosing.

Water

Water was available ad libitum throughout the study via an automatic watering system fitted with stainless lixit valves. The water source was the municipal drinking water as provided by the Town of Blacksburg.

Bedding

Animal bedding consisted of 100% reclaimed virgin wood pulp. Bedding was supplied by Tek-Fresh and was changed twice per week.

Dosing

Dosing was accomplished by oral gavage with a stainless steel gavage needle. Test chemicals were mixed with 100% Mazola corn oil. Corn oil was also used as the dosing agent for all control animals and for dose equalization. Animals were fasted five or more hours prior to dosing. The total dose was manipulated so that all animals received the identical volume of liquid each day. The total dose given to the animals ranged from 1.9 ml of liquid at the onset of the study to 1.6 ml of liquid at the study's conclusion.

Day-Night Schedule

The day night cycle in the animal holding room was manipulated so that the active (night) phase of the animals occurred from 2:00 p.m. to 2:00 a.m. each day. Fluorescent bulbs supplied lighting.

Functional Observational Batteries

Functional observational batteries, FOB's, were utilized to measure the toxicity of the test compounds. FOB's were utilized due to their ability to detect a great variety of nervous system effects on the rat. Such testing is straightforward and non-invasive in nature and was perfectly suited for this study as it allows detection of whole-body and focused changes or deficiencies in both the central and peripheral nervous systems (Moser et al., 1988).

FOB measurements included qualitative and quantitative scoring of the animals in a variety of behavioral and physiologic measures. Scoring took place while the animal was in his “home cage”, during handling, while on a slowly rotating rod (rotorod), and in an open field environment. Scoring categories included, but were not limited to, posture, clonic and tonic movement, vocalizations, lacrimation, palpebral closure, piloerection of the fur, visual placing, handling reactivity and overall arousal. Reflex measurements included approach, touch, click, and tail pinch responses, as well as the righting and tail-limb reflex. Physiologic measurements included body weight, rectal temperature, rotorod agility and forelimb/hindlimb grip strength. Attachment A to this document contains a list of the behavioral and physiologic measures taken during each FOB.

FOB's were performed prior to initial dosing, on day nine prior to the onset of TCE dosing, and finally at the study's conclusion during the evening prior to animal sacrifice. All neurological testing took place during the active (dark) phase of the research animals' day-night cycle and generally followed procedures specified in Section 7 - Functional Observational Battery (FOB), Code 11-07 of the Unit Laboratory for Neurotoxicity Studies, Veterinary Medical Experiment Station, Virginia Polytechnic Institute and State University, dated 10/25 and 11/9/95. Exceptions included adjustments to the suggested FOB schedule, a modified dark-light schedule, and the addition of the pinna reflex test as a measure of facial nerve deficiencies, to the behavior data sheet.

Blinding

The investigator was blinded during FOB testing with the assistance of the LAR staff. Cage numbers were randomly drawn and the LAR staff arranged the cages in the random order selected prior to performance of each test. Upon conclusion of the FOB testing for the day, the investigator would rearrange the

cages into their original order according to animal number and dosage group. Animals were not randomized for the initial FOB, as dosing had not yet begun. Although randomized, it was generally apparent during subsequent FOB's which rats were receiving lead dosages. Cage removal ease, reduced body weight, enlarged abdomens, and pale eyes were among the observable changes seen in the lead and lead/TCE dosed animals.

Statistical Evaluation

The Virginia Tech Statistics Department performed statistical evaluation for the FOB as per Moser et al. (1988), except for repeated measures. Repeated measures could not be used due to the confounding effect of multiple toxicants provided at different times during the study. Continuous data were analyzed by a general linear model (SAS, 1985) using each rat's initial FOB as a covariate. Values were then adjusted to pre-dosing levels and then were subjected to a two-way analysis of variance (ANCOVA). Descriptive and rank data were analyzed using the categorical data modeling procedure CAT-MOD (SAS, 1985). . All forty animals, ten per group, were included in the FOB statistical evaluation. An alpha level of 0.05 was used to determine significance for all statistical measures

Pathological Examination

Tissues were collected from all forty animals (ten per group) at the termination of the study. Tissues were initially placed in buffered formalin and kept for later processing. The tissue samples were then processed with a hemotoxylin and eosin stain for examination via light microscopy.

Tissue weights were obtained during tissue harvest to allow for organ-to-body, organ-to- brain and organ weight calculations to be made.

Blood samples were obtained at the studies conclusion immediately after decapitation. Blood from the animal was allowed to fill a 5-milliliter eppendorph tube inoculated with heparin. The Veterinary Medicine Toxicology Laboratory analyzed the lead samples by atomic absorption spectroscopy following perchloric acid digestion.

Test Chemicals

Trichloroethylene (TCE, CAS# 79-01-6)

The TCE used in the project was obtained from Southchem Inc. (Bedford, VA) and manufactured by Dow Chemical Company (Midland, MI) under the trade name Neu-Tri (R) Solvent. Industrial grade solvent was used rather than laboratory grade as the industrial grade material better represents real world exposure conditions. Lot 8780, T-72834-97 D24 was used. TCE was mixed with corn oil to make a 1,000 mg/ml stock solution for dosing all animals.

Whitney et al. (1983) report that TCE absorption is slower when administered with corn oil as the oil acts as a reservoir for lipophilic compounds in the gut. Nonetheless, Prout et al. (1985) reported absorption levels of up to 90% in rats dosed with corn oil. Delivery in an aqueous emulphor has been shown to be more lethal but less hepatotoxic than administration using corn oil (Merrick et al., 1989).

Lead Carbonate (CAS # 1319-46-6)

The lead carbonate utilized in this study was obtained from Southchem Inc. and manufactured by Halstab Division of Hammond Group Inc. (Hammond, IN). The lot number of the compound used was 982/7. This compound was chosen over other lead compounds due to its common use in electroplating operations and its ability to readily dissolve and emulsify in corn oil, the dosing vehicle used in this study. The lead carbonate content of the material used was reported to be 100%

by Southchem Incorporated, the distributor of this compound. The lead carbonate was mixed with corn oil to make 350 and 500 mg/ml stock solutions for dosing. Stock solution concentration began at 350 mg/ml but was increased to 500 mg/ml when concurrent TCE administration began. This change was necessary to keep the total volume of liquid administered by oral gavage to the rats within lab animal guidelines.

General Statistical Analysis

Statistical analysis of organ weights, were analyzed by a general linear model (SAS, 1985). Univariate analysis, comparing each group to the control group was performed. Organ-to-body weight, organ-to-brain weight, and lead blood value analysis was performed using the Student's t-Test. The replicate size for each analysis was ten animals per group with the exception of the lead blood analysis, in which only five lead and five lead/TCE animals were tested for the presence of blood lead.

RESULTS

General

Lead toxicity was found to be predominant in both the lead and lead/TCE test groups. Typical lead induced changes included decreases in body weight, rectal temperature, and grip strength. However, the lead/TCE test group did exhibit differences as compared to the lead group. Included were additional body weight decreases, increased cage removal ease, as well as statistically significant changes in organ-to-body weight and organ-to-brain weight ratios. The TCE dosed animals exhibited minor physiological effects, though the behavioral reactivity (e.g. aggressiveness and vocalizations) of these animals did increase substantially.

NEUROLOGICAL RESULTS

Lead Dosed Group

Functional observational batteries of lead dosed rats yielded the expected behavioral and physiological changes. Body weight decreased 127 grams ($P=0.0001$) between the control and lead dosed groups. The first subjectively identified behavioral change seen by the investigator was reduced muscle tone and ease of handling of the lead dosed rats. The ease of handling was initially noticed after only two days of lead dosing. The lead-dosed rats were much easier to remove from the cage, and were more easily dosed and otherwise manipulated. They were generally docile and would sit quietly in the investigator's hand. Remarkably, some lead-dosed animals seemed to initially improve their ability to stay aloft on the rotorod. This was most likely due to their slow methodical movements being favorable for staying on a slowly revolving rod. This effect was short-lived and did not last through subsequent FOB testing. Rotorod agility was negatively affected by lead. The rotorod agility of the lead dosed animals decreased considerably after lead dosing as borne out by

statistical analysis from pre to post and mid to post FOB's, where P equaled 0.0015 and 0.0034 respectively. No such changes were seen in the control (corn oil treated) rats.

Reduced scrotal size and bloated stomachs were also observed in the lead dosed animals. Post study autopsy revealed severely bloated stomachs in the test animals as well as retracted testicles. Testicular retraction was most likely not a direct response to lead administration but a secondary adaptation by the animals whose body temperature has dropped to keep the testicles at the appropriate temperature. The stomach changes indicate a severe reaction of the stomach tissues to lead carbonate administration, an unexpected effect.

Other significant lead-based changes were seen in the lead-dosed animals. Deleterious effects include abnormal posture, piloerection of the fur, a body weight reduction in excess of ninety grams, a core body temperature drop as much as a five degree Celsius, and a loss of foregrip and hindgrip strength. Other indications of lead's negative effect noted in the test animals included an increase in foot splay, a decreased ability to stay on the rotarod, a reduction in activity, an increase in palpebral closure, a decrease in handling reactivity, and abnormal gait (arched back) and arousal behaviors (stupor).

The following pages contain tables listing the behavioral and physiologic measures from the FOB and organ weight data (Table 1). Listings include comparison from the pre to mid study FOB's, pre to post FOB's, and mid-study to post FOB's. Included for each are the significance values. Data tables are included in Attachment E of Appendix I.

Organ Weight Results

Organ weights were affected by lead exposure. Testis weight was substantially decreased ($P=0.0031$), as was spleen ($P=0.0001$), heart ($P=0.0001$), and liver

($P=0.0001$) as compared to the control group. Organ-to-body weight analysis showed the testes, spleen, kidney/adrenals, and brain to be significantly different from the control group. Organ-to-brain weight analysis, a measure that may be more relevant in this study due to lead-induced weight loss, revealed similar results with body, spleen, heart, and liver weights being statistically significant, and testes weight approaching significance ($P=0.07$), (Table 2).

TABLE 1

FOB STATISTICAL EVALUATION

LEAD GROUP

<u>Measure</u>	<u>Significance Values</u>		
	A-B	A-C	B-C
Body weight decrease	0.0001	0.0001	0.0001
Posture changes	0.1418	0.0001	0.3016
Piloerection of fur	0.3711	0.0001	0.0001
Rectal temperature decrease	0.0001	0.0001	0.0001
Foregrip strength decrease	0.0614	0.0001	0.0012
Hindgrip strength decrease	0.0643	0.0001	0.0055
Footsplay changes	0.0058	0.0051	NC
Rotorod time decrease	0.0121	NC	NC
Activity decrease	0.0001	0.0001	0.0005
Removal ease increase	NC	0.0001	0.0001
Palpebral closure	NC	0.0149	0.0149
Handling reactivity decrease	0.0006	0.0001	0.0092
Gait score	NC	0.0009	0.0009
Arousal decrease	0.0338	NC	NC
Rotorod agility decrease	NC	0.0015	0.0034

Note: A= pre-study FOB
 B= mid-study FOB
 C= end-study FOB
 NC= not calculated

TABLE 2

LEAD GROUP

ORGAN WEIGHT STATISTICAL EVALUATION

(Click on box to view table)

ORGAN-TO-BODY WEIGHT RATIOS

(Click on box to view table)

ORGAN-TO-BRAIN WEIGHT RATIOS

(Click on box to view table)

TCE Dosed Group

Trichloroethylene-induced changes in behavior and the physiologic parameters observed in the FOB were very few in comparison with the changes noted in the lead dosed animals. Body weight decreased significantly ($P=0.0013$) from the mid-study to the post-study FOB.

Behaviorally, the TCE dosed animals revealed statistically significant changes in their ability to stay on the rotorod, foot splay distance, touch reactivity, and tail pinch response, with click reactivity approaching significance. The TCE rats were generally more vocal, more difficult to handle, and more aggressive than the other animals.

Reaction to dosing, though not included as a FOB observation, was noticed by the investigator to be much more robust in the TCE group. Dosing in this group elicited much more reaction than corn oil, lead, and TCE/lead dosed animals. The typical response of the TCE animals after being placed back into their cage was to run hurriedly around their cage, sometimes digging under the bedding only to come out from under the bedding and repeat the process. A few instances of hopping or jumping behavior were also noted in the animals that had just been dosed with TCE. Of course, once the TCE began to enter the animals' bloodstream, the typical intoxicant response was seen as TCE ultimately causes a depressant or anaesthetic effect. Prior to their impending sleep, many of the animals were found to be scratching their midsection with their rear paws.

Table 3 contains the behavioral and physiologic measures from the FOB. Listings include comparison from the pre to mid-study FOB's, pre to post FOB's and mid-study to post FOB's. Included for each are the significance values. Actual data are included in Attachment E of Appendix I to this document.

Organ Weight Analysis

Organ weights were statistically similar to the control group except for the liver. Liver weight was shown to be significantly increased by organ weight and organ-to-brain weight analysis. Organ-to-body weight analysis, however, also showed the spleen to be significantly reduced in weight versus the control group, (Table 4).

TABLE 3

FOB STATISTICAL EVALUATION

TCE GROUP

<u>Measure</u>	<u>Significance Values</u>		
	A-B	A-C	B-C
Rotorod agility decrease	NC	NC	0.0254
Weight reduction	NC	NC	0.0013
Footsplay increase	NC	NC	0.0027
Rotorod time decrease	NC	0.0221	NC
Touch reactivity increase	NC	0.0038	0.0392
Click reactivity increase	NC	0.0663	NC
Tail pinch response increase	NC	0.0053	NC

Note: A= pre-study FOB
 B= mid-study FOB
 C= end-study FOB
 NC= not calculated

TABLE 4

TCE GROUP

ORGAN WEIGHT STATISTICAL EVALUATION

(Click on box to view table)

ORGAN-TO-BODY WEIGHT RATIOS

(Click on box to view table)

ORGAN-TO-BRAIN WEIGHT RATIOS

(Click on box to view table)

Lead / Trichloroethylene Dosed Group

FOB's performed on this group showed few significant changes related to the dual administration of both lead carbonate and trichloroethylene (Table 5). Most changes observed are what would have been expected from exposure to each compound alone. Two measures were found to be of significance compared to the lead and TCE only groups. A ninety-one gram reduction in body weight of the animals dosed with both substances was observed at autopsy ($P=0.0171$). Reduced body weight was also noted as compared from the pre to post and mid to post FOB's ($P=0.0276$ and 0.013 respectively). A decrease in removal ease reluctance was the only other significant change seen in this test group ($P=0.0207$).

Table 5 shows the significant behavioral and physiologic measures from the FOB. Listings include comparison from the pre to mid-study FOB's, pre to post FOB's and mid-study to post FOB's. Included for each are the significance values. Actual data are included in Attachment E of Appendix I to this document.

Organ Weight Analysis

Organ weights of the ten animals in this test group were similar to the lead dosage group except that the kidneys and brain were statistically different in weight from the control group and, unlike the lead group, heart weight did not differ from the control weight. Also, organ-to-body weight analysis showed both the liver and brain to be significantly different from the lead-dosed animals. Organ-to-brain weight analysis, being less sensitive to body weight changes, revealed statistical differences in body, liver, and kidney/adrenal weights as compared to the lead dosed group. Table 6 provides organ, organ-to-body and organ-to-brain weight analysis data as compared to the control group. Table 7 and Table 8 are also provided to facilitate comparison of the lead and lead/TCE dosage groups.

TABLE 5

FOB STATISTICAL EVALUATION

LEAD / TCE GROUP

<u>Measure</u>	<u>Significance Values</u>		
	A-B	A-C	B-C
Body weight decrease	NC	0.0276	0.0143
Removal ease increase	NC	0.0207	NC

Note: A= pre-study FOB
 B= mid-study FOB
 C= end-study FOB
 NC= not calculated

TABLE 6

LEAD / TCE GROUP

ORGAN WEIGHT STATISTICAL EVALUATION

(Click on box to view table)

ORGAN-TO-BODY WEIGHT RATIOS

(Click on box to view table)

ORGAN-TO-BRAIN WEIGHT RATIOS

(Click on box to view table)

TABLE 7

Lead vs. Lead / TCE

ORGAN WEIGHT STATISTICAL EVALUATION:

(Click on box to view table)

ORGAN-TO-BODY WEIGHT COMPARISON: SIGNIFICANT MEASURES

(Click on box to view table)

ORGAN-TO-BRAIN WEIGHT COMPARISON: SIGNIFICANT MEASURES

(Click on box to view table)

TABLE 8

Lead and Lead / TCE vs. Control Comparisons
(Click on box to view table)

Tissue Pathology

Testes, spleen, heart, liver, kidneys/adrenals, and brain were harvested, weighed, and preserved in buffered formalin immediately after sacrifice.

Pathologist John Robertson (VMD, MS, Ph.D.) offered instruction on the proper harvest and handling procedures of the tissues. He also provided pathology expertise to the project by examining all of the tissues.

Gross Pathology

Oil Group:

Animals in the oil group showed similar gross anatomy upon dissection. All were generally normal with no remarkable observations except for one animal exhibiting a lung hemorrhage, possibly due to a dosing error, and another animal with brown colored kidneys that were not in character with the kidney color seen in the other oil dosed rats.

Oil / TCE Group:

The animals dosed with both corn oil and TCE showed no remarkable pathology. All were observed to be normal except one animal that had atypical brown kidneys.

Lead / Oil Group:

All animals dosed with lead exhibited similar atypical gross anatomical changes. Included were one to two retracted testes, a marked decrease in testicular size, severely bloated stomachs (many that were four to five times normal in size), a nearly complete absence of mesenteric fat, palely colored livers, and hemorrhaging of the testicles. One lead-dosed animal was found dead the morning of the scheduled sacrifice.

TCE / Lead Group

The gross pathology of animals dosed with both lead and TCE was identical to the observations mentioned above in the lead/oil group. One animal from this group was found dead the morning before its scheduled sacrifice.

Histopathological Examination

Pathological examination revealed evidence of tissue toxicity in lead dosed and lead/TCE dosed animals, with control and TCE dosed animals exhibiting no discernible changes in the studied tissues. The lead and lead/TCE animal tissues showed few discernible differences. Testes, spleen, heart, liver, kidney/adrenal gland, and brain were harvested. Selected photographs of kidney, testes, and stomach may be found in Attachment B of Appendix I. The Pathology data tables may be found in Attachment C. A summary of this data is included in the text as Table 9. The pathologist's summary follows:

Lung: Seemingly random lung hemorrhages were seen in all test groups with five of ten, seven of ten, six of ten, and nine of ten animals exhibiting hemorrhages in the oil, TCE, lead, and lead/TCE dosage groups, respectively. These hemorrhages are believed to be associated with dose administration or dose volume.

Arterial Wall: The TCE dosed animals exhibited an increased incidence of chronic inflammation. This inflammation was observed in four of the ten TCE dosed animals, but was also seen in two of the control animals. The lead and lead/TCE dosed animals exhibited no such arterial wall changes.

Heart: No changes in the heart tissues were observed during pathological examination.

Kidneys: Morphological changes seen in the lead and lead/TCE animals include kidney inclusions that were not seen in the oil or TCE dosed animals, an increased incidence of coagulated proteins, and tubal dilation which was generally more severe in the medullary segments.

Adrenals: No changes were observed in the adrenal glands of any of the test or control animals.

Liver: Liver tissues were generally normal for all dosage groups. The lead/TCE group did exhibit vacuolization, generally in the periportal hepatocytes, in five of the ten animals, while two out of ten animals showed vacuolization in the oil and TCE groups, and one animal of the lead group showed vacuolization of the cells.

Single cell necrosis was observed in two out of ten animals in both the lead and lead/TCE test groups, with no such necrosis being observed in the liver tissue of the TCE and Oil dosed groups.

Glycogen stores were observed in the liver tissue of all dosage groups, with four of the ten oil dosed animals showing such storage and one animal each in the TCE, lead, and lead/TCE dosage groups.

Brain: Scattered vacuoles of the gray and white matter, presumably from lead administration, were observed in four of the lead dosed animals and three of the lead/TCE dosed animals. The Oil and TCE dosed animals had no observed vacuoles.

Stomach: Glandular dilation was found in all dosage groups with a more severe dilation seen in the lead and lead / TCE dosed animals. Massive necrosis of the stomach tissue was found to be exclusive to the lead and lead/TCE dosage groups with seven and eight animals exhibiting necrosis in the lead and lead/TCE groups respectively. Gastritis, hyperplasia, and peritonitis was observed in two of the ten animals in the lead/TCE dosage group. This was the only group demonstrating these lesions.

Small Intestine: Multifocal epithelial vacuoles were seen in two animals of the Lead dosage group. No other group had observable vacuolization.

Pancreas: No observable pathological change was seen in any dosage group.

Testis: Testicular atrophy was observed in one control animal, one Lead dosed animal, and three Lead/TCE dosed animals. Testicular necrosis was found in four of ten rats in the Lead group and two of ten rats in the Lead/TCE group. Neither the oil nor the TCE dosage groups exhibited testicular necrosis.

Lead Blood Values

The VMRCVM Toxicology Lab analyzed whole blood samples from twenty animals, five from each of the four dosage groups, for lead. The mean lead values of the five lead dosed rats tested was 5,034 ug/ml (503.4 ug/dl) with individual values ranging from 2,802 to 6,162 ug/ml. The mean lead value of the five lead/TCE-dosed rats was 4,226 ug/ml (422.6 ug/dl) with individual values ranging from 3,096 to 6,207 ug/ml. Mean blood lead levels in the corn oil treated group and TCE only group were 82 ug/ml (range 52-105 ug/ml) and 161 ug/ml (86-490 ug/ml), respectively. Actual data may be seen in Attachment F of Appendix 1.

TABLE 9

Pathology Summary Data: Score Averages

(Click on box to view table)

DISCUSSION

General

Lead and TCE both have toxic effects on many of the same organs. These include the kidneys, liver, and nervous system. It was expected that dosing of animals with lead and TCE in combination would exacerbate these toxic effects. Besides mutual target organs, other interactions led the investigator to believe that the combined dosing of these two compounds would have an additive or synergistic effect. For example, lead has been found to inhibit the formation of cytochrome P-450 in children (Alvares et al., 1975). Cytochrome P-450 is utilized in the metabolism of TCE. When this pathway is saturated, it is believed that a switch to glutathione conjugation may occur that could lead to production of a renal toxicant (Toxicological Profile for Trichloroethylene, 1995). The interactions expected, and the organ toxicity due to the dual administration of lead and TCE were not clearly seen in this study. However, the statistical differences found in the liver, brain, body, and kidney/adrenal weights does indicate the presence of both additive and protective interactions, the nature of which are unknown. Therefore, the hypothesis that lead and TCE would produce additive effects could not be clearly supported or refuted by the experimental results. Lead toxicity seems to have caused the vast majority of the toxic consequences seen in this study, overshadowing any minor effects that the concurrent addition of TCE may have caused. The Lead and Lead/TCE dosage groups did not substantially differ histopathologically or behaviorally. This indicates that lead is the principal toxicant that is accountable for most of the morphological changes in the observed tissues, although concurrent administration of TCE may have both increased and reduced the toxicity of lead on various organs.

Both lead and TCE are known to cause nerve damage. TCE in particular seems to target the cranial and facial nerves, as indicated by facial numbness, jaw weakness and facial discomfort (Toxicological Profile for Trichloroethylene, 1995). However, no indication of facial nerve numbness or dysfunction was found in either the TCE or the lead/TCE dosage groups as determined by vibrissae assessment and the pinna reflex .

Lung hemorrhages were seen in all dosage groups. These hemorrhages are believed to be dosing or dose volume related.

TCE and Lead

Changes in both the lead dosed and lead / TCE dosed rats were mostly associated with lead toxicity. Lead toxicity is known to cause decreases in body weight, and in the sizes of certain organs such as the testis. Lead can also cause the general malaise and listlessness seen in the study animals. The addition of trichloroethylene to the lead dosed animals on day nine of the study had a negative effect in body weight and cage removal ease. Further study would be necessary to ascertain whether or not continued administration of both compounds would have any significant effect beyond what was observed in this study. It is readily apparent to the author that a longer-term study would necessitate reduction of the lead carbonate dose in order to avoid lead-induced morbidity or mortality. It is not apparent what physiological changes caused the decreased body weight and cage removal ease findings although each compound clearly could be expected to cause changes in both of these measurements. It is also not clear what interactions are taking place that would account for the organ weight findings noted in the general discussion. These findings are conflicting as they are indicative of both additive and protective toxicity mechanisms.

No definitive pathological changes were seen in the lead/TCE dosage group that were attributable to the interaction of both lead and TCE in the body. The gastritis and peritonitis seen exclusively in two animals of this group could possibly be due to the interaction of both lead and TCE. TCE is a skin irritant and could be expected to cause irritation of the stomach lining while lead carbonate was found to have a definite negative reaction on stomach tissues.

Lead and TCE share similar target organs. These include the liver, kidneys, and nervous system. It was believed that these organs would be more susceptible to dual administration of these compounds due to their effect on the same organs. Despite common target organs, pathological data did not fully support the additive effect hypothesis.

TCE Group

Acute TCE intoxication was very apparent but had no discernible effect except in the measures of increased liver weight (a reversible hypertrophy adaptation), reduced time on the rotorod, increased foot splay, and general irritation as borne out by increased touch reactivity, click reaction, and tail pinch response. Sax (1997) reports that TCE can cause hallucinations or altered perceptions. Such altered perceptions could have played a role in these responses. Another possible cause of the reactivity changes may be due to the intoxicating effect of TCE and subsequent "hangover" stage normally associated solvent or alcohol intoxication. Prolonged exposure to TCE can cause headaches in humans (Sax NI, 1997). Such effects could have played a role in the irritability of the TCE dosed rats.

The excitement (running, hopping, and scratching) after dosing could be due to the irritating effect of the TCE on the throat and stomach. The human analogy

would be the burning sensation felt after swallowing a liquid with a very high alcohol content.

Fatalities due to increased cardiac excitement seen in studies where high doses of TCE were administered were not seen in this study as the doses provided were not within the range to elicit that particular response. The dose required to elicit cardiac arrhythmias is not exactly known. Morreale (1978) reported that one woman who accidentally swallowed 20 ml of TCE suffered a myocardial infarction within two hours. Dhuner et al., (1959) reported cardiac arrhythmias in two males after they ingested 350 and 500 ml of TCE. In this case, the arrhythmias continued for up to three days. Merck (1989) lists the LD₅₀ for TCE in rats at 4.92 ml/kg, with the cause of death being ventricular fibrillation.

A second facial nerve function test, the pinna reflex, was added to the FOB as human studies had implicated facial nerve paralysis with administration of high levels of TCE (Toxicological Profile for Trichloroethylene, 1995). Loss of facial nerve function was not noted, indicating that the dose or duration of TCE administration was not sufficient to elicit a response, that facial nerve paralysis is not a toxicity indicator in rats, or that lead administration overshadows any effect TCE may have on the facial nerve.

Hearing loss has been found in lab animals exposed to TCE at 2,500 ppm or higher in the air. Hearing loss was not expected in this study due to the utilization of oral exposure rather than atmospheric or inhalatory exposure. Thus, focused TCE exposure did not occur to the ear. The rats in the study showed no subjective signs of hearing loss.

The liver weight of animals dosed exclusively with TCE showed a statistical increase in weight. The cause of this enlargement was not apparent during

histopathologic examination. Research indicates that such an increase after TCE exposure is normally due to cellular hypertrophy, an adaptive rather than adverse effect, (Toxicological Profile for Trichloroethylene, 1995).

Liver damage is associated with chronic TCE exposure. Previous research has shown that liver enlargement is largely reversible (due to enlarged and vacuolated hepatocytes or peroxisomal proliferation) with no permanent cytotoxic effects. Hepatorenal failure was reported in one human overdose. Kidney nephrosis has only been reported following chronic exposure (Toxicological Profile for Trichloroethylene, 1995).

Lead Group

The changes seen in the present experiment were typical of lead change expected in lead dosed animals. Handling ease changes, reduced activity, posture changes, weakness in the limbs, and drooping eyelids are all typical symptoms of mid to advanced lead poisoning (Halstab MSDS, 1994, Toxicological Profile for Lead, 1993).

Reduced core body temperature punctuated lead's effect on the central nervous system of the lead-dosed animals. Changes in core temperature as a response to lead intoxication are not well documented in the literature. The reduction of core body temperature is most likely a symptom of advanced encephalopathy (Moore, 1997).

Food intake decreased markedly for the lead dosed animals, which hastened the animals' emaciated and weakened state. This was expected as the earliest symptoms of lead poisoning include poor appetite, GI disturbances, dullness, and general malaise (Halstab MSDS, 1994; Kumar, 1992; Lewis, 1991; OSHA lead standard; Toxicological Profile for Lead, 1993). By the end of the study the food

intake of the lead rats was near zero. Fat stores, including mesenteric fat, were most likely the only "food source" utilized by the lead-dosed animals during the final days of the experiment. This accounts for the absence of mesenteric fat seen during tissue harvest.

Pathologically, the lead dosage groups exhibited expected lead induced effects. The targets of lead include the blood, nervous system, GI tract, kidneys, male reproductive organs and liver (Kumar, 1992; Lewis, 1991). Signs of kidney effects were seen in this study, although no effects were seen in the liver other than a change to a pale color and reduced liver-to-brain weight. Toxicity to the testicles included hemorrhaging and necrosis. The hemorrhaging is not well documented in the literature, though atrophy and necrosis of the testicles is (Toxicological Profile for Lead, 1993).

The stomach tissues unexpectedly reacted to the administration of lead carbonate. The pathologist saw massive stomach necrosis in 70% and 80% of the Lead and Lead/TCE dosed rats, respectively. It is unclear how this adverse reaction was elicited as little information exists regarding the effects of lead carbonate on the gastrointestinal tract.

Lead carbonate caused a pre-cancerous condition within the tissues of the stomach in the lead-dosed animals. The pathologist who examined the tissues remarked that such lesions were extraordinary due to the short period of dosing. He further stated that these tissue anomalies were similar to those he had seen in animals dosed for great periods of time with cancer causing agents. Lead has been shown to be carcinogenic in animal models by oral administration (Amdur et al., 1991) though the IARC lists animal evidence as inadequate to identify lead as a cancer causing agent (Aldrich MSDS, 1997). Lead was listed as a

carcinogen in Hungary in 1993 (Aldrich MSDS, 1997). Further study of lead carbonate is needed regarding its role as a carcinogen by oral administration.

The reduction in core body temperature of the lead-dosed animals was the result of lead toxicity to the central nervous system and the encephalopathy that was well underway. The reduction in core body temperature was the likely cause of the testicular retraction. Another manifestations of lead's effect on the nervous system were vacuoles in the brain tissues of the lead and lead/TCE dosage groups respectively. The significance of these vacuoles is not understood, although they are believed to be a consequence of lead induced toxicity. Lead's deleterious effects on the nervous system, brain function included, are well-documented (Amdur et al., 1991).

Limitations of the FOB

The FOB checklist was the standard test of Moser et al. (1988), and was not custom tailored to the test chemicals utilized in this project. In hindsight, a more suitable list of observations could have revealed additional information. This list could have contained additional tests of the nervous system including the hopping and flexor tests, extensor thrust and olfactory reflexes, and cognitive or memory tests as all are potentially toxic endpoints for both lead and TCE.

Tests of short and long term memory would have been a valuable addition to the study since both lead and TCE have a record of affecting perception, memory, and reaction time. A maze or some other test for perception, memory, and/or reaction time would have been useful in determining whether the concurrent dosing of both of these neurotoxicants had any short or long term effect beyond the impact of each compound provided separately. The hopping reflex, being one of the most sensitive tests for detecting deficits (Oliver and Lorenz, 1983),

could have revealed interactions that were not detected by utilization of the reflex and reaction tests in the experimental protocol.

Certain observations, such as cage removal ease and handling reactivity, would also have benefited by alteration of the grading scale, as these measures did not include a median or normal value. Acceptable choices in the middle of the cage removal ease scale were either "easy" or "moderately difficult". Choices in the middle of the handling reactivity were similar with "moderately low" or "moderately high" listed. The standardized grading scale gave no options for "normal". The outcome of this quandary was that animals were scored the same even though the investigator could discern differences between test animals. In hindsight, a separate FOB measure for muscle tone, rather than relying solely on cage removal ease, may have also been beneficial.

Experimental Shortfalls & Enhancements for Future Study

As with any experiment, certain unforeseen problems arise during the course of a study that could impact the results obtained in a variety of ways. These shortfalls and problems are discussed below to assist anyone interpreting the information generated from this experiment and to assist future investigations.

Better control could be added. These controls could include the use of a certified diet, utilization of water that had been recently analyzed, and rotating the cages within the rack to minimize the effects of light exposure. Also, testing of the lead and TCE stock solutions prior to the onset of the study, once during the study, and at the conclusion of the study would verify the concentration of toxicants and offer additional study control. Testing of blood lead and TCE values during the study would also have some added value as well as liver and urinary chemistry

values. However, such testing could effect behavioral observations and would obviously add additional cost to the project in regard to both time and finances.

A change in the dosing regimen would have been useful in this project. Dosing of the rats should have been staggered into four groups rather than two. Having four groups of animals would reduce the quantity of time needed to perform FOB's on any given day. This would decrease error on the part of a tired investigator. Limiting the duration one person spent performing FOB's was one item listed in the FOB protocol that was not always followed.

A repeat of the study using much lower lead concentrations and or another lead compound would provide valuable information. The lead carbonate used in the study overshadowed any effects of the TCE and produced both mortality and morbidity.

Using separate gavage needles and syringes rather than merely washing them once per day would be a worthwhile study enhancement. Due to the lack of extra syringes and gavage needles, the syringes used for the lead and TCE rats were shared once concurrent administration began. Sharing also occurred due to syringe malfunctions late at night, with new syringes being unavailable at that time. Any TCE residue remaining in the lumen of the gavage needle or syringe would be expected to volatilize rapidly and not impact the next day's dosing. The lead carbonate however, tended to coat the inside of the needle and syringe and was difficult to remove by washing. Small traces of lead could potentially remain in the needle lumen that could be passed on to non-lead treated animals during the next dosing. While the quantity of lead delivered in this fashion would be negligible as compared to the quantities received by the animals in the lead-treated group, small elevations in blood lead values could potentially impact a project.

Due to miscommunication, the fact that the test animals had arrived at the facility and were out of quarantine was not conveyed to the investigator. The ultimate outcome of this miscommunication was that the study began several weeks later than anticipated. The older rats were larger, and therefore, required larger doses. Dose volume was at or near the acceptable limit for the test animals. Doses larger than the capacity of the animal's stomach would logically back-flow into the animal's mouth, resulting in incomplete dose administration, or could be aspirated into the lung, resulting in adverse consequences to the lung tissue.

The increased age of the rats may also have had some effect on the outcome of the study. Younger rats, like younger humans, are generally more susceptible to neurotoxic compounds, and therefore, may have exhibited more susceptibility to the joint administration of TCE and lead. The study protocol mandated the use of 60-day-old rats. The rats in the study were approximately 82 days of age at the beginning of the dosing period.

Due to the scope of the project, male rats were used for this experiment. A more comprehensive experiment would utilize both male and female animals to discern whether or not sex related differences exist in regards to the toxic consequences of lead and TCE.

The toxicity of lead carbonate itself may deserve additional testing as little information exists on this particular lead compound. On the other hand, the likelihood of exposure to large quantities of lead carbonate, as compared to other lead species, is minimal except for occupational exposure.

CONCLUSIONS AND IMPLICATIONS

Risk, defined here as the dose-response relationship of TCE and lead and the possibility of exposure (exposure risk), to the human population would be the same as the risk of succumbing to the toxic effects of either chemical alone. From this study, there is no definitive evidence that toxicity is increased during concurrent oral exposure of TCE and lead.

Trichloroethylene and lead do not appear to have a synergistic relationship and at best have a weak additive effect. An increase in cage removal ease and a decrease in body weight were the only statistically significant measures noted during the FOB's that were attributable to the concurrent oral administration of TCE and lead. However, organ-to-body and organ-to-brain weight analysis of the lead/TCE group revealed statistically significant changes in the weights of the liver, kidney, and brain that may indicate both an additive and protective toxicity mechanism.

SUMMARY

Forty albino rats were orally dosed with corn oil, corn oil and lead carbonate, corn oil and trichloroethylene, or corn oil, lead carbonate and trichloroethylene for approximately three weeks. The vast majority of the toxic effects seen during the study were due to the administration of lead carbonate, with an increase in cage removal ease and a decrease in body weight the being the only clear additive toxic consequence seen when trichloroethylene was given concurrently with lead. Organ weight ratio analysis did show differences between the lead and lead/TCE dosage groups for body, liver, kidney/adrenal, and brain weights. This indicates that the additional of TCE alters the effect of lead toxicity, both in an additive and protective fashion. The chemical interactions that caused these changes and their ultimate biological significance is not known.

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