Examination of the Acute Hepatic and Renal Toxicity from the Concurrent Oral Exposure to Chloroform and Trichloroethylene.

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Increased hepatic and renal toxicity occurs following concurrent exposure to carbon tetrachloride (CCl_4) and trichloroethylene (TCE) relative to that seen from each chemical alone. Consequently, the interaction of TCE and chloroform ($CHCl_3$), a close structural analogue of CCl_4, was examined. Adult male F-344 rats were gavaged with 0, 0.5 or 1.0 ml $CHCl_3$/kg, 0 or 1.0 ml TCE/kg or their factorial combinations in corn oil. Urine was collected at 0, 6, 12, 24, 36 and 48 hrs, and hepatotoxicity was assessed at 24 and 48 hrs. To examine the influence of gavage vehicle, additional rats were gavaged with 0, 0.5 or 1.0 ml $CHCl_3$/kg, 0 or 1.0 ml TCE/kg or their factorial combinations in an aqueous vehicle (10% Emulphor 620). Urine was collected at 0, 6, 12, 24, 36 and 48 hrs, and hepatotoxicity was assessed at 24 and 48 hrs. $CHCl_3$ alone caused hepatic and renal toxicity. At 48 hours post dosing, serum AST and LDH were observed to increase 88-146 fold and 45-48 fold, respectively, when CHCl3 was administered in corn oil. Increases in AST and LDH were significant when CHCl3 was administered in the aqueous solution; however, elevations were no more than 3-fold for either enzyme. Vehicle differences were also observed in increases of ALT and bile acids. Both were significantly elevated when administered in oil compared to the aqueous vehicle. Peak hepatotoxicity was observed to be 48 hours when CHCl3 was administered in oil whereas in the aqueous vehicle, 24 is greater or equal to 48 hours, based on elevations in ALT and bile acids.

CHCl3 appeared to be more nephrotoxic when administered in the oil vehicle compared to the aqueous. Histopathological lesions (both renal tubule degeneration and renal tubule necrosis) were more severe in the groups receiving CHCl3 in oil compared to the aqueous vehicle. In contrast to CHCl3, TCE alone was not overtly toxic to either organ. Concurrent exposure to both $CHCl_3$ and TCE produced significantly less toxicity relative to $CHCl_3$ alone. This was observed in serum enzymes at 24 and 48 hours and in urinary enzymes at 36 and 48 hours. Additionally, histological lesions were consistently less severe when CHCl3 was administered in the presence of TCE compared to CHCl3 alone. In conclusion, hepatic and renal toxicity were observed following $CHCl_3$ exposure and no effects were observed from TCE alone. CHCl3 was significantly more acutely hepatotoxic when administered in an oil than in an aqueous vehicle. Relative to $CHCl_3$ alone, serum and urinary indicators were significantly decreased following concurrent administration of $CHCl_3$ and TCE which appeared to be independent of the dosing vehicle.

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I. Introduction

Human contact with chemicals, environmentally or occupationally, seldom occurs as a single exposure to an individual substance. It is well known that we are exposed to many hundreds of compounds, both synthetic and natural, on a daily basis. As a result, knowledge of the toxicity from single chemical exposures, based on animal toxicity assessments, may not accurately predict the effect of multiple exposures to chemicals in conjunction with other confounding environmental factors. The pursuit of understanding and characterizing such interactions is an important one if we are to ultimately, accurately predict the human health consequences of exposure to multiple chemicals in the environment.

There is a pressing need to evaluate the toxicity of complex chemical mixtures and since drinking water is a potential media for multiple exposure to potentially large populations, assessing the public health risk associated with drinking water and the maintenance of drinking water quality are high priorities for environmental regulatory and public health agencies. Contaminants associated with the disinfectant process such as trihalomethanes (THMs) as well as other volatile organic compound (VOC) contaminants commonly found in water have been the focus of much interactive toxicity investigations.

Concurrent exposure to a binary mixture of carbon tetrachloride (CCl_4) and trichloroethylene (TCE) is known to result in a potentiation of toxicity (Simmons et al., 1992; Pessayre et al., 1982; Borzelleca et al., 1990; Steup et al., 1991). Also, simultaneous exposure to CCl_4 and chloroform ($CHCl_3$) has been shown to exert a more than additive effect (Borzelleca et al., 1990; Steup et al., 1991; Harris et al., 1982). Chlordecone (Hewitt et al., 1980) and ethanol (Strubelt, 1980) have both been found to potentiate the toxicity of CCl_4; additionally, both compounds are known to potentiate the toxicity of $CHCl_3$

(Hewitt et al., 1980).

Both $CHCl_3$ and TCE are commonly found at hazardous waste sites (Grisham, 1986) and as waste sites are potential sources of groundwater contamination, the simultaneous occurrence of the two compounds in drinking water is probable. Additionally, since $CHCl_3$ is formed by chlorination of surface waters for disinfection, this increases the importance of studying this particular VOC and its potential interactive capabilities with other drinking water contaminants.

TCE is known to potentiate CCl_4, and since CCl_4 and $CHCl_3$ are structurally similar compounds, it was the objective of this study to examine the possible interaction of $CHCl_3$ and TCE, to characterize the dose dependence of any observed interaction and to examine the effect over time. This would provide us with information on the behavior of two structurally similar binary mixtures.

Digestible oils are the classical vehicle for volatile, lipophilic compounds which are poorly soluble in water (Kim et al.,1990a). As a result, most health risk assessments are based on data derived from orally gavaged animal studies that utilized oil as the vehicle of administration. The oil vehicles were previously thought to be inert but there is increasing evidence that it may be a confounding factor. Alterations in physiological effects, target organ dose, absorption, distribution, metabolism, elimination and toxicity may result from the vehicle used to administer the chemical (Kim et al., 1990a; Condie et al., 1986). In addition, corn oil may alter the nutritional status of the animal which might alter the toxicity of the compound of interest (Condie et al., 1986). It has recently been shown that corn oil affects the activity of P450IIE by increasing the concentrations of the isozyme (Yoo et al., 1990). IIE is known to be the isozyme responsible for the metabolism of $CHCl_3$ (Brady et al., 1989) as well as other

VOCs.

Drinking water is one of the exposure routes of interest for these chemicals and evidence exists that the degree of toxicity observed from administration in an oil vehicle is different from that observed following administration in an aqueous vehicle in aliphatic halogenated hydrocarbon studies (Chieco et al., 1981; Kim et al., 1990a; Condie et al., 1986). Differences in rate and extent of uptake in pharmacokinetic studies have been observed for chlorinated hydrocarbons when administered in oil solutions as compared to aqueous solutions (Withey et al., 1983); thus, another aspect of this study was to compare the toxicity produced by CHCl3 and TCE alone and in combination when given in both oil and aqueous vehicles.

II. Literature Review

A. Introduction

The National Research Council (NRC)(1984) proposed a scheme to prioritize and identify chemicals for toxicity testing and hazard ranking. Important factors included in the prioritizing procedure include:

1. a chemical being present at the disposal site
2. its potential for release and migration through the environment
3. its potential for resulting in adverse human health effects
4. its recognition by the scientific community and or general public as being a chemical causing concern.

In addition, the Mitre Corporation (1983) compiled a list of chemicals or groups of chemicals that were reported as being present in the environment in the vicinity of the then 546 National Priority List (NPL) sites which had been designated by the Environmental Protection Agency (U.S. EPA) as mandated under Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) and are identified by U.S. EPA as being disposal sites causing documented environmental concern.

According to the Mitre study, trichloroethylene (TCE) was found at 129/546 NPL sites and chloroform (CHCl3) was found at 68/546. When ranked by frequency of occurrence, TCE was first followed by toluene, benzene, lead with chloroform ranked 5th. The Conservation Foundation (1984) also reported that EPA had evaluated approximately 900 sites for possible inclusion on the NPL for cleanup and CHCl3 and TCE were among the ten most commonly reported substances at these sites.

Considering the NRC criteria and the information regarding the prevalence of chloroform and trichloroethylene in the environment, these are two of the many contaminants which merit further toxicological evaluation.

Knowledge of the toxicity based on exposure via drinking water is of interest since it is a potential route for human exposure. In addition, the interaction of the two compounds is of importance since in many instances simultaneous exposure is plausible.

B. Chloroform: Occurrence in the Environment

CHCl3 occurs naturally; however, the majority in the environment is due to anthropogenic activities. In 1989, 587.6 million lbs of CHCl3 was produced in the U.S. (SOC 1955-1990). CHCl3 is primarily used to make fluorocarbon-22, a component of the cooling fluid for air-conditioners (ATSDR, 1989a). In addition it is used in the manufacturing of pesticides, dyes, fire extinguishers, spot removers, solvents for penicillin, vitamins, alkaloids, lacquers, floor polishes, waxes and adhesives (ATSDR, 1989a).

Releases into the environment occur from the pulp and paper manufacturing process, pharmaceutical and chemical manufacturing plants and from the chlorination of waste and drinking water (ATSDR, 1989a). CHCl3 was widely used in drug, cosmetic and food products until it was banned for those purposes by EPA in 1976 (Grisham, 1986).

Routes of Exposure

Human exposure to CHCl3 can occur via ingestion, inhalation or dermal contact; however, the exposure pathway of primary concern for the general population is ingestion. CHCl3 enters drinking water from the chlorination disinfection process as well as contamination of surface and groundwater from chemical spills and waste disposal sites. The major exposure is via ingestion of contaminated ground water (Grisham, 1986); however, occupationally, inhalation may be a major route. Inhalation and dermal, in addition to oral exposures, in the domestic environment are now of increasing concern for health-based risk assessments (Jo et al., 1990).

CHCl3 has been found in concentrations ranging from 0.7 to 540 ug/L with a mean concentration in all waters of 14 ug/L (U.S.EPA 1990). In a study monitoring CHCl3 in surface water supplies of 80 cities, values ranged from less than 0.3 to 311 ug/L (Grisham, 1986). A U.S. EPA survey of finished

drinking water found a 70.3% occurrence in samples taken from ground water supplies (Grisham, 1986). Higher concentrations of CHCl3 are found in finished drinking water that has high levels of humic/fulvic acids and algal growth, an alkaline pH, and relatively warm temperatures (U.S.EPA 1981). Alternate disinfection processes are available to lower the CHCl3 levels; however, they can be costly and unavailable to smaller water treatment facilities.

CHCl3 is one of several trihalomethanes (THMs) which are currently regulated collectively by EPA. In efforts to protect public health from drinking water exposure, EPA has promulgated a drinking water maximum contaminant level (MCL) for total THMs of 100 ppb (ug/L) as technically and economically feasible for municipal water supplies serving 10,000 people or more (ATSDR, 1989a).

Toxicological Profile

For both inhalation and ingestion routes of exposure, the systemic target organs for toxicity are the liver, kidneys and central nervous system (CNS) (ATSDR, 1989a). The liver and kidney are the most sensitive target organs with CNS toxicity observed only at very high exposures. Toxic hepatitis and liver enlargement were found in workers exposed by inhalation to CHCl3 at levels of 2 to 205 ppm over a period of 1 to 4 years (Bomski et al., 1967). A fatal oral dose of CHCl3 may be as little as 10 ml (211 mg/kg) for a 70 kg human according to Schroeder (1965). A wide range of oral LD_{50} values (lethal dose that kills 50% of the treated animals) have been reported; the ATSDR reports that the LD_{50} values for rats are 444 to 2000 mg CHCl3/kg and 118 to 1400 mg CHCl3/kg for mice depending on age, sex and strain.

In a chronic study, rats were gavaged with dosages greater or equal to 90 mg CHCl3/kg/day for 78 weeks and then observed for the following 33

weeks. Mortality was found to be dose related, due perhaps to liver toxicity (NCI 1976). In mice, gavaged with CHCl3 for 78 weeks and observed for 14-15 weeks, a dosage of 477 mg/kg/day resulted in decreased survival whereas dosages less than 238 mg CHCl3/kg/day did not (NCI 1976).

Levels of acute oral exposures found to produce any liver and kidney effects in humans are not known; however, a patient who ingested 2.6 to 11.6 g CHCl3/day in cough medicine for approximately 10 years developed hepatitis and necrosis (Wallace, 1950). It has been noted that confounding factors make these results difficult to interpret. The NOAEL (no observable adverse effect level) for humans is currently set at 1mg CHCl3/kg/day for liver and kidney toxicity (ATSDR, 1989a). The acute oral NOAEL and LOAEL (lowest observable adverse effect level) for target organ toxicity in mice are set at 18 and 30 mg CHCl3/kg, respectively (ATSDR, 1989a). Rats have been found to more resistant to CHCl3 toxicity with the acute NOAEL and LOAEL being 30 and 150 mg CHCl3/kg respectively (ATSDR, 1989a).

An epidemiological study uncovered a possible relationship between human exposure to chlorinated drinking water and cancer of the bladder, large intestine and rectum (U.S.EPA, 1985). CHCl3 is one of several volatile organic contaminants (VOCs) considered to have carcinogenic potential, but has yet to be identified as the main cause of cancer associated with chlorinated drinking water (ATSDR, 1989a).

Metabolism and Hepatotoxicity

When orally ingested, CHCl3 is absorbed rapidly through the intestinal mucosa, a dose-dependent first-pass effect occurs with pulmonary elimination of the unchanged compound (ATSDR, 1989a). Mink et al., (1986) administered single oral doses of 100 mg ^{14}C-CHCl3/kg in corn oil (16uCi/kg) to rats and mice. In rats the total recovery was 78% after 8 hours, 65% of which was expired as

unmetabolized CHCl3, 6.5% was expired as CO2, 2.6 % was excreted in urine and 3.6% was recovered in organs. A greater extent of metabolism was found in mice with 26% expired as unchanged CHCl3 and 49.6% as CO2 with total recovery of 94%.

Large interspecies and sex differences in metabolism, tissue distribution and covalent binding to tissue macromolecules make dose and species extrapolations difficult (ATSDR, 1989a). Dogs appeared to be the most sensitive species to CHCl3 induced hepatotoxicity. The liver is generally the most sensitive target organ but in some male mice strains such as the ICR, the kidneys were more susceptible.

In order to exert toxicity, CHCl3 must first be metabolized (Pohl, 1979). This occurs primarily in the liver and kidneys and has been found to be a saturable process. The exhalation of CO_2 is a major route of elimination with the concentration of CO_2 in the exhaled breath being dose dependent. The liver converts CHCl3 to CO2 approximately 5 times more rapidly than the kidney with the adipose, blood and muscle tissue exerting little influence on CHCl3 metabolism (Paul and Rubenstein, 1963; Pohl, 1979). The metabolic activity is localized in the microsomal fraction of liver homogenates and depends upon the presence of pyridine nucleotides (Pohl, 1979). The pretreatment of animals with inducing agents of liver microsomal cytochrome P-450 such as ethanol, DDT and phenobarbital markedly increased the hepatotoxic response to CHCl3 (Pohl, 1979). In contrast, when animals are pretreated with inhibitors of liver cytochrome P-450 such as SKF 525-A or piperonyl butoxide, a decrease in hepatotoxicity is found .

CHCl3 is known to deplete liver glutathione (GSH) in rats by a process which appears to involve a CHCl3 metabolite based on the observation that an increased amount of GSH depletion was found in rats pretreated with

phenobarbital (Pohl, 1979). GSH, a scavenger of electrophillic compounds which can potentially bind to tissues may react with the $CHCl_3$ metabolites and protect against any resulting hepatotoxicity. This role is supported by the observation that hepatotoxicity is potentiated when liver GSH is depleted by pretreatment with diethyl maleate (Pohl, 1979). GSH depletion in the liver is a rapid process. Within 1 hour after the interperitoneal (ip) administration of 0.2 ml $CHCl_3$/kg to phenobarbital-treated rats, it was observed that approximately 59% of the GSH was depleted (Ilett et al., 1973).

$CHCl_3$ metabolites have been reported to bond covalently in vivo to macromolecules such as protein, lipids or nucleic acids in the target organs where it causes tissue damage (Ilett et al., 1973). A correlation between the extent of covalent binding of $CHCl_3$ metabolites to tissue macromolecules and tissue damage and toxicity has been reported (Pohl, 1979). Pretreatment with inhibitors or inducers of P-450 decrease or increase, respectively, the amount of liver damage and the amount of covalent binding (Pohl, 1979). Pohl reports that the covalent binding of $CHCl_3$ metabolites displays a striking preference for the accumulation in the centrilobular hepatocyctes and in the proximal convoluted tubular cells in the kidney.

Histological studies have shown that $CHCl_3$ causes marked alterations of cellular organelles. The rough endoplasmic reticulum shows fragmentation and loss of ribosomes and a depression in protein synthesis is observed (Smuckler, 1976). This functional loss is believed to be in part responsible for the accumulation of fat seen in the liver based on the knowledge that excretion of lipid from the liver requires a carrier protein.

As early as 1915 it was suggested that phosgene ($COCl_2$) was a metabolite of $CHCl_3$ (Pohl, 1979). The potential mechanism for the biological formation of $COCl_2$ from $CHCl_3$ is outlined in Figure I. The trichloromethanol

(Cl3-C-OH) would not be expected to be stable and would spontaneously dechlorinate to COCl2 since the only trihalomethanol derivative found in the literature is trifluoromethanol (F3COH) a compound which spontaneously dehydrofluorinated at -20^0 C (Pohl, 1979). The COCl2 is extremely electrophillic since it is a di-acyl chloride and consequently will react with any nucleophile, nucleophilic thiol or hydroxyl and amino groups in protein and lipids and therefore be responsible for the covalent binding and the depletion of hepatic GSH.

Cysteine is known to react rapidly with COCl2 to form 2-oxothiazolidine-4-carboxylic acid (see Figure 2). Thus, using cysteine as a trapping agent, since COCl2 is reactive and volatile, it was concluded that phosgene was in fact a metabolite of CHCl3 (Pohl et al., 1977).

CDCl3 is known to be metabolized more slowly than CHCl3 suggesting that the cleavage of the C-H bond of the CHCl3 was the rate determining step in this enzymatic process (Pohl, 1979). This observation supported the proposed oxidative dechlorination mechanism for CHCl3. Other potential toxic metabolites of CHCl3 include the trichloromethyl radical and the dichloromethyl carbene (Pohl, 1979).

Nephrotoxicity

In addition to eliciting hepatotoxicity, CHCl3 is known to be nephrotoxic. The lesion is localized primarily in the proximal tubule and characterized by a marked increase in kidney weight and swelling of the tubular epithelium, which is associated with marked necrosis and tubular casts (Lock, 1989). Species differences have been observed in the renal metabolism of CHCl3 by the rat in contrast to the mouse or rabbit. In a study by Smith et al. (1985) rat renal cortical tissue was found to metabolize CHCl3 to CO2 and a reactive intermediate capable of binding to macromolecules. The extent of metabolism

in the rat tissue was substantially less than that observed previously with mice and rabbit renal tissue. In addition, an atmosphere of CO:oxygen (80:20) did not alter CHCl3 toxicity in rat renal cortical slices whereas CO did reduce CHCl3 toxicity in mouse renal cortical slices (Smith and Hook,1983).

Hepatic vs Renal Toxicity and Metabolism

It has been suggested that the renal metabolism of CHCl3 may not involve cytochrome P-450 as much as the hepatic metabolism due to the inability of CO to reduce CHCl3 toxicity in rat renal cortical slices (Pohl, 1979). This suggestion was supported by the observation that the severity of CHCl3 nephrotoxicity in vitro did not differ between Fischer-344 and Sprague-Dawley rats although the concentration of renal cytochrome P-450 has been shown to be two-fold greater in the Fischer-344 rat strain (Smith et al., 1985). Consequently, there appears to be a difference in the metabolism of CHCl3 in the kidney and liver microsomes. This is supported by the finding that in vivo CHCl3 did not deplete GSH in the kidney of the rat as it did in the liver (Docks and Krishna, 1976). Thus, the activation of CHCl3 by oxidative dechlorination may not occur in the rat kidney as it does in the liver since no kidney glutathione is depleted and it does not appear to be catalyzed by P-450 (Docks and Krishna, 1976).

Various ketonic solvents are capable of potentiating CHCl3-induced renal and hepatic toxicity in rats (Hewitt et al., 1983). In Sprague-Dawley rats, the potentiation of hepatic CHCl3 toxicity by 2-hexanone (methyl-n-butyl ketone) is associated with increased concentration of hepatic cytochrome P-450, increased bioactivation of CHCl3 to phosgene, and increased depletion of hepatic GSH (Branchflower and Pohl, 1981).

The findings that 2-hexanone treatment did not increase the total level of renal microsomal cytochrome P450 or lead to a depletion of total renal GSH

after the administration of CHCl3 suggested that the metabolism of CHCl3 to COCl2 may not occur in the kidney (Branchflower and Pohl, 1981). These data suggested that the mechanism of CHCl3-induced nephrotoxicity in rats may differ from that of hepatotoxicity (Smith et al., 1985). CHCl3-induced murine nephrotoxicity was only observed in males (Smith and Hook, 1983). The renal metabolism of CHCl3 to phosgene (trapped as 2-oxothiazolidine-4-carboxylic acid) requires the presence of O2 and NADPH and can be inhibited by CO supporting a role for cytochrome P-450 in this species (Lock, 1989). Administration of CHCl3 to male mice produced a depletion of renal GSH, indicating that phosgene is also formed in vivo as a metabolite in mouse kidney (Lock, 1989). Incubation of CHCl3 under an atmosphere of CO diminished the toxic effects showing further evidence of renal metabolism suggesting oxidative metabolism of CHCl3 by cytochrome P-450. The levels of renal cytochrome P-450 and associated monooxygenases were 3 to 5 times higher in the male vs female mice and pretreatment with diethyl maleate a GSH depletor, increased male but not female susceptability, suggesting sex differences in kidney metabolizing enzymes (Smith and Hook, 1983).

Reductive Metabolism

A large number of studies have suggested that the CHCl3 activation process depends on the microsomal drug metabolizing system and is influenced by the presence of oxygen (Pohl, 1979). In a study by Tomasi et al., (1985) about 30% of the metabolism of CHCl3 still occurs under anaerobic conditions, suggesting the presence of a reductive metabolic pathway. It is known that halomethanes compete with oxygen for electrons supplied at the cytochrome P-450 locus, thus metabolic reduction is favored at a low O2 tension (Tomasi et al., 1985). Tomasi found that several THMs (CHCl3 and chlorodibromomethane (CHClBr2) undergo reductive metabolism in isolated

hepatocytes incubated under nitrogen and give rise to free radical intermediates (Tomasi et al., 1985). Additionally, Testai and Vitozzi, (1986) recently demonstrated that the induction of rats with phenobarbital dramatically stimulates the hypoxic reductive bioactivation of CHCl3. The investigators concluded that the hypoxic bioactivation may have a role in CHCl3 toxicity. Testai and Vitozzi, (1992) have extensively studied both reductive and oxidative metabolism of CHCl3. The investigators have proposed 3 processes that produce chemically reactive species from chloroform. Two of these are oxidative, but differ in their affinity for CHCl3 and O_2. The products formed by these processes are not different and both bind to lipids and proteins and are efficiently scavenged by GSH. The difference is that one has been observed to be inhibited by lower O_2 tension than the other. The third process is inhibited by oxygen and gives rise to a protein-lipid binding ratio similar to that attributable to trichloromethyl radicals. This may represent the reductive formation of radicals in agreement with Tomasi (1985). The investigators propose that in the centrilobular region of the liver, the oxidative process is limited because of the low O_2 tension and allows the reductive pathway to proceed at the maximum level.

C. Trichloroethylene: Occurrence in the Environment

Approximately 200 million lbs of TCE are used annually in the United States (ATSDR, 1989b). Roughly 80% is used as a solvent for the vapor degreasing of fabricated metal parts and the remaining 20% for chemical intermediates, miscellaneous use and exports (ATSDR, 1989b). Most of the TCE is released into the atmosphere by evaporative losses from degreasing uses and leaching to groundwater from waste disposal landfills (Sabel and Clark,1984).

According to the Mitre study, trichloroethylene (TCE) was found at 129/546 NPL sites. When ranked by frequency of occurrence, TCE was first followed by toluene, benzene, lead with chloroform ranked 5th. The U.S. EPA Groundwater Supply survey of 945 water supplies nationwide using groundwater sources found TCE in 91 of the 945; the median level of the positive samples was ~1ppb with a single maximum level of 130 ppb (Westrick et al.,1984). Various federal and state surveys indicate that between 9 and 34% of the water supply sources in the U.S. may be contaminated with TCE (ATSDR, 1989b); thus, a main source of exposure occurs via ingestion of drinking water.

EPA has established a drinking water standard of 5 ppb which applies to community water systems and those which serve the same 25 or more persons for at least 6 months (ATSDR, 1989b). Occupational exposure is also of major concern and OSHA regulates exposure at an average concentration of 50 ppm in the air for an 8 hour day over a 40 hour week (ATSDR, 1989b).

Pharmacokinetics

TCE is an uncharged, nonpolar and highly lipophilic compound that is readily absorbed across the gastrointestinal mucosal barrier (ATSDR, 1989b). Absorption following oral exposure in both humans and animals is rapid and extensive. In a study by Prout et al., (1985), male Osborne-Mendel and Alderly

Park Wistar-derived rats and Swiss-Webster and B6C3F1 mice were exposed via a single intragastric administration to 10, 500, 1000, 2,000 mg/kg (^{14}C)TCE in corn oil, (dosing volume was 1.0 ml for rats and 0.5 ml for mice). Metabolism of TCE in the mouse was linear over the range of dosages whereas in the rat it became constant and independent of dosage at 1000 mg/kg and above. At 2000 mg/kg TCE, 78 % of the dose was eliminated unchanged in the rat, but only 14 % in the mice. Mice appear to have a greater capacity to metabolize TCE, explaining their greater susceptability to TCE toxicity and carcinogenicity (ATSDR, 1989b). No significant strain differences were observed throughout the study other than the B6C3F1 mice having a higher tolerance to doses over 1000 mg/kg TCE.

The absorption characteristics of TCE are different depending on whether the compound is administered to fasted or nonfasted animals. D'Souza et al., (1985) found that in fasted male Sprague-Dawley rats (300-350g), TCE administered in 50% aqueous suspension (PEG 400) was rapidly and completely absorbed with peak blood concentrations 6-10 minutes after dosing. In nonfasted animals the peak blood TCE concentration occurred at the same time but the peak levels were 2-3 times lower than those observed in fasted animals. Vehicle effects on absorption and peak blood concentration have also been observed with TCE (Withey et al.,1983).

Toxicological Profile and Hepatotoxicity

Animal studies indicate that the liver and kidney are the principal target organs of oral exposure to TCE (ATSDR, 1989b). In an acute study by Tucker et al., (1982) to determine the LD50 in male and female CD-1 mice, TCE was administered in 10% Emulphor by gavage in a volume of 0.01 ml/g body weight. No deaths occurred at dosages up to 750 mg/kg for females and 1250 mg/kg for males. There was 100% mortality at 5500 mg/kg for females and 6000 mg/kg in

males. Consequently, the oral LD50s were determined to be 2443 mg/kg for female mice and 2402 mg/kg for male mice (ATSDR, 1989b). Tucker et al.,1982, also conducted a subchronic study in which male weanling CD-1 mice were exposed to 24 or 240 mg TCE/kg/day in 10% Emulphor (0.01ml/g body wt) for 14 days by oral gavage. Increased liver weight was the only treatment-related effect. Based on this study, 240 mg/kg represents the NOAEL for hepatic and renal effects for acute oral exposure (ATSDR, 1989b). The dosages of 5,620 mg/kg/day and 3,160 mg/kg/day represent the LOAEL and NOAEL respectively for intermediate oral lethality in rats (ATSDR, 1989b).

In intermediate duration oral studies using male B6C3F1 mice, 2,400 mg TCE/kg by oral gavage in 10 ml/kg corn oil for 3 days caused centrilobular hepatocellular swelling with focal hepatocellular necrosis. Dosages of 500-1200 mg TCE/kg by oral gavage in 10 ml/kg corn oil caused increased relative liver weights in male B6C3F1 mice when administered 5 days/week for 3 weeks (Stott et al., 1982). The highest NOAEL for liver effects in mice is 1200 mg TCE/kg for intermediate duration oral exposure and the only dosage associated with necrosis was 2400 mg TCE/kg which is designated as the LOAEL (ATSDR, 1989b).

TCE is extensively metabolized (40-75% of the retained dose in humans) to trichloroethanol (TCE-OH), trichloroethanol-glucuronide and trichloroacetic acid (TCA) with minor metabolites including chloral hydrate monochloroacetic acid and n(hydroxyacetyl) aminoethanol.(ATSDR, 1989b) Three urinary metabolites account for ~90 % of the total TCE, TCA (15%), TCE-OH (12%) and conjugated TCE-OH(62%). (DeKant et al., 1984).

Rouisse and Chakrabarti (1986) pretreated adult male Sprague-Dawley rats with phenobarbital then administered 0 to 2 ml TCE/kg in corn oil ip 16 hours after fasting. A maximum of only 29% depletion of hepatic GSH occurred

within 2 hours when 1 ml TCE/kg was administered to phenobarbital-pretreated rats with concentrations returning to control values within 6 hours and increasing significantly 24 hours after the dose. These results suggest that the conjugation of hepatic glutathione with the electrophilic intermediate of TCE does not appear to be a major detoxification pathway for TCE therefore unimportant in TCE hepatotoxicity. In addition, administration of TCE resulted in a dose-dependent decrease of liver microsomal cytochrome P-450 content, reaching an apparent plateau at about 1.0 ml TCE/kg. At this saturation dosage the concentration of P-450 was decreased to 51% of controls. Rouisse and Chakrabarti (1986) suggested that the reactive intermediate produced by metabolic activation through the MFO system may form ligand complexes with microsomal cytochrome P-450 with simultaneous loss of mixed function oxidase activity. This study indicated a good correlation between decreased MFO activity or cytochrome P-450 content and the extent of liver injury. It was also shown that there exists an apparent saturable metabolism of TCE including its activation deactivation pathways which correspond to an apparent threshold (or minimal) toxic dose (eg ~1ml TCE/kg for its hepatotoxicity).

Nephrotoxicity

Chakrabarti and Tuchweber (1988) demonstrated that TCE exerts its acute nephrotoxic potential only at a very high dose level and produces nephrotoxic insult at the proximal tubule and possible glomerular regions of the rat kidney when exposed by inhalation or ip routes.

Treatment of male Fischer-344 rats (150-180g) with up to 11 mmol TCE/kg (0.99 ml TCE/kg) in corn oil per kg body weight (ip) did not influence any of the measured biochemical parameters of nephrotoxicity. Also significantly elevated levels of urinary N-acetyl-b-D-glucosaminidase (NAG) as well as serum urea nitrogen were observed at 24 hours only at the highest dose

level, 22mmol TCE/kg (1.97 ml TCE/kg) (Chakrabarti and Tuchweber, 1988). Consequently, TCE is known to exert hepatotoxicity well before any nephrotoxic damage is evident.

D. Oral Gavage Vehicles for Lipophilic Compounds

Health risk assessments of drinking water contaminants have typically been based on the results of toxicity studies in which the chemical is administered to the animal orally in an oil vehicle. There is increasing concern that the administration of an oil bolus will not only produce physiological effects but alter pharmacokinetic parameters such as the absorption or target organ dose and in so doing possibly alter the effects or introduce confounding factors which could significantly affect the accuracy and relevance of oral toxicity studies (Kim et al.,1990a).

Digestible oils have classically been used as vehicles or diluents for the volatile lipophilic VOCs because of their poor solubility in oil at concentrations used for toxicity tests (Kim et al.,1990a). An alternative to corn oil is the use of emulsifying agents to solubilze hydrophobic compounds in aqueous vehicles for oral gavage or drinking water exposure. Emulphor, for example, is a nontoxic liquid (Oral LD50=40g/kg in rats) prepared by polyoxyethylation of castor oil (GAF corp). It serves as a nonionic surfactant to produce an oil in water emulsion.

Following is a discussion of several studies demonstrating that the toxicity or pharmacokinetics of certain VOCs can be significantly affected by the vehicle in which the chemical is administered.

Withey et al. (1983) evaluated the relative uptake of equivalent doses in vegetable oil and aqueous dosing vehicles of CHCl3, TCE, dichloroethane and methylene chloride in male Wistar rats (~400g) in a dosing volume of 3-5ml.

Area under the curve (AUC) blood-concentration time curves and maximum concentration (Cmax) following intragastric dosing were assessed. Absorption was rapid in both vehicles but, the rate and extent of absorption varied greatly with vehicle. The peak concentration of CHCl3 in blood after administration was 39.3 ug/ml when administered in water and 5.9 ug/ml when administered in corn oil. This could be due to the faster partitioning of the CHCl3 with mucosal lipids from the aqueous vehicle relative to the oil vehicle since CHCl3 is a lipophilic compound. The time to peak blood concentration occurred slightly more rapidly with the aqueous vehicle (5.6 min) than the oil (6.0 min).

An even greater difference was observed in the case of TCE where the AUC ratio of aqueous to oil was 218 (CHCl3 was 8.70). Witheys study revealed that the most similar uptake between oil and aqueous, based on AUC ratios, occurred with methylene chloride which had the highest aqueous solubility whereas TCE was the most different and had the lowest aqueous solubility. Clearly the oil-water partition coefficient had an important bearing on the uptake from an oil solution into the GI tract showing that the vehicle can produce substantial effects by altering the systemic bioavailability of a lipophilic substance. Also the uptake of the VOCs was more pulsed from the oil than the aqueous possibly due to the dose breaking up into immiscible globules.

Chieco et al., (1981) studied the effects of different vehicles (corn oil and aqueous emulsion) when 200 mg 1,1 DCE/kg (dichloroethylene) was given to fed and fasted adult male Sprague-Dawley rats. In the fasted rats massive injury [(>100 fold increase of alanine aminotransferase (ALT) and aspartate aminotransferase (AST)] was observed in corn-oil treated animals with moderate injury (~15 fold increases of ALT and AST) in rats receiving the aqueous solution. In contrast the fed groups showed only slight liver injury in groups receiving both vehicles.

Exhalation concentrations were also measured every 15 min for 5 hours. In both fed and fasted animals, the most rapid decline occurred when the compound was administered in aqueous vehicle. The exhalation of 1,1 DCE has an initial rapid phase followed by a slower period occurring one hour following exposure. Exhalation was slightly delayed in the fasted groups in the early phase whereas in the later phase, the vehicle appeared to have a marked influence on 1,1 DCE exhalation. The half life of the 1,1 DCE after the 2nd hour was 103 min for the corn oil vs 42 min for the aqueous. There was little influence of the vehicle on the total percent of the dose exhaled; however, the half life ($t_{1/2}$) values reflect a marked influence of the administrative vehicle.

Chieco found that the hepatotoxicity of 1,1 DCE in fasted animals was diminished when the compound was given in the aqueous solution, and based on the exhalation of the parent compound can be correlated with the more rapid clearance of 1,1 DCE from the body. Thus Chieco demonstrated that both fasting and vehicles can greatly affect the absorption and toxicity.

Condie et al. (1986) evaluated the effects of gavage vehicle on the severity of the subchronic hepatotoxicity of CCl4 in male and female CD-1 mice gavaged with 0, 1.2, 12 or 120 mg CCl4/kg in either corn oil or 1% Tween-60 for 90 days (5 days/week). The study revealed greater hepatotoxicity in the mice that received CCl4 administered in corn oil. In this study the data indicate the NOAEL based on subchronic data in corn oil was 1.2 mg CCl4/kg whereas the NOAEL for aqueous was 12 mg CCl4/kg. In a subchronic study by Bull et al. (1986), 270 mg/kg/day CHCl3 was administered in corn oil or 2% Emulphor for 90 days in 1ml/100g dosing volume by stomach tube to male and female B6C3F1 mice. CHCl3 administered in the corn oil vehicle resulted in greater hepatotoxicity than when the chemical was given in an aqueous vehicle.

In a subacute study by Merrick et al. (1989) concentrations up to 2400

mg/kg/day of TCE were administered to male and female B6C3F1 mice in either corn oil or a 20% aqueous solution of emulphor (1ml/100g body weight) for 4 weeks. Within the first week, all male mice exposed to 2400 mg/kg/day died and 8/12 of the female mice died when exposed to 1800 mg/kg/day administered in Emulphor. In contrast, only a few male mice died when exposed to TCE in oil. TCE in Emulphor proved to be more lethal but less hepatotoxic because those that survived for 4 weeks appeared to have less hepatocellular necrosis compared to the animals receiving TCE in oil. Additionally, serum enzyme levels (AST, ALT and LDH) were significantly elevated in the corn oil group compared to the aqueous group. Merrick concludes that the high oil water partition coefficient of TCE for corn oil delays the rate and extent of GI uptake reducing the peak blood levels over administration in aqueous vehicle, affecting both bioavailability and toxicity.

Kim et al. (1990a) assessed the acute hepatotoxicity of 0-1000 mg CCl4/kg in male Sprague-Dawley rats (200-230g). CCl4 was administered by oral gavage in corn oil or 0.25% Emulphor (5ml/kg) as well as pure chemical (3-4 ul) and water (6-8 ml dosing volume). Animals were fasted 18 hours prior to chemical administration. Dose response increases in serum enzyme levels were observed in each vehicle; however, serum enzymes were consistently lower in groups given CCl4 in oil. Kim noted that the use of aqueous Emulphor emulsions appears more appropriate in acute toxicity studies of VOC drinking water contaminants since similar responses of toxicity were observed in the groups receiving CCl4 in Emulphor, when compared to the pure chemical or the administration in water. In an additional study by Kim et al. (1990b), the effects of dosing vehicle on the pharmacokinetics of orally administered CCl4 in rats were examined. Fasted 200-230g male Sprague-Dawley rats received 25 mg CCl4/kg in corn oil, Emulphor, water and as pure chemical, in the same

dosing volumes as the previous study. Differences in the acute hepatotoxicity had been observed in the companion study by Kim et al (1990a) and the objective was to relate the pharmacokinetics of the vehicle effects, bioavailability and absorption to the effects seen in the acute study. Corn oil markedly delayed the absorption of CCl4 from the GI tract and produced secondary peaks in the blood concentration vs time profile. There was a high degree of correlation of both Cmax and AUC measured from 0 to 120 min with hepatotoxicity. Kim found that CCl4 was less acutely hepatotoxic in corn oil due to delay and prolongation of CCl4 absorption resulting in a marked decrease in the concentration of the chemical in the arterial blood and liver.

Corn oil likely acts as a reservoir in the GI tract slowing the systemic absorption and consequently altering the overall pharmacokinetics, metabolism and resulting toxicity in comparison to chemical administration in an aqueous solution. An understanding of the importance of studying the toxic effects of a contaminant in the media in which exposure is most likely to occur is becoming a more relevant issue as the influences of vehicles are being observed.

E. Chemical Interactions

Toxicological assessment of chemical interactions is a difficult task. It is important not only to assess the biological effects of exposure to chemical mixtures but also the pharmacokinetic influences as well as determining the mechanisms of interaction. This information could aid in the interpretation of the chemical interactions which would allow for tissue dose and species extrapolations and ultimately more accurate assessments of the health risk of multiple chemical exposure.

It has been noted that CHCl3 toxicity is influenced by substances that alter microsomal enzyme activity or hepatic GSH levels (ATSDR, 1989a). Disulfuram, an inhibitor of microsomal drug metabolizing enzymes has been observed to decrease the hepatotoxicity of chloroform (Jorgensen et al., 1988).

Andersen et al. (1987) evaluated the in vivo interaction of TCE and 1,1 dichloroethylene (DCE) in vivo in male Fischer-344 rats. Both compounds are metabolized by single saturable oxidative pathways with high affinity substrate binding. Rats were exposed for six hours to 0, 100, 200, 300 and 400 ppm 1,1 DCE. A dramatic increase in AST levels was observed above the 100 ppm concentration. When coexposed to both 1,1 DCE and TCE, TCE was held constant at 500 ppm and 1,1 DCE concentrations were 300, 713 and 1718 ppm. In the presence of TCE, the concentration response curves were shifted to the right indicating a inhibition of 1,1 DCE metabolism. Consequently, higher concentrations of 1,1 DCE in the presence of TCE were required to produce the same elevation in AST compared to 1,1 DCE alone. An excellent correspondence between predicted and observed behavior was noted when the inhibition was assumed to be purely competitive.

Interactive hepatotoxicity of CHCl3 and CCl4 has been examined in male Long-Evans rats (210-300g) by Harris et al. (1982). Chemicals were given ip as

30% solutions in corn oil and rats were killed 24 hours later. When administered alone 0.1 ml CCl4/kg lowered hepatic P450 concentrations and 0.2 ml CHCl3/kg had no effect. Marked histological changes were seen in animals receiving both chemicals while only modest changes were seen in animals receiving either chemical alone. Both compounds, singly and in combination, failed to alter hepatic GSH concentrations. Harris concluded that by several criteria of hepatotoxicity including serum ALT, triglycerides, calcium concentration and histopathological changes, that subthreshold doses of CHCl3 and CCl4 are hepatotoxic when given together.

Borzellecca et al. (1990) examined the interactions of two pairs of VOCS using male Sprague-Dawley rats (200-250g). CCl4 (0-400 mg/kg) and CHCl3 (0-700 mg/kg) as well as CCl4 (0-400 mg/kg) and TCE (0-400 mg/kg) or the individual chemicals were administered by oral gavage in 5% Emulphor. Borzellecca found that the chemicals in combination displayed a synergistic hepatic response for sorbitol dehydrogenase (SDH), ALT and AST, with the peak plasma enzyme activity occurring at approximately 36 hours. In this study, animals were fasted for 16-24 hours presurgery, then cannulated via the carotid artery to allow for serial blood sampling. A total of 8 samples were obtained (650ul each). Blood withdrawal and fasting may have stressed the animals causing additional elevations in enzyme levels.

Steup et al. (1991) examined the interactions of CCl4 and CHCl3 as well as CCl4 and TCE using drinking water for the route of exposure for TCE and CHCl3 and following with an ip administration of CCl4. Male Fischer-344 rats (175-250g) were pretreated for 3 days by administration of TCE or CHCl3 in their drinking water followed by a challenge dose of 1mmol CCl4/kg in corn oil via ip injection. TCE and CHCl3 alone produced no significant increase in serum ALT activity. When rats were pretreated with TCE and CHCl3, significant

increases in the plasma ALT were observed in response to the CCl4 challenge. Steup also conducted strain comparisons and investigated the most appropriate plasma enzyme marker for hepatotoxicity. His results suggest that the Fischer-344 rat is more sensitive to VOCs and VOC interactions than Sprague-Dawley rats. The indicator most accurately representing hepatotoxicity and tissue injury was ALT, which provided the clearest evidence of the interactive toxicity consistent with the histological evidence.

Hewitt et al. (1983) demonstrated that the exposure of male Sprague-Dawley rats (150-300g) to a variety of ketones can potentiate the hepatotoxicity of a subsequent exposure to a haloalkane such as CHCl3. Animals were given 15 mmole/kg po of ketones (acetone, 2-butanone, 2-pentanone, 2-hexanone or 2-hepatatone) in corn oil (10 ml/kg) then challenged 18 hours later with 0.5 or 0.75 ml CHCl3/kg ip in corn oil (4ml/kg); liver injury was examined 24 hours later. The relationship between carbon skeleton length of ketonic solvents and potentiation of CHCl3 induced hepatotoxicity was examined. None of the ketones from acetone to heptanone produced appreciable liver injury as neither did CHCl3 alone. A marked degree of liver injury was produced by CHCl3 in ketone pretreated rats. The severity of ketone potentiated CHCl3-induced liver damage correlated with the ketone carbon chain length. The authors suggest that while carbon chain length is a factor in determining the potentiating capacity of a ketone, other factors are likely to be involved such as metabolite biotransformation. Additionally, a positive correlation between liver weight and ketone chain length was observed. It has been shown that several of the ketonic solvents are capable of inducing hepatic mixed function oxidase activity (Branchflower and Pohl, 1981) which might produce hypertrophy of the endoplasmic reticulum and increase relative liver weight. Branchflower and Pohl (1981) proposed that methyl n-butyl ketone potentiated chloroform

hepatotoxicity by increasing P450 levels thereby enhancing chloroform metabolism to phosgene and decreasing GSH levels.

Ethanol pretreatment is known to enhance the toxicity of hepatotoxic agents (Strubelt, 1980). Substances that are potentiated by ethanol are metabolized in the liver to toxic metabolites and include CCl4, CHCl3 and TCE (Strubelt , 1980). This supports the hypothesis that an induction or activation of the hepatic microsomal drug metabolizing system is responsible for ethanol-induced potentiation of hepatotoxicity. Acute or chronic ethanol administration is known to enhance the MFO activity, to enhance the in vitro and in vivo covalent binding of $^{14}CCl4$ to liver microsomal protein and to accelerate the in vitro biotransformation of $^{14}CCl4$ to $^{14}CO2$ (Strubelt, 1980).

Pessayre et al. (1982) reported that ip administration of 64 ul CCl4/kg or 1ml TCE/kg (in 0.5 ml liquid paraffin), did not significantly increase serum ALT levels and did not significantly decrease hepatic cytochrome P-450 concentrations measured 24 hours later in male Sprague-Dawley rats (180-220g). However, when CCl4 and TCE were administered simultaneously, ALT activity increased markedly and a 55% decrease in the hepatic cytochrome P-450 concentrations was observed. The mechanism of TCE potentiation of CCl4 has been under investigation. Pessayre found an association between lipid peroxidation and hepatotoxicity which was proposed as the mechanism by which TCE potentiates CCl4. In contrast, a study by Keflas and Stacy (1989) showed that lipid peroxidation was not responsible for the TCE-induced potentiation.

F. Methods of Toxicological Evaluation: Indicators of Hepatic Damage

Toxicological damage to organs can manifest itself in both functional and morphological changes. The morphological or pathological processes involved in the injury will largely determine the functional or biochemical alterations as a result of injury (Plaa and Hewitt,1982). The four categories of tests that are useful specifically for evaluation of hepatic injury include histological analysis, serum enzymes, hepatic excretory function and alterations in chemical constituents (Plaa and Hewitt, 1982).

The hepatic lesions induced by cytotoxic injury occur primarily in the hepatocyte (Tyson et al., 1985). The injury may be characterized by steatosis, the accumulation of lipids or necrosis, the appearance of degenerative processes leading to the death of the cell, a small group of cells (focal necrosis) or zonal damage or damage to virtually all cells in the lobule (massive necrosis) (Plaa and Hewitt, 1982). Cholestatic responses also may occur involving changes in the bile flow resulting in alterations of bile salts and bilirubin concentrations (Plaa and Hewitt, 1982). Hepatomegaly, enlargement of the liver, may be due to increased numbers of cells (hyperplasia) or an increase in cell volume (hypertrophy) (Tyson et al., 1985) Many, but not all, compounds cause hepatomegaly and there is not always a correlation of hepatomegaly and hepatotoxicity; thus, liver enlargement is viewed by some as an adaptive, functional response rather than a toxic response (Mitoma, 1985).

In order to classify hepatic lesions, two schemes of the parenchymal mass have been used to relate the hepatocytes with the vascular supply and biliary system (Plaa and Hewitt, 1982). Based on the classical scheme, the hexagonal lobule, hepatic lesions have been classified as centrilobular, midzonal or periportal according to location relative to the terminal hepatic

venule and the portal triad (Plaa and Hewitt, 1982). The acinar model has corresponding regions of functional specificity and metabolic activity designated as zones that are not incompatible with the classical description.

Serum enzyme levels have become the standard measure of hepatic injury (Zimmerman, 1982). They are useful for early detection of liver damage and for serial sampling over time. The significance of biochemical abnormalities as indices of hepatic injury should be judged by the supporting evidence of histological damage since there are limitations to their uses. Levels may increase as a result of leakage from cells due to systemic effects other than hepatic necrosis and in addition some forms of hepatic injury do not produce elevations in serum enzyme levels (Zimmerman, 1982).

Zimmerman proposed the following classification for serum enzymes in the detection of hepatic injury:

1. Those reflecting cholestatic alterations such as 5' Nucleotidase (5'NUC) and Alkaline phosphatase (ALK PH).
2. Those reflecting cytotoxic injury subdivided as follows into those:
   a) that are nonspecific and can reflect extrahepatic damage, such as aspartate aminotransferase (AST) and lactate dehydrogenase (LDH).
   b) that are mainly in liver, such as alanine aminotransferase (ALT).
   c) that are exclusively in liver, such as sorbitol dehydrogenase (SDH).

The investigator states that the enzymes 5'Nuc, AST, LDH and ALT are the most useful for the detection of hepatic toxicity. They are sensitive to hepatic injury and reach high concentrations in serum in response to parenchymal injury.

The transaminases are the most widely employed and generally accepted serum measure of hepatic injury despite equal or greater hepatospecificity of several other enzymes released to blood as a result of hepatic injury. They are easily measured and have high degree of sensitivity to

acute hepatic injury which accounts for the general dependence on the transaminases as a means of detecting toxic liver injury. AST, a cytosolic enzyme is found in several tissues besides liver, including muscle, myocardial and kidney whereas ALT occurs mainly or almost exclusively in the liver (Zimmerman, 1982). In virtually all mammalian species, AST is a sensitive measure of acute hepatic necrosis when accompanied by the elevation of other liver specific enzymes (Zimmerman, 1982). Plaa and Hewitt (1982) note that ALT shows a very good correlation between serum levels and severity of histological lesion since agents that produce severe necrotic lesions also produce pronounced ALT elevation. Thus, ALT allows detection of the presence of liver injury in addition to an estimation of the severity of the lesion by the degree of ALT elevation.

SDH, like AST, is a cytoplasmic enzyme and is relatively specific for hepatocellular damage (Plaa and Hewitt, 1982). In several studies, elevation of SDH has been detected before other enzymes, thus it appears to be one of the most sensitive enzymatic indexes when damage is minimal; however, it appears to be less sensitive than histopathological evaluation (Plaa and Hewitt, 1982).

LDH is widely distributed in mammalian tissue with the myocardium, kidney, liver, and muscle being especially rich. It is ubiquitous and it can be released from erythrocytes with hemolysis; thus, it is highly variable in response to a variety of conditions (Tyson et al., 1985). Total serum enzyme activity of LDH may not be as useful as isozyme analysis because changes in the isozymes may go undetected. More specificity can be achieved but it does require specific equipment and a more complex analysis (Mitoma, 1985). LDH isoenzymes have been used in the evaluation of organ damage and are specific to liver and kidney tissue thus providing the benefit of differentiation of

organ damage (Plaa and Hewitt, 1982). LDH5 is found in rats with liver injury and LDH1 and 2 are found in rats with renal damage (Plaa and Hewitt, 1982).

Gopinath et al. (1980) reported on the usefulness of bile acid clearance and serum bile acid concentrations in rats with acute liver injury for detection of biliary tract proliferation or obstruction. 5' NUC is another useful indicator of obstructive liver injury as it is localized in the membranes of the hepatocyte and bile ductular cells (Plaa and Hewitt, 1982). Alkaline phosphatase is found in the kidney cortex, intestinal mucosa, bone, placenta and the liver which contains relatively little in a variety of species including rats (Dooley, 1982).

Chemicals that are hepatotoxic can alter the activity of the hepatic mixed function oxidase system, thereby changing the rate at which xenobiotics are metabolized (Plaa and Hewitt, 1982). P450 plays a central role in drug oxidation and thus serves as the binding site for several chemicals that are oxidized in the liver to reactive metabolites (Plaa and Hewitt, 1982) including $CHCl_3$ (Brady et al., 1989) and TCE (Moslen, et al., 1990). The terminal oxidase of the mixed function oxidase system, cytochrome P-450, is particularly susceptible to damage and depletion and is an increasingly popular index of damage to the endoplasmic reticulum (Plaa and Hewitt, 1982).

Finally, the measurement of urea, cholesterol, plasma protein and blood glucose have been found to be insensitive and nonspecific in the monitoring of hepatic injury (Zimmerman, 1982).

Indicators of Renal Damage

Histological examination of the kidney has been considered a useful method for detecting chemically induced renal damage and is important in determining the site of damage (Ohata et al., 1987). Renal pathological lesions caused by chemical or biological agents primarily result in nephrosis encompassing a range of changes from cell swelling to overt necrosis and

desquamation of tubule cells (Tyson et al., 1985). Seventy-five percent of the water the kidney filters is reabsorbed in the proximal convoluted tubule of the nephron and consequently is found to be particularly vulnerable (Price, 1982). The resulting concentration of the remaining fluid, coupled with the high blood flow thru the kidney (25% cardiac output) makes it particularly vulnerable to toxic attack (Price, 1982).

Two of the more commonly used **serum indicators** for renal injury in animal toxicity studies are BUN and creatinine. Increases in BUN concentrations can result from any damage to the nephron whether the lesion occurs in the glomeruli or tubules or whether it affects renal blood supply and thus indirectly the glomerular filtration rate (Tyson et al., 1985) BUN provides no insight into site or nature of the lesion and is generally considered to be a relatively insensitive marker for renal injury (Tyson et al., 1985). Berndt (1976) noted that measurements of BUN are commonly used to assess glomerular function. BUN increases as filtration slows or ceases. Serum creatinine is derived from creatinine in skeletal muscle and is excreted by the kidneys mainly by glomerular filtration Serum creatinine values do not increase significantly in renal disease until kidney function is considerably impaired such as severe toxic nephrosis (Tyson et al., 1985). Plasma creatinine levels parallel those of BUN and are frequently used as a marker for glomerular filtration for acute renal failure due to xenobiotic-induced damage as well as chronic failure (Berndt, 1976).

Ohata et al. (1987) noted that increases in **urinary enzyme** excretion precedes other common indicators of nephrotoxicity including the increase in urinary protein, the decrease in creatinine clearance, the elevation of serum creatinine and blood urea nitrogen (BUN) and that the increase in urinary excretion of enzymes correlate well with the onset of histological changes in

renal damage caused by several nephrotoxic agents. Compared to serum enzymes, urinary indicators in general have received little attention as diagnostic indicators (Price, 1982). The principal reason for the slow development is the difficulty involved with assays of enzymes in a fluid which varies in volume, composition and which is a hostile environment for many enzymes (Price, 1982).

The principal source of urinary enzymes is the kidney while the bladder and urogenital tract contribute comparatively little (Price, 1982). Urinary enzymes have been used in nephrotoxicity studies as noninvasive tests for renal damage and elucidation of the primary site of damage along the nephron. In addition, as excretion is dose related they can be used to assess the nephrotoxicity of xenobiotics (Price, 1982). Urinary enzymes are most valuable during the early acute stages of renal disease but also can provide information on the rate of recovery (Price, 1982). Periodic determination by urinalysis is required because the increases are transient (Ohata et al., 1987). When an enzyme is chosen as a diagnostic indicator, its subcellular and regional location should be known, it should be stable in urine and maintain activity under storage conditions for a reasonable time at 4^0C (Price, 1982). In addition, control levels of enzymes in normal serum and urine should be known.

Typically, urine is collected from animals housed in metabolism cages and should be collected over ice. The samples should be filtered or centrifuged to remove hair, debris, fecal and food contamination because of the presence of enzymes in gut flora and commercial food pellets (Price, 1982). Most importantly urinary enzyme and constituent analysis should be done on samples collected over a finite period of time and related to urinary creatinine to reduce intra- and inter-individual variability (Tyson et al., 1985).

It is important to be aware of conditions that may affect results such as

collection and sampling site for serum enzyme analysis (Neptun et al., 1985) and collection conditions for urine enzyme analysis. The routine use of urinalysis in rodent studies for the assessment of kidney function continues to be limited by a need to improve collection methods and handling (Tyson et al., 1985; Sey et al., 1991).

It is known that there are changes in cortical and medullary metabolism which suggests the differentiation of enzymes along the nephron (Guder and Ross, 1984); however, no enzyme has proven to be entirely specific to a particular region (Price, 1982). AST activity is greatest in the ascending limb and distal convoluted tubule with considerable activity also present in the proximal tubule. AST, a mitochondrial enzyme is found in the cytosol and follows the mitochondrial density along the nephron (Guder and Ross, 1984). Mitochondria are unevenly distributed along the nephron with the greatest density in the thick ascending limb of the loop of Henle and the proximal convoluted tubule (Guder and Ross, 1984). ALT is present in all structures in relatively small amounts except the proximal straight tubule where it is much higher and exceeds that of AST (Guder and Ross, 1984).

ALK PH and 5' NUC are brush border enzymes in the proximal tubule. Their role is not well understood; it seems to be similar to that of intestinal brush border hydrolases, namely to split peptides polysaccharides and other molecules into reabsorbable fragments. Activity increases towards the end of the proximal tubule (Guder and Ross, 1984). In addition, LDH is associated with the brush border of the proximal tubular cells (Guder and Ross, 1984). Elevation in LDH isoenzymes in the rat kidney are useful for determining renal damage at an early stage. The distribution of isoenzymes is not homogeneous along the nephron allowing selectively damaged regions to be differentiated (Ohata et al., 1987).

In a study by Stonard et al. (1987) the urinary enzyme NAG among others was measured as an indicator of papillary damage. When damage occurred, NAG was elevated; thus, it is a useful diagnostic enzymes for that region (Price, 1982). Sustained increases in urine volume and NAG activity suggest a defect in concentrating ability and therefore also indicative of papillary damage (Price, 1982).

Measurement of **nonenzymatic urinary indicators** such as total protein, glucose, pH, albumin and osmolality can prove useful information in assessing the functional, as opposed to biochemical, state of the kidney (Price, 1982). Urine volume is used to indicate changes in kidney load and function. In acute renal malfunction, urine volume is either normal or reduced (oliguria) and many well known chemicals such as CCl4 can produce this effect. Frequently however, the initial oliguria may be superceded by a diuretic phase (Tyson et al., 1985). Urine volume, specific gravity and color are somewhat interdependent in that as volume increases color is paler and specific gravity decreases (Tyson et al., 1985). In the assessment of the toxicological significance of biochemical changes, it is important to have sufficient background data on normal ranges of values to be expected in the strain and species used (Tyson et al., 1985). Also, controls should accompany samples and laboratory historical data can provide a good reference for normal ranges.

The use of urinary enzymes to monitor glomerular function suffers two disadvantages. First there are few examples of enzymes which are specific markers for glomeruli. Second, glomerular damage allows the passage of normally excluded plasma enzymes into the tubular fluid which, if not reabsorbed, appear in the urine (Price, 1982). The measurement of urinary excretion of proteins is, however, a useful procedure for the assessment of the glomerular integrity. The selective permeability of the glomerular membrane is

an important aspect of normal renal function. When the selective permeability is decreased, a common occurrence in renal failure, excessive amounts of plasma proteins are excreted (Berndt, 1976). The first proteins to leak through the glomerulus are albumin and alpha-globulin followed by other serum proteins as the damage increases (Tyson et al., 1985). Not all proteinurias should be interpreted as evidence of glomerular dysfunction, although any time an experimental animal excretes increased amounts of protein in urine, glomerular dysfunction is suspected (Berndt, 1976). In addition, increased protein could be due to failure of tubular reabsorption and not glomerular damage. The differentiation of glomerular from tubular proteinuria may be accomplished by separation of high and low molecular weight proteins by gel filtration (Tyson et al., 1985).

Changes in urinary pH can also be nonspecific reflecting adaption to nonrenal physiologic abnormalities. Exposure of urine to air for extended periods of time can render pH measurements useless (Tyson et al., 1985). Consequently, care should be taken in the measurement and interpretation of urine pH. It has been noted that nephrotoxic agents reduce the animal's ability to concentrate urine, changes in urine osmolality occur early but osmolality is not nephro-specific and usually occurs as a prelude to other events (Berndt, 1976).

The kidney contains the highest activities of enzymes involved with GSH synthesis and degradation, all nephron cells studied contain millimolar concentration of GSH with highest levels in the proximal straight tubule (Guder and Ross, 1984). Thus the proximal tubule is thought to be the site of GSH linked reactions. Cytochrome P-450 is also most prevalent in the proximal straight tubule. Thus the coincidence of enzymes and cofactors together with enzymes of GSH metabolism, points to the proximal tubule as an important site

of renal drug metabolism (Guder and Ross, 1984).

III. Materials and Methods

Animals

Male Fischer-344 rats (Charles River Laboratories, Raleigh, NC), 62-65 days old on arrival were housed 2/cage in polyethylene shoebox cages with heat-treated pine-shavings as bedding. Two days later rats were moved to individual plastic Nalgene metabolism cages (Nalgene Corp., Rochester, NY) for the duration of the experiment including a 3 day acclimation period prior to chemical treatment.

Tap water and feed (Rodent Chow no. FO165 BioServ, Results Brand, Frenchtown NJ) were provided *ad libitum*. Animals were maintained under conditions of controlled temperature of 22 +/- 2^0 C, relative humidity of 50 +/- 10% and a 12 hour light-dark cycle with light from 0600 to 1800. Feed and water consumption and body weight were monitored daily. At the end of the 3 day acclimation period, rats were assigned by body weight to one of 12 treatment groups.

Dosing

The treatment groups included dosages of 0, 0.5 or 1.0 ml CHCl3/kg, 0 or 1.0 ml TCE/kg or their factorial combinations in either corn oil or aqueous vehicle. The solutions were administered at a constant volume of 10 ml/kg for both the oil and aqueous vehicles. The individual chemicals and their binary combinations were administered via a single oral gavage. CHCl3 (Aldrich, Milwaukee, WI, 99.8+% spectrophotometric grade, Lot No. 04201CX) and TCE (Aldrich, Milwaukee, WI, 99+% spectrophotometric grade, Lot No. 00723AP) were prepared in either corn oil (Sigma, St. Louis, MO, Lot No. 80H0835) or 10% Emulphor (GAF Corp., Linden, NJ, EL-620 Lot No. 3053) in deionized water.

Control animals received only corn oil or 10% Emulphor. One day prior to dosing, Emulphor-620 and deionized water were heated separately to 40^0 C. A 10% solution of Emulphor was prepared by shaking the deionized water and Emulphor vigorously and maintaining the solution at 40^0 C. Dosing solutions were prepared in crimp top vials, sealed and shaken then placed in the water bath for 2 hours before being removed, covered and left at room temperature until the following morning when animals were dosed. Chemicals were administered in a constant volume of 10 ml/kg by oral gavage with 20-gauge, 2.5 inch ball-tipped gavage needles attached to a 3.0 ml disposable syringe. Control animals received vehicles in the same volume.

Safety Precautions

All dosing solutions were made under the ventilated hood in the laboratory. During animal dosing, personnel were required to wear respirators with cartridges for removing organic vapors in addition to protective lab coats and gloves. Dosing was performed in an animal room under negative pressure ventilation which is restricted for VOC studies.

Experimental Design

Twenty-four hours post exposure, rats were tail bled. Blood was collected using serum separator tubes (Microtainer, Becton-Dickinson, Lincoln Park, NJ Lot No. 1A671) held on ice for 30 minutes then centrifuged at 17,000 x g for 4 minutes. Serum was collected and frozen at -80^0 C until analyzed. At 48 hours rats were weighed then anesthetized with sodium pentobarbital (Nembutal, Abbot Lab., Chicago, Ill), injected at 50 mg/kg ip. They were bled through the abdominal aorta using serum separator tubes (Becton-Dickinson, Lincoln Park, NJ). The blood was held on ice for 30 minutes then centrifuged at 1000 x g for 30 minutes. Serum was collected and frozen at -80^0 C until analyzed.

Urine Clinical Chemistry

Animals were housed in stainless-steel wire-bottomed plastic Nalgene metabolism cages (Nalgene Corp., Rochester, NY) with a separating funnel below to separate feces and allow urine to run down into a collecting tube. Refrigerant ice packs (Polyform Packer Corp., Wheeler, IL) surrounded the collection tube to keep the samples cold. A baseline urine sample was collected for 12 hours prior to dosing in addition to collection from 0 to 6 hours, 6 to 12 hours, 12 to 24 hours 24 to 36 hours and 36 to 48 hours post gavage. Samples were centrifuged at 800 x g for 10 minutes at 4^0C then poured into clean tubes.

Osmolality, pH and volume were measured immediately following urine collection. Remaining urine samples were held at 4^0C and within 24 hours of the urine collection, urine chemistry profiles were determined by automated procedures using a Centrifichem-500 centrifugal analyzer (Baker Instruments Co., Allentown, PA) and appropriate reagent kits. Analysis inlcuded determination of activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALKPH) and lactate dehydrogenase (LDH) and the concentrations of creatinine (CRE), urea nitrogen (UN) and total protein. Remaining urine samples were frozen at -40^0C as is or in 10% glycerol (Sigma St Louis, MO, Lot No. 10H0549).

Serum Clinical Chemistry

Serum chemistry profiles were determined by automated procedures using a Centrifichem-500 centrifugal analyzer. Analysis included determination of activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALKPH), lactate dehydrogenase (LDH), sorbitol dehydrogenase (SDH), 5' nucleotidase (5' NUC) and the concentrations of

creatinine (CRE), blood urea nitrogen (BUN), bile acids, triglycerides (TRIG), total billirubin (TBILI) on the 48 hour samples and the determination of ALT and bile acids on the 24 hour samples. Parameters for the 24 hour samples were selected based on expected sensitivity from preliminary experimental data and quantity of serum required for the analysis.

Histology

The liver and kidneys of each rat were excised, rinsed in physiological saline, blotted gently and weighed. Relative organ weights (ratio of organ weight to sacrifice body weight) were calculated for each rat. Horizontal and longitudinal sections of the right and left kidney, respectively, and a sample from the left hepatic lobe of the liver were taken for open histopathological examination (Society of Toxicologic Pathologists, 1986; Prasse et al., 1986). Tissue sections were fixed in 10% phosphate-buffered formalin then hematoxylin- and eosin- stained. Hepatocellular degeneration and necrosis were evaluated and graded separately for lobular location (centrilobular, periportal, mid-zonal) and severity according to the following criteria: none; minimal (one to several hepatocytes affected); mild (no more than one-fourth of the hepatocytes in the affected zone involved); moderate (expansion of the number of damaged hepatocytes up to one-half of the affected zone); and marked (over one-half of the lobular architecture involved) (Simmons et al., 1988). Kidney nephropathy was graded on a scale of increasing severity as none, minimal (scattered foci of tubular cell regeneration with affected tubules having an increased number of cells with intense cytoplasmic and nuclear staining with up to 25% of the cortex affected); mild (increased extent and number of regenerative foci, basement membranes surrounding regenerative tubules and within glomeruli thickened, tubular protein casts with 25-50% of the cortex affected); moderate (further increases in number and severity of

regenerative foci, increased number of tubular protein casts, increased glomerular damage with adhesions between the glomerular tuft and perietal epithelial cytoplasm with 50-75% of the cortex affected); and marked (diffuse involvement of cortex, "end-stage" kidney with sufficient impairment to result in either secondary hyperparathyroidism or contribute to death).

Statistical Methods

Analysis of variance (ANOVA) (SAS Institute, Inc., 1989) was used to assess the statistical significance of CHCl3, TCE and vehicle effects on serum and urinary indicators of toxicity and organ and body weights. In all cases, 0.05 was the level of significance. A Bartlett's test was performed prior to analysis to test for the assumption of homogeneity of variance. The criterion of significance for the homoscedasticity tests was $p \leq 0.001$ (Anderson and McLean, 1974). If the assumption was not satisfied, a log scale transformation was performed to correct for heterogeneity.

A four-way analysis of variance with the factors CHCl3, TCE, vehicle and experiment and their interactions was performed on each variable. Experiment was included as a blocking factor to remove variability due to the effect of experimental replication. In the majority of analyses of serum and urinary indicators and organ and body weights, at least one interaction with vehicle was significant. Therefore, it was necessary to perform a three-way analysis of variance with CHCl3, TCE and experiment factors on the data for each vehicle.

In the three-way analyses of variance where CHCl3 and TCE did not interact significantly, a Ryan(1959 and 1969)-Einot(1975)-Welsh(1977) (REW) multiple comparison procedure was performed to assess the differences among CHCl3 doses if that main effect was significant ($p<0.05$).

In the cases where CHCl3 and TCE interacted significantly, additional analyses were performed. A two-way analysis of variance was done on the

data at each level of CHCl3 to determine if the difference in TCE was significant. Also, a two-way analysis of variance was done on the data at each level of TCE to assess differences in CHCl3. In these last two analyses if the CHCl3 effect was significant, a REW multiple comparison procedure was done to determine which dose levels were different from each other.

The serum indicators LDH, CRE, BUN, NUC and AST in addition to body and organ weights were single independent measurements whereas the bile acids and ALT were repeated measures at both 24 and 48 hours. All urinary indicators were measured over 48 hours at a total of 6 time intervals. These repeated measures parameters were treated separately at each time point and consequently analyzed using the same approach as the single measure serum indicators.

A 5-way multivariate analysis of variance for repeated measures with the factors CHCl3, TCE, vehicle, time, experiment and their interactions was performed on both ALT and bile acids which were measured at 24 and 48 hours. Experiment was included as a blocking factor to remove variability due to the effect of experimental replication. Vehicle interactions were significant; therefore, it was necessary to perform a three-way analysis of variance to examine the effects of time on each combination of CHCl3 and TCE for each vehicle.

IV. Results

Acute Hepatic Response

Chloroform Toxicity

The administration of 0.5 and 1.0 ml $CHCl_3$/kg alone in corn oil produced a significant decrease, compared to controls, in animal body weight 48 hours post dosing (Figure 3). Consequently relative liver weight (liver weight/body weight) was assessed rather than absolute liver weight. In both the 0.5 and 1.0 ml $CHCl_3$/kg dosage groups there was a significant elevation in relative liver weight over the control animals (Figure 5). The administration of 0.5 and 1.0 ml $CHCl_3$/kg in the aqueous vehicle produced a significant decrease in body weight at 48 hours with 1.0 ml causing a significant decrease over 0.5 ml $CHCl_3$/kg (Figure 4). Relative liver weight, in the aqueous group, was significantly decreased by 1.0 ml $CHCl_3$/kg (Figure 6).

Serum enzyme indicators used for the detection of hepatic damage included LDH, AST and 5'Nuc which were measured at 48 hours post dosing. Additionally, ALT and bile acids were measured at both 24 and 48 hours post dosing in order to assess the effect of time on the observed toxicity. LDH and 5' Nuc were significantly elevated at 48 hours when 0.5 and 1.0 ml $CHCl_3$/kg alone were administered in oil (Figures 7 and 11). AST enzyme levels were also significantly elevated over the control group at 0.5 and 1.0 ml $CHCl_3$/kg in oil with 0.5 ml $CHCl_3$/kg causing significantly greater elevations of AST than 1.0 ml $CHCl_3$/kg (Figure 9). When administered in the aqueous vehicle, significant elevations in LDH and AST resulted from the 1.0 ml $CHCl_3$/kg dosage only (Figures 8 and 10). No significant differences in 5' Nuc were observed following administration of $CHCl_3$ in the aqueous vehicle (Figure 12).

At 24 hours, ALT was significantly elevated over the control group by 0.5 and 1.0 ml $CHCl_3$/kg alone in oil (Figures 13 and 14). Similarly, at 48 hours, ALT was significantly elevated by both 0.5 and 1.0 ml $CHCl_3$/kg in oil with 0.5 ml $CHCl_3$/kg causing significantly greater levels of ALT than 1.0 ml $CHCl_3$/kg (Figures 13 and 14). In the aqueous vehicle, both 0.5 and 1.0 ml $CHCl_3$/kg alone, compared to the controls, caused significantly greater levels of ALT at 24 hours (Figures 15 and 16). At 48 hours, 1.0 ml $CHCl_3$/kg produced a significant elevation over controls (Figure 16). Bile acids were significantly elevated by 0.5 and 1.0 ml $CHCl_3$/kg at both 24 and 48 hours when administered in oil. Conversely, in the aqueous vehicle, $CHCl_3$ caused no significant elevations in bile acids at either time (Figures 17 - 20).

Trichloroethylene Toxicity

The administration of 1ml TCE/kg alone caused no significant changes in body weight in either oil or aqueous solutions (Figures 3 and 4). Relative liver weight was significantly elevated over controls in those groups receiving TCE alone in oil (Figure 5). However, TCE had no effect on relative liver weight following administration in the aqueous vehicle (Figure 6).

Levels of LDH, AST and 5'Nuc were not altered by 1.0 ml TCE/kg alone in either oil or aqueous vehicle when compared to the control group (Figures 7-12). No significant differences in ALT levels at 24 and 48 hours were observed following administration of 1.0 ml TCE/kg alone in either vehicle when compared to the control group (Figures 13-16). Bile acids showed no significant differences at 24 or 48 hours relative to controls when 1.0 ml TCE/kg was administered in the aqueous vehicle (Figures 19 and 20) or at 24 hours in the oil vehicle (Figures 17 and 18). However, 1.0 ml TCE/kg alone caused a significant elevation over the controls at 48 hours in the oil vehicle (Figures 17 and 18). In addition, both 0.5 and 1.0 ml TCE/kg at 48 hours were significantly

elevated compared to the 24 hour values when the compound was administered in the aqueous vehicle only (Figures 19-20).

Vehicle Effects

Direct vehicle comparisons were performed on the levels of ALT and bile acids at 24 and 48 hours following administration of CHCl3 alone in oil and aqueous vehicles. At 24 hours, ALT levels as a result of 1.0 ml CHCl3/kg were significantly different in the two vehicles with oil causing higher elevations. (Figure 21). At 48 hours, ALT was significantly greater when CHCl3 was administered in the oil than in the aqueous vehicle at both 0.5 and 1.0 ml CHCl3/kg (Figure 22). Significant vehicle differences were observed in bile acids at 24 hours with 0.5 ml CHCl3/kg in oil causing greater toxicity (Figure 23). At 48 hours both 0.5 and 1.0 ml CHCl3/kg administered in oil produced significantly greater levels of bile acids than aqueous vehicle administration (Figure 24).

Differences in vehicle effects were examined indirectly in the additional serum enzymes by examining the ratio of the response in oil to the response in aqueous administration. Oil to aqueous ratios for LDH, AST and 5'Nuc are presented in Table 2. Overall, the oil/aqueous ratios for AST and LDH show greater elevation in these enzymes by all dose groups administered in the oil vehicle. CHCl3 alone resulted in significant increases when compared to controls in 5'Nuc when administered in oil but not when administered in the aqueous vehicle (Figures 11 and 12). At 0.5 ml CHCl3/kg oil gavage resulted in LDH levels more than 40-fold greater than aqueous gavage. At 1.0 ml CHCl3/kg in oil, LDH was elevated by almost 18-fold relative to aqueous. Similarly AST was elevated more than 110-fold when 0.5 ml CHCl3 was administered, and 26-fold when 1.0 ml CHCl3/kg was administered, in oil

compared to aqueous. TCE alone produced almost a 4-fold elevation in LDH when administered in oil than aqueous; however, vehicle differences were not apparent in AST and 5'Nuc levels when 1.0 ml TCE was administered in either vehicle. Vehicle effects were not as great when CHCl3 is administered in the presence of TCE; however, LDH and AST were elevated by CHCl3 in the presence of TCE in the oil compared to the aqueous vehicle.

Interactions of Chloroform and Trichloroethylene

When both CHCl3 and TCE were administered there were no significant differences from control animals in body weight at either concentration of CHCl3 or in either vehicle (Figures 3 and 4). Similarly with relative liver weight, when both chemicals were administered in either oil or aqueous vehicles no differences from controls were observed (Figures 5 and 6).

Serum levels of LDH, AST and 5'Nuc were significantly decreased when both CHCl3 and TCE were administered in oil relative to CHCl3 alone in oil. This occurred at both 0.5 and 1.0 ml CHCl3/kg (Figures 7, 9 and 11). In the aqueous vehicle, LDH, AST and 5'Nuc levels following both CHCl3 and TCE administration were not significantly different from CHCl3 alone (Figures 8, 10 and 12).

ALT levels at 24 hours were significantly lower in the groups receiving both CHCl3 and TCE in oil than CHCl3 alone in oil. This was observed in both the 0.5 and 1.0 ml CHCl3/kg dosage groups (Figures 13 and 14). At 48 hours, 0.5 ml CHCl3/kg plus TCE in oil produced a significant decrease in ALT levels relative to CHCl3 alone in oil. When administered in the aqueous vehicle, both 0.5 and 1.0 ml CHCl3/kg plus TCE significantly decreased ALT levels at both 24 and 48 hours compared to CHCl3 alone (Figures 15 and 16).

In oil, 0.5 ml CHCl3/kg plus TCE caused a significant decrease in bile acids at both 24 and 48 hours relative to CHCl3 alone. Administration of 1.0 ml

$CHCl_3$/kg plus TCE produced a significant decrease at 48 hours compared to $CHCl_3$ alone. Although not statistically significant, the trend of decreased toxicity was also observed at 24 hours (Figures 17 and 18). In the aqueous vehicle, a similar trend was apparent although none of the changes were significant (Figures 19 and 20).

Histopathological Results

When $CHCl_3$ alone was administered in oil, the histopathological examination at 48 hours revealed that 0.5 ml $CHCl_3$/kg caused mild (4/6) and moderate (1/6) centrilobular hepatic necrosis. In oil, 1.0 ml $CHCl_3$/kg caused mild (6/6) centrilobular hepatic necrosis. In contrast, both 0.5 and 1.0 ml $CHCl_3$/kg administered in the aqueous vehicle produced no observable hepatic necrosis (Table 5).

In oil, 0.5 ml $CHCl_3$ caused mild (1/6), moderate (3/6) and marked (2/6) centrilobular vacuolar degeneration and 1.0 ml $CHCl_3$/kg caused moderate (3/6) and marked (3/6) damage. When administered in the aqueous vehicle, 0.5 ml $CHCl_3$/kg caused minimal (5/6) vacuolar degeneration and 1.0 ml $CHCl_3$/kg caused minimal (4/5) and mild (1/5) damage (Table 6). TCE alone did not produce any hepatic necrosis or vacuolar degeneration in either vehicle (Tables 5 and 6).

When animals received oil administration of the chemicals, an apparent reduction in lesion severity was observed in the dose groups receiving both $CHCl_3$ and TCE when compared to the $CHCl_3$ alone dose group. When 0.5 ml $CHCl_3$/kg is administered in oil in the presence of TCE no (3/6) and minimal (3/6) lesions were observed whereas in the absence of TCE, mild (4/6) and moderate (1/6) hepatic necrotic lesions were observed. Exposure to 1.0 ml $CHCl_3$/kg in the presence of TCE produced minimal (6/6) lesions whereas in the absence of TCE, mild (6/6) necrotic lesions were observed (Table 5).

In the presence of TCE, 0.5 ml CHCl3/kg in oil produced lesions ranging form minimal to moderate whereas in the absence of TCE, mild to marked centrilobular vacuolar degeneration was observed. In the presence of TCE, 1.0 ml CHCl3/kg in oil produced mild (3/6) and moderate (3/6) lesions whereas in the absence of TCE, moderate (3/6) and marked (3/6) lesions were observed (Table 6). Centrilobular necrosis and vacuolar degeneration caused by the aqueous administration of all dose groups is less severe with no apparent observable interaction of CHCl3 and TCE (Tables 5 and 6).

In all dose groups, the vehicle effect is apparent. CHCl3, both in the absence and presence of TCE appears to produce more severe centrilobular necrosis and vacuolar degeneration when administered in the oil than aqueous vehicle. This is most apparent in the observed hepatocellular necrosis where minimal, mild and moderate lesions were observable when the chemicals were administered in oil whereas no necrosis was observed when chemicals were administered in the aqueous vehicle (Tables 5 and 6).

B. Acute Renal Response

Chloroform Toxicity

When CHCl3 alone was administered in the oil vehicle, both 0.5 and 1.0 ml/kg resulted in significant increases in relative kidney weight over the control group (Figure 25). When CHCl3 alone was administered in the aqueous vehicle, only 1.0 ml CHCl3/kg resulted in a significant elevation in relative kidney weight (Figure 26).

Serum indicators used for the detection of renal damage included BUN and CRE which were measured at 48 hours. Both CRE and BUN were significantly elevated by the 0.5 and 1.0 ml CHCl3/kg dosage when administered in the oil vehicle (Figures 27 and 29). In the aqueous vehicle,

BUN was significantly elevated only by the 1.0 ml CHCl3/kg dosage (Figure 28) while CRE levels were not elevated at either CHCl3 dosage (Figure 30).

Due to the investigative nature of this study a number of urinary enzymes and indicators of renal damage were measured, including urinary AST, ALT and LDH as well as urea nitrogen (UN), urinary creatinine (UCRE), total protein (TPR) and osmolality plus urine volume and pH.

Each of these parameters was measured in a baseline sample in addition to 6, 12, 24, 36 and 48 hours post dosing. As a first step in determining the significant differences between dosing groups, an initial analysis of variance was performed to determine if there was any significant effect due to the vehicle of administration. In order to reduce the number of analyses conducted, if vehicle was not significant, further analysis was done without distinguishing between vehicles, giving an effective N of 12/group. In those cases where vehicle was a significant factor, dose groups were separated by vehicle before proceeding with analysis (N=6/group). Consequently, in reporting the results, if no vehicle distinction is made, it can be assumed that any findings apply to both vehicles combined, otherwise, vehicle differences will be discussed.

AST was significantly elevated by 0.5 and 1.0 ml CHCl3/kg alone administered in both oil and aqueous vehicles at 36 and 48 hours post dosing (Figures 31 and 32). ALT was significantly elevated at 36 and 48 hours (vehicle combined) by both 0.5 and 1.0 ml CHCl3/kg alone (Figures 33 and 34). LDH was significantly elevated over control values at 24 hours in both vehicles, 36 hours (vehicle combined) and 48 hours in both vehicles by 0.5 and 1.0 ml CHCl3 (Figures 35 and 36).

UCRE (Figures 37 and 38) was significantly decreased by 0.5 ml CHCl3/kg alone (vehicle combined) at 6 hours. At 12 hours in both oil and aqueous vehicles, both 0.5 and 1.0 ml CHCl3/kg caused a decrease compared

to the control group. At 24, 36 and 48 hours both 0.5 and 1.0 ml CHCl3/kg decreased the UCRE level as compared to the control values (vehicle combined).

UN (Figures 39 and 40) was significantly elevated at 6 hours by the 1.0 ml CHCl3/kg dose (vehicle combined). At 12 hours, both 0.5 and 1.0 ml CHCl3/kg caused an elevation in UN when administered in oil whereas in aqueous vehicle only 1.0 ml CHCl3/kg elevated the UN level significantly above control. At 24 hours, oil administration of 1.0 ml CHC3/kg caused a significant elevation of UN while aqueous administration of 0.5 ml CHCl3/kg caused a significant decrease. At 36 hours, 1.0 ml CHCl3/kg in both oil and aqueous vehicles produced a significant elevation.

TPR (Figures 41 and 42) increased at 36 and 48 hours in the dose groups receiving 0.5 and 1.0 ml CHCl3/kg alone in oil. At 48 hours 0.5 ml CHCl3/kg resulted in significantly greater TPR than 1.0 ml CHCl3/kg. When administered in the aqueous vehicle, 0.5 ml CHCl3/kg caused a significant decrease at 24 hours in TPR when compared to controls. At 36 and 48 hours 1.0 ml CHCl3/kg in aqueous vehicle produced a significant elevation.

The osmolality (Figures 43 and 44) was decreased by 0.5 and 1.0 ml CHCl3/kg alone at 24 and 36 hours (vehicle combined) and at 48 hours in both vehicles. In addition, in the aqueous vehicle, decreases occurred at 12 hours in the 0.5 and 1.0 ml CHCl3/kg dosage groups. Urine volume (Figures 45 and 46) was significantly increased by 0.5 and 1.0 ml CHCl3/kg alone at 6 hours when CHCl3 was administered in oil. Elevations occurred by 0.5 and 1.0 ml CHCl3/kg at 12 and 24 hours (vehicle combined). At 48 hours when 0.5 ml CHCl3/kg was administered in an aqueous vehicle, a significant increase in volume was observed. pH (Figures 47 and 48) was decreased at 24 hours by 0.5 and 1.0 ml CHCl3/kg alone administered in oil and aqueous vehicles.

Additionally, similar results were observed at 6, 36 and 48 hours in the vehicle combined analysis.

Trichloroethylene Toxicity

In both oil and aqueous vehicles, 1.0 ml TCE/kg resulted in significant increases in relative kidney weight (Figures 25 and 26). No differences in CRE and BUN were observed at 48 hours in either vehicle when TCE alone was administered relative to control (Figures 27-30). Administration of 1.0 ml TCE/kg alone in either vehicle did not alter the enzyme levels of AST or ALT at any time (Figures 31-34). LDH was unaffected by TCE when administered in oil; however, a significant elevation occurred at 24 hours when TCE alone was administered in the aqueous vehicle (Figure 36).

Administration of 1.0 ml TCE/kg alone caused minimal or no response in the urinary indicators at each time point when compared to the control group. The following are the indicators which were significantly altered by TCE administration: UCRE at 12 hours when TCE was administered in aqueous vehicle (Figure 38); TPR at 36 and 48 hours when TCE was administered in an oil vehicle (Figure 41); osmolality at 12 hours when TCE was administered in aqueous vehicle (Figure 44); and, pH at 6 and 24 hours when TCE was administered in an aqueous vehicle (Figure 48).

Vehicle Comparisons

No direct vehicle comparisons were made on the indicators of renal damage; however, oil/aqueous ratios of urinary enzymes AST and LDH are presented in Tables 3 and 4, respectively. The urine data were variable, even in the control values; consequently, the following is a discussion of the ratios obtained; however, the investigator feels that vehicle effects as observed in the urinary enzymes are very weak. At 24 hours, LDH was elevated more than 2-fold in the aqueous vehicle when compared to the oil administration of 0.5 and

1.0 ml CHCl3/kg alone. At 36 hours however, the vehicle effect was reversed with 0.5 ml CHCl3/kg alone resulted in almost a 2-fold elevation in LDH when administered in oil. The response of LDH at 36 hours to 1.0 ml CHCl3 alone did not appear to be affected by vehicle. At 48 hours, 0.5 ml caused a 4-fold elevation and 1.0 ml CHCl3/kg resulted in a 1.3-fold elevation in LDH when administered in oil vs aqueous vehicle. Levels of LDH did not appear to be greatly affected by vehicle when TCE was administered. The interaction of CHCl3 and TCE resulted in slightly higher elevations when administered in oil vs aqueous vehicles at 36 hours and 48 hours when 0.5 and 1.0 ml CHCl3/kg were administered. Conversely, at 24 hours the aqueous vehicle produced a greater elevation in LDH from exposure to 0.5 and 1.0 ml CHCl3 in the presence of TCE.

AST was elevated by 0.5 and 1.0 ml CHCl3/kg alone by almost 3-fold when administered in aqueous compared to oil at 24 hours. In contrast, 0.5 and 1.0 ml CHCl3/kg elevated AST at both 36 and 48 hours by 1.3 to 4.5-fold when administered in the oil vehicle. TCE alone appeared to elevate AST at 24, 36 and 48 hours when administered in the oil compared to the aqueous vehicle (Table 3). At 24 and 36 hours, 0.5 ml CHCl3/kg plus TCE in either vehicle caused a similar response in AST whereas 1.0 ml in oil in the presence of TCE elevated AST by more than 2-fold. At 48 hours, the administration of 0.5 ml CHCl3 in the presence of TCE resulted in a 2-fold elevation in AST when administered in the aqueous vehicle, whereas 1.0 ml CHCl3 plus TCE produced a greater than 4-fold elevation, occurring when the chemicals were administered in oil (Table 3).

Interactions of Chloroform and Trichloroethylene

In the presence of TCE, 0.5 ml CHCl3 in oil significantly increased relative kidney weight when compared to controls; however, 0.5 ml CHCl3/kg in

oil in the absence of TCE caused a significantly greater increase when compared to CHCl3 plus TCE (Figure 25). In the presence of TCE, BUN was elevated following 1.0 ml CHCl3/kg in oil; however, this was not significantly different from 1.0 ml CHCl3 in the absence of TCE (Figure 27). In contrast, when CHCl3 was administered in oil in the absence of TCE, both 0.5 and 1.0 ml CHCl3/kg were significantly elevated relative to controls (Figure 27). CHCl3, in the presence of TCE, did not elevate BUN relative to controls when administered in the aqueous vehicle; however, 1.0 ml CHCl3/kg in the absence of TCE elevated BUN significantly relative to controls (Figure 28).

When administered in oil in the presence of TCE, no significant elevations in CRE resulted from 0.5 ml CHCl3/kg when compared to controls. In contrast, in the absence of TCE, 0.5 ml CHCl3/kg significantly elevated CRE relative to controls. In addition, the CRE concentration resulting from CHCl3 in the presence of TCE was significantly less than the CRE concentration resulting from CHCl3 in the absence of TCE (Figure 29). In the presence of TCE, CRE was significantly elevated by 1.0 ml CHCl3/kg in oil relative to controls; however, this was not significantly different from 1.0 ml CHCl3 in the absence of TCE (Figure 29). The aqueous administration of CHCl3 either in the presence or absence of TCE had no significant effect on CRE (Figure 30).

In the presence of TCE, AST was elevated by 1.0 ml CHCl3/kg in oil at 48 hours when compared to controls. At 36 hours in both oil and aqueous vehicles and at 48 hours in oil vehicle, AST levels resulting from 0.5 and 1.0 ml CHCl3/kg in the presence of TCE were significantly decreased relative to CHCl3 in the absence of TCE. Similarly at 48 hours in the aqueous vehicle, ALT levels following 1.0 ml CHCl3/kg in the presence of TCE were significantly decreased when compared to 1.0 ml CHCl3/kg in the absence of TCE (Figures 31 and 32).

In vehicle combined analysis, 0.5 and 1.0 ml CHCl3/kg in the presence of TCE resulted in significantly decreased ALT at 36 hours when compared to CHCl3 in the absence of TCE . Although not statistically significant, similar trends were seen in ALT at 48 hours (Figures 33 and 34).

At 24 hours, 0.5 ml CHCl3 in oil in the presence of TCE, resulted in significantly lower LDH levels than 0.5 ml CHCl3/kg in the absence of TCE At 36 hours (vehicle combined), in the presence of TCE, 0.5 and 1.0 ml CHCl3/kg resulted in significantly lower LDH levels when compared to 0.5 and 1.0 ml CHCl3/kg in the absence of TCE (Figures 35 and 36). At 48 hours, administration of 0.5 and 1.0 ml CHCl3/kg in oil in the presence of TCE resulted in significantly decreased LDH relative to CHCl3 in the absence of TCE. At 48 hours, when CHCl3 in the presence of TCE was administered in the aqueous vehicle, no significant elevations in LDH were observed, whereas CHCl3 in the absence of TCE produced significant increases in LDH (Figures 35 and 36).

At all time points, CHCl3 doses administered in the presence of TCE did not produce a significantly different response from control values or CHCl3 in the absence of TCE in UCRE and volume in both vehicles and osmolality in oil whereas in the absence of TCE, CHCl3 administration produced significant differences from the control values in these parameters (Figures 37, 38, 43, 45 and 46).

In the presence of TCE, 0.5 and 1.0 ml CHCl3/kg in oil produced a significant elevation of UN over control values at 36 hours At 0.5 ml CHCl3 in the presence of TCE, a significantly greater elevation of UN occurred than in the absence of TCE. When administered in the aqueous vehicle, 1.0 ml CHCl3/kg in the presence of TCE resulted in significantly lower levels of UN at 36 hours when compared to 1.0 ml CHCl3 in the absence of TCE (Figures 39 and 40).

Total protein was significantly decreased at 12 hours by 1.0 ml CHCl3/kg in the presence of TCE (vehicles combined) when compared to both the TCE control (0.0 ml CHCl3/kg/1.0 ml TCE/kg) and 1.0 ml CHCl3 in the absence of TCE. At 36 hours in both oil and aqueous vehicles and 48 hours in oil, in the presence of TCE, both 0.5 and 1.0 ml CHCl3/kg caused a significant decrease in TPR compared to the CHCl3 doses alone. In contrast, 0.5 and 1.0 ml CHCl3/kg in the absence of TCE caused a significant elevation of TPR over controls. At 36 hours, 0.5 and 1.0 ml CHCl3/kg in oil, in the presence of TCE, significantly decreased TPR compared to 1.0 ml TCE/kg alone. When administered in the aqueous vehicle, 1.0 ml CHCl3 in the presence of TCE, produced significantly lower TPR levels than CHCl3/kg in the absence of TCE which resulted in significant elevations in TPR at 48 hours compared to controls (Figures 41 and 42).

Administration of 1.0 ml CHCl3/kg in aqueous vehicle in the presence of TCE, resulted in a significant increase in osmolality at 12 hours compared to CHCl3 alone. In contrast, 1.0 ml CHCl3/kg in the aqueous vehicle, in the absence of TCE, resulted in a significant decrease in osmolality compared to controls. No other differences in the CHCl3 doses at the TCE levels were observed in the measurement of osmolality (Figures 43 and 44).

At 24 hours, in the presence of TCE, 0.5 ml CHCl3/kg in oil resulted in a significant elevation in pH compared to this CHCl3 dosage alone. In contrast, administration of 0.5 ml CHCl3/kg in oil, in the absence of TCE, produced a significant decrease in pH compared to controls. Additionally, 1.0 ml CHCl3/kg in oil in the presence of TCE produced a significantly lower pH than the TCE control. When administered in the aqueous vehicle, 1.0 ml CHCl3/kg in the presence of TCE, resulted in a significant elevation in pH relative to the CHCl3 alone. In contrast, 1.0 ml CHCl3/kg in the absence of TCE resulted in a

significant decrease in pH at 24 hours relative to controls. At 36 hours, 0.5 and 1.0 ml CHCl3/kg (vehicles combined), in the presence of TCE, caused a significant decrease in pH when compared to the control. In the absence of TCE, 0.5 and 1.0 ml CHCl3/kg also resulted in decreases in pH; however, these were not significantly less than the decreases resulting from CHCl3 in the presence of TCE. At 48 hours, 0.5 and 1.0 ml CHCl3/kg (vehicle combined) in the presence of TCE produced a significant decrease in pH compared to controls. In the absence of TCE, 0.5 and 1.0 ml CHCl3/kg also resulted in decreases in pH; these decreases were significantly less those resulting from CHCl3 in the presence of TCE. In summary, in the presence of TCE, CHCl3 produced a significantly higher pH (closer to controls) when compared to CHCl3 in the absence of TCE (Figures 47 and 48).

Histopathological Results

In oil, administration of 0.5 ml CHCl3/kg alone, resulted in mild (2/6), moderate (1/6) and marked (2/6) renal tubule degeneration. Moderate (4/6) and marked (2/6) damage occurred following 1.0 ml CHCl3/kg. When administered in the aqueous vehicle, 0.5 ml CHCl3/kg resulted in no (3/6) minimal (2/6) and mild (1/6) lesions with 1.0 ml CHCl3/kg causing minimal (2/5), mild (1/5) and moderate (2/5) damage (Table 7). In oil, 0.5 ml CHCl3/kg resulted in minimal (3/6), mild (1/6) and moderate (2/6) renal tubule necrosis. At 1.0 ml CHCl3/kg minimal (2/6), mild (2/6) and moderate (1/6) damage occurred. When administered in the aqueous vehicle, 0.5 ml CHCl3/kg resulted in no (5/6) and mild (1/6) lesions with 1.0 ml CHCl3/kg causing minimal (3/5), mild (1/5) and moderate (1/5) damage (Table 8). TCE alone was not observed to produce any renal tubule necrosis or degeneration in either vehicle (Tables 7 and 8).

Little if any effect of CHCl3 and TCE interactions are observed in renal tubule degeneration; however, the interaction is evident in renal necrotic

lesions. In the absence of TCE, 0.5 ml $CHCl_3$/kg produced minimal (3/6), mild (1/6), and moderate (2/6) renal tubule necrosis whereas in the presence of TCE a minimal lesion was observed in only one animal and 5 animals had no necrosis. In the absence of TCE, 1.0 ml $CHCl_3$/kg in oil produced minimal (2/6), mild (2/6) and moderate (1/6) renal tubule necrosis whereas in the presence of TCE, no (3/6) minimal (2/6) and mild (1/6) necrotic lesions were observed (Table 8).

In the aqueous vehicle in the absence of TCE, 0.5 ml $CHCl_3$/kg resulted in mild renal tubule necrosis in one animal. In the presence of TCE, no necrotic lesions were observed. In the aqueous vehicle in the absence of TCE, 1.0 ml $CHCl_3$/kg produced minimal (3/5), mild (1/5) and moderate (1/5) necrosis whereas in the presence of TCE no necrotic lesions were observed (Table 8).

Vehicle effects are apparent in the renal histological damage. Vehicle differences appear to be more apparent in renal tubule degeneration than necrosis. However $CHCl_3$, both in the absence and presence of TCE, appears to produce more severe renal tubule necrosis and degeneration when administered in the oil than in the aqueous vehicle. One exception is the renal tubule necrosis at 1.0 ml $CHCl_3$/kg alone where the lesions produced by the chemical administration in both vehicles are similar.

V. Discussion

A. Chloroform Hepatotoxicity

Observations from the present study indicate that exposure of male F-344 rats by oral gavage to 0.5 and 1.0 ml CHCl3/kg alone in both oil and aqueous vehicles resulted in acute hepatotoxicity. ALT, a reliable and sensitive indicator of hepatotoxicity, found to correlate well with histological lesions (Zimmerman 1982, Plaa, 1982), was elevated more than 8-fold over controls at 24 hours when 0.5 and 1.0 ml CHCl3/kg were administered in oil. At 48 hours, ALT levels exceeded those at 24 hours, with almost a 200-fold elevation resulting from 0.5 ml CHCl3/kg in oil and over a 100-fold elevation from 1.0 ml CHCl3/kg in oil. When CHCl3 was administered in the aqueous vehicle increases of ALT ranged from 3- to almost 5-fold at 24 hours. ALT levels produced by 1.0 ml CHCl3/kg at 48 hours, although significantly elevated over controls, were less than those at 24 hours indicating that the peak hepatotoxicity, when CHCl3 was administered in the aqueous vehicle, appeared to be 24 hours. Conversely, toxicity was greatest at 48 hours when CHCl3 was administered in oil. In addition, increased serum bile acids, an indicator of cholestatic damage (Gopinath et al., 1980) occurred at 24 hours (9-12 fold) and to a greater extent at 48 hours (58-73 fold) when oil was the vehicle of administration. However, no elevations in serum bile acids occurred when CHCl3 was administered in the aqueous vehicle.

Additionally, other serum enzymes measured at 48 hours including LDH and AST, enzymes reflecting cytotoxic damage (Zimmerman, 1982) and 5'Nuc (indicative of cholestasis) (Plaa and Hewitt, 1982) were elevated when CHCl3 was administered in an oil vehicle when compared to aqueous vehicle administration. LDH, AST and 5' Nuc were elevated 45- to 48-fold, 88- to 146-

fold and 2- to 3-fold, respectively, over controls when CHCl3 was administered in oil. However, in the aqueous vehicle, elevations were 2.8 fold for LDH, 1- to 3-fold for AST with no elevation in 5' Nuc.

Borzelleca et al. (1990) administered 0.47 ml CHCl3/kg (700 mg/kg) in 5 % Emulphor (10ml/kg dosing volume) to male Sprague-Dawley rats and took serial blood samples over 72 hours. They noted an approximate 2.5-fold elevation in ALT and an approximate 1.75-fold elevation in AST and that peak toxicity occurred at 36 hours. In the aqueous vehicle, elevations of ALT and AST at 24 hours in the present study were similar to the values obtained by Borzelleca et al. at 36 hours. Differences may be the result of the animals being cannulated for serial blood sampling or the fasting of animals prior to dosing in the study by Borzelleca; additionally, they used a different strain of rats. In another study, Steup et al. (1991) examined the toxicity of an ip administration of 0.2 ml CHCl3/kg (298 mg/kg) in oil (2.0 ml/kg dosing volume) in F-344 rats. The investigators observed an approximately 8-fold elevation in levels of ALT 24 hours post dosing. This value is similar to that observed for 0.5 ml CHCl3 in the present study. Higher values may have been due to the ip injection route of exposure vs oral gavage used in this study. Harris et al. (1982) administered 0.2 ml CHCl3/kg ip as a 30% solution in corn oil to male Long Evans rats and observed no significant elevations in ALT 24 hours post dosing.

Serum enzyme indicators as well as histopathological evidence, useful for specific evaluation of liver injury (Plaa and Hewitt, 1982), confirm the acute hepatotoxicity of CHCl3 in this study and suggest that the severity of hepatotoxicity is dependent upon the vehicle of administration.

Vehicle effects were of interest since these chemicals are common drinking water contaminants. Also, the majority of toxicity studies have previously been carried out in oil-based solutions; thus, it was thought that a

direct vehicle comparison of CHCl3 toxicity and CHCl3 and TCE interactions would be of interest. Additionally, recent findings indicate that the dosing vehicle may significantly influence the acute toxicity of halocarbon VOCs including carbon tetrachloride (Kim et al., 1990a) and 1,1 dichloroethylene (Chieco et al., 1981).

Withey et al. (1983) studied the effects of vehicles on the uptake of TCE and CHCl3 among other VOCs from the GI tract of the rat. Higher Cmax values were observed for both TCE and CHCl3 when administered in the aqueous vehicle vs an oil administration (Table 9). Thus, uptake was observed to be more rapid from aqueous solutions when equivalent amounts were administered in oil and aqueous solutions. Additionally if unmetabolized, CHCl3 could be eliminated by exhalation. Consequently, exhalation of unmetabolized CHCl3 may occur more readily from the aqueous solution accounting for the reduced toxicity when administered in the aqueous vehicle. Whereas, when administered in oil, the compounds are not as readily exhaled allowing a slow absorption of the chemicals into the system resulting in metabolism of a larger portion of the dose (Burnett et al., 1992).

Kim et al. (1990b) studied the pharmacokinetics of the uptake of CCl4 from different vehicles of administration (Table 10). Kim et al. noted significantly higher Cmax values when CCl4 was administered in the aqueous vehicles vs the oil and concluded that an overall delay in the uptake occurred when the chemical was administered in corn oil. In a companion study by Kim et al. (1990a), it was observed that acute hepatic toxicity from administration of CCl4 was more pronounced when given in an aqueous vehicle than in the oil vehicle. Substantial vehicle effects were observed in the present study; however, in contrast to the increased hepatotoxicity of CCl4 observed in the aqueous vehicle, CHCl3 was significantly more acutely hepatotoxic when administered

in the oil vehicle. Thus, CCl4 and CHCl3 appear to have opposing correlations with blood concentrations (Cmax) and resulting acute hepatotoxicity. This may be a result of the specific pathways of metabolism and detoxification involved in CHCl3 and CCl4 metabolism. Additionally, the relationship between the kinetics of chemical absorption into the systemic circulation and saturation of metabolic pathways may aid in predicting vehicle effects on toxicity. Consequently, it appears that the influence of vehicles on uptake into the systemic circulation, the application of pharmacokinetic data such as Cmax and the correlation between blood concentration and hepatotoxicity require careful examination.

B. Chloroform Nephrotoxicity

Many in vitro studies have addressed CHCl3 nephrotoxicity (Smith et al. 1985; Smith and Hook, 1983); however, little information is available in the literature on CHCl3 nephrotoxicity in oil and aqueous vehicles in vivo. A study by Branchflower and Pohl (1981) examined CHCl3 nephrotoxicity in F-344 rats. Twenty-four hours after administration of 0.5 ml CHCl3/kg ip to sesame oil pretreated rats no significant elevation in BUN over oil controls was evident.

In the present study, acute dose-dependent CHCl3 nephrotoxicity was observed in both oil and aqueous vehicles. Histological examination, a useful method for detecting chemically-induced renal damage and for elucidating the site of damage, was used to evaluate nephrotoxicity (Ohata, 1987). Renal tubule degeneration and necrosis were apparent in both vehicles; however, oil administration appeared to be more acutely nephrotoxic. Ohata et al. (1987) also noted that increases in urinary enzymes precede other common indicators of toxicity such as creatinine clearance, serum creatinine and BUN and that enzymes correlate well with histological changes. Thus, in addition to the

histological lesions, other indicators of renal damage including urinary AST, LDH and TPR are suggestive of distal convoluted tubule, proximal straight tubule and glomerular damage respectively (Guder and Ross,1984; Berndt, 1976)

The influence of vehicles on the urinary enzymes was minimal if any. However, when examining the severity of the histopathological lesions including both renal tubule degeneration and necrosis, it is apparent that CHCl3 was more acutely nephrotoxic when administered in oil compared to the aqueous vehicle.

C. Trichloroethylene Hepatotoxicity

In the present study, no hepatic toxicity was observed from the administration of TCE. A significant elevation over control in relative liver weight was observed when TCE was administered in oil; however, no necrosis was observed upon histological evaluation of the liver tissue. This was expected as it was the objective of this study to examine the interaction of a nonhepatotoxic dose of TCE on both the hepato- and nephro-toxicity of CHCl3. Similarly, Pessayre et al. (1982) noted that liver histology was normal 24 hours after the administration of 1 ml TCE/kg ip in liquid parafin to male Sprague-Dawley rats; additionally, no significant elevation in ALT was observed.

D. Trichloroethylene Nephrotoxicity

In the present study, elevations in urinary enzymes or indicators of nephrotoxicity were not observed when TCE was administered. Relative kidney weight was significantly increased over control in both vehicles; however, no histological evidence of renal toxicity was observed.

Chakrabarti and Tuchweber (1988) studied the acute nephrotoxic potential of TCE in male F-344 rats. Rats were pretreated with phenobarbital (80mg/kg ip for 3 days) then 0, 0.49, 0.99 and 1.97 ml TCE/kg was administered

ip in corn oil. Treatment of rats with up to 0.99 ml/kg did not influence any of the measured biochemical parameters at 24 hours. TCE at 1.97 ml /kg was observed to elevate NAG, BUN and glucose and an apparent saturation of metabolism was observed. Thus, the investigators concluded that TCE exerts acute nephrotoxicity at very high dose levels.

E. Interactions of Chloroform and Trichloroethylene on Hepatotoxicity

There are a number of studies in the literature discussing the synergistic interaction of CCl4 and CHCl3 (Borzelleca et al., 1990; Harris et al., 1982; Steup et al., 1991) and the synergistic interaction of CCl4 and TCE (Borzelleca et al., 1990; Steup et al., 1991); however, no studies were found in which the interaction of CHCl3 and TCE was examined. In contrast to the increased hepatotoxicity seen with CCl4 and TCE it was observed in this study that the concurrent administration of CHCl3 and TCE resulted in significantly less hepatotoxicity when compared to that caused by CHCl3 alone. The inhibition of toxicity by TCE can be observed in the decreased levels of AST, LDH, 5'Nuc at 48 hours, ALT and bile acid at 24 and 48 hours and in the histological lesions. Additionally, the inhibition was observed to be independent of dose, time or vehicle.

Both CHCl3 and TCE are known to be metabolized by the liver mixed-function oxidase system (Pohl, 1979; Miller and Guengerich, 1983; Rouisse and Chakrabarti, 1986). Moslen et al. (1977) studied the deactivation of P-450 by TCE in phenobarbital-pretreated Sprague-Dawley rats exposed to 1 % (10,000 ppm) TCE by inhalation for 24 hours. The investigators found significantly decreased P-450 in the pretreated rats after 1, 2 and 8 hours of exposure by inhalation to TCE. Brady et al. (1989) performed a study elucidating that the specific P450 isozyme responsible for the oxidative metabolism of CHCl3 is IIEl (CYP2E1). Brady et al. demonstrated that CHCl3 metabolism was reduced

when IIEI was inhibited by diallyl sulfide. Andersen et al. (1987) examined the metabolic interactions of TCE and 1,1 dichloroethylene, two compounds metabolized by single saturable oxidative pathways with high-affinity substrate binding. It was observed that the kinetic interactions in the metabolic pathways were strictly competitive thus explaining the inhibition of metabolism when coexposed. Andersen noted that the efficacy as an inhibitor in vivo is dependent both on binding constants and tissue solubilities and that TCE has high solubility in all tissues and blood. In a study by Gargas et al. (1988) on the solubilities of halomethanes, he reported tissue:air partition coefficients of several compounds (Table 11). TCE and CHCl3 have similar values for blood and liver tissue indicating that both compounds readily enter the tissue.

Therefore, a possible explanation for the inhibition of CHCl3 toxicity in the presence of TCE is that the P450 is deactivated by TCE thus inhibiting the CHCl3 metabolism to its reactive intermediates. Another possibility is that the TCE competitively binds to the P450; thus, blocking the CHCl3 metabolism. If TCE competitively binds or deactivates P450, this could lead to increased exhalation of unmetabolized CHCl3 and consequently, decreased toxicity.

F. Interactions of Chloroform and Trichloroethylene on Nephrotoxicity

The inhibition of CHCl3 toxicity by TCE observed in the liver was also apparent in the kidney. Renal tubule necrosis was more severe when CHCl3 was administered in the absence of TCE when compared to the presence of TCE. Urinary enzymes AST and LDH were also observed to significantly increase when CHCl3 was administered in the absence of TCE. Conversely, a significant decrease, relative to CHCl3 alone, is observed when CHCl3 was administered in the presence of TCE. Total protein concentrations were elevated over controls when CHCl3 was administered alone compared to no significant elevation in the presence of TCE. These interactions were observed

to be independent of vehicle and occurred primarily at 36 and 48 hours in both vehicles.

It has been noted that the activation of CHCl3 by oxidative dechlorination to phosgene may not occur in the kidney as it does in the liver since kidney GSH is not depleted (Docks and Krishna, 1976). Additionally, CHCl3 does not appear to be metabolized as extensively by P450 in the kidney when compared to the liver (Docks and Krishna, 1976). This suggestion was supported by the observation that the severity of CHCl3 nephrotoxicity in vitro did not differ between the F-344 and Sprague Dawley rats although the concentration of renal P450 has been shown to be two-fold greater in the F-344 strain (Smith et al., 1985).

Consequently, it was thought that the metabolism of CHCl3 and TCE in the liver in the present study could be responsible for the observed effects in the kidney; however, in a study by McMartin et al. (1981) that issue was addressed. The investigator administered 0.45 g CHCl3/kg in corn oil (6% v/v) by gavage to examine the effects of P450 on hepatic and renal necrosis. McMartin found no correlation between the effects of cadmium, phenobarbital or fasting pretreatments in increasing or decreasing hepatic cytochrome P450 concentrations and increasing or decreasing renal necrosis. Thus, suggesting that the renal necrosis is a result of CHCl3 metabolism in the kidney rather than the liver.

These possible differences of CHCl3 metabolism in the liver and kidney complicates the explanation of the interactive effects seen by the inhibition of CHCl3 toxicity by TCE in both organs. This is an area that warrants further study in order to explain the interactive toxicity observed in both liver and kidney tissue.

VI. Conclusions

CHCl3 was observed to produce a concentration-dependent increase in hepatic and renal toxicity while TCE alone produced no overt toxicity in either organ. It was also noted that corn oil appeared to enhance the acute hepatic and nephrotoxicity of CHCl3. Vehicle effects appeared to be independent of dose and time as they were observed at both 24 and 48 hours with 0.5 and 1.0 ml CHCl3/kg in both the liver and kidneys. Greater hepatotoxicity appeared to occur at 48 hours when CHCl3 was administered in oil whereas the aqueous vehicle appears to exhibit more or equal toxicity at 24 compared to 48 hours. Additionally, based on severity of the histopathological lesions CHCl3 appeared to be more acutely nephrotoxic when administered in the oil compared to the aqueous vehicle.

In the studies by Kim et al. (1990a and b), a correlation between a high Cmax and aqueous administration of CCl4-induced hepatotoxicity was evident. Cmax was also high when CHCl3 was administered in an aqueous vehicle when compared to oil (Withey et al., 1983); however, it was found to be more hepato- and nephrotoxic when administered in the oil vehicle. It appears that the rate of uptake and absorption plays an important role in the toxicity produced when a compound is administered. As a result of the apparent opposing correlations of blood concentrations and acute hepatotoxicity of CCl4 and CHCl3, two structurally similar halocarbons, it seems elucidation of the underlying mechanism of vehicle effects is crucial to more accurate risk assessment and that further assessment of the relationship between blood concentration and both hepatic and renal toxicity is of interest.

It is becoming more apparent that the potentially confounding effects of the vehicle of chemical administration and route of exposure should be

examined. Additionally, an understanding of the importance of studying the toxic effects of a contaminant in the media in which exposure is most likely to occur is becoming a more relevant issue as the influences of vehicles are being observed. This is especially applicable to the VOCs since they are very lipophilic and recent findings indicate significant vehicle alterations in pharmacokinetics and toxicity.

The purpose of this work is to ultimately lead to an better understanding of the risks of human exposure to interactions of VOCs in drinking water. Mixture toxicology is a new and complex field. There are infinite combinations of mixtures that could be tested and it would an impossibility to begin to do so. Thus, if the mechanism(s) of the toxicity can be elucidated or if chemicals can be grouped according to structural properties or toxic endpoint(s), it would reduce the amount of testing required.

Examples of binary mixture interactions include the potentiation of CCl4 by TCE and the synergism of CCl4 and CHCl3 (Borzelleca et al., 1990; Steup et al., 1991). In the present study, concurrent exposure to CHCl3 and TCE produced significant decreases in toxicity relative to CHCl3 alone. This inhibition of CHCl3 toxicity by TCE was observed to be independent of dose, vehicle and time and was observed in both the liver and kidney. Thus, this study in combination with others (Borzelleca et al., 1990; Steup et al., 1991; Simmons et al., 1992) provides an example of the opposing interaction of TCE on both CHCl3 and CCl4 and the opposite responses of two structurally similar binary mixtures.

Toxicological assessment of chemical interactions is a difficult task. It is important not only to assess the biological effects of exposure to chemical mixtures but also the pharmacokinetic influences (Andersen et al., 1987) as well as determining the mechanism(s) of interaction. This information could aid in

the interpretation of the chemical interactions which ultimately may lead to more accurate assessments of the health risk associated with multiple chemical exposures.

Table 1 Legend for Explanation of Statistical Symbols
* In the absence of TCE, the CHCl3 dose is significantly different from the controls (0.0 ml CHCl3/kg/0.0 ml TCE/kg).
< In the absence of TCE, the CHCl3 dose is significantly different from both the control (0.0 ml CHCl3/kg/0.0 ml TCE/kg) and the low CHCl3 dose.
+ In the presence of TCE, the CHCl3 dose is significantly different from the controls (0.0 ml CHCl3/kg/1.0 ml TCE/kg).
In the presence of TCE, the CHCl3 dose is significantly different from both the control (0.0 ml CHCl3/kg/1.0 ml TCE/kg) and the low CHCl3 dose.
! The TCE levels, at the same level of CHCl3, are significantly different.
= There is a significant difference between the vehicles.
$ 24 hour value is significantly different from the 48 hour value..
TIME Vehicle was not significantly different and statistical analysis was performed without making that distinction.

Table 2. Vehicle comparison of LDH in serum at 48 hours

CHCl3/TCE (ml/kg)	oil vehicle (IU/l)	aqueous vehicle (IU/l)	oil/aqueous ratio
0.0/0.0	154.7	149.2	1.0
0.0/1.0	498.5	135.3	3.7
0.5/0.0	6958.3	155.3	44.8
0.5/1.0	389.3	166.5	2.3
1.0/0.0	7387.7	420.0	17.6
1.0/1.0	529.3	168.2	3.2

Vehicle comparison of AST in serum at 48 hours

CHCl3/TCE (ml/kg)	oil vehicle (IU/l)	aqueous vehicle (IU/l)	oil/aqueous ratio
0.0/0.0	60.2	61.3	1.0
0.0/1.0	74.2	56.5	1.3
0.5/0.0	8756.7	79.5	110.1
0.5/1.0	367.5	54.0	6.8
1.0/0.0	5267.2	200.8	26.2
1.0/1.0	980.3	74.0	13.2

Vehicle comparison of 5'Nucleotidase in serum at 48 hours

CHCl3/TCE (ml/kg)	oil vehicle (IU/l)	aqueous vehicle (IU/l	oil/aqueous ratio
0.0/0.0	48.7	87.50	0.6
0.0/1.0	89.4	96.2	0.9
0.5/0.0	120.9	63.7	1.9
0.5/1.0	63.0	56.0	1.1
1.0/0.0	163.4	58.0	2.8
1.0/1.0	80.0	53.4	1.5

Table 3. Normalized AST* in Urine: Oil/Aqueous Ratios							
CHCl3/TCE (ml/kg)		0 hr	6 hr	12 hr	24 hr	36 hr	48 hr
0.0/0.0	oil	0.09	0.12	1.24	0.21	0.12	0.19
	aqueous	0.24	0.41	0.17	4.22	0.13	0.16
	ratio	**0.38**	**0.30**	**7.20**	**0.05**	**0.87**	**1.19**
0.0/1.0	oil	0.10	0.15	0.11	0.33	0.16	0.27
	aqueous	0.11	0.17	0.16	0.22	0.11	0.14
	ratio	**0.94**	**0.91**	**0.68**	**1.49**	**1.43**	**1.90**
0.5/0.0	oil	0.10	0.23	0.21	0.47	2.75	4.00
	aqueous	0.11	0.14	0.20	1.12	0.87	0.89
	ratio	**0.90**	**1.68**	**1.05**	**0.42**	**3.16**	**4.48**
0.5/1.0	oil	0.24	0.60	0.14	0.18	0.16	0.33
	aqueous	0.15	0.15	0.13	0.19	0.16	0.59
	ratio	**1.54**	**3.94**	**1.05**	**0.99**	**1.01**	**0.57**
1.0/0.0	oil	0.10	0.16	0.16	0.18	3.46	3.74
	aqueous	0.06	0.15	0.21	0.49	2.63	2.95
	ratio	**1.48**	**1.03**	**0.75**	**0.37**	**1.32**	**1.27**
1.0/1.0	oil	0.06	0.56	0.29	0.42	0.29	0.92
	aqueous	0.17	0.12	0.11	0.19	0.14	0.20
	ratio	**0.36**	**4.87**	**2.52**	**2.26**	**2.02**	**4.56**

*Expressed as IU/10 mg UCRE

Table 4. Normalized LDH* in Urine: Oil/Aqueous Ratios							
CHCl3/TCE (ml/kg)		0 hr	6 hr	12 hr	24 hr	36 hr	48 hr
0.0/0.0	oil	0.338	0.347	0.778	0.424	0.294	0.285
	aqueous	0.375	0.442	0.393	0.310	0.321	0.333
	ratio	**0.901**	**0.785**	**1.980**	**1.368**	**0.916**	**0.856**
0.0/1.0	oil	0.355	0.427	0.520	0.454	0.364	0.304
	aqueous	0.381	0.477	0.678	0.488	0.323	0.324
	ratio	**0.932**	**0.895**	**0.767**	**0.930**	**1.127**	**0.938**
0.5/0.0	oil	0.357	0.491	0.551	1.019	3.128	6.364
	aqueous	0.361	0.438	0.624	2.408	1.778	1.520
	ratio	**0.989**	**1.121**	**0.883**	**0.423**	**1.759**	**4.187**
0.5/1.0	oil	0.355	0.487	0.556	0.417	0.681	0.809
	aqueous	0.351	0.527	0.570	0.577	0.434	0.379
	ratio	**1.011**	**0.924**	**0.975**	**0.723**	**1.569**	**2.135**
1.0/0.0	oil	0.367	0.550	0.576	0.620	3.770	6.241
	aqueous	0.374	0.532	0.699	1.413	4.203	4.495
	ratio	**0.981**	**1.034**	**0.824**	**0.439**	**0.897**	**1.388**
1.0/1.0	oil	0.367	0.445	0.519	0.515	0.708	1.337
	aqueous	0.303	0.432	0.626	0.595	0.452	0.572
	ratio	**1.211**	**1.030**	**0.829**	**0.866**	**1.567**	**2.337**

*Expressed as IU/10 mg UCRE

Table 5. Centrilobular Hepatocellular Necrosis		
CHCl3/TCE (ml/kg)	Oil Vehicle	Aqueous Vehicle
0.0/0.0	000000*	000000
0.0/1.0	000000	000000
0.5/0.0	022223	000000
0.5/1.0	000111	000000
1.0/0.0	222222	00000**
1.0/1.0	111111	000000

* Each digit is the lesion score from one animal and indicates severity of damage as described in the methods section: 0=no lesions observed; 1=minimal; 2=mild; 3=moderate; 4=marked.
**One animal died

Table 6. Centrilobular Vacuolar Degeneration		
CHCl3/TCE (ml/kg)	Oil Vehicle	Aqueous Vehicle
0.0/0.0	000000*	000000
0.0/1.0	000000	000000
0.5/0.0	233344	011111
0.5/1.0	112233	000114
1.0/0.0	333444	11112**
1.0/1.0	222333	111122

* Each digit is the lesion score from one animal and indicates severity of damage as described in the methods section: 0=no lesions observed; 1=minimal; 2=mild; 3=moderate; 4=marked.
**One animal died

Table 7. Renal Tubule Degeneration		
CHCl3/TCE (ml/kg)	Oil Vehicle	Aqueous Vehicle
0.0/0.0	000000*	000000
0.0/1.0	000000	000000
0.5/0.0	022344	000112
0.5/1.0	233333	000011
1.0/0.0	333344	11233**
1.0/1.0	333444	011112

* Each digit is the lesion score from one animal and indicates severity of damage as described in the methods section: 0=no lesions observed; 1=minimal; 2=mild; 3=moderate; 4=marked.
**One animal died

Table 8. Renal Tubule Necrosis		
CHCl3/TCE (ml/kg)	Oil Vehicle	Aqueous Vehicle
0.0/0.0	000000*	000000
0.0/1.0	000000	000000
0.5/0.0	111233	000002
0.5/1.0	000001	000000
1.0/0.0	011223	11123**
1.0/1.0	000112	000000

* Each digit is the lesion score from one animal and indicates severity of damage as described in the methods section: 0=no lesions observed; 1=minimal; 2=mild; 3=moderate; 4=marked.
**One animal died

Table 9. Withey et al. 1983, Pharmacokinetic Data on 75 mg CHCl3/kg and 18 mg TCE/kg in Oil and Aqueous Vehicles

CHCl3	Corn Oil	Std Error or 95% CI	Aqueous	Std Error or 95% CI
Cmax (ug/ml)	5.9	1.2	39.3	6.8
Tmax (min)	6.0	0.0	5.6	1.1
AUC (ug min/ml)	199	93,426	1735	1280,2351
TCE				
Cmax (ug/ml)	<1.0	-	15.9	1.1
Tmax (min)	-	-	10.6	3.7
AUC (ug min/ml)	1.11*	0.5,2.46	241.5	196,298

*Obtained by extrapolation.

Table 10. Kim et al. 1990, Pharmacokinetic Data on 25 mg CCl4/kg in Different Vehicles

	Corn Oil	Std Error	Aqueous	Std Error	Pure Chemical	Std Error	Water	Std Error
Cmax (ng/ml)	371	30	3814	1012	1084	145	3447	654
Tmax (min)	184	66	6	0.5	20.5	1.9	3.5	0.7
AUC ug min/ml	164	8	166	23.4	114.2	14.9	175	22.7

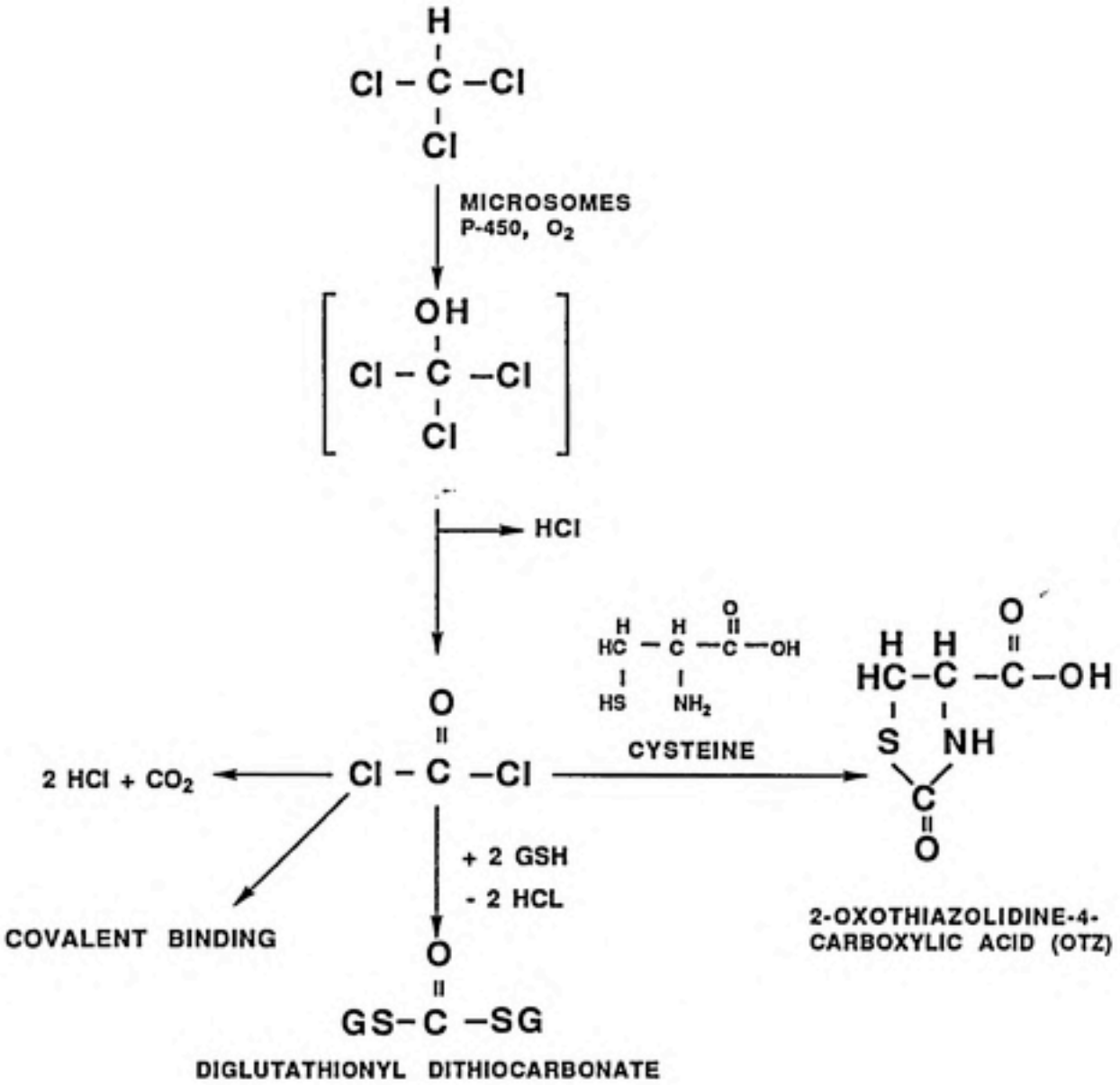
Table 11 Blood:saline and oil partition coefficients Gargas et al. (1988)

Chemical	Saline	Std Error	Olive Oil	Std Error
CHCl3	3.38	0.09	402	12
TCE	0.83	0.30	553	46
CCl4	0.35	0.03	374	11

Tissue:Air Partition Coefficients. Gargas et al. (1988)

	Blood	Std Error	Liver	Std Error	Muscle	Std Error	Fat	Std Error
CHCl3	20.8	0.1	21.1	1.5	13.9	1.9	203	5
TCE	21.9	1.4	27.2	3.4	10.1	2.7	554	21
CCl4	4.52	0.35	14.2	0.97	4.54	0.59	359	11

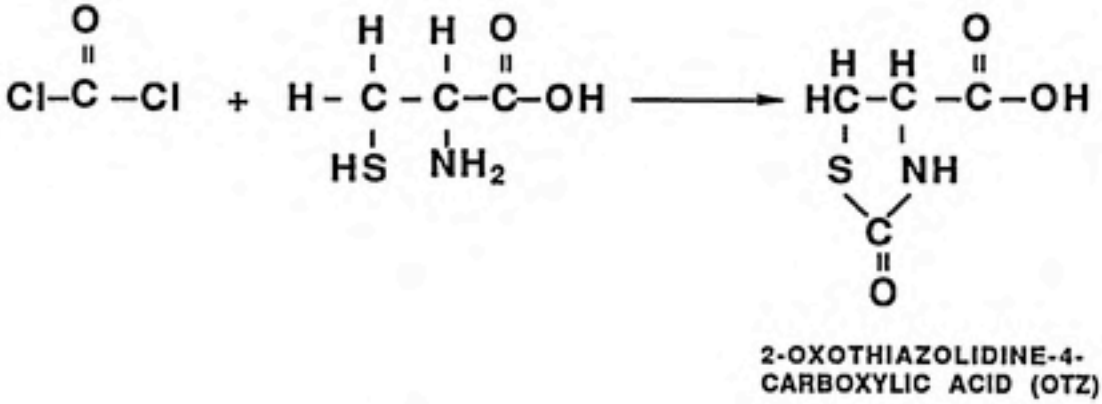
Figure 1



Hepatic Metabolism and Activation of Chloroform.

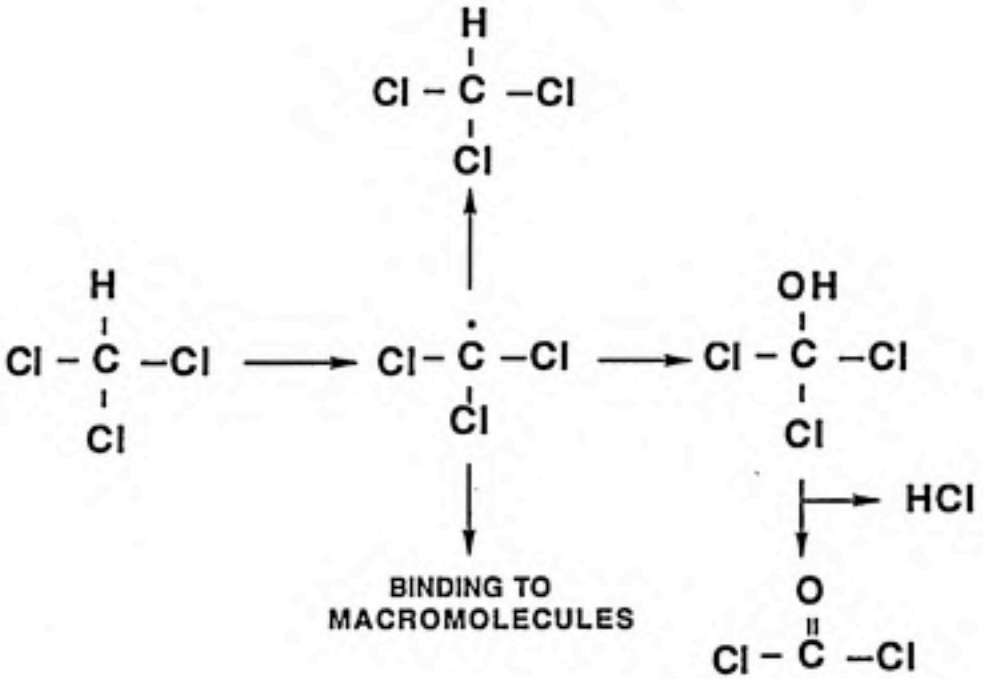
(From Pohl, L.R. Reviews of Biochemical Toxicology, Bend, J. and Philpot, R.M., Eds., Elsevier/North-Holland, New York, 1979.

Figure 2



Reaction of Phosgene with Cysteine

(From Pohl, L.R. Reviews of Biochemical Toxicology, Bend, J. and Philpot, R.M., Eds., Elsevier/North-Holland, New York, 1979.



Potential Metabolic Oxidative Dehalogenation Pathway for CHCl3 Involving a Free Radical Intermediate

(From Pohl, L.R. Reviews of Biochemical Toxicology, Bend, J. and Philpot, R.M., Eds., Elsevier/North-Holland, New York, 1979.

FIG. 3. BODY WEIGHT AT 48 HRS - OIL VEHICLE

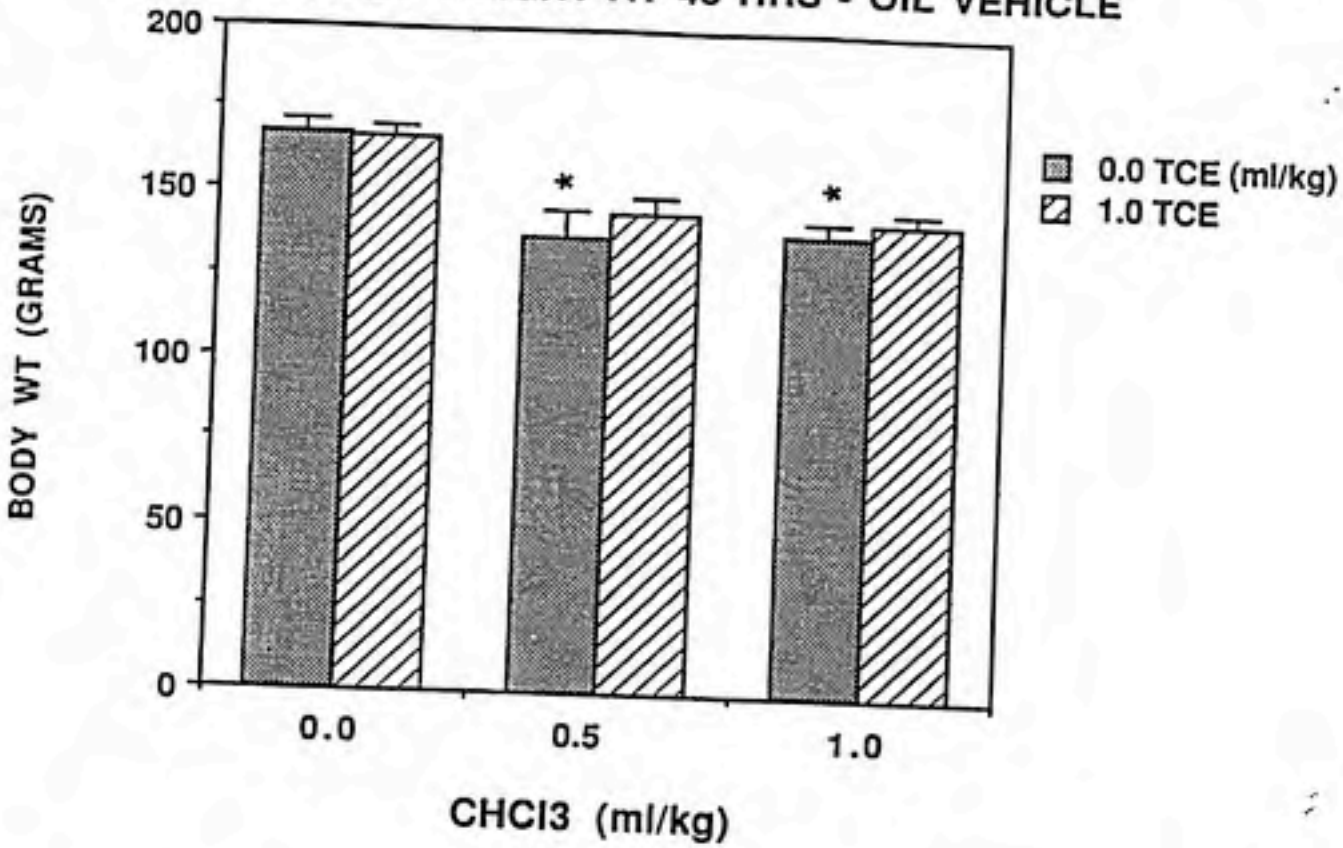


FIG. 4. BODY WEIGHT AT 48 HRS - AQUEOUS VEHICLE

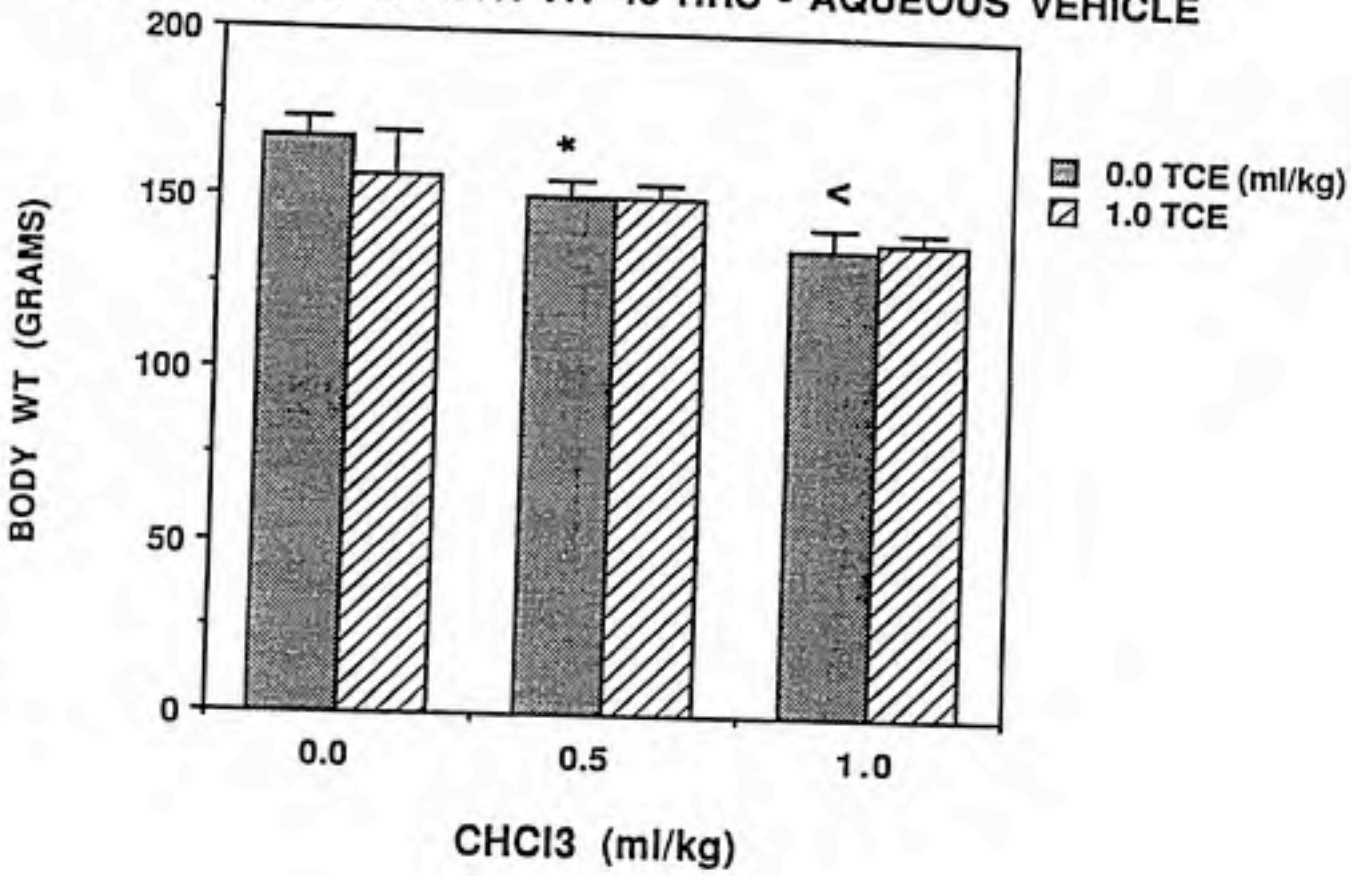


FIG. 5. RELATIVE LIVER WEIGHT - OIL VEHICLE

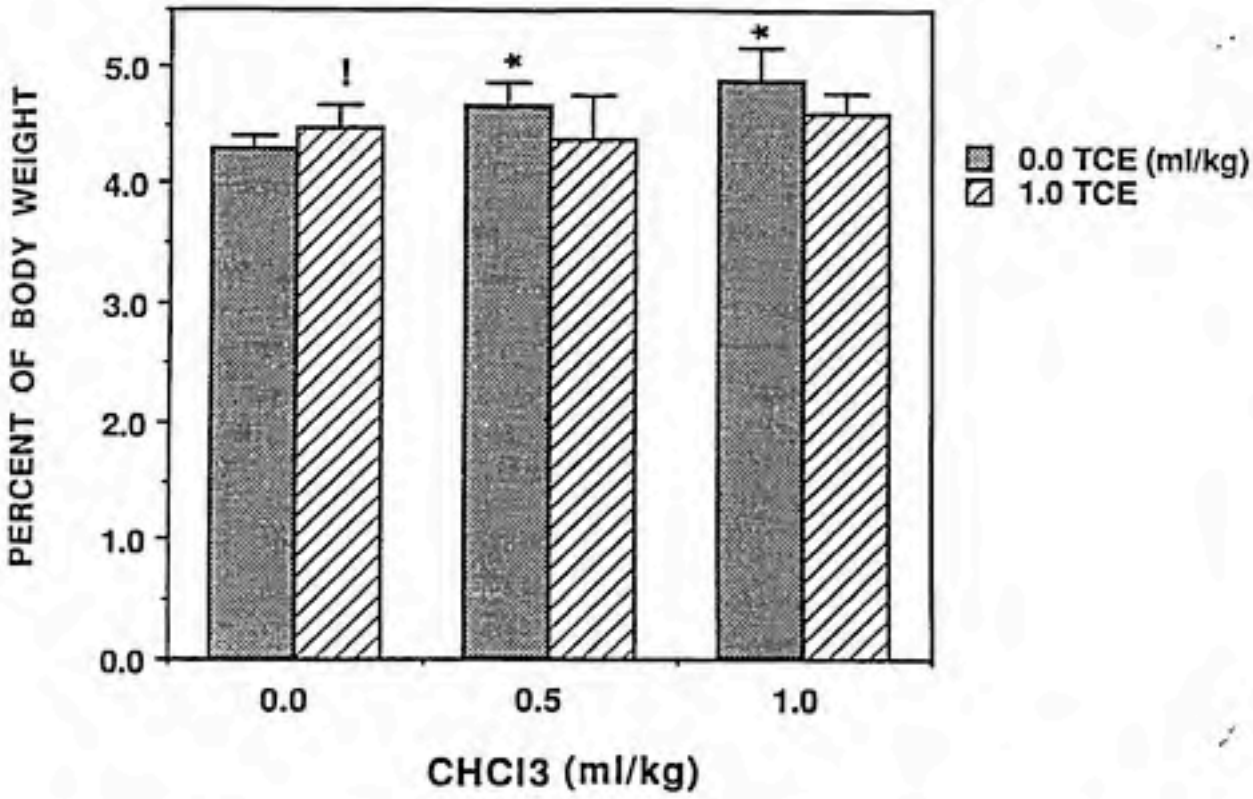


FIG. 6. RELATIVE LIVER WEIGHT - AQUEOUS VEHICLE

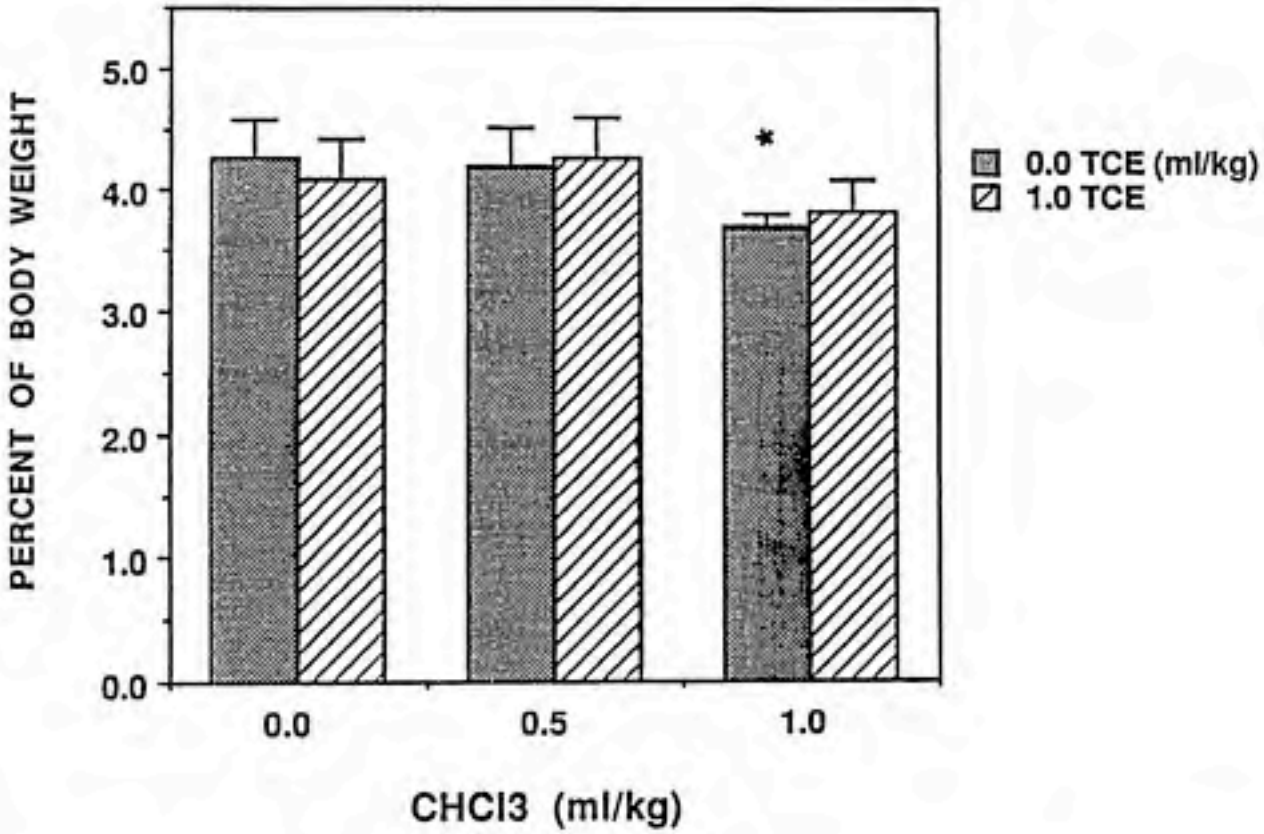


FIG. 7. LDH AT 48 HRS - OIL VEHICLE

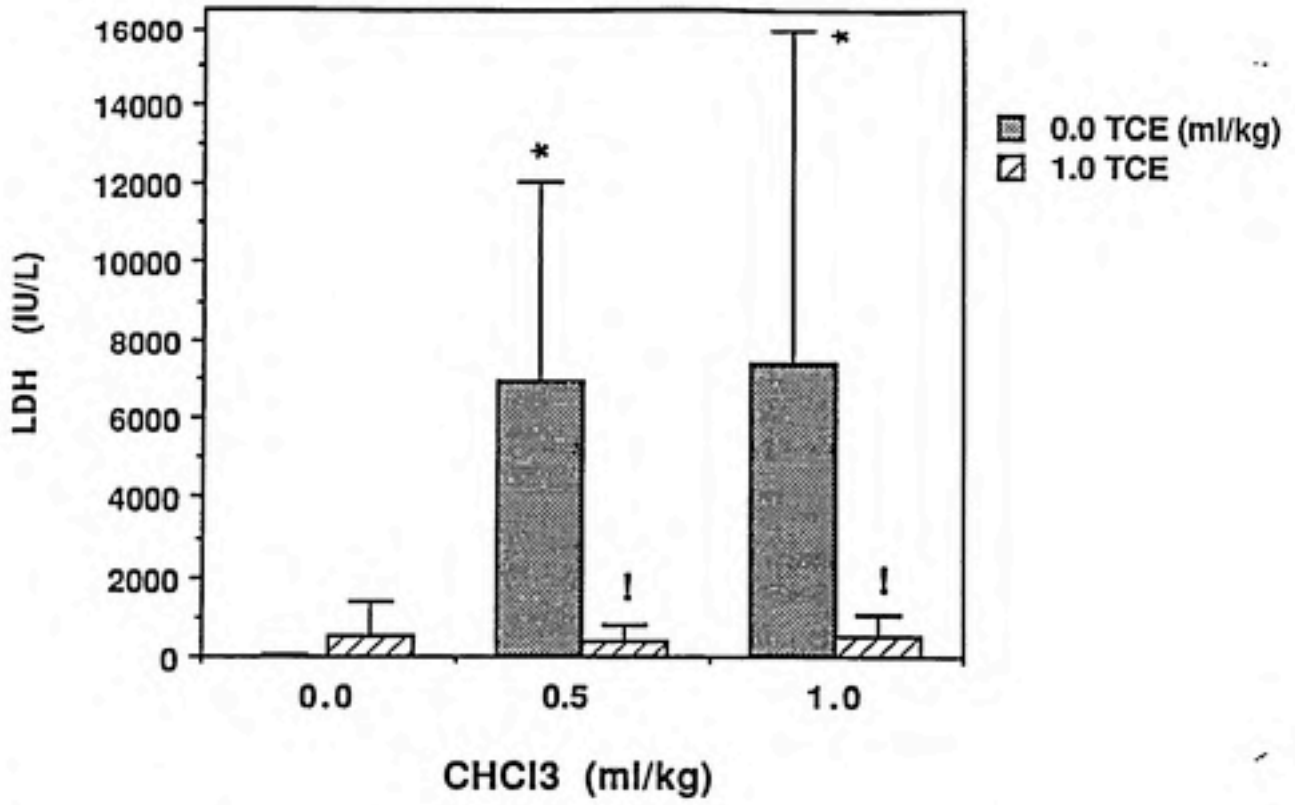


FIG. 8. LDH AT 48 HRS - AQUEOUS VEHICLE

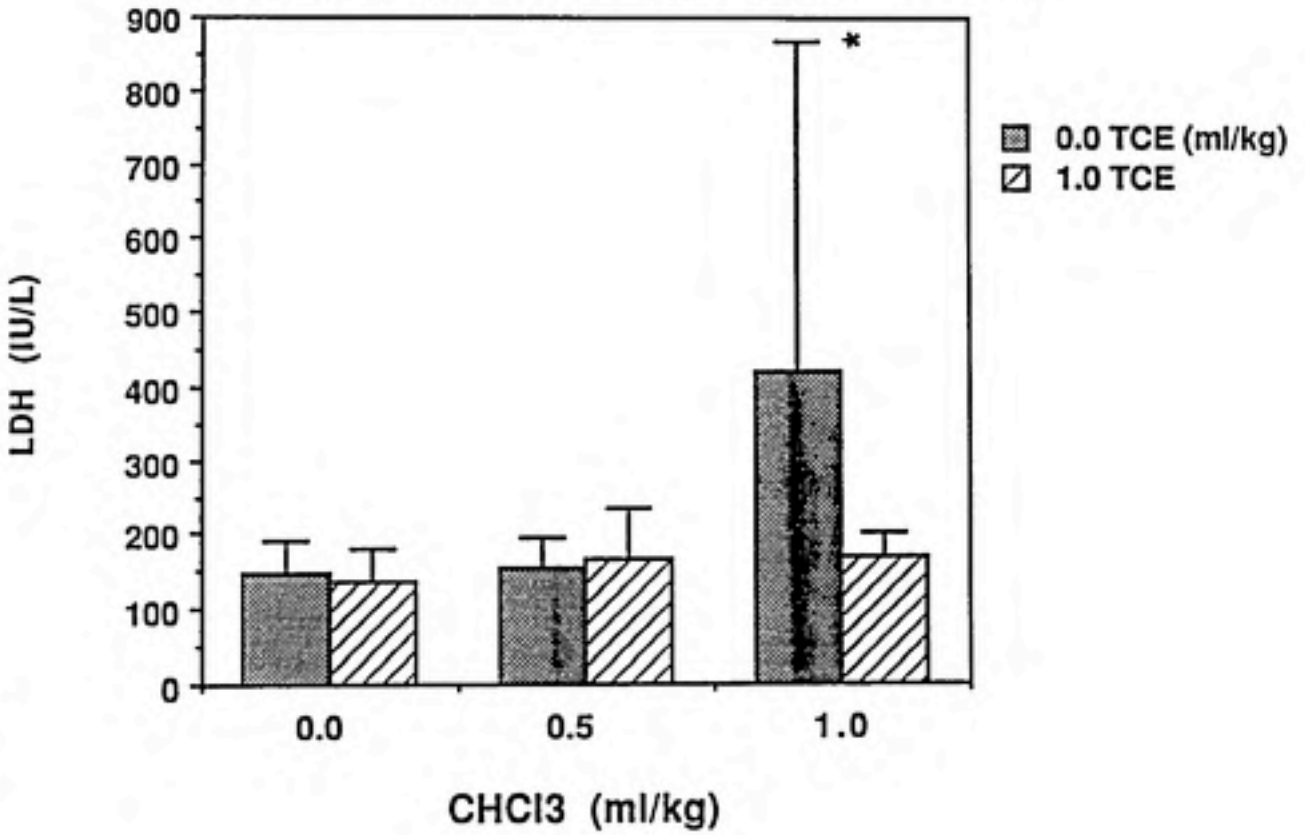


FIG. 9. AST AT 48 HRS - OIL VEHICLE

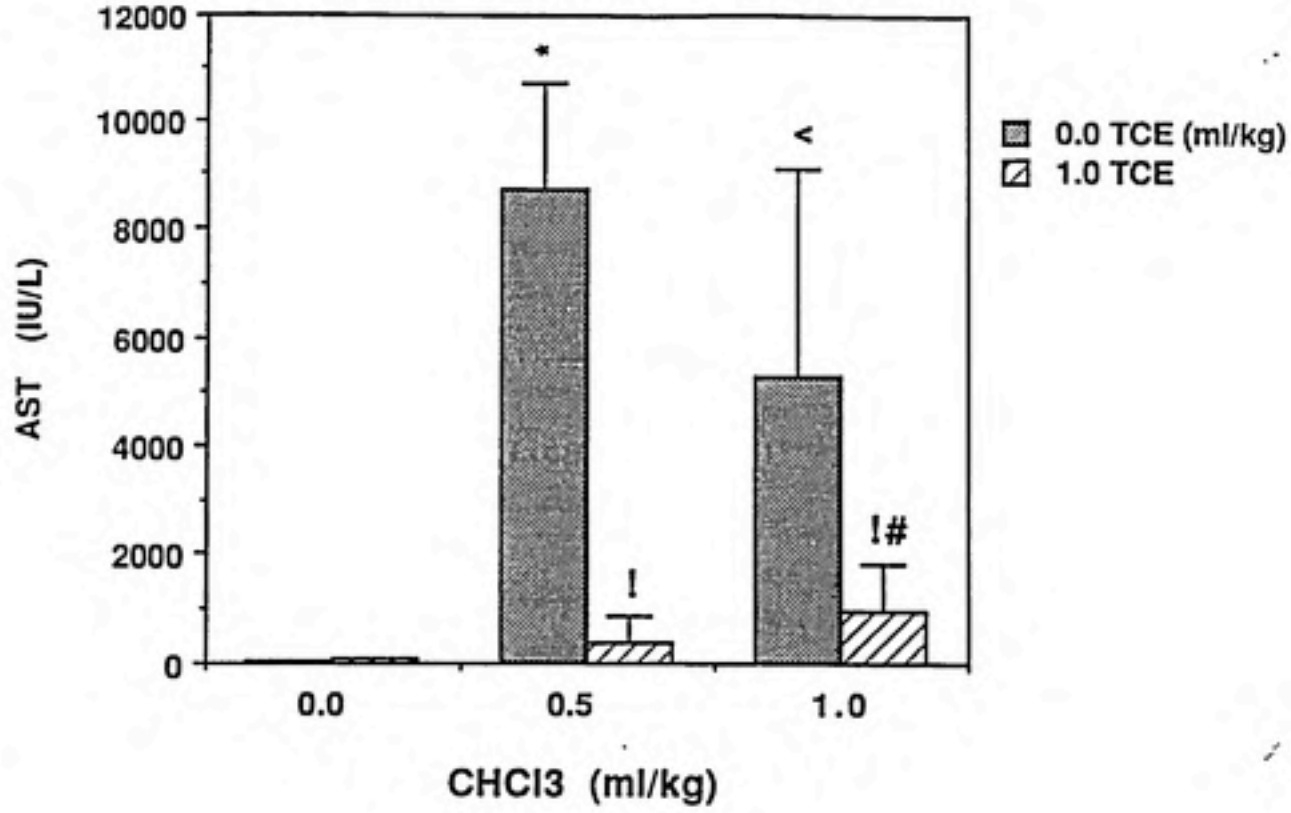


FIG. 10. AST AT 48 HRS - AQUEOUS VEHICLE

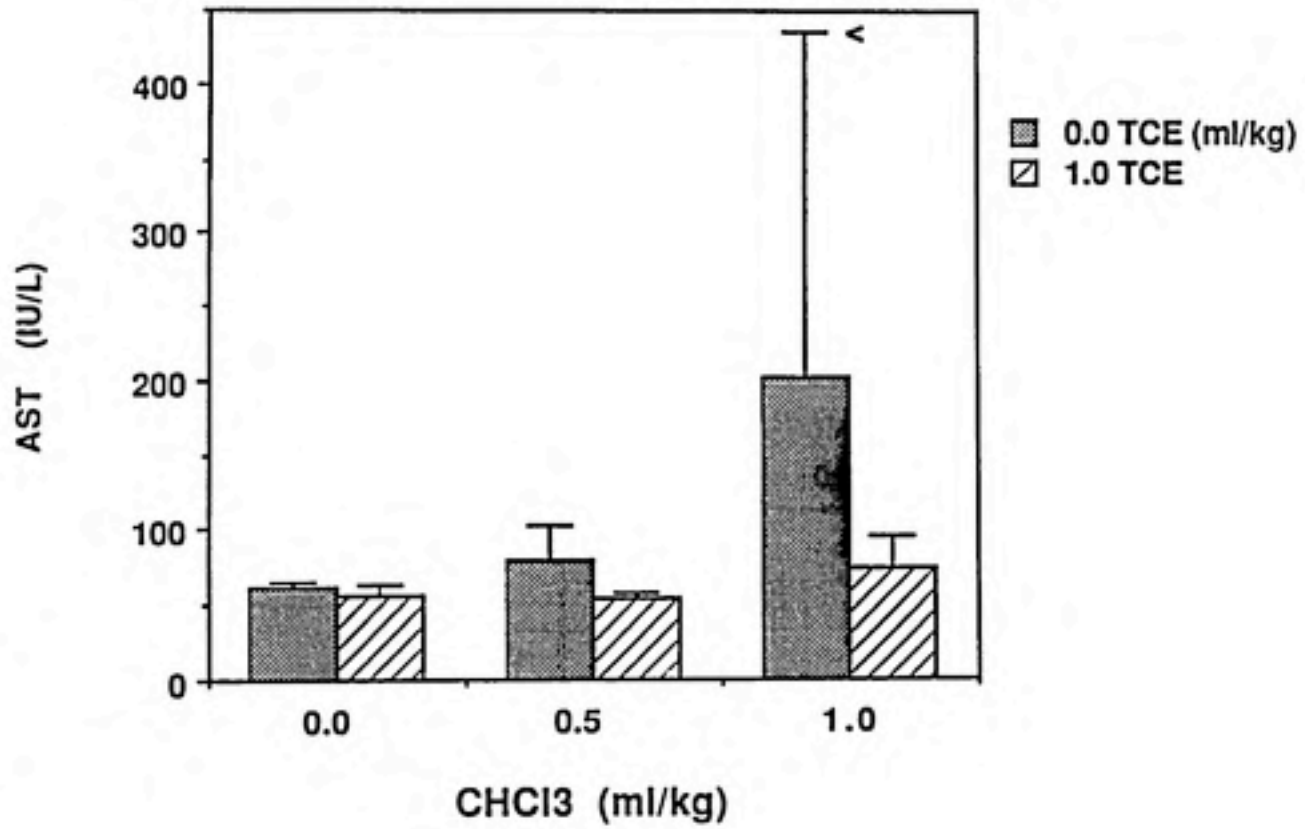


FIG. 11. 5' NUC AT 48 HRS - OIL VEHICLE

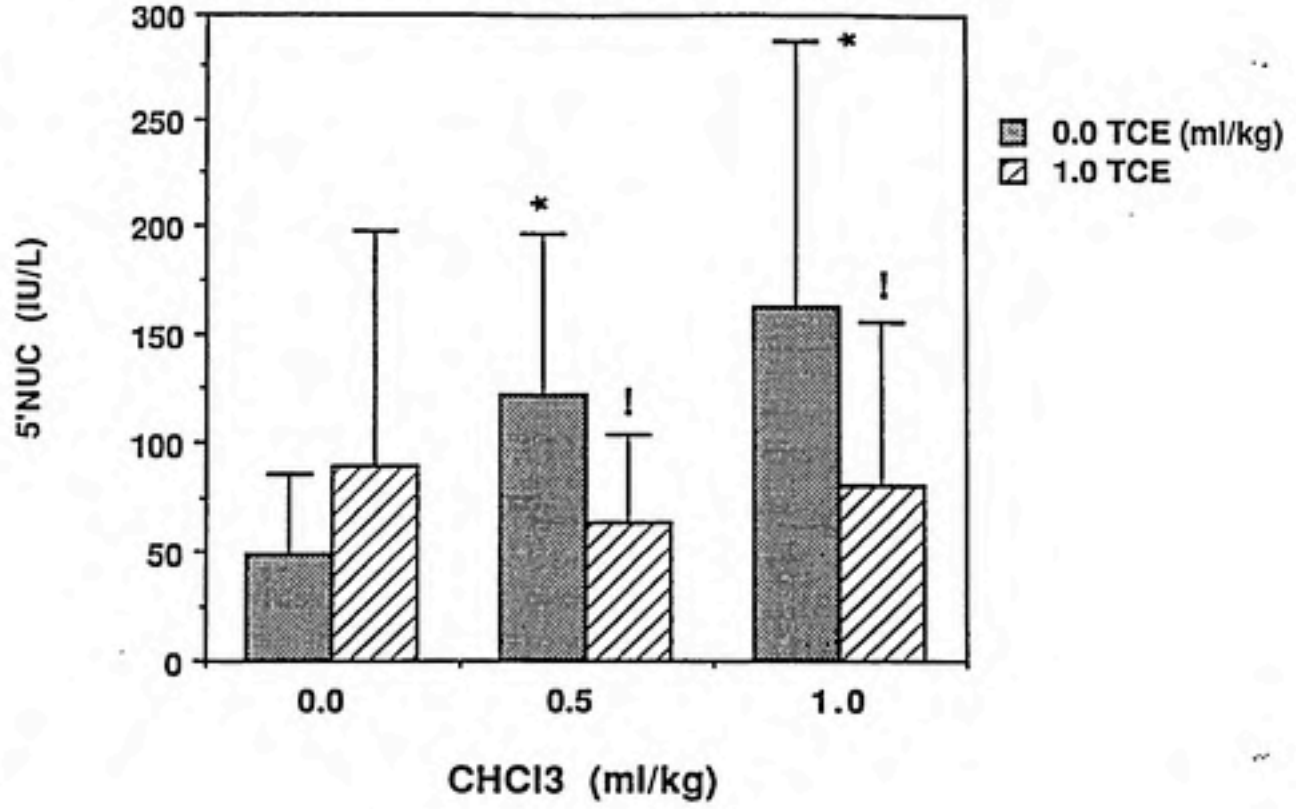


FIG. 12. 5' NUC AT 48 HRS - AQUEOUS VEHICLE

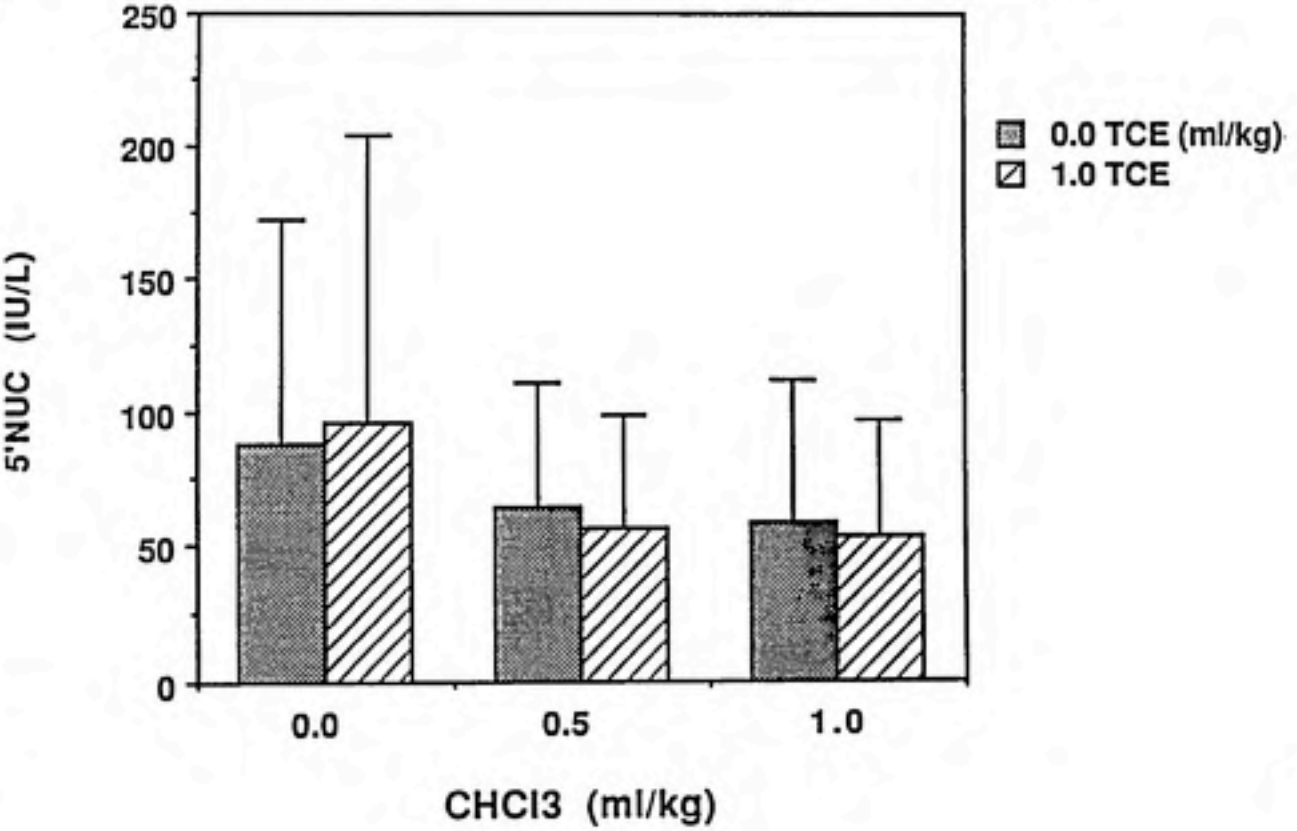


FIG. 13. HEPATOTOXICITY OF 0.5 ml CHCl3/kg AND TCE IN OIL VEHICLE

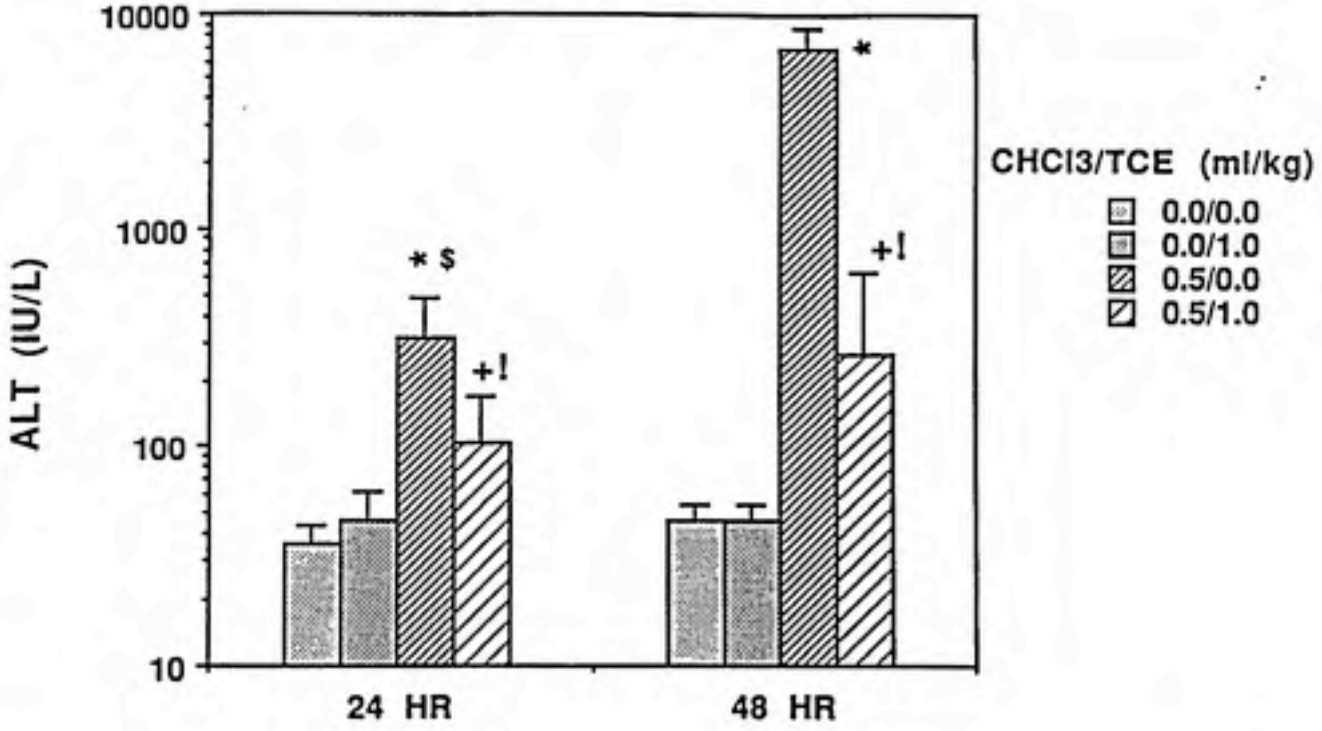


FIG. 14. HEPATOTOXICITY OF 1.0 ml CHCl3/kg AND TCE IN OIL VEHICLE

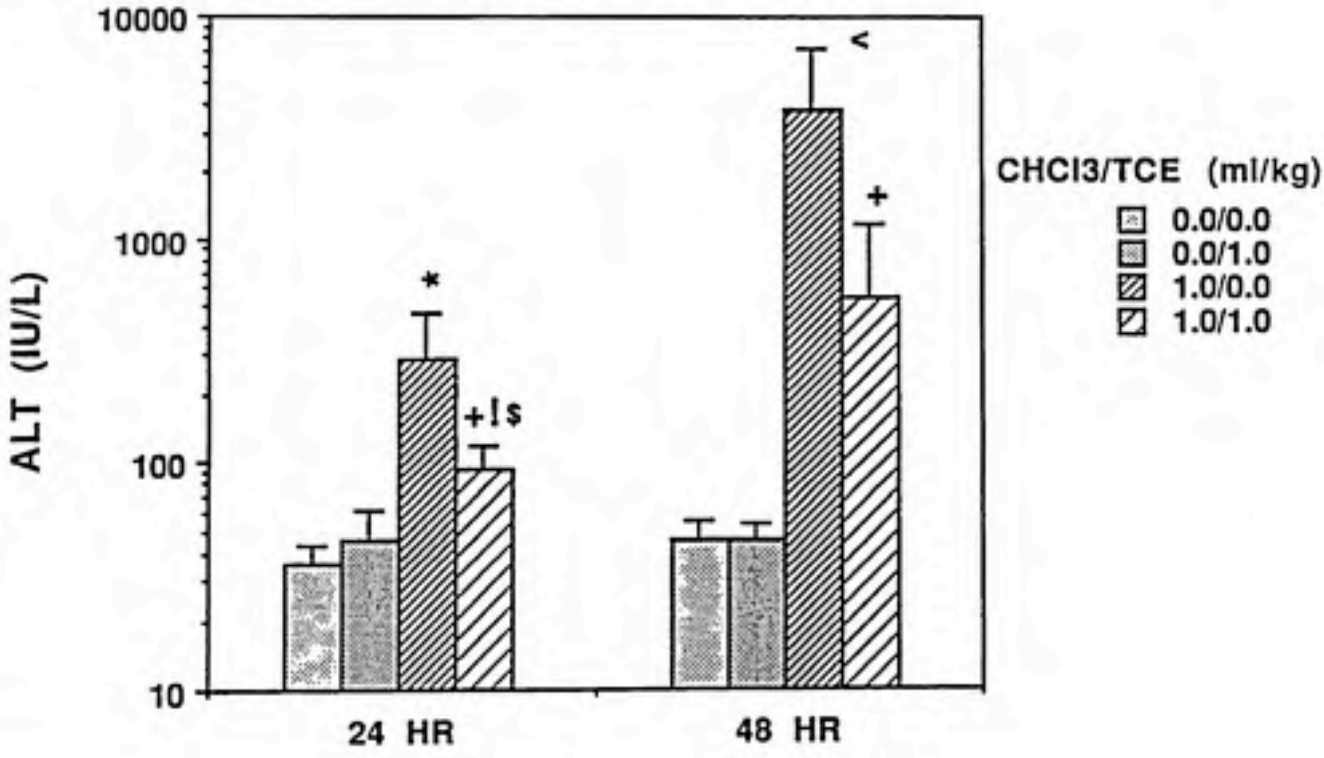


FIG. 15. HEPATOTOXICITY OF 0.5 ml CHCl3/kg AND TCE IN AQUEOUS VEHICLE

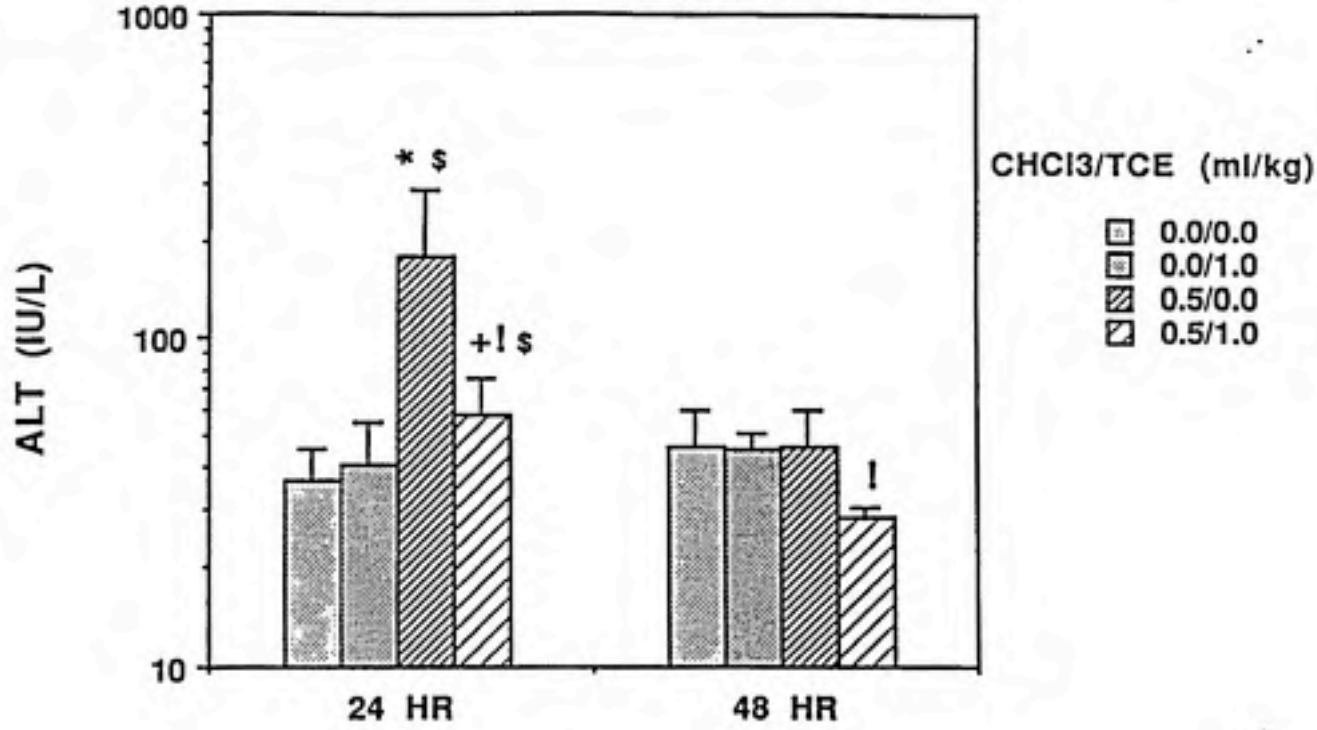


FIG. 16. HEPATOTOXICITY OF 1.0 ml CHCl3/kg AND TCE IN AQUEOUS VEHICLE

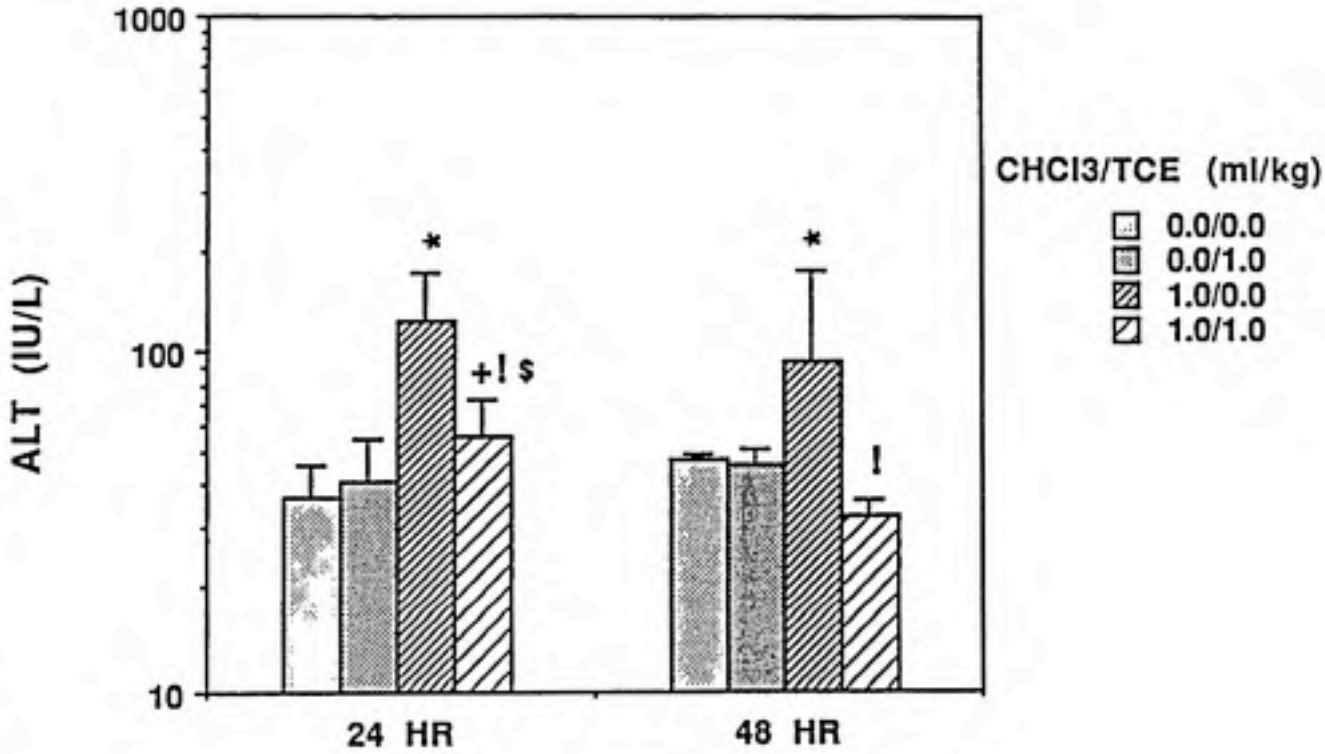


FIG. 17. HEPATOTOXICITY OF 0.5 ml CHCl3/kg AND TCE IN OIL VEHICLE

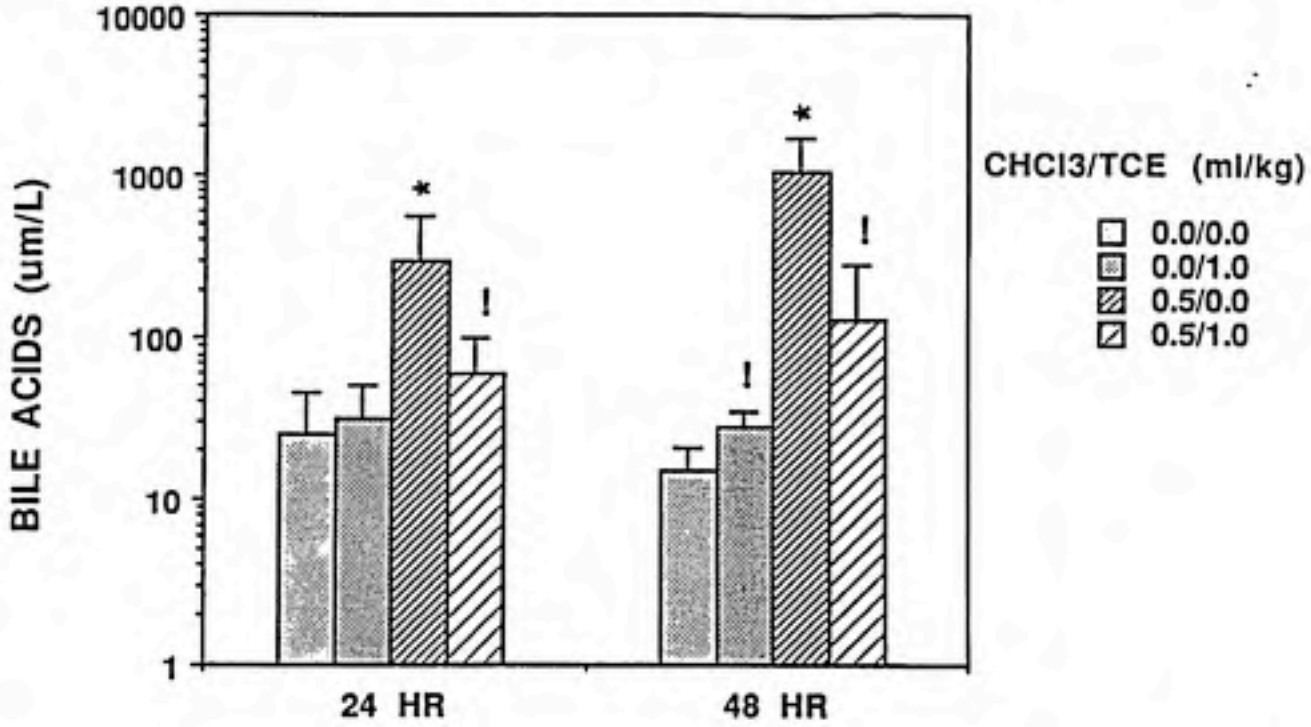


FIG. 18. HEPATOTOXICITY OF 1.0 ml CHCl3/kg AND TCE IN OIL VEHICLE

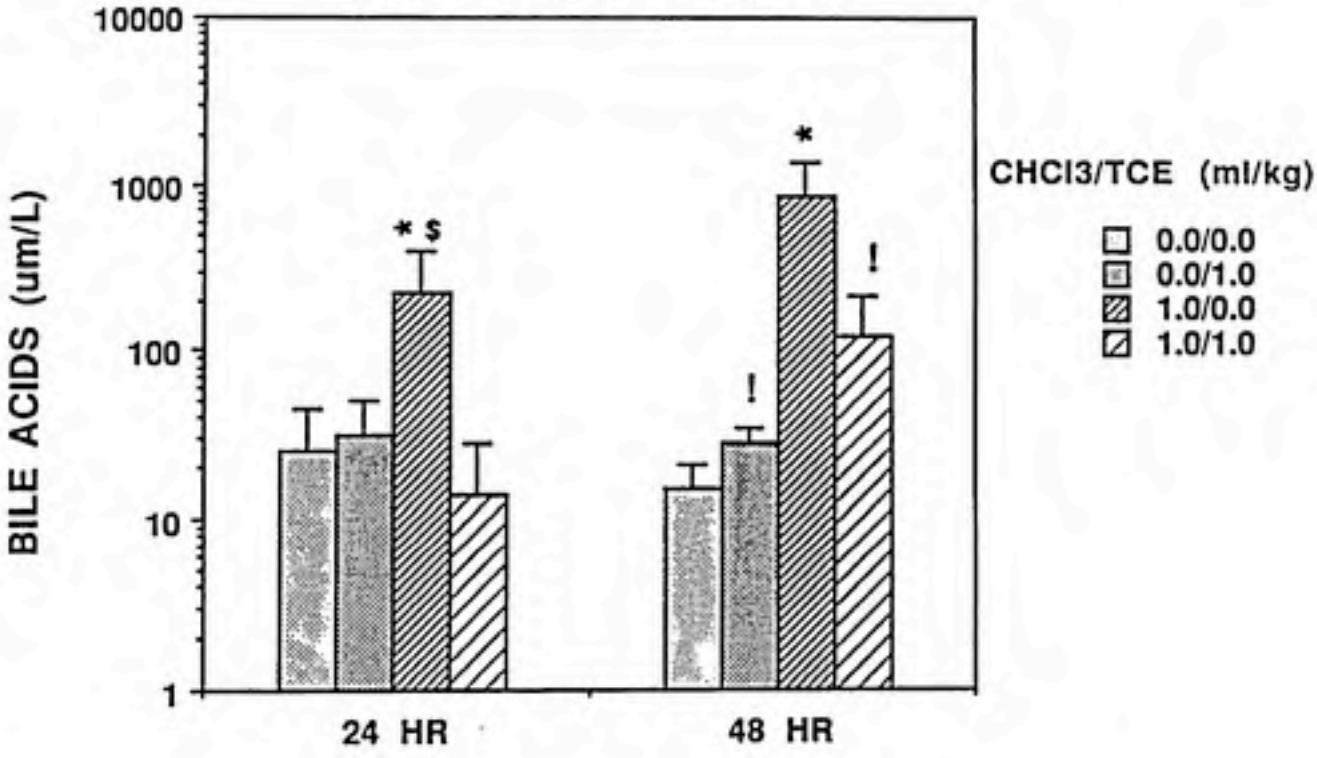


FIG. 19. HEPATOTOXICITY OF 0.5 ml CHCl3/kg AND TCE IN AQUEOUS VEHICLE

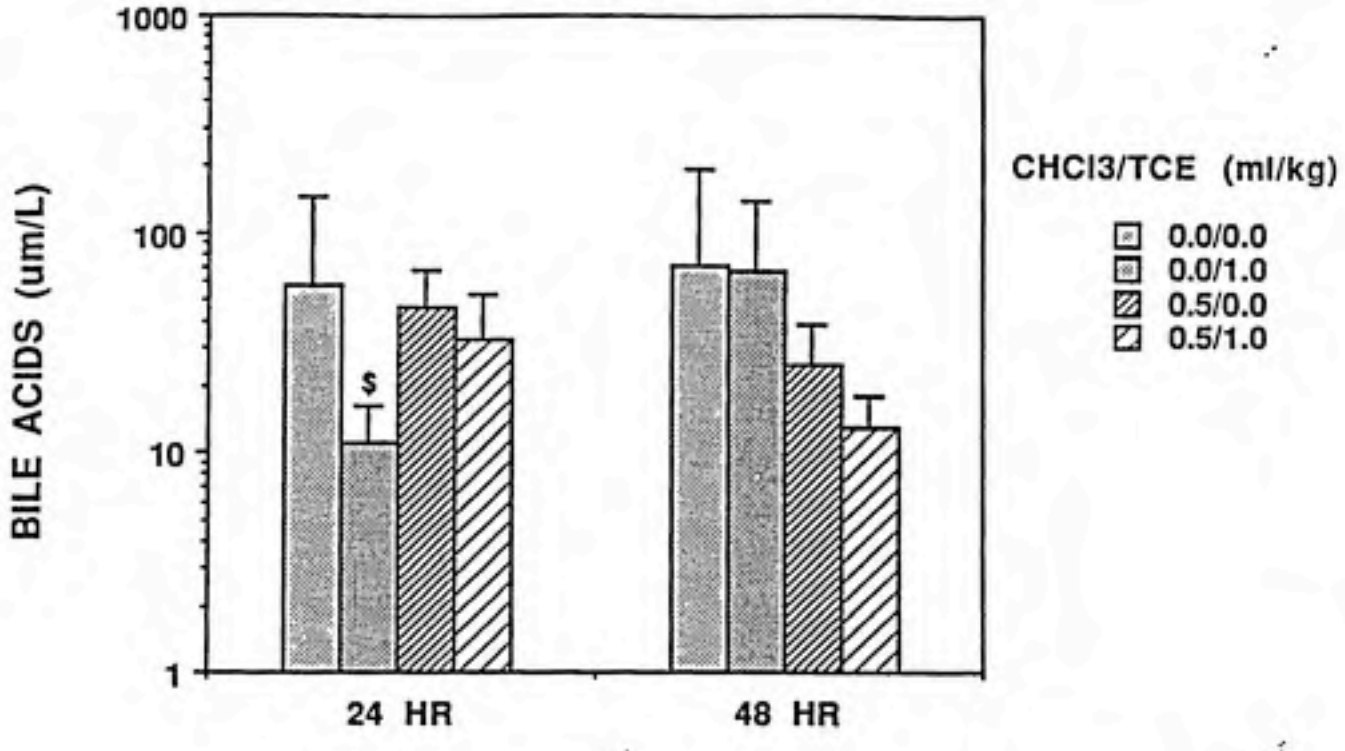


FIG. 20. HEPATOTOXICITY OF 1.0 ml CHCl3/kg AND TCE IN AQUEOUS VEHICLE

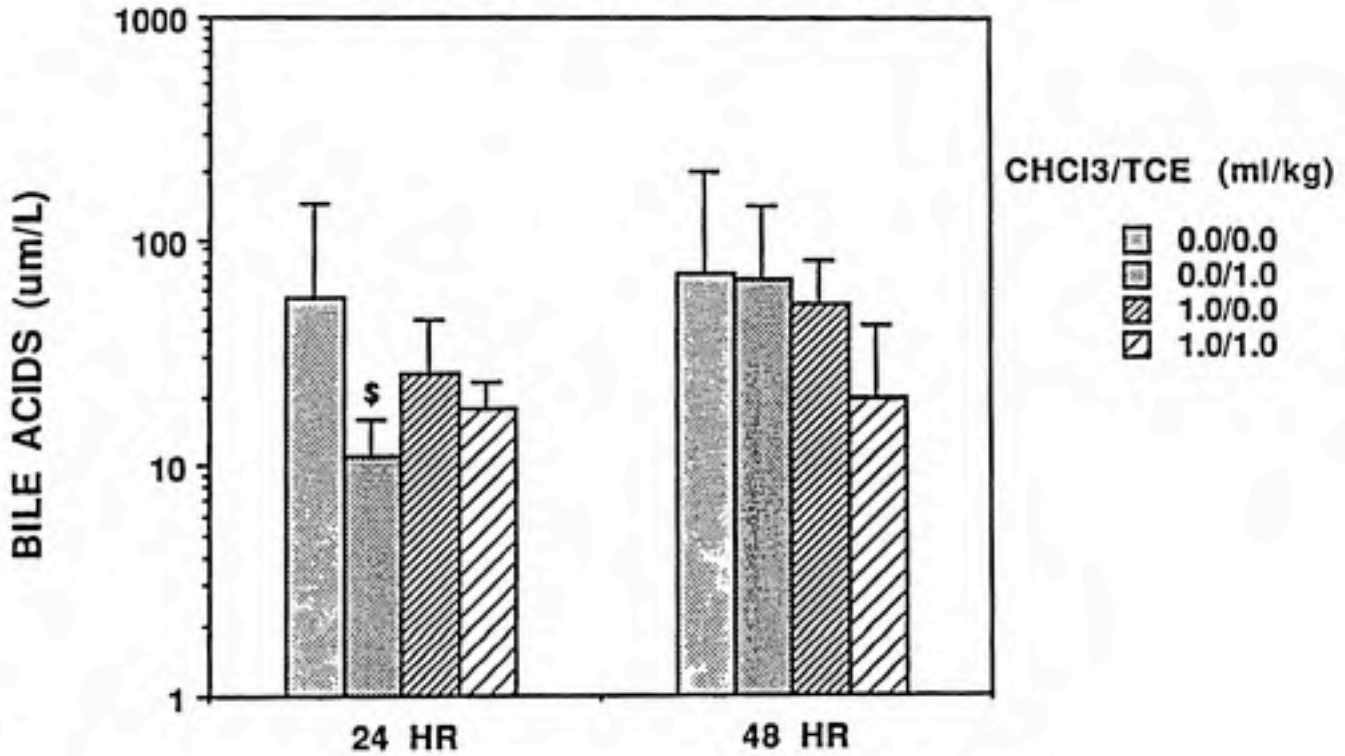


FIG. 21. CHCl3 HEPATOTOXICITY IN OIL AND AQUEOUS VEHICLES AT 24 HOURS

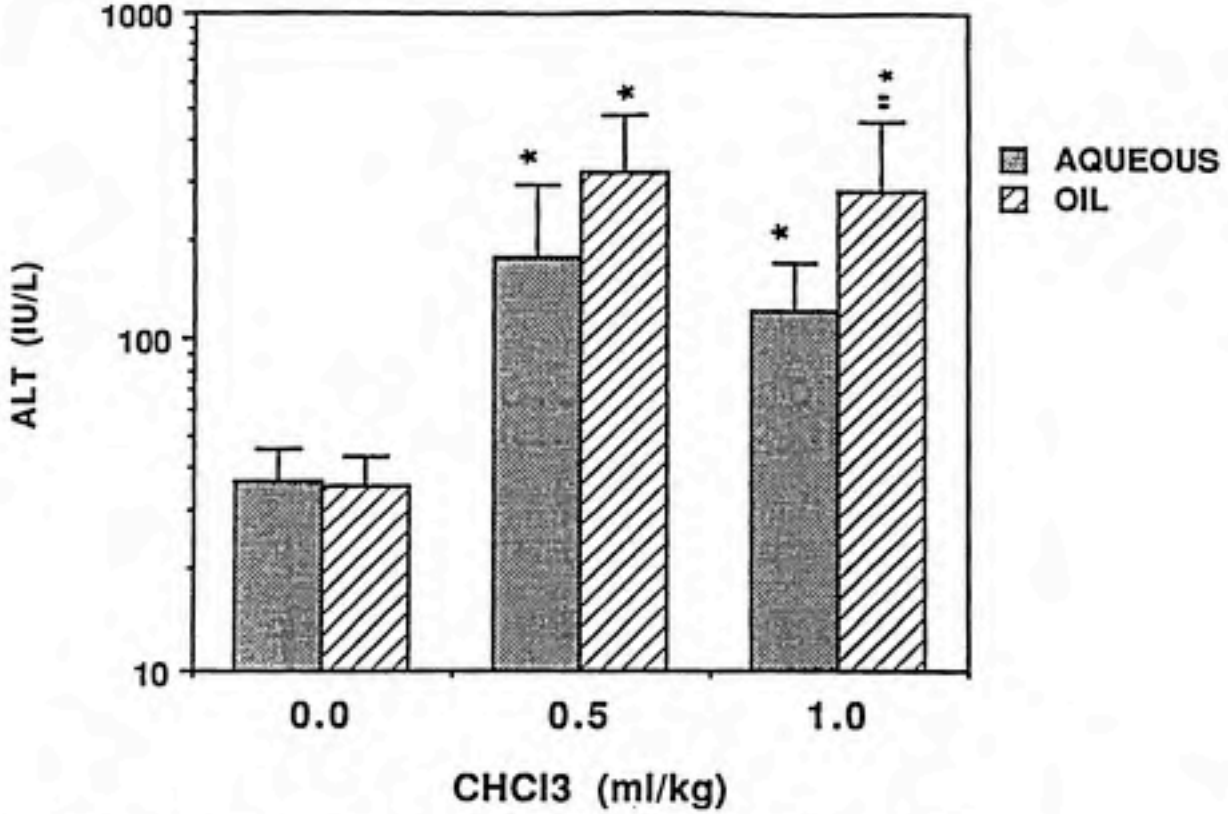


FIG. 22. CHCl3 HEPATOTOXICITY IN OIL AND AQUEOUS VEHICLES AT 48 HOURS

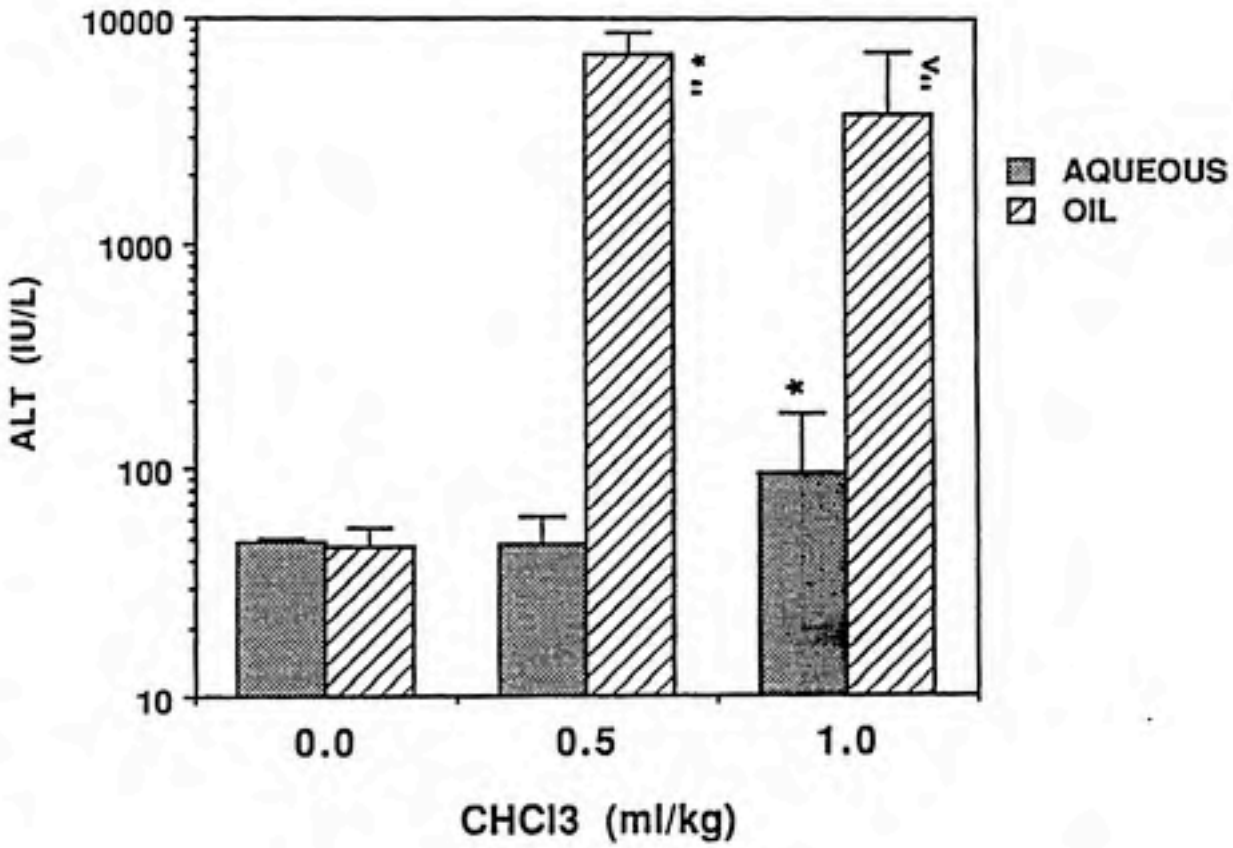


FIG. 23. CHCl3 HEPATOTOXICITY IN OIL AND AQUEOUS VEHICLES AT 24 HOURS

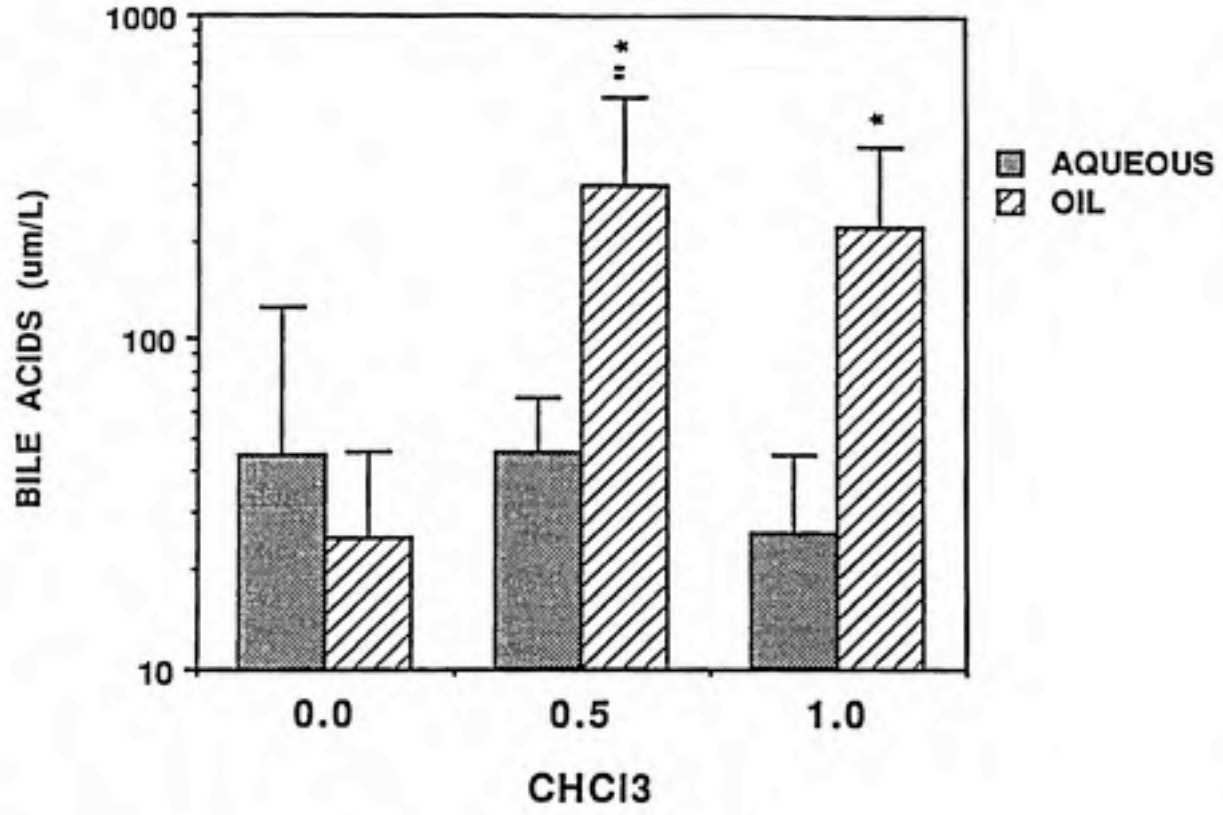


FIG. 24. CHCl3 HEPATOTOXICITY IN OIL AND AQUEOUS VEHICLES AT 48 HOURS

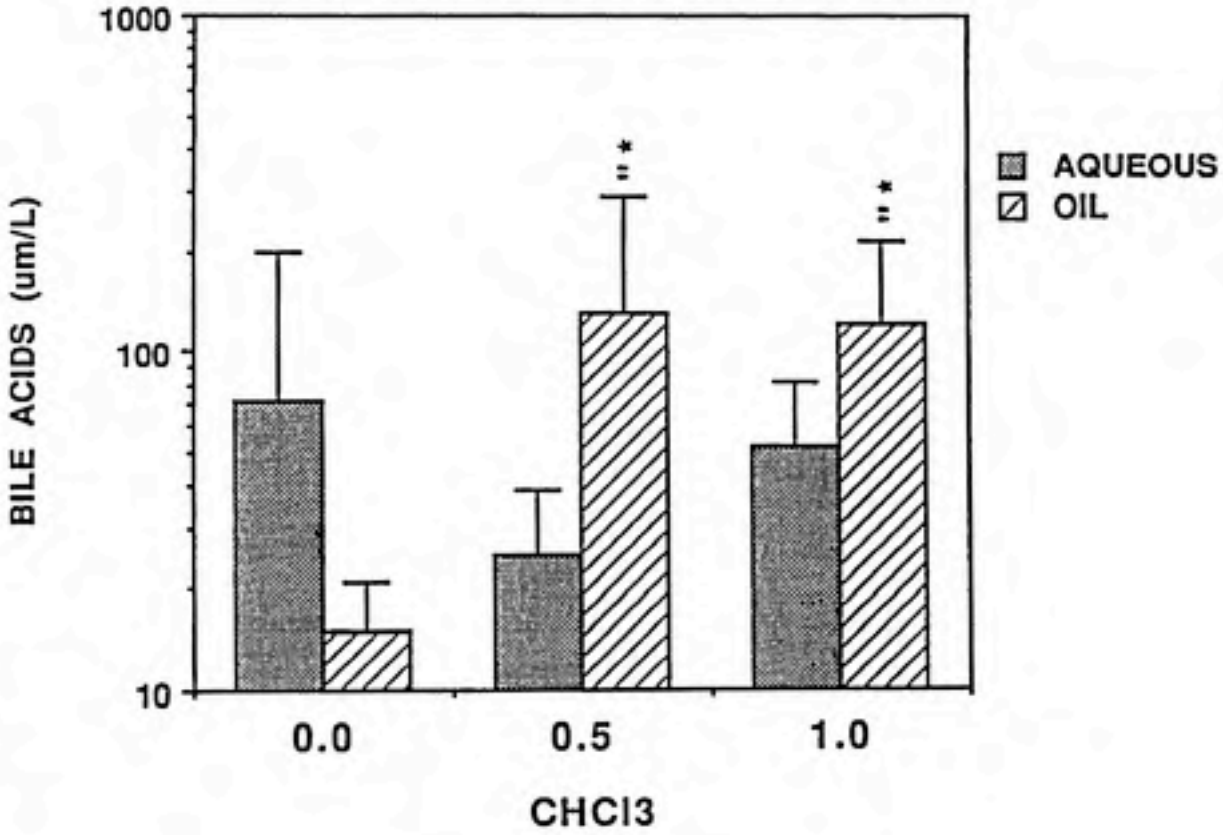


FIG. 25. RELATIVE KIDNEY WEIGHT - OIL VEHICLE

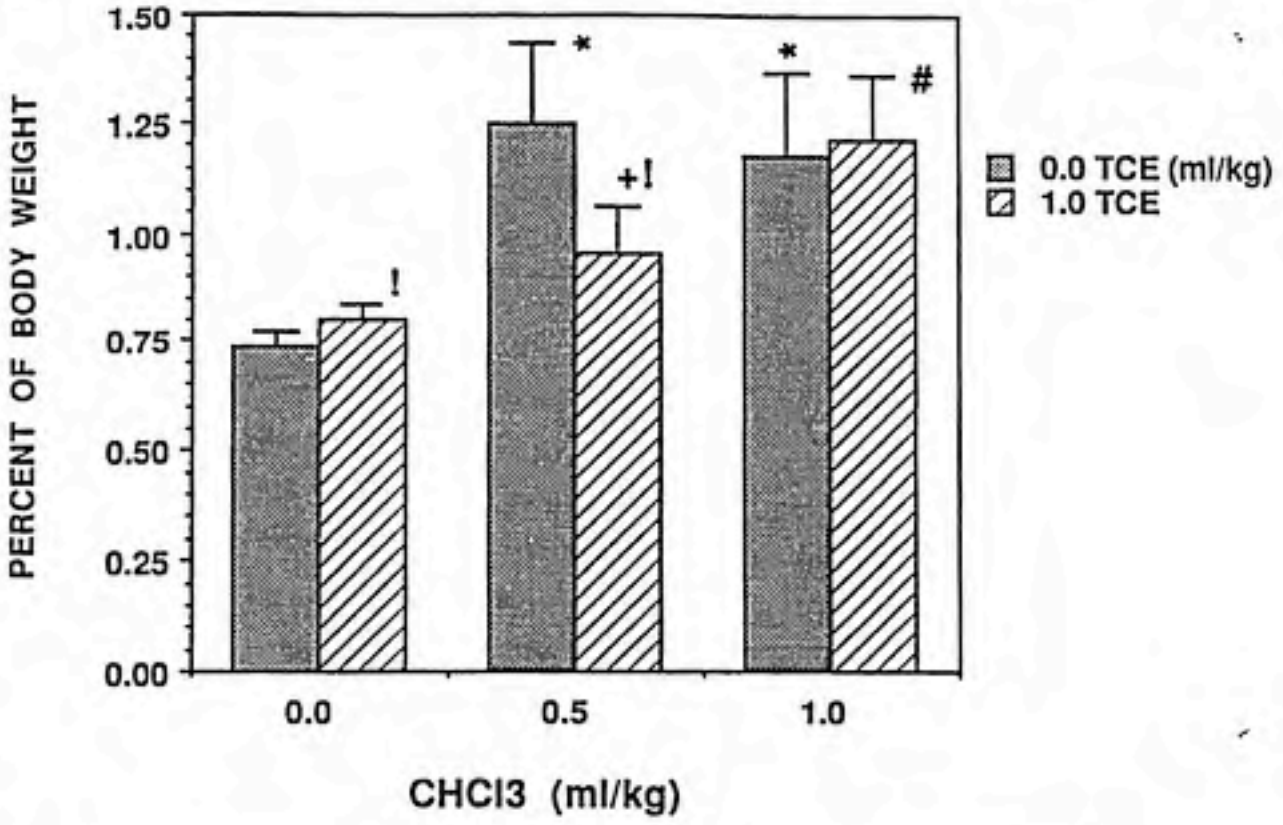


FIG. 26. RELATIVE KIDNEY WEIGHT - AQUEOUS VEHICLE

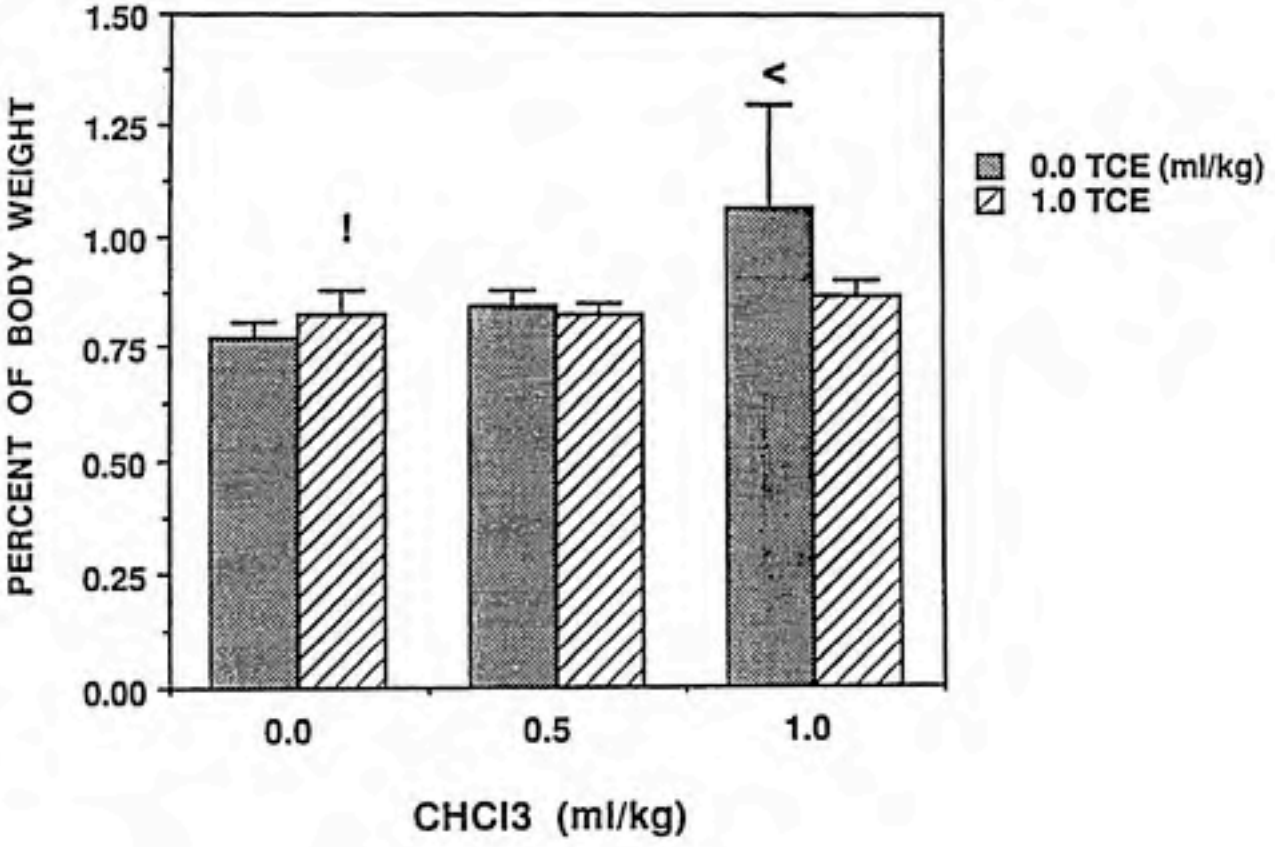


FIG. 27. BUN AT 48 HRS - OIL VEHICLE

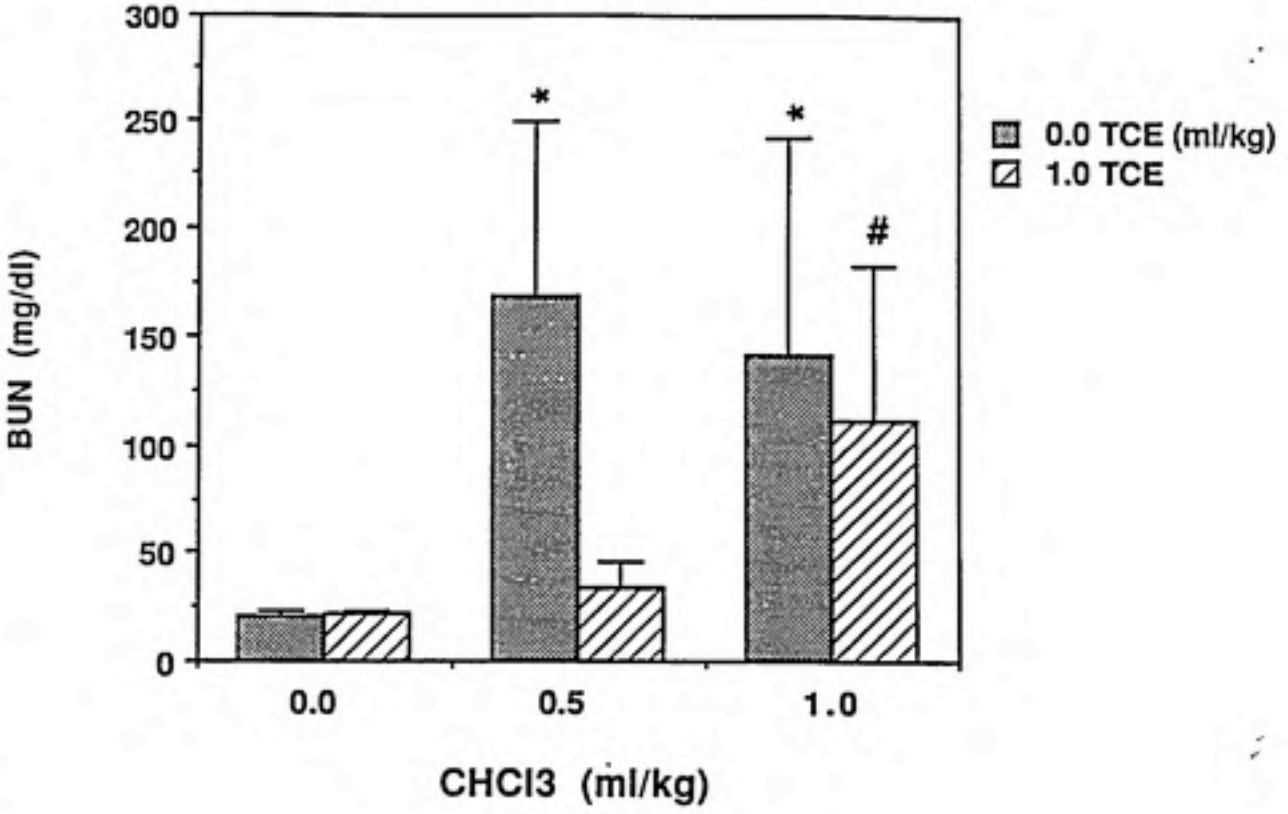


FIG. 28. BUN AT 48 HRS - AQUEOUS VEHICLE

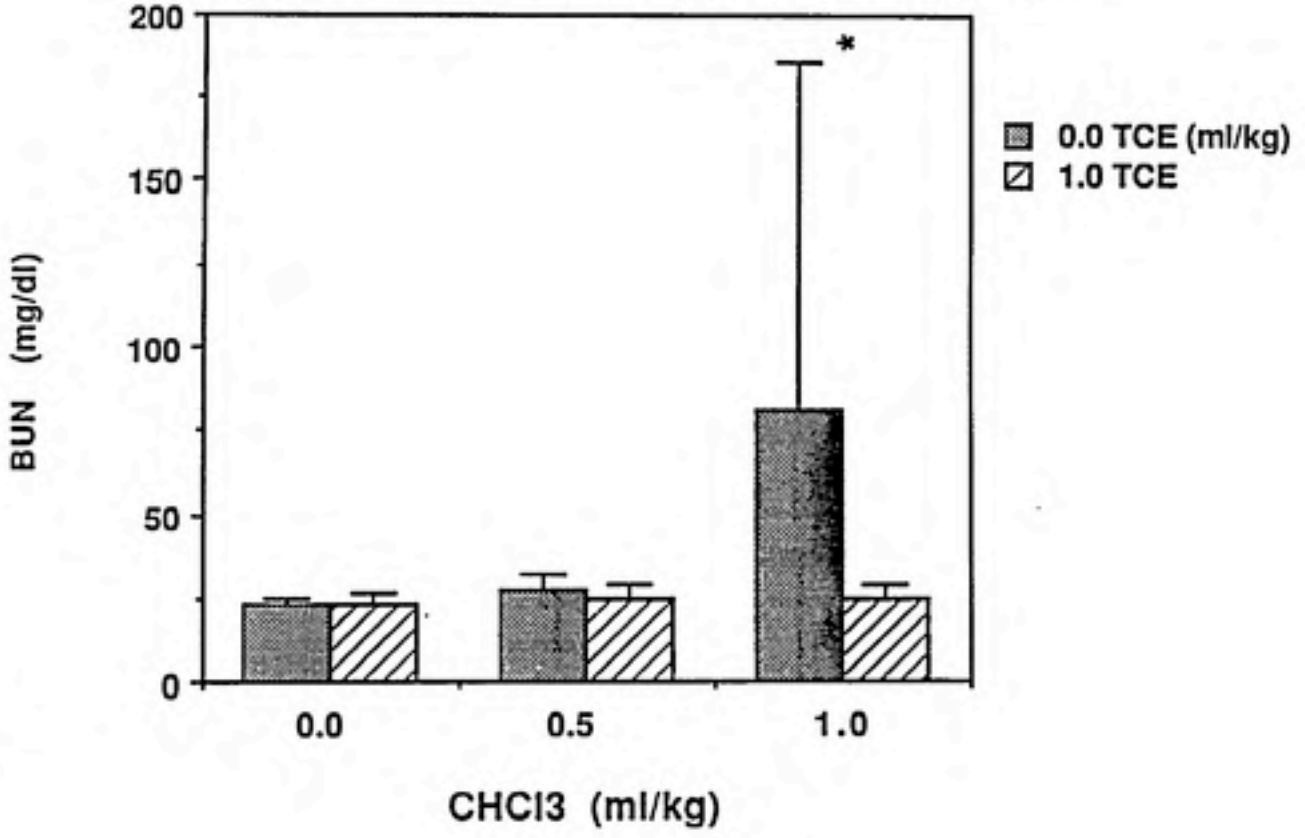


FIG. 29. CRE AT 48 HRS - OIL VEHICLE

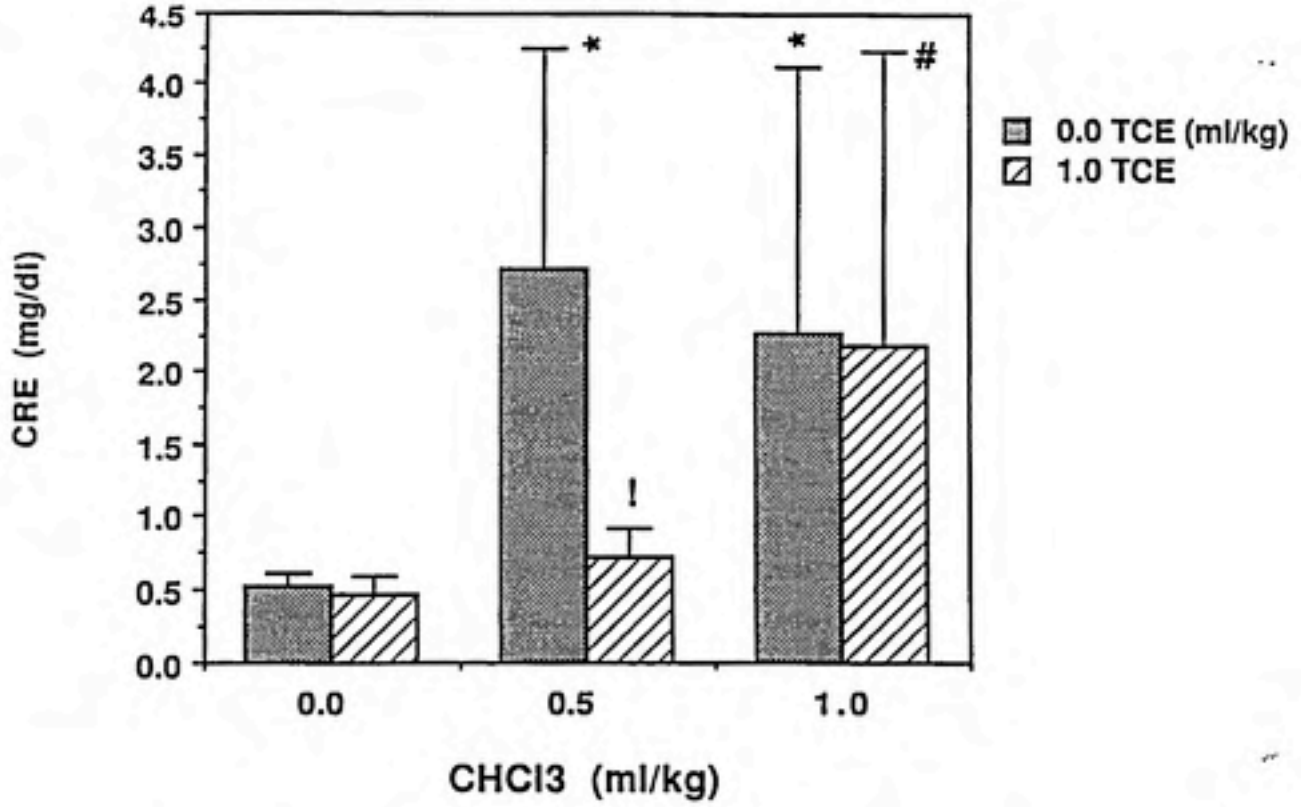


FIG. 30. CRE AT 48 HRS - AQUEOUS VEHICLE

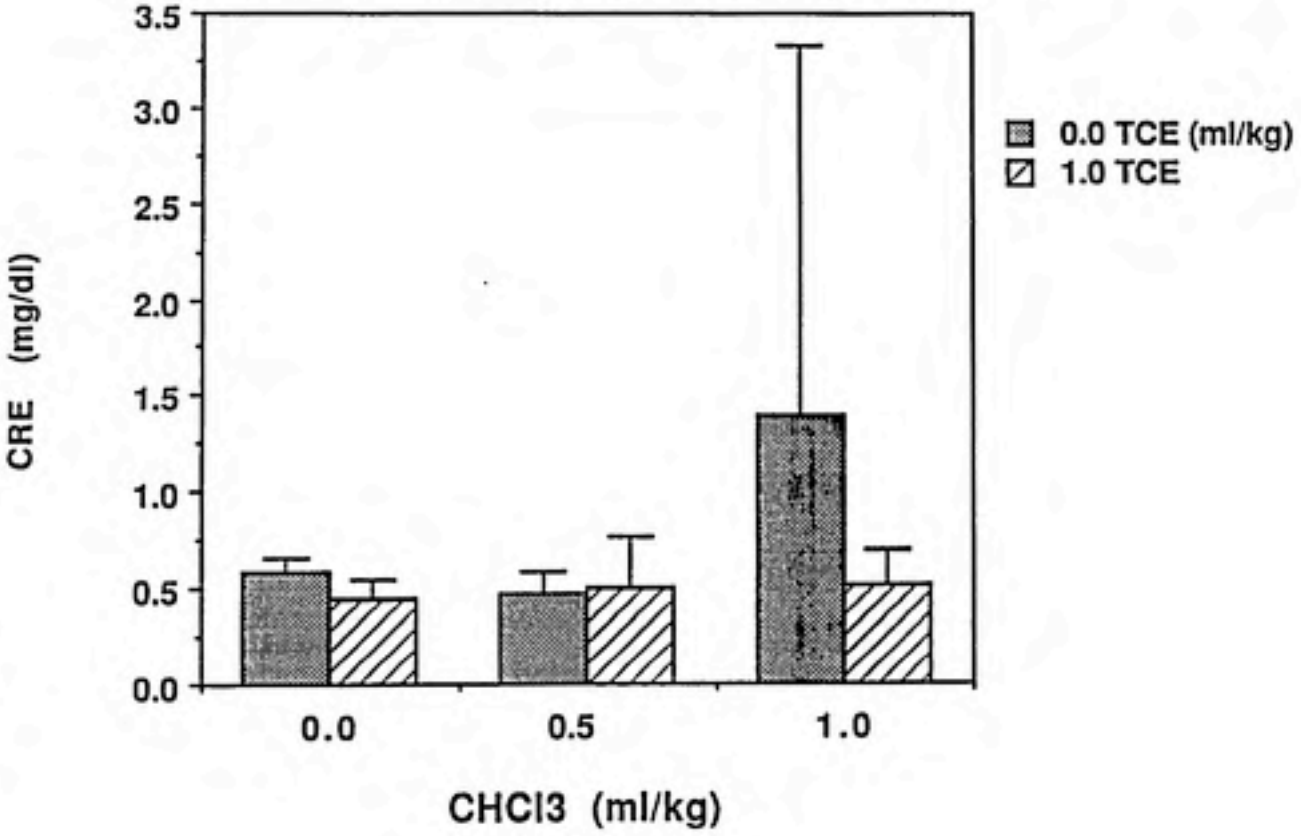


FIG. 31. NEPHROTOXICITY OF CHCl3 AND TCE IN OIL VEHICLE

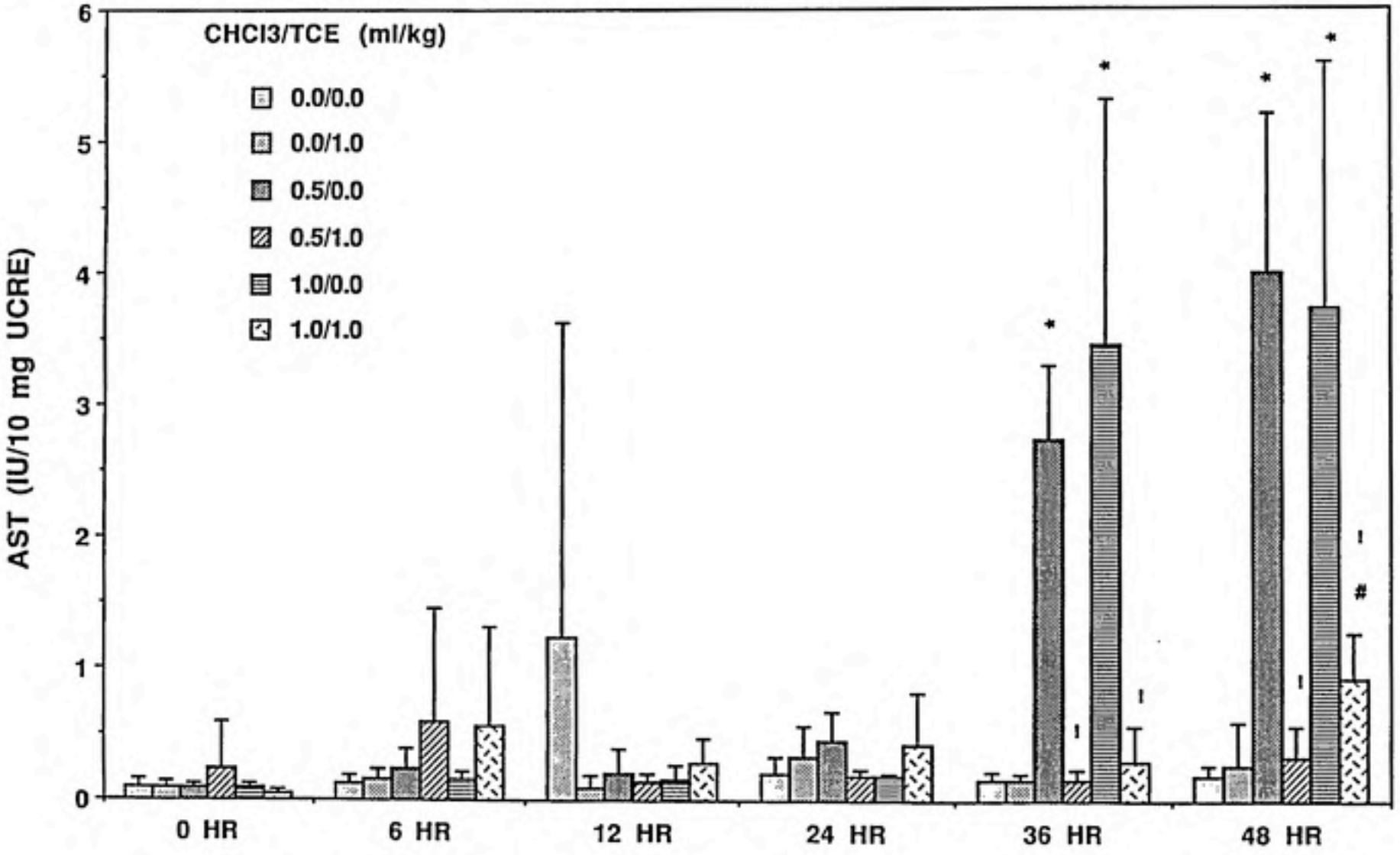


FIG. 32. NEPHROTOXICITY OF CHCl3 AND TCE IN AQUEOUS VEHICLE

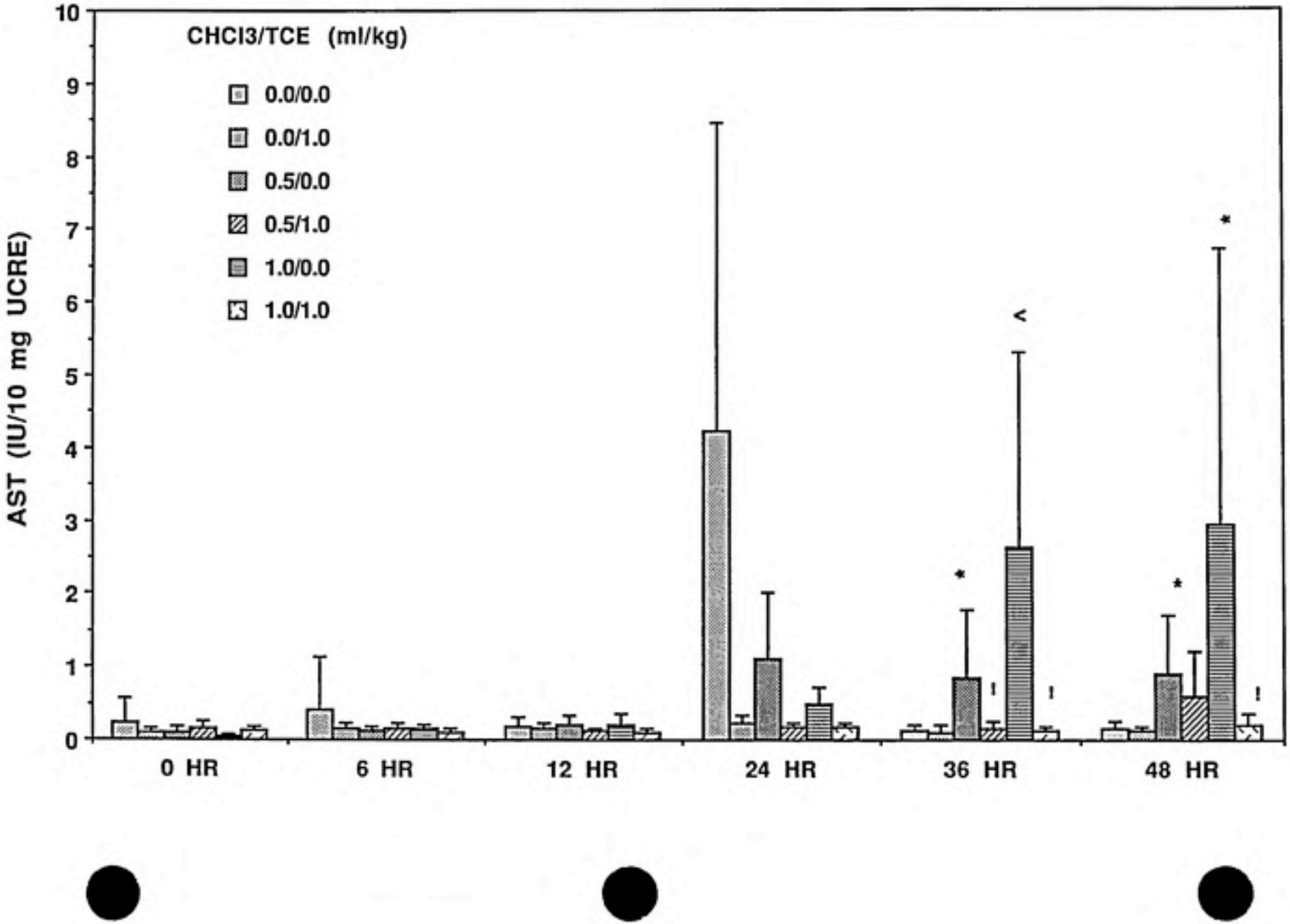


FIG. 33. NEPHROTOXICITY OF CHCl3 AND TCE IN OIL VEHICLE

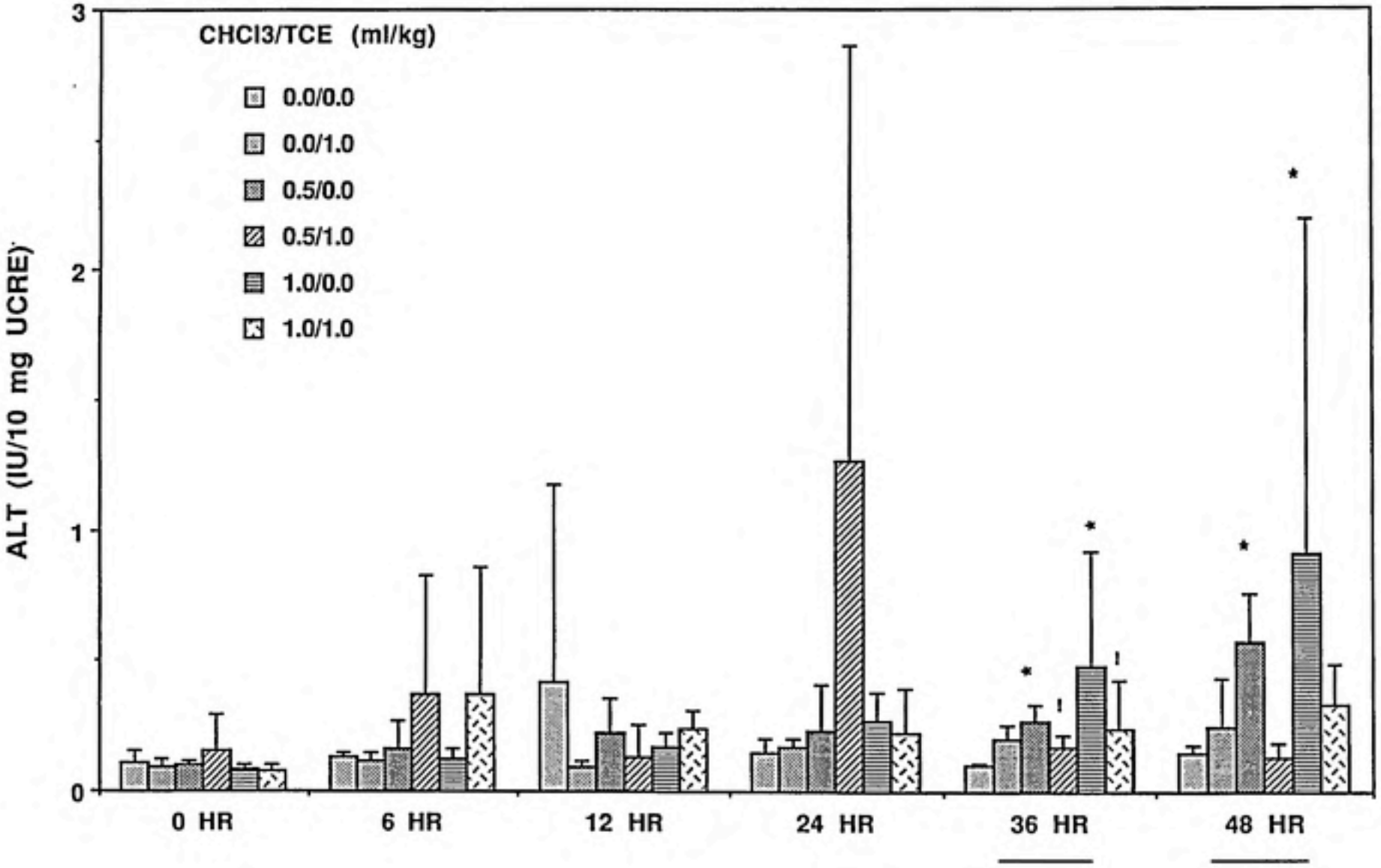


FIG. 34. NEPHROTOXICITY OF CHCl3 AND TCE IN AQUEOUS VEHICLE

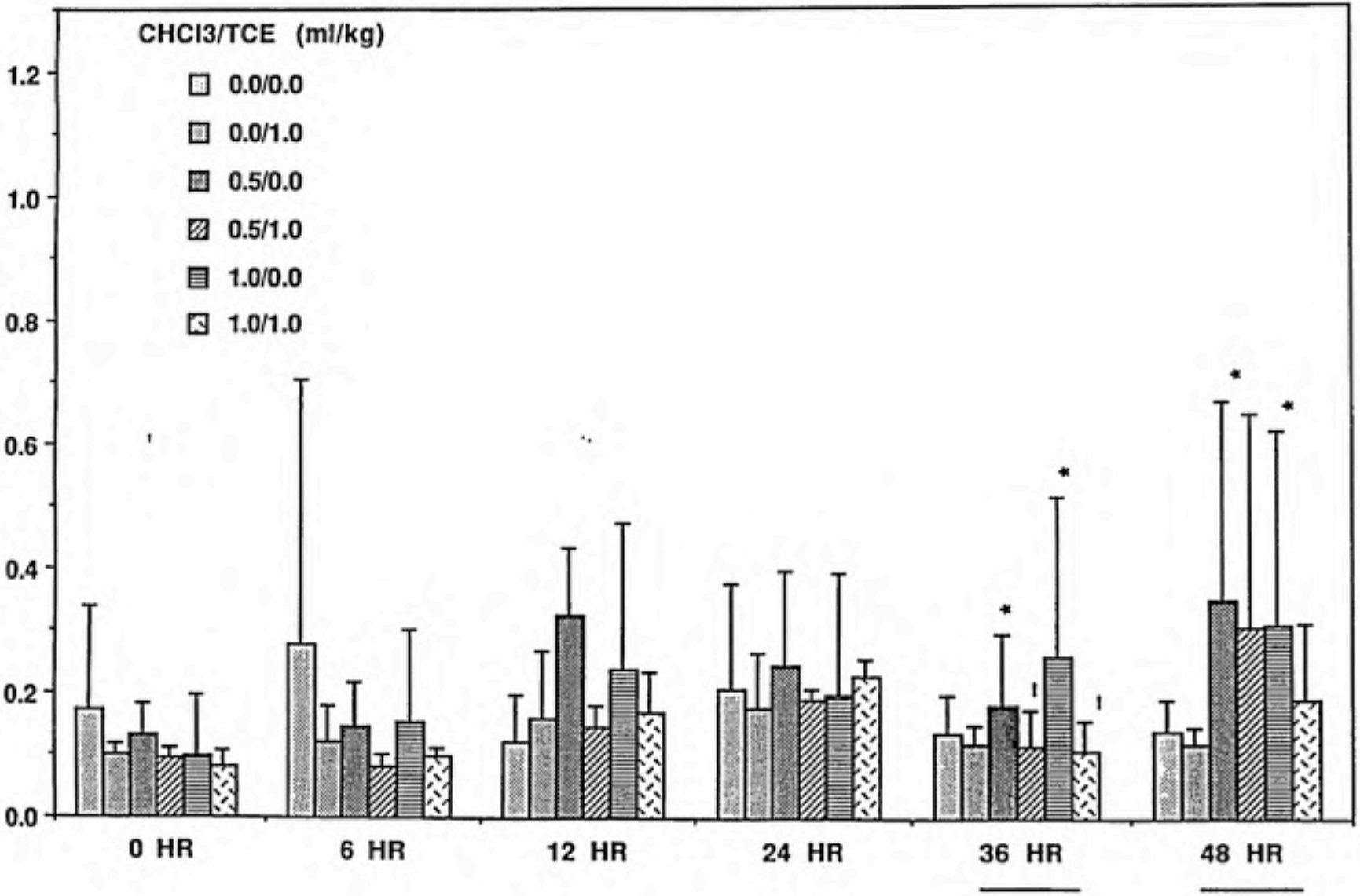


FIG. 35. NEPHROTOXICITY OF CHCl3 AND TCE IN OIL VEHICLE

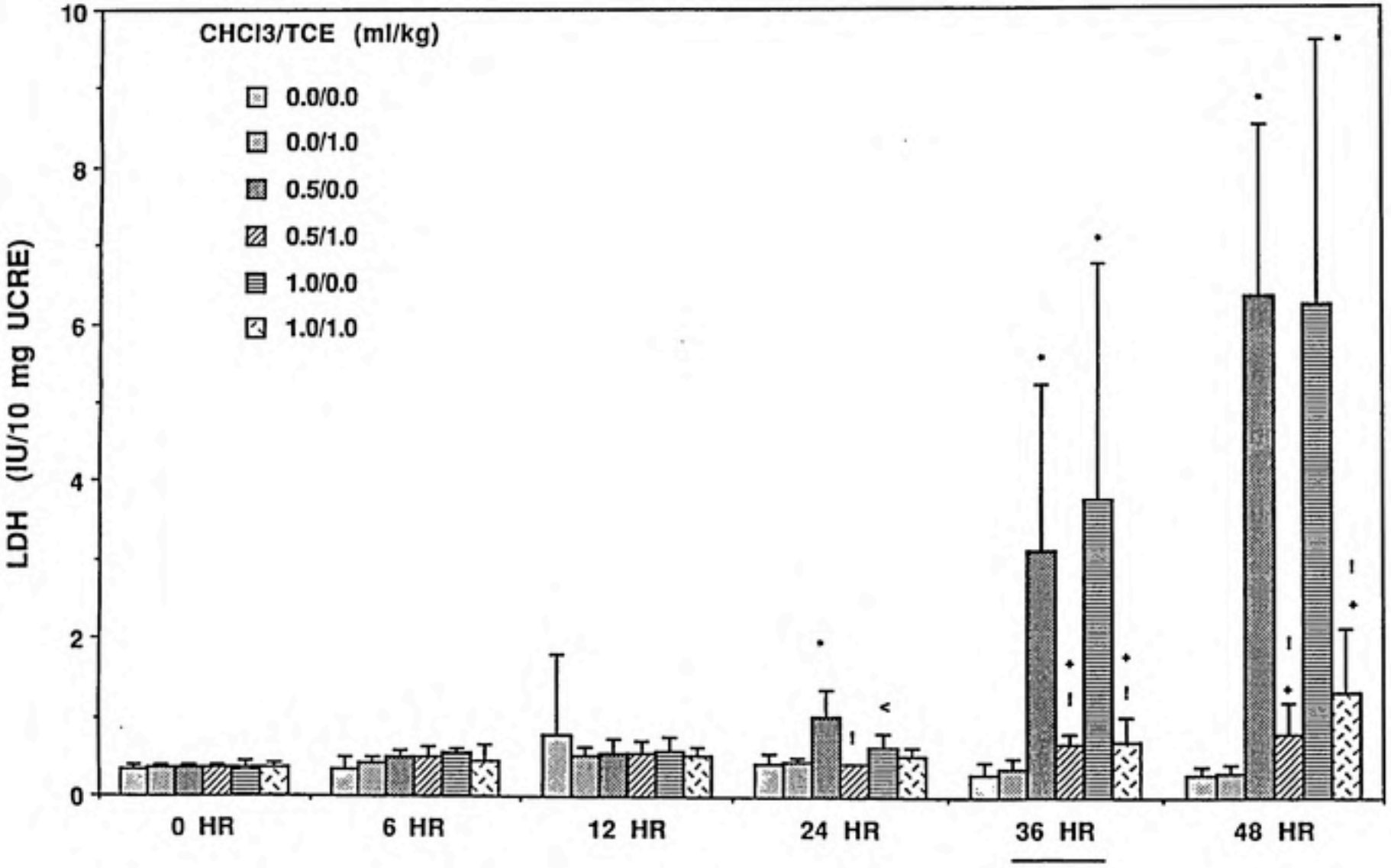


FIG. 36. NEPHROTOXICITY OF CHCl3 AND TCE IN AQUEOUS VEHICLE

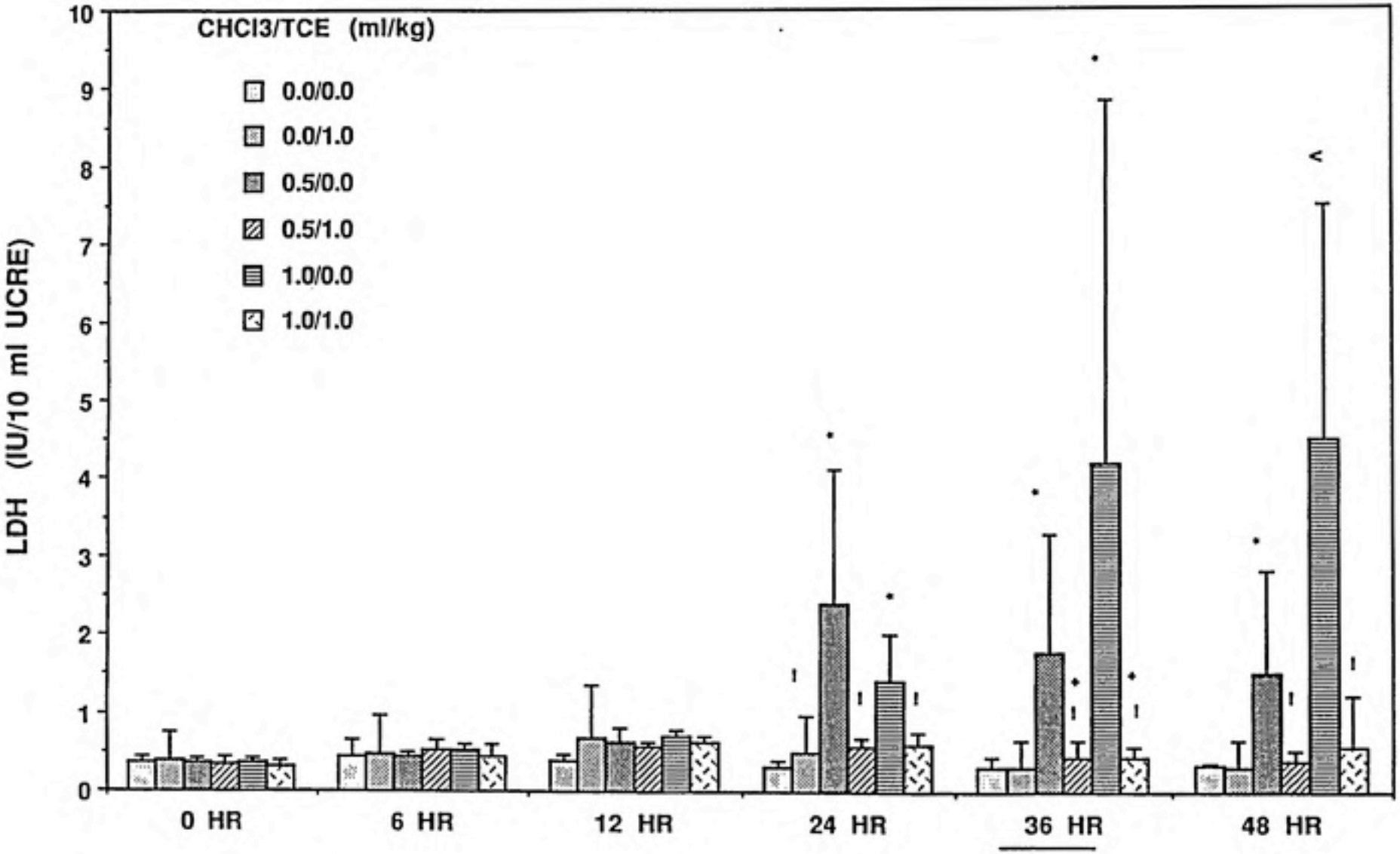


FIG. 37. NEPHROTOXICITY OF CHCl3 AND TCE IN OIL VEHICLE

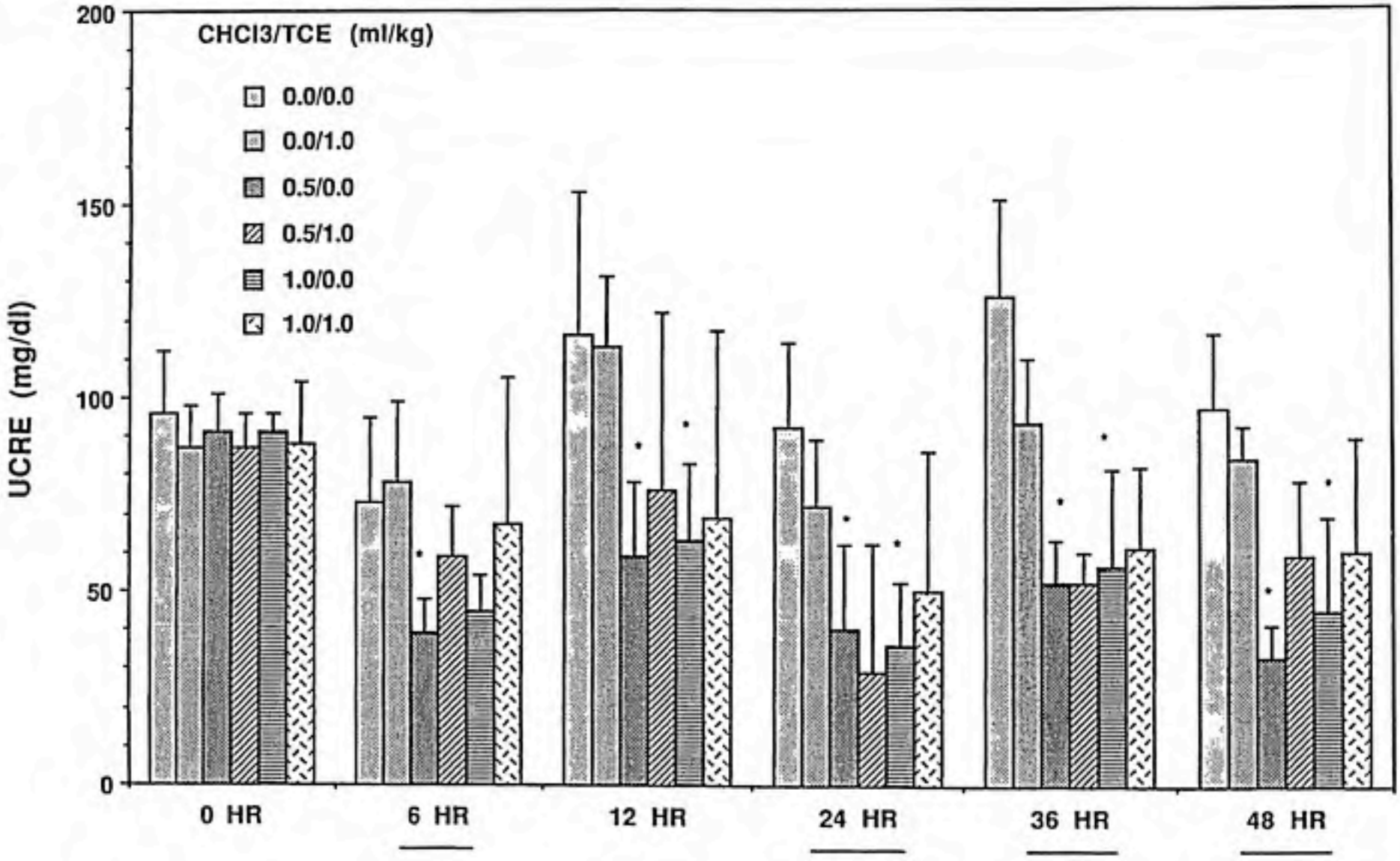


FIG. 38. NEPHROTOXICITY OF CHCl3 AND TCE IN AQUEOUS VEHICLE

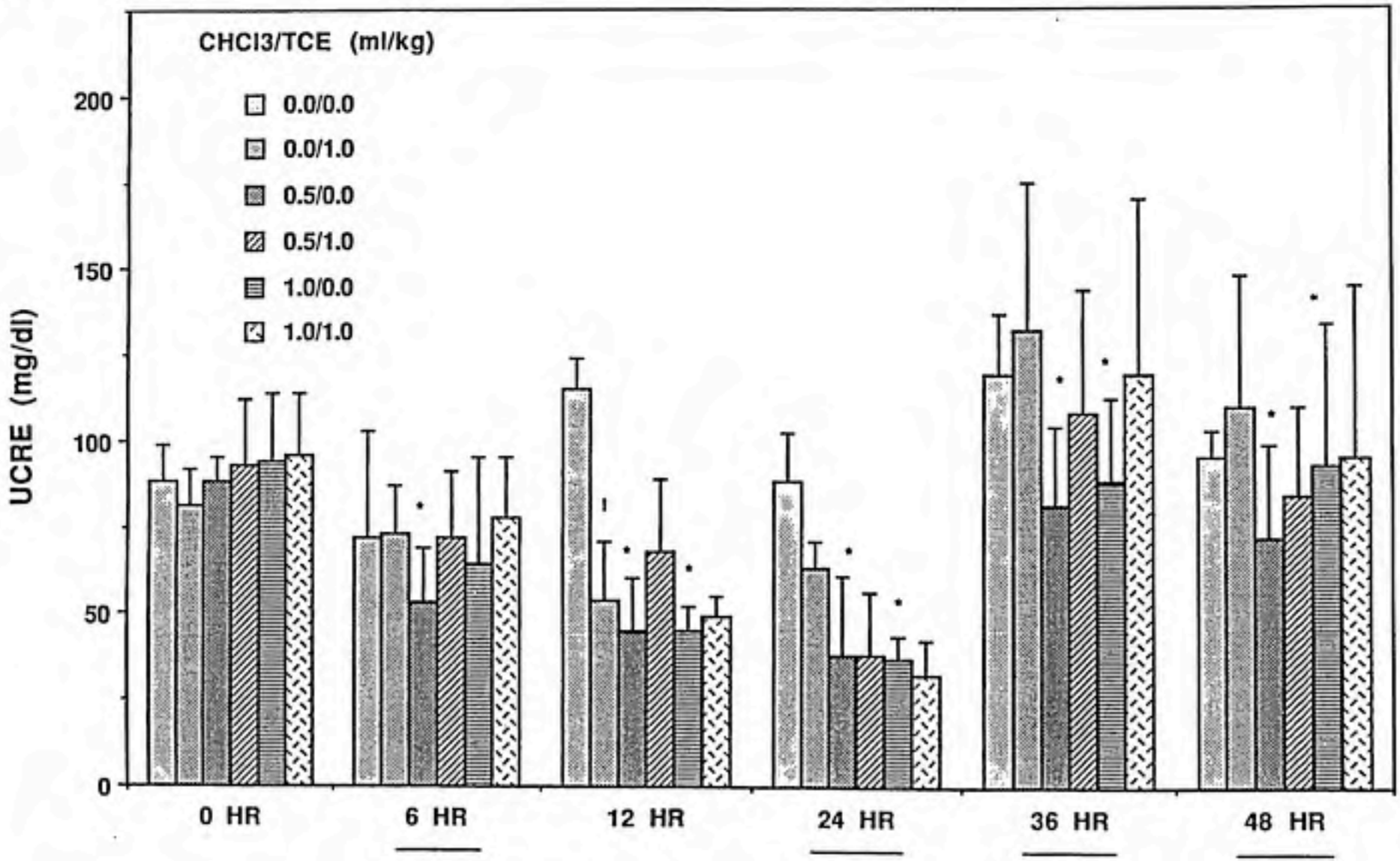


FIG. 39. NEPHROTOXICITY OF CHCl3 AND TCE IN OIL VEHICLE

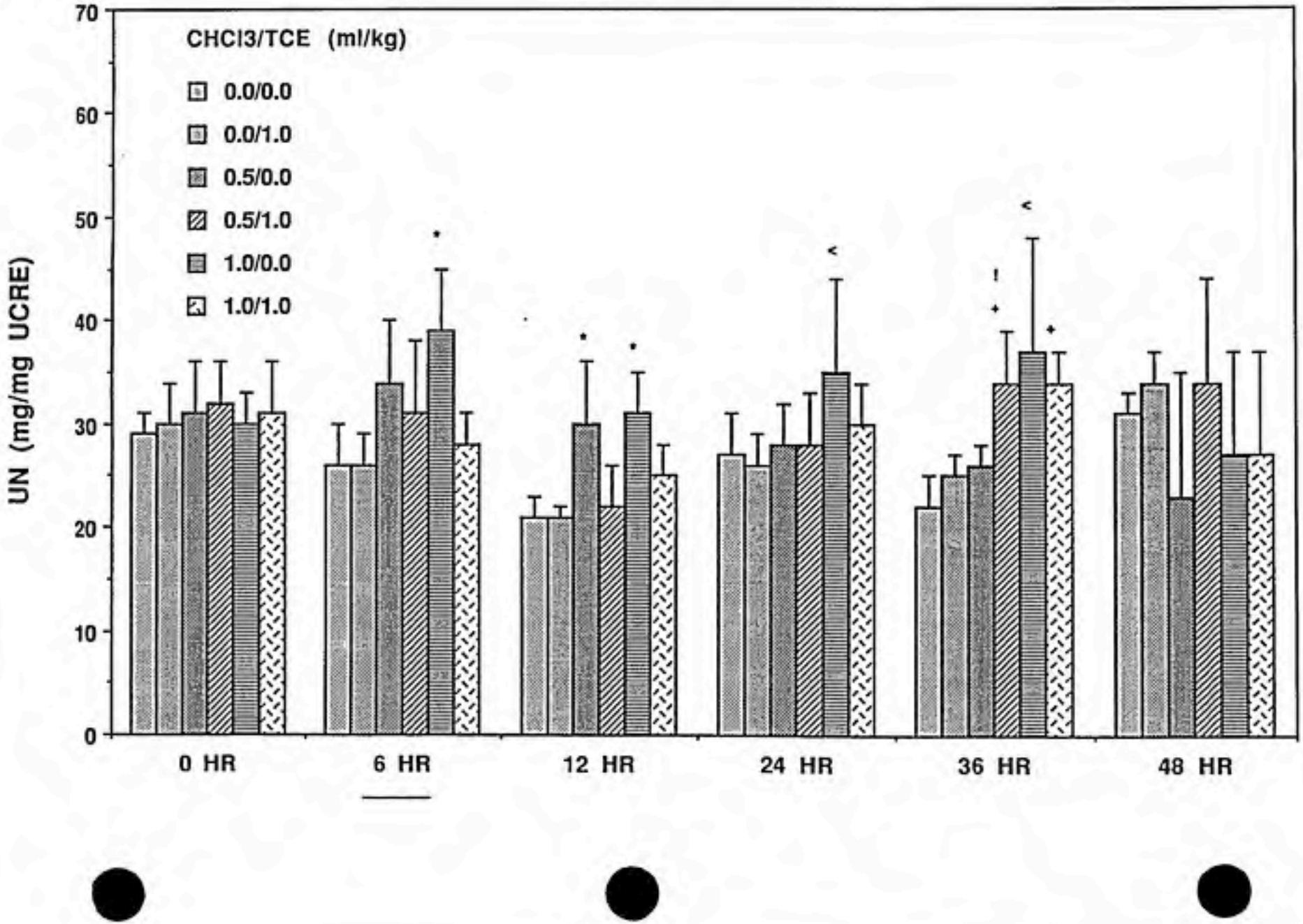


FIG. 40. NEPHROTOXICITY OF CHCl3 AND TCE IN AQUEOUS VEHICLE

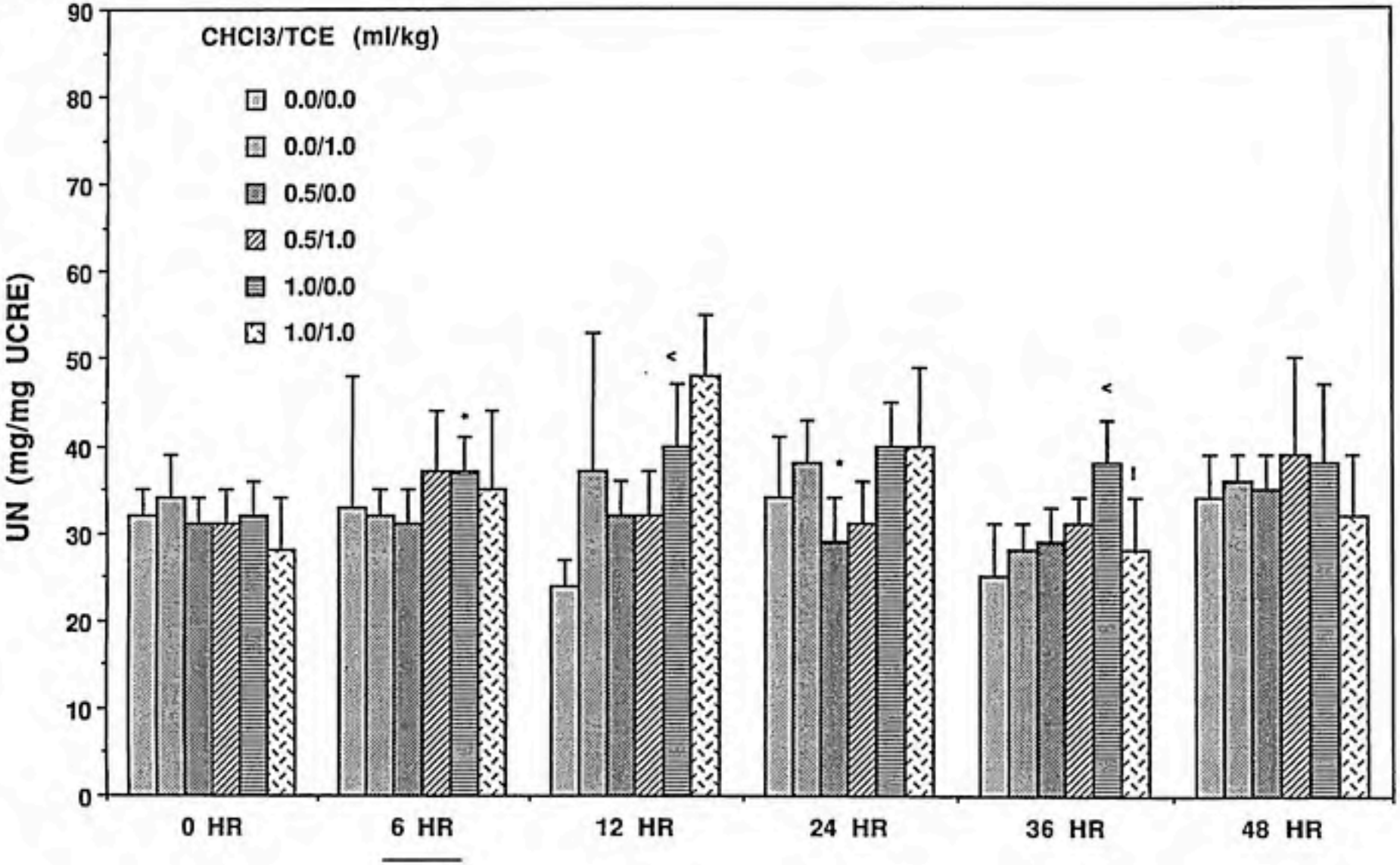


FIG. 41. NEPHROTOXICITY OF CHCI3 AND TCE IN OIL VEHICLE

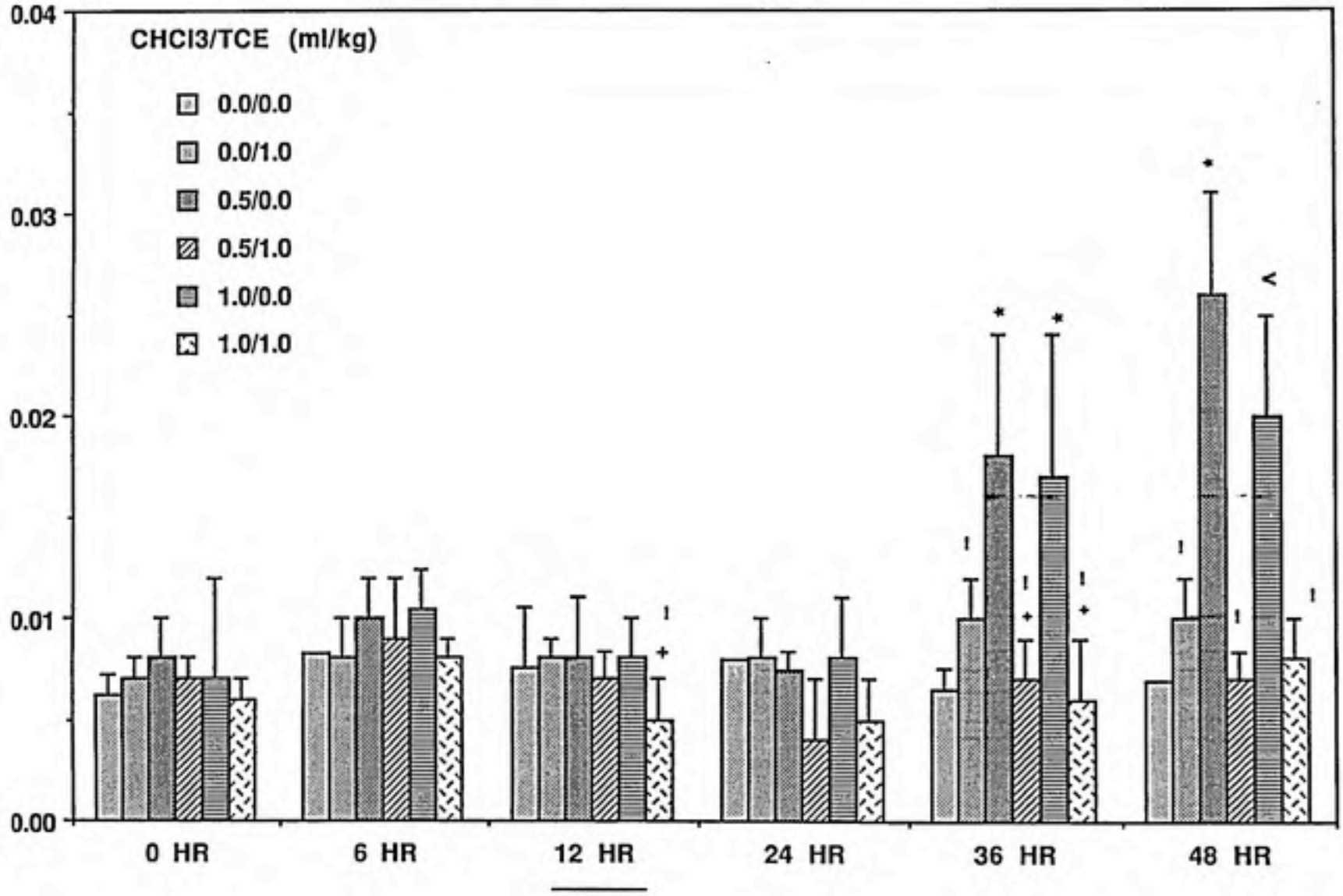


FIG. 42. NEPHROTOXICITY OF CHCl3 AND TCE IN AQUEOUS VEHICLE

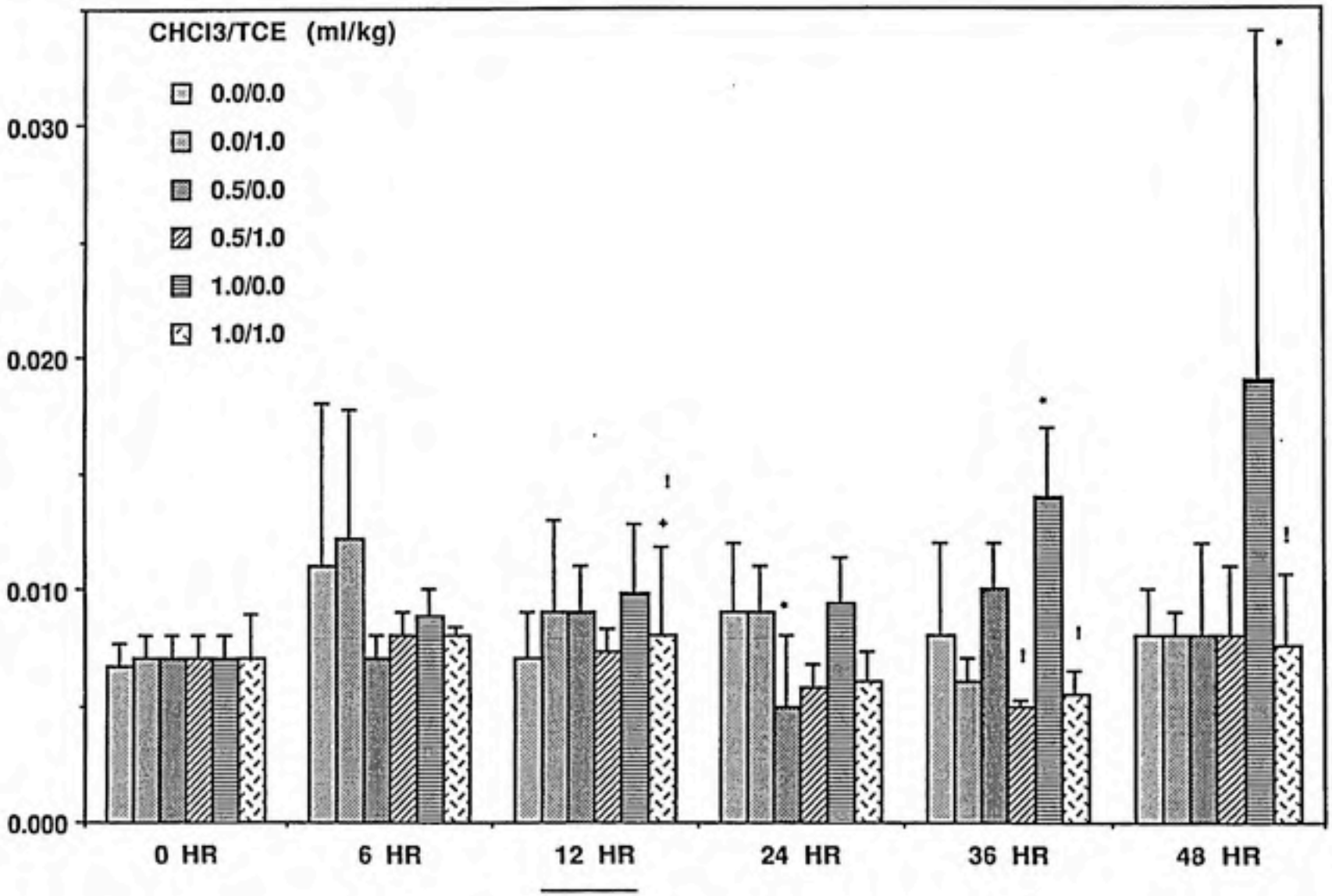


FIG. 43. NEPHROTOXICITY OF CHCl3 AND TCE IN OIL VEHICLE

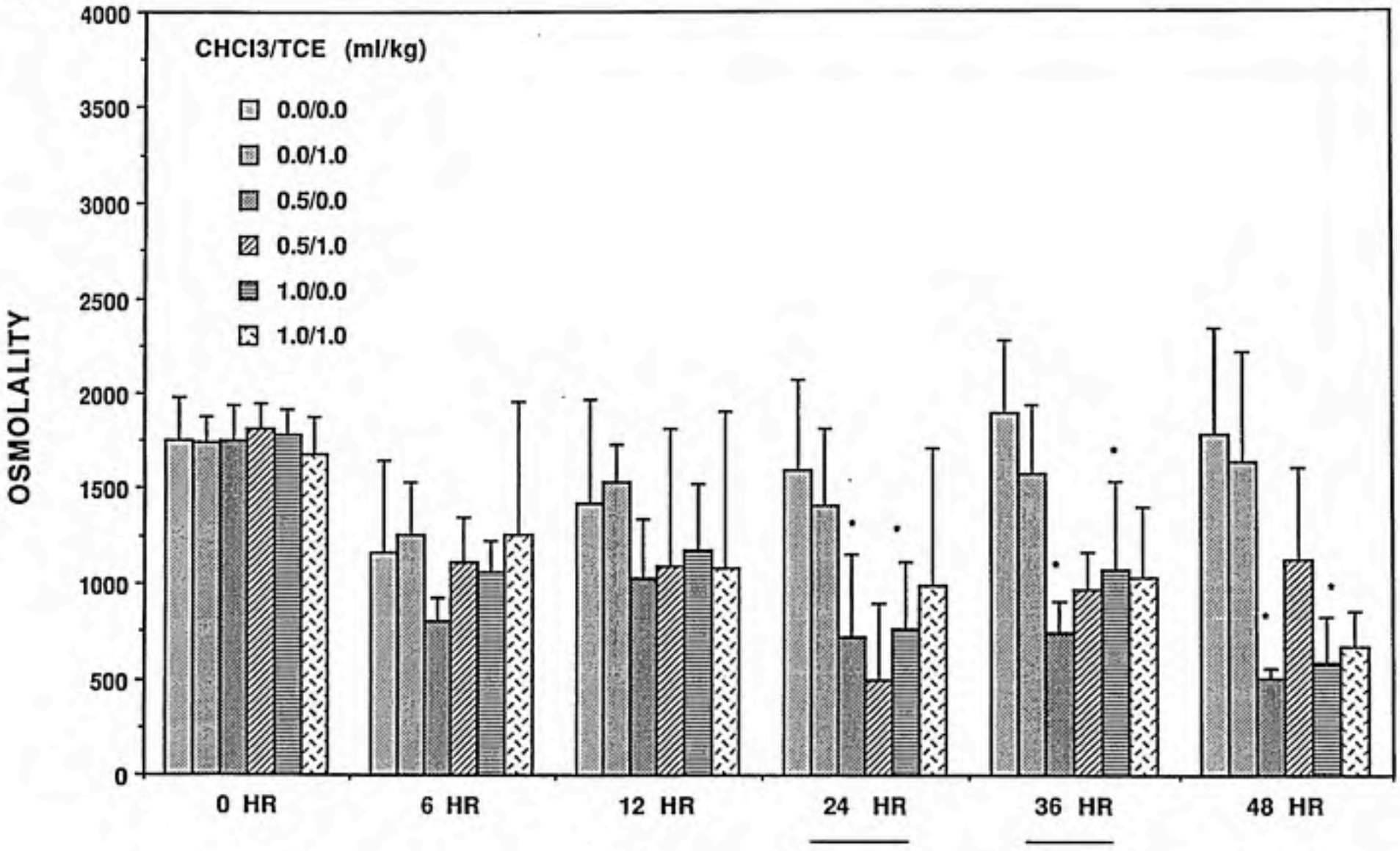


FIG. 44. NEPHROTOXICITY OF CHCl3 AND TCE IN AQUEOUS VEHICLE

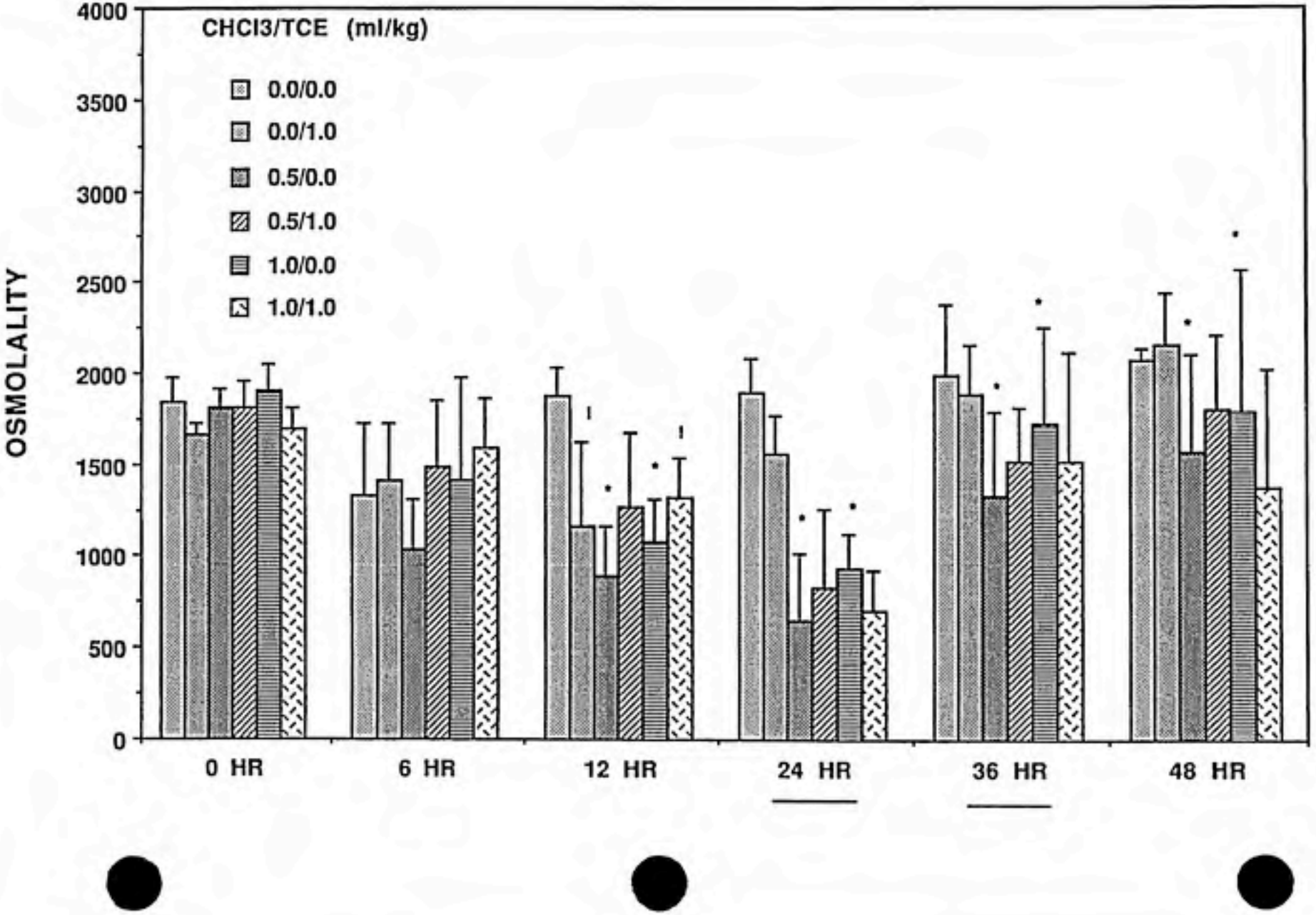


FIG. 45. NEPHROTOXICITY OF CHCl3 AND TCE IN OIL VEHICLE

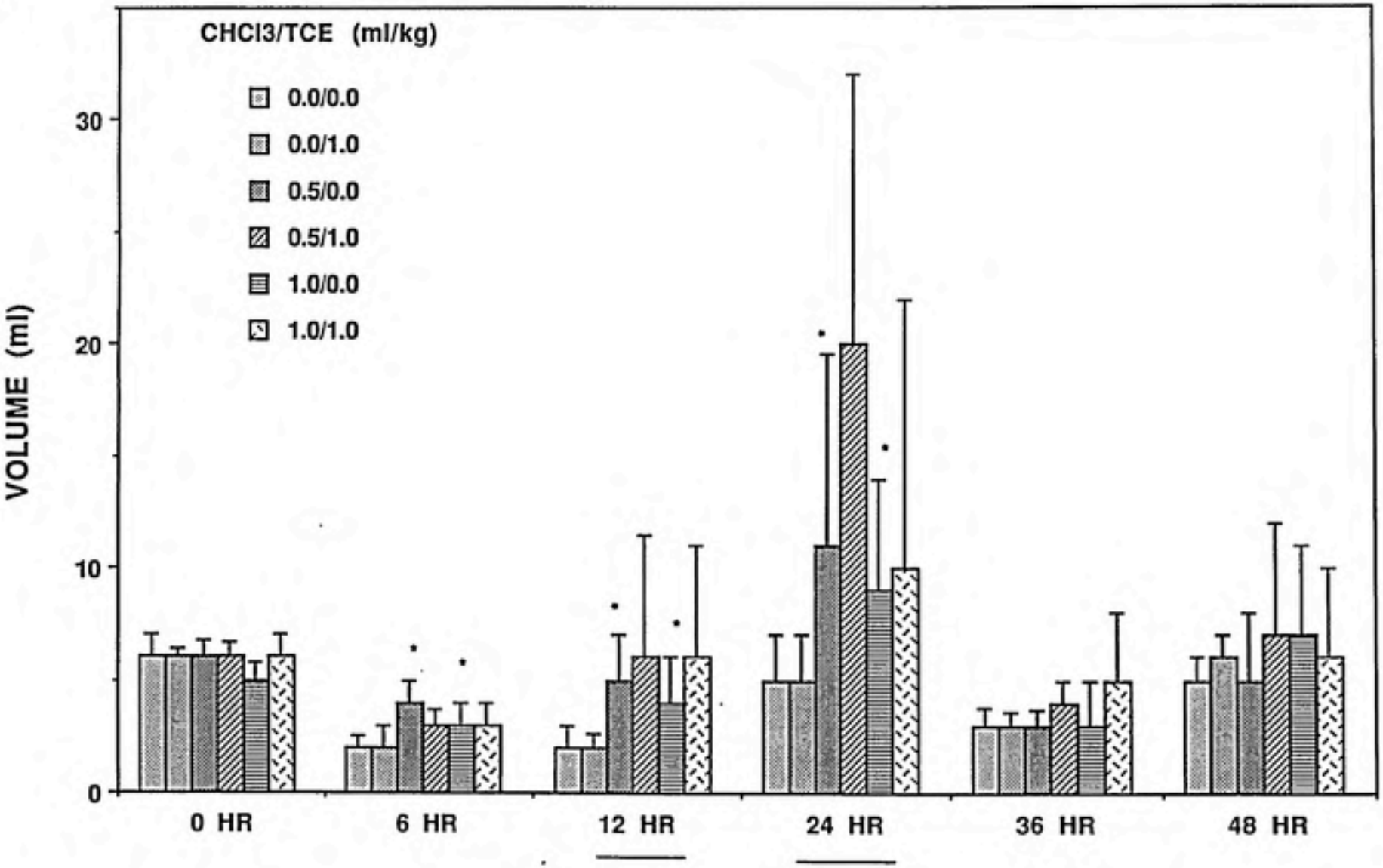


FIG. 46. NEPHROTOXICITY OF CHCl3 AND TCE IN AQUEOUS VEHICLE

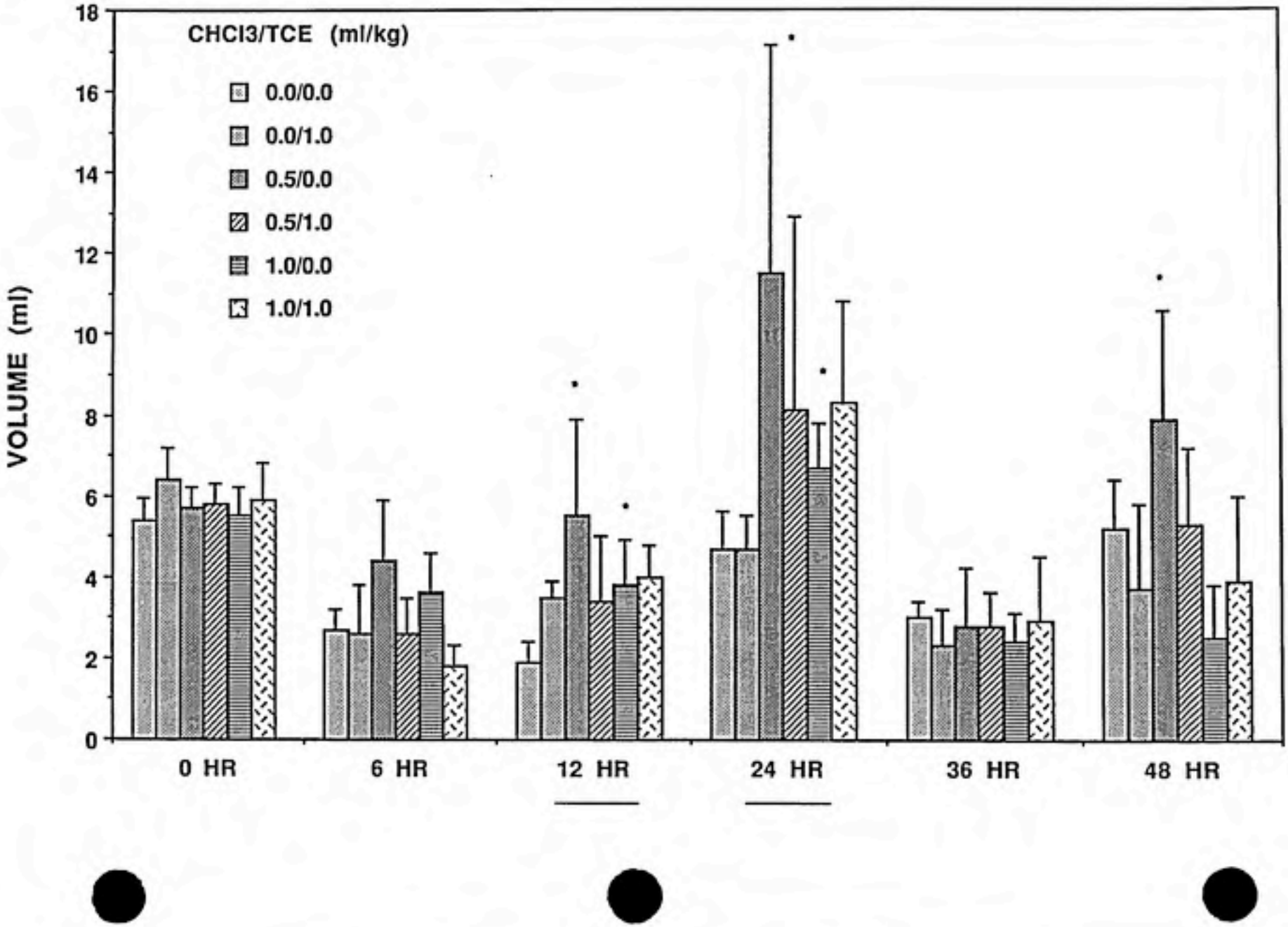


FIG. 47. NEPHROTOXICITY OF CHCl3 AND TCE IN OIL VEHICLE

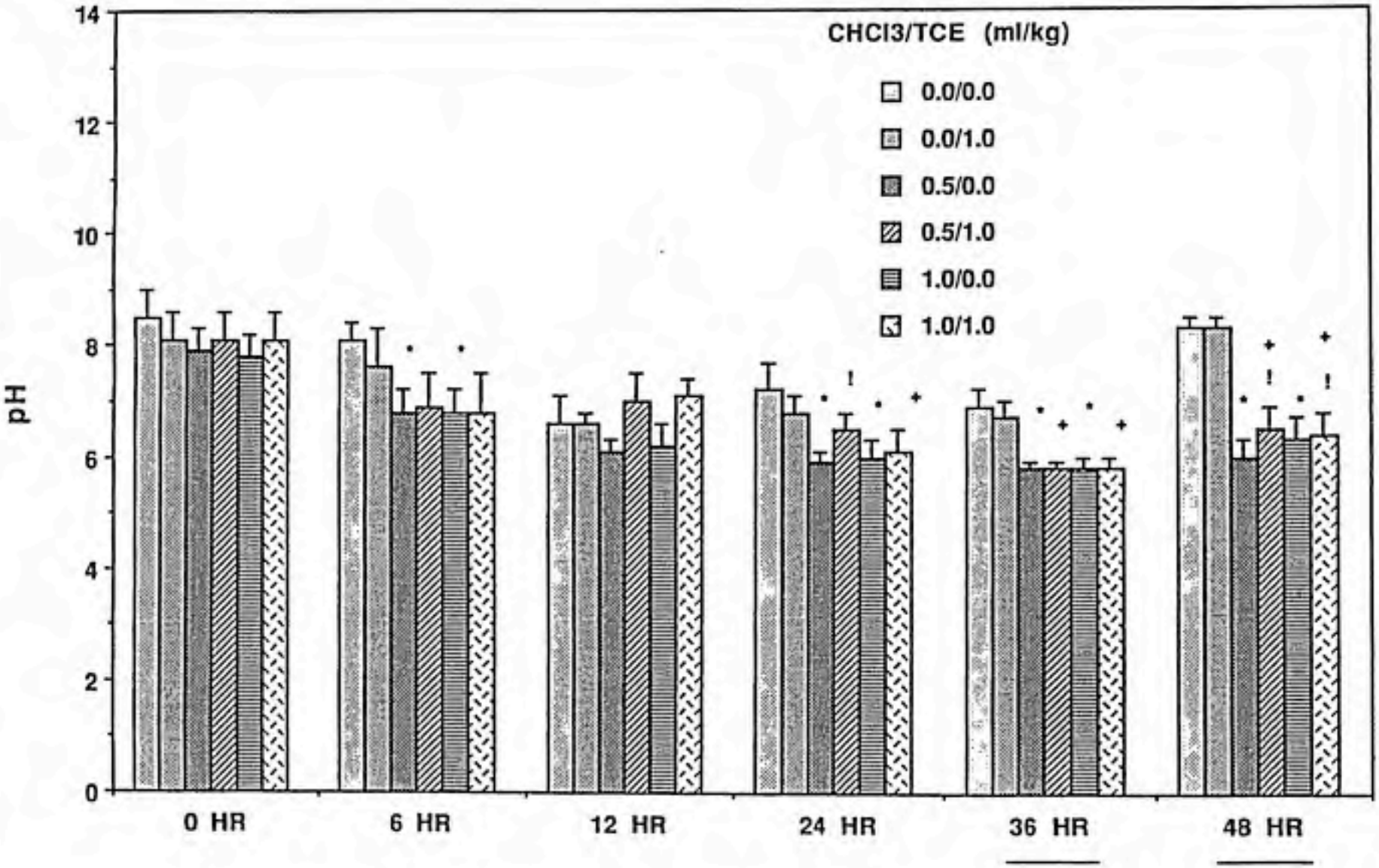
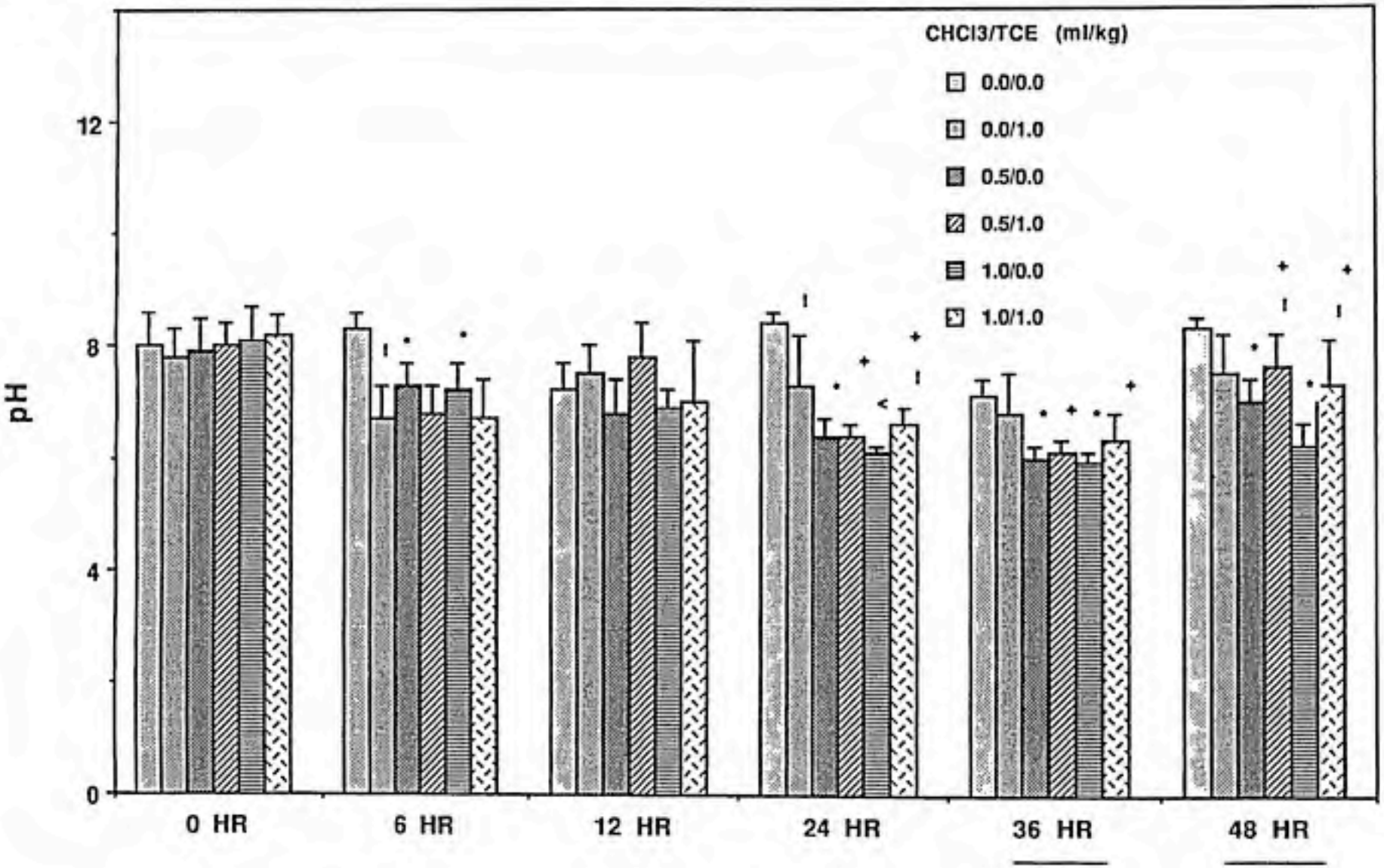


FIG. 48. NEPHROTOXICITY OF CHCl3 AND TCE IN AQUEOUS VEHICLE



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VIII. Appendix

A. Statistical Tables

BODY WEIGHT OIL VEHICLE

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	35		38.77	<0.001
EXP	1	41.82		
CHCl3	2	2487.77	111.23	<0.001
TCE	1	94.74	4.24	0.049
CHCl3 x TCE	2	45.18	2.02	0.151
ERROR	29	22.37		

BODY WEIGHT OIL VEHICLE

CHCl3 (ml/kg)[a]	MEAN (grams)	STD DEV	N	TCE (ml/kg)	MEAN (grams)	STD DEV	N
0.0	166.6	3.6	12	0.0	148.3	14.6	18
0.5	141.8	7.0	12	1.0	151.6	11.2	18
1.0	141.5	4.3	12				

[a] 0.5 and 1.0 ml CHCl3/kg are significantly lower than 0.0 ml CHCl3/kg ($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.

BODY WEIGHT AQUEOUS VEHICLE

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	34		12.73	<0.001
EXP	1	1.58		
CHCl3	2	1713.74	34.70	<0.001
TCE	1	52.16	1.06	0.313
CHCl3 x TCE	2	147.11	2.98	0.067
ERROR	28	49.39		

BODY WEIGHT AQUEOUS VEHICLE

CHCl3 (ml/kg)[a]	MEAN (grams)	STD DEV	N	TCE (ml/kg)	MEAN (grams)	STD DEV	N
0.0	162.9	10.9	12	0.0	153.0	14.0	18
0.5	151.4	4.4	12	1.0	149.7	10.7	18
1.0	138.5	4.8	11				

[a] 1.0 ml CHCl3/kg is significantly lower than 0.5 ml CHCl3/kg ($p<0.05$), 0.5 ml CHCl3/kg is significantly lower than 0.0 ml CHCl3/kg ($p<0.05$) and 1.0 ml CHCl3/kg is significantly lower than 0.0 ml CHCl3/kg ($p<0.05$).

RELATIVE LIVER WEIGHT OIL VEHICLE

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	35		5.65	<0.001
EXP	1	0.280		
CHCl3	2	0.401	8.03	0.002
TCE	1	0.152	3.04	0.092
CHCl3 x TCE	2	0.229	4.58	0.019
ERROR	29	0.050		

RELATIVE LIVER WEIGHT OIL VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN % of body wt	STANDARD DEVIATION	N
0.0	0.0	4.29	0.12	6
0.0	1.0	4.48	0.19	6
0.5	0.0	4.66	0.21	6
0.5	1.0	4.38	0.38	6
1.0	0.0	4.89	0.28	6
1.0	1.0	4.60	0.18	6

[a]In the absence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg ($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different. In the presence of TCE, there were no significant differences in the CHCl3 levels.

[b]The TCE levels are significantly different at 0.0 ml CHCl3/kg ($p=0.019$)

RELATIVE LIVER WEIGHT AQUEOUS VEHICLE

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	34		7.15	<0.001
EXP	1	0.966		
CHCl3	2	0.822	13.48	<0.001
TCE	1	0.005	0.09	0.766
CHCl3 x TCE	2	0.087	1.42	0.258
ERROR	28	0.061		

RELATIVE LIVER WEIGHT AQUEOUS VEHICLE

CHCl3 (ml/kg)[a]	MEAN % of body wt	STD DEV	N	TCE (ml/kg)	MEAN % of body wt	STD DEV	N
0.0	4.19	0.31	12	0.0	4.08	0.09	17
0.5	4.24	0.31	12	1.0	4.07	0.08	18
1.0	3.77	0.21	11				

[a]1.0 ml CHCl3/kg is significantly lower than 0.0 and 0.5 ml CHCl3/kg ($p<0.05$). 0.5 and 1.0 ml CHCl3/kg.are not significantly different .

RELATIVE KIDNEY WEIGHT OIL VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	35		17.88	<0.001
EXP	1	0.003		
CHCl3	2	0.634	45.55	<0.001
TCE	1	0.020	1.41	0.244
CHCl3 x TCE	2	0.101	7.28	0.003
ERROR	29	0.014		

[a]Based on LOG(PCKID)

RELATIVE KIDNEY WEIGHT OIL VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN % of body wt	STANDARD DEVIATION	N
0.0	0.0	0.74	0.03	6
0.0	1.0	0.80	0.03	6
0.5	0.0	1.25	0.19	6
0.5	1.0	0.96	0.10	6
1.0	0.0	1.18	0.19	6
1.0	1.0	1.21	0.15	6

[a]In the absence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg ($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.
In the presence of TCE, 1.0 ml CHCl3/kg is significantly higher than 0.5 ml CHCl3/kg ($p<0.05$), 0.5 ml CHCl3/kg is significantly higher than 0.0 ml CHCl3/kg (ie TCE alone) ($p<0.05$) and 1.0 ml CHCl3/kg is significantly higher than 0.0 ml CHCl3/kg (ie TCE alone)($p<0.05$).
[b]The TCE levels are significantly different at 0.0 ml CHCl3/kg ($p=0.005$) and 0.5 ml CHCl3/kg ($p=0.012$).

RELATIVE KIDNEY WEIGHT AQUEOUS VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	34		6.06	<0.001
EXP	1	0.001		
CHCl3	2	0.087	10.97	0.003
TCE	1	0.015	1.97	0.171
CHCl3 x TCE	2	0.049	6.22	0.006
ERROR	28	0.007		

[a]Based on LOG(PCKID)

RELATIVE KIDNEY WEIGHT AQUEOUS VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN % of body wt	STANDARD DEVIATION	N
0.0	0.0	0.77	0.04	6
0.0	1.0	0.83	0.05	6
0.5	0.0	0.84	0.04	6
0.5	1.0	0.82	0.03	6
1.0	0.0	1.06	0.24	5
1.0	1.0	0.87	0.04	6

[a]In the absence of TCE, 1.0 ml CHCl3/kg is significantly higher than 0.0 and 0.5 ml CHCl3/kg($p<0.05$). 0.0 and 0.5 ml CHCl3/kg are not significantly different.
In the presence of TCE, there were no significant differences in the CHCl3 levels.
[b]The TCE levels are significantly different at 0.0 ml CHCl3/kg ($p=0.030$).

SERUM LDH OIL VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	35		14.18	<0.001
EXP	1	4.46		
CHCl3	2	14.85	16.93	<0.001
TCE	1	22.81	26.01	<0.001
CHCl3 x TCE	2	8.83	10.07	0.001
ERROR	29	0.78		

[a]Based on Log(LDH)

SERUM LDH OIL VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (IU/L)	STANDARD DEVIATION	N
0.0	0.0	154.7	55.2	6
0.0	1.0	498.5	883.8	6
0.5	0.0	6958.3	5135.3	6
0.5	1.0	389.3	380.5	6
1.0	0.0	7387.7	8563.1	6
1.0	1.0	529.3	544.9	6

[a]In the absence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different . In the presence of TCE, there were no significant differences in the CHCl3 levels.

[b]The TCE levels are significantly different at 0.5 ml CHCl3/kg ($p<0.001$) and 1.0 ml CHCl3/kg ($p=0.010$).

SERUM LDH AQUEOUS VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	34		2.20	0.073
EXP	1	0.176		
CHCl3	2	0.716	3.97	0.030
TCE	1	0.421	2.34	0.138
CHCl3 x TCE	2	0.314	1.75	0.193
ERROR	28	0.180		

[a]Based on Log(LDH)

SERUM LDH AQUEOUS VEHICLE

CHCl3 (ml/kg)[a]	MEAN (IU/L)	STD DEV	N	TCE (ml/kg)	MEAN (IU/L)	STD DEV	N
0.0	142.3	41.6	12	0.0	231.0	258.1	17
0.5	160.9	53.1	12	1.0	156.7	49.8	18
1.0	282.6	312.2	11				

[a]1.0 ml CHCl3/kg is significantly higher than 0.0 ml CHCl3/kg ($p<0.05$). No other differences were significant.

SERUM AST OIL VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	35		40.23	<0.001
EXP	1	0.15		
CHCl3	2	39.70	73.20	<0.001
TCE	1	28.92	53.33	<0.001
CHCl3 x TCE	2	11.21	20.68	<0.001
ERROR	29	0.54		

[a]Based on Log(AST)

SERUM AST OIL VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (IU/L)	STANDARD DEVIATION	N
0.0	0.0	60.2	6.7	6
0.0	1.0	74.2	42.4	6
0.5	0.0	8756.7	1967.4	6
0.5	1.0	367.5	487.9	6
1.0	0.0	5267.2	3847.5	6
1.0	1.0	980.3	841.1	6

[a]In the absence of TCE, 1.0 ml CHCl3/kg is significantly lower than 0.5 ml CHCl3/kg ($p<0.05$), 0.5 ml CHCl3/kg is significantly higher than 0.0 ml CHCl3/kg ($p<0.05$) and 1.0 ml CHCl3/kg is significanlty higher than 0.0 ml CHCl3/kg ($p<0.05$). In the presence of TCE, 1.0 ml CHCl3/kg is significantly higher than 0.5 and 0.0 ml CHCl3/kg. 0.5 and 0.0 ml CHCl3/kg are not significantly different .

[b]The TCE levels are significantly different at 0.5 ml CHCl3/kg ($p<0.001$) and 1.0 ml CHCl3/kg ($p=0.008$).

AST AQUEOUS VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	34		4.54	0.003
EXP	1	0.305		
CHCl3	2	0.927	7.31	0.003
TCE	1	1.198	9.45	0.005
CHCl3 x TCE	2	0.240	1.89	0.170
ERROR	28	0.127		

[a]Based on Log(AST)

AST AQUEOUS VEHICLE

CHCl3 (ml/kg)[a]	MEAN (IU/L)	STD DEV	N	TCE (ml/kg)	MEAN (IU/L)	STD DEV	N
0.0	58.9	5.9	12	0.0	108.7	132.9	17
0.5	66.8	20.7	12	1.0	61.5	15.3	18
1.0	131.6	162.8	11				

[a]1.0 ml CHCl3/kg is significantly higher than 0.0 and 0.5 ml CHCl3/kg ($p<0.05$). 0.0 and 0.5 ml CHCl3/kg are not significantly different.

SERUM 5' NUCLEOTIDASE OIL VEHICLE

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	35		9.80	<0.001
EXP	1	122710		
CHCl3	2	8352	2.80	0.077
TCE	1	10127	3.40	0.076
CHCl3 x TCE	2	12884	4.32	0.023
ERROR	29	2980		

SERUM 5' NUCLEOTIDASE OIL VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN	STANDARD DEVIATION	N
0.0	0.0	48.7	36.1	6
0.0	1.0	89.4	109.1	6
0.5	0.0	120.9	76.1	6
0.5	1.0	63.0	40.6	6
1.0	0.0	163.4	124.0	6
1.0	1.0	80.0	76.3	6

[a]In the absence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg($p<0.05$). 0.5 and1.0 ml CHCl3/kg are not significantly different. In the presence of TCE, there were no significant differences in the CHCl3 levels.

[b]The TCE levels are significantly different at 0.5 ml CHCl3/kg ($p=0.046$) and 1.0 ml CHCl3/kg ($p=0.019$).

SERUM CREATININE OIL VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	35		7.15	<0.001
EXP	1	1.93		
CHCl3	2	4.53	12.60	<0.001
TCE	1	2.01	5.60	0.025
CHCl3 x TCE	2	1.23	3.38	0.048
ERROR	29	0.36		

[a]Based on Log(CRE)

SERUM CREATININE OIL VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (mg/dl)	STANDARD DEVIATION	N
0.0	0.0	0.53	0.08	6
0.0	1.0	0.47	0.12	6
0.5	0.0	2.72	1.52	6
0.5	1.0	0.72	0.20	6
1.0	0.0	2.27	1.86	6
1.0	1.0	2.17	2.06	6

[a]In the absence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different. In the presence of TCE, 1.0 ml CHCl3/kg is significantly higher than 0.0 and 0.5 ml CHCl3/kg. 0.0 and 0.5 ml CHCl3/kg are not significantly different.

[b]The TCE levels are significantly different at 0.5 ml CHCl3/kg ($p=0.003$).

BLOOD UREA NITROGEN OIL VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	35		14.37	<0.001
EXP	1	1.40		
CHCl3	2	8.04	28.22	<0.001
TCE	1	2.71	9.50	0.005
CHCl3 x TCE	2	2.19	7.68	0.002
ERROR	29	0.29		

[a]Based on Log(BUN)

BLOOD UREA NITROGEN OIL VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (mg/dl)	STANDARD DEVIATION	N
0.0	0.0	20.8	2.4	6
0.0	1.0	20.5	2.3	6
0.5	0.0	169.5	80.5	6
0.5	1.0	33.2	12.2	6
1.0	0.0	141.0	101.7	6
1.0	1.0	112.5	72.1	6

[a]In the absence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different . In the presence of TCE, 1.0 ml CHCl3/kg is significantly higher than 0.0 and 0.5 ml CHCl3/kg ($p<0.05$). 0.0 and 0.5 ml CHCl3/kg are not significant.

BLOOD UREA NITROGEN AQUEOUS VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	34		3.67	0.008
EXP	1	0.593		
CHCl3	2	0.626	4.79	0.016
TCE	1	0.693	5.30	0.029
CHCl3 x TCE	2	0.368	2.82	0.077
ERROR	28	0.131		

[a]Based on LOG(BUN)

BLOOD UREA NITROGEN AQUEOUS VEHICLE

CHCl3 (ml/kg)[a]	MEAN (mg/dl)	STD DEV	N	TCE (ml/kg)	MEAN (mg/dl)	STD DEV	N
0.0	23.3	2.8	12	0.0	41.8	59.1	17
0.5	25.9	4.5	12	1.0	24.4	3.9	18
1.0	50.8	73.0	11				

[a]1.0 ml CHCl3/kg is significantly higher than 0.0 ml CHCl3/kg ($p<0.05$). No other differences were significant.

SERUM ALT 24 HR OIL VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	35		24.80	<0.001
EXP	1	0.03		
CHCl3	2	7.95	51.33	<0.001
TCE	1	3.79	24.44	<0.001
CHCl3 x TCE	2	1.67	10.75	<0.001
ERROR	29	0.15		

[a]Based on Log(ALT24)

SERUM ALT 24 HR OIL VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (IU/L)	STANDARD DEVIATION	N
0.0	0.0	35.7	7.6	6
0.0	1.0	45.0	16.2	6
0.5	0.0	322.2	157.5	6
0.5	1.0	105.2	65.9	6
1.0	0.0	284.0	175.4	6
1.0	1.0	94.5	24.6	6

[a]In the absence of TCE, 0.5 and1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different . In the presence of TCE, 0.5 and1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg. 0.5 and 1.0 ml CHCl3/kg are not significantly different ($p<0.05$).

[b]The TCE levels are significantly different at 0.5 ml CHCl3/kg ($p=0.001$) and 1.0 ml CHCl3/kg ($p=0.003$).

SERUM ALT 24 HR AQUEOUS VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	29		8.62	<0.001
EXP	1	0.60		
CHCl3	2	2.45	13.89	<0.001
TCE	1	2.46	13.97	0.001
CHCl3 x TCE	2	0.86	4.86	0.017
ERROR	23	0.18		

[a]Based on Log(ALT24)

SERUM ALT 24 HR AQUEOUS VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (IU/L)	STANDARD DEVIATION	N
0.0	0.0	37.0	8.7	6
0.0	1.0	41.0	13.7	5
0.5	0.0	179.8	110.0	6
0.5	1.0	57.8	17.3	6
1.0	0.0	124.0	48.5	3
1.0	1.0	56.5	15.8	4

[a]In the absence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different. In the presence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg. 0.5 and 1.0 ml CHCl3/kg are not significantly different.

[b]The TCE levels are significantly different at 0.5 ml CHCl3/kg ($p=0.019$) and 1.0 ml CHCl3/kg ($p=0.052$).

SERUM ALT 48 HR OIL VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	35		19.78	<0.001
EXP	1	2.80		
CHCl3	2	39.15	34.86	<0.001
TCE	1	29.03	25.85	<0.001
CHCl3 x TCE	2	11.60	10.33	<0.001
ERROR	29	1.12		

[a]Based on Log(ALT48)

SERUM ALT 48 HR OIL VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (IU/L)	STANDARD DEVIATION	N
0.0	0.0	35.8	1.7	6
0.0	1.0	37.2	11.4	6
0.5	0.0	6961.7	1853.8	6
0.5	1.0	271.0	364.5	6
1.0	0.0	3802.7	3294.6	6
1.0	1.0	534.5	621.0	6

[a]In the absence of TCE, 1.0 ml CHCl3/kg is significantly lower than 0.5 ml CHCl3/kg ($p<0.05$), 0.5 ml CHCl3/kg is significantly higher than 0.0 ml CHCl3/kg ($p<0.05$) and 1.0 ml CHCl3/kg is significanlty higher than 0.0 ml CHCl3/kg ($p<0.05$). In the presence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.

[b]The TCE levels are significantly different at 0.5 ml CHCl3/kg ($p<0.001$).

SERUM ALT 48 HR AQUEOUS VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	34		8.42	<0.001
EXP	1	0.22		
CHCl3	2	0.56	7.25	0.003
TCE	1	2.29	29.52	<0.001
CHCl3 x TCE	2	0.36	4.57	0.019
ERROR	28	0.08		

[a]Based on Log(ALT48)

SERUM ALT 48 HR AQUEOUS VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (IU/L)	STANDARD DEVIATION	N
0.0	0.0	36.5	4.5	6
0.0	1.0	30.2	3.5	6
0.5	0.0	46.3	14.5	6
0.5	1.0	28.3	2.2	6
1.0	0.0	94.6	80.2	5
1.0	1.0	32.3	4.1	6

[a] In the absence of TCE, 1.0 ml CHCl3/kg is significantly higher than 0.5 and 0.0 ml CHCl3/kg($p<0.05$). 0.0 and 0.5 ml CHCl3/kg are not significantly different. In the presence of TCE, there were no significant differences in the CHCl3 levels.

[b]The TCE levels are significantly different at 0.5 ml CHCl3/kg ($p=0.007$) and 1.0 ml CHCl3/kg ($p=0.005$).

SERUM BILE ACIDS 24 HR OIL VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	21		6.17	0.002
EXP	1	1.13		
CHCl3	2	4.21	5.90	0.013
TCE	1	8.83	12.38	0.003
CHCl3 x TCE	2	4.22	5.91	0.013
ERROR	15	0.71		

[a]Based on Log(BAC24)

SERUM BILE ACIDS 24 HR OIL VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (um/L)	STANDARD DEVIATION	N
0.0	0.0	24.8	20.7	4
0.0	1.0	31.8	17.6	4
0.5	0.0	296.3	263.2	4
0.5	1.0	59.5	43.3	4
1.0	0.0	223.0	166.6	4
1.0	1.0	14.0	14.1	2

[a] In the absence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different. In the presence of TCE, there were no significant differences in the CHCl3 levels.

[b]The TCE levels are significantly different at 0.5 ml CHCl3/kg ($p=0.038$).

SERUM BILES ACID 48 HR OIL VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	34		13.71	<0.001
EXP	1	1.60		
CHCl3	2	23.90	25.23	<0.001
TCE	1	10.34	10.92	0.003
CHCl3 x TCE	2	7.32	7.73	0.002
ERROR	28	0.95		

[a]Based on Log(BAC48)

SERUM BILE ACIDS 48 HR OIL VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (um/L)	STANDARD DEVIATION	N
0.0	0.0	14.5	5.9	6
0.0	1.0	27.6	7.3	5
0.5	0.0	1054.2	654.1	6
0.5	1.0	132.0	157.5	6
1.0	0.0	848.2	543.2	6
1.0	1.0	122.3	92.5	6

[a] In the absence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different. In the presence of TCE, there were no significant differences in the CHCl3 levels.

[b]The TCE levels are significantly different at 0.0 ml CHCl3/kg ($p=0.018$), 0.5 ml CHCl3/kg ($p=0.041$) and 1.0 ml CHCl3/kg ($p=0.001$).

URINE AST 36 HR AQUEOUS VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	34		12.12	<0.001
EXP	1	0.54		
CHCl3	2	7.86	13.73	<0.001
TCE	1	17.51	30.60	<0.001
CHCl3 x TCE	2	3.69	6.44	0.005
ERROR	28	0.57		

[a]Based on Log(NAST36)

URINE AST 36 HR AQUEOUS VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (IU/10mg CRE)	STANDARD DEVIATION	N
0.0	0.0	0.13	0.08	6
0.0	1.0	0.11	0.10	6
0.5	0.0	0.87	0.93	6
0.5	1.0	0.16	0.10	5
1.0	0.0	2.63	2.68	6
1.0	1.0	0.15	0.05	6

[a]In the absence of TCE, 1.0 ml CHCl3/kg is significantly higher than 0.5 ml CHCl3/kg ($p<0.05$), 0.5 ml CHCl3/kg is significantly higher than 0.0 ml CHCl3/kg ($p<0.05$) and 1.0 ml CHCl3/kg is significantly higher than 0.0 ml CHCl3/kg ($p<0.05$). In the presence of TCE, there were no significant differences in the CHCl3 levels.

[b]The TCE levels are significantly different at 0.5 ml CHCl3/kg ($p=0.021$) and 1.0 ml CHCl3/kg ($p<0.001$).

URINE AST 36 HR OIL VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	35		34.77	<0.001
EXP	1	<0.01		
CHCl3	2	12.18	36.17	<0.001
TCE	1	25.74	74.45	<0.001
CHCl3 x TCE	2	10.08	29.92	<0.001
ERROR	29	0.34		

[a]Based on Log(NAST36)

URINE AST 36 HR OIL VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (IU/10mg CRE)	STANDARD DEVIATION	N
0.0	0.0	0.12	0.07	6
0.0	1.0	0.16	0.06	6
0.5	0.0	2.75	0.06	6
0.5	1.0	0.16	0.07	6
1.0	0.0	3.46	1.87	6
1.0	1.0	0.29	0.28	6

[a]In the absence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg. 0.5 and 1.0 ml CHCl3/kg are not significantly different ($p<0.05$).
In the presence of TCE, there were no significant differences in the CHCl3 levels.
[b]The TCE levels are significantly different at 0.5 ml CHCl3/kg ($p<0.001$) and 1.0 ml CHCl3/kg ($p<0.001$).

URINE AST 48 HR AQUEOUS VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	29		5.18	0.002
EXP	1	<0.01	-	
CHCl3	2	5.48	6.14	0.007
TCE	1	5.90	6.60	0.017
CHCl3 x TCE	2	3.36	3.76	0.039
ERROR	23	0.89		

[a]Based on Log(NAST48)

URINE AST 48 HR AQUEOUS VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (IU/10mg CRE)	STANDARD DEVIATION	N
0.0	0.0	0.16	0.10	6
0.0	1.0	0.14	0.05	5
0.5	0.0	0.89	0.80	6
0.5	1.0	0.59	0.62	3
1.0	0.0	2.95	3.77	6
1.0	1.0	0.20	0.17	4

[a]In the absence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.
In the presence of TCE, there were no significant differences in the CHCl3 levels.
[b]The TCE levels are significantly different at 1.0 ml CHCl3/kg ($p=0.033$).

URINE AST 48 HR OIL VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	32		24.12	<0.001
EXP	1	0.01		
CHCl3	2	14.11	36.91	<0.001
TCE	1	14.66	38.35	<0.001
CHCl3 x TCE	2	4.83	12.62	<0.001
ERROR	26	0.38		

[a]Based on Log(NAST48)

URINE AST 48 HR OIL VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (IU/10mg CRE)	STANDARD DEVIATION	N
0.0	0.0	0.19	0.07	6
0.0	1.0	0.27	0.32	4
0.5	0.0	4.00	1.22	6
0.5	1.0	0.33	0.24	6
1.0	0.0	3.74	1.85	5
1.0	1.0	0.92	0.34	6

[a]In the absence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.
In the presence of TCE, 1.0 ml CHCl3/kg is significantly higher than 0.0 and 0.5 ml CHCl3/kg($p<0.05$). 0.0 and 0.5 ml CHCl3/kg are not significantly different .
[b]The TCE levels are significantly different at 0.5 ml CHCl3/kg ($p<0.001$) and 1.0 ml CHCl3/kg ($p=0.003$).

URINE LDH 24 HR AQUEOUS VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	33		15.67	<0.001
EXP	1	0.28		
CHCl3	2	3.20	20.65	<0.001
TCE	1	2.39	15.44	<0.001
CHCl3 x TCE	2	2.34	15.12	<0.001
ERROR	27			

[a]Based on Log(NLDH24)

URINE LDH 24 HR AQUEOUS VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (IU/10mg CRE)	STANDARD DEVIATION	N
0.0	0.0	0.31	0.08	6
0.0	1.0	0.49	0.05	6
0.5	0.0	2.41	1.71	6
0.5	1.0	0.58	0.11	4
1.0	0.0	1.41	0.59	6
1.0	1.0	0.60	0.15	6

[a]In the absence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.
In the presence of TCE, there were no significant differences in the CHCl3 levels.
[b]The TCE levels are significantly different at 0.0 ml CHCl3/kg ($p=0.006$), 0.5 ml CHCl3/kg ($p=0.006$) and 1.0 ml CHCl3/kg ($p=0.006$).

URINE LDH 24 HR OIL VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	28		7.93	<0.001
EXP	1	0.14		
CHCl3	2	0.25	3.91	0.035
TCE	1	0.51	7.96	0.010
CHCl3 x TCE	2	0.34	5.37	0.013
ERROR	22	0.06		

[a]Based on Log(NLDH24)

URINE LDH 24 HR 0IL VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (IU/10mg CRE)	STANDARD DEVIATION	N
0.0	0.0	0.42	0.11	6
0.0	1.0	0.45	0.05	6
0.5	0.0	1.02	0.33	6
0.5	1.0	0.42	-	1
1.0	0.0	0.62	0.17	6
1.0	1.0	0.52	0.12	4

[a]In the absence of TCE, 1.0 ml CHCl3/kg is significantly lower than 0.5 ml CHCl3/kg ($p<0.05$), 0.5 ml CHCl3/kg is significantly higher than 0.0 ml CHCl3/kg ($p<0.05$) and 1.0 ml CHCl3/kg is significanltly higher than 0.0 ml CHCl3/kg ($p<0.05$).
In the presence of TCE, there were no significant differences in the CHCl3 levels.
[b]The TCE levels are significantly different at 0.5 ml CHCl3/kg ($p=0.050$).

URINE LDH 36 HR[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	71		18.28	<0.001
EXP	1	8.03		
TCE	1	14.96	52.28	<0.001
CHCl3	2	13.36	46.66	<0.001
VEHICLE	1	1.03	3.60	0.063
TCE x CHCl3	2	5.20	18.19	<0.001
TCE x VEH	1	0.29	1.00	0.322
VEH x CHCl3	2	0.56	1.95	0.151
TCE x CHCl3 x VEH	2	0.13	0.44	0.647
ERROR	59	0.29		

[a]Based on Log(NLDH36)

URINE LDH 36 HR

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (IU/10mg CRE)	STANDARD DEVIATION	N
0.0	0.0	0.31	0.13	12
0.0	1.0	0.34	0.12	12
0.5	0.0	2.45	1.89	12
0.5	1.0	0.56	0.21	12
1.0	0.0	3.98	3.72	12
1.0	1.0	0.58	0.26	12

[a]In the absence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.
In the presence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.
[b]The TCE levels are significantly different at 0.5 ml CHCl3/kg ($p<0.001$) and 1.0 ml CHCl3/kg ($p<0.001$).

URINE LDH 48 HR AQUEOUS VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	34		10.91	<0.001
EXP	1	0.44		
CHCl3	2	4.48	10.28	<0.001
TCE	1	11.32	25.96	<0.001
CHCl3 x TCE	2	3.40	7.79	0.002
ERROR	28	0.44		

[a]Based on Log(NLDH48)

URINE LDH 48 HR AQUEOUS VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (IU/10mg CRE)	STANDARD DEVIATION	N
0.0	0.0	0.33	0.03	6
0.0	1.0	0.32	0.03	6
0.5	0.0	1.52	1.30	6
0.5	1.0	0.38	0.14	6
1.0	0.0	4.50	3.00	6
1.0	1.0	0.57	0.64	5

[a]In the absence of TCE, 1.0 ml CHCl3/kg is significantly higher than 0.5 ml CHCl3/kg ($p<0.05$), 0.5 ml CHCl3/kg is significantly higher than 0.0 ml CHCl3/kg ($p<0.05$) and 1.0 ml CHCl3/kg is significantly higher than 0.0 ml CHCl3/kg ($p<0.05$).
In the presence of TCE, there were no significant differences in the CHCl3 levels ($p<0.05$).
[b]The TCE levels are significantly different at 0.5 ml CHCl3/kg ($p=0.011$) and 1.0 ml CHCl3/kg ($p=0.007$).

URINE LDH 48 HR OIL VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	34		32.17	<0.001
EXP	1	0.20		
CHCl3	2	17.50	61.51	<0.001
TCE	1	13.26	46.61	<0.001
CHCl3 x TCE	2	3.81	13.38	<0.001
ERROR	28	0.28		

[a]Based on Log(NLDH48)

URINE LDH 48 HR OIL VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (IU/10mg CRE)	STANDARD DEVIATION	N
0.0	0.0	0.29	0.10	6
0.0	1.0	0.30	0.12	6
0.5	0.0	6.36	2.14	6
0.5	1.0	0.81	0.39	6
1.0	0.0	6.24	3.33	5
1.0	1.0	1.34	0.81	6

[a]In the absence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.
In the presence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.
[b]The TCE levels are significantly different at 0.5 ml CHCl3/kg ($p<0.001$) and 1.0 ml CHCl3/kg ($p=0.006$).

URINE ALT 36 HR [a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	58		4.28	<0.001
EXP	1	0.28		
TCE	1	1.39	7.35	0.009
CHCl3	2	1.01	5.35	0.008
VEHICLE	1	2.46	13.02	0.001
TCE x CHCl3	2	1.36	7.18	0.002
TCE x VEH	1	0.36	1.90	0.175
VEH x CHCl3	2	0.19	0.99	0.379
TCE x CHCl3 x VEH	2	0.17	0.92	0.407
ERROR	46	0.19		

[a]Based on LOG(NALT36)

URINE ALT 36 HR

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (IU/10mg CRE)	STANDARD DEVIATION	N
0.0	0.0	0.12	0.04	11
0.0	1.0	0.15	0.06	9
0.5	0.0	0.22	0.09	10
0.5	1.0	0.14	0.06	10
1.0	0.0	0.35	0.29	10
1.0	1.0	0.17	0.14	9

[a]In the absence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different. In the presence of TCE, there were no significant differences in the CHCl3 levels.

[b]The TCE levels are significantly different at 0.5 ml CHCl3/kg ($p=0.025$) and 1.0 ml CHCl3/kg ($p=0.018$).

URINE ALT 48 HR[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	71		3.28	0.002
EXP	1	0.89		
TCE	1	2.10	5.20	0.027
CHCl3	2	2.46	6.10	0.005
VEHICLE	1	2.11	5.23	0.027
TCE x CHCl3	2	1.15	2.85	0.068
TCE x VEH	1	0.33	0.83	0.367
VEH x CHCl3	2	0.28	0.69	0.509
TCE x CHCl3 x VEH	2	0.73	1.79	0.178
ERROR	59			

[a]Based on LOG(NALT48)

URINE ALT 48 HR

CHCl3 (ml/kg)[a]	MEAN (IU/10mg CRE)	STD DEV	N	TCE (ml/kg)	MEAN (IU/10 mg CRE)	STD DEV	N
0.0	0.16	0.10	24	0.0	0.41	0.57	36
0.5	0.36	0.29	24	1.0	0.22	0.20	36
1.0	0.47	0.73	24				

[a] 0.5 and 1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg ($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.

TOTAL PROTEIN 12 HR[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	71		5.46	<0.001
EXP	1	2.05		
TCE	1	0.37	5.49	0.023
CHCl3	2	0.04	0.68	0.510
VEHICLE	1	0.57	8.42	0.005
TCE x CHCl3	2	0.37	5.50	0.007
TCE x VEH	1	0.14	2.05	0.157
VEH x CHCl3	2	0.20	2.94	0.060
TCE x CHCl3 x VEH	2	0.03	0.38	0.689
ERROR	59	0.07		

[a]Based on Log(NTPR12)

TOTAL PROTEIN 12 HR

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (mg/mg CRE)	STANDARD DEVIATION	N
0.0	0.0	0.007	0.002	12
0.0	1.0	0.008	0.003	12
0.5	0.0	0.008	0.003	12
0.5	1.0	0.007	0.001	12
1.0	0.0	0.009	0.003	12
1.0	1.0	0.007	0.003	12

[a]In the absence of TCE, there were no significant differences in the CHCl3 levels.
In the presence of TCE, 1.0 ml CHCl3/kg is significantly lower than 0.0 ml CHCl3/kg ($p<0.05$). No other differences were significant.
[b]The TCE levels are significantly different at 1.0 ml CHCl3/kg ($p=0.006$).

TOTAL PROTEIN 24 HRS AQUEOUS VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	35		3.27	0.014
EXP	1	0.26		
CHCl3	2	1.46	6.66	0.004
TCE	1	0.03	0.16	0.694
CHCl3 x TCE	2	0.54	2.47	0.102
ERROR	29	0.22		

[a]Based on Log(NTPR24)

TOTAL PROTEIN 24 HRS AQUEOUS VEHICLE

CHCl3 (ml/kg)[a]	MEAN (mg/mg CRE)	STD DEV	N	TCE (ml/kg)	MEAN (mg/mg CRE)	STD DEV	N
0.0	0.009	0.002	12	0.0	0.008	0.003	18
0.5	0.005	0.002	12	1.0	0.007	0.002	18
1.0	0.008	0.002	12				

[a]0.5 ml CHCl3/kg is significantly lower than 0.0 and 1.0 ml CHCl3/kg ($p<0.05$). 0.0 and 1.0 ml CHCl3/kg are not significantly different.

TOTAL PROTEIN 36 HR AQUEOUS VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	35		11.82	<0.001
EXP	1	0.03		
CHCl3	2	0.25	4.20	0.025
TCE	1	2.86	47.06	<0.001
CHCl3 x TCE	2	0.45	7.45	0.002
ERROR	29	0.06		

[a]Based on Log(NTPR36)

TOTAL PROTEIN 36 HR AQUEOUS VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (mg/mg CRE)	STANDARD DEVIATION	N
0.0	0.0	0.008	0.004	6
0.0	1.0	0.006	0.001	6
0.5	0.0	0.010	0.002	6
0.5	1.0	0.005	0.000	6
1.0	0.0	0.004	0.003	6
1.0	1.0	0.006	0.001	6

[a]In the absence of TCE, 1.0 ml CHCl3/kg is significantly different than 0.0 ml CHCl3/kg. No other differences were significant.
In the presence of TCE, there were no significant differences in the CHCl3 levels ($p>0.05$).

[b]The TCE levels are significantly different at 0.5 ml CHCl3/kg ($p<0.001$) and 1.0 ml CHCl3/kg ($p<0.001$).

TOTAL PROTEIN 36 HR OIL VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	35		24.52	<0.001
EXP	1	1.03		
CHCl3	2	0.31	5.52	0.009
TCE	1	2.71	48.38	<0.001
CHCl3 x TCE	2	1.94	34.68	<0.001
ERROR	29	0.06		

[a]Based on Log(NTPR36)

TOTAL PROTEIN 36 HR OIL VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (mg/mg CRE)	STANDARD DEVIATION	N
0.0	0.0	0.007	0.001	6
0.0	1.0	0.010	0.002	6
0.5	0.0	0.018	0.006	6
0.5	1.0	0.007	0.002	6
1.0	0.0	0.017	0.007	6
1.0	1.0	0.006	0.003	6

[a]In the absence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.
In the presence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly lower than 0.0 ml CHCl3/kg($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different .

[b]The TCE levels are significantly different at 0.0 ml CHCl3/kg ($p<0.001$), 0.5 ml CHCl3/kg ($p<0.001$) and 1.0 ml CHCl3/kg ($p<0.001$).

TOTAL PROTEIN 48 HR OIL VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	33		33.96	<0.001
EXP	1	0.06		
CHCl3	2	0.85	19.01	<0.001
TCE	1	3.48	78.01	<0.001
CHCl3 x TCE	2	2.03	45.54	<0.001
ERROR	27	0.04		

[a]Based on Log(NTPR48)

TOTAL PROTEIN 48 HR OIL VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (mg/mg CRE)	STANDARD DEVIATION	N
0.0	0.0	0.007	0.000	6
0.0	1.0	0.009	0.002	6
0.5	0.0	0.026	0.005	6
0.5	1.0	0.007	0.001	6
1.0	0.0	0.019	0.005	5
1.0	1.0	0.008	0.002	5

[a]In the absence of TCE, 1.0 ml CHCl3/kg is significantly lower than 0.5 ml CHCl3/kg ($p<0.05$), 0.5 ml CHCl3/kg is significantly higher than 0.0 ml CHCl3/kg ($p<0.05$) and 1.0 ml CHCl3/kg is significantly higher than 0.0 ml CHCl3/kg ($p<0.05$).
In the presence of TCE, there were no significant differences in the CHCl3 levels.
[b]The TCE levels are significantly different at 0.0 ml CHCl3/kg ($p=0.002$), 0.5 ml CHCl3/kg ($p<0.001$) and 1.0 ml CHCl3/kg ($p=0.003$).

TOTAL PROTEIN 48 HR AQUEOUS VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	34		3.11	0.018
EXP	1	0.16		
CHCl3	2	0.44	2.87	0.073
TCE	1	0.55	3.60	0.068
CHCl3 x TCE	2	0.58	3.80	0.035
ERROR	28	0.15		

[a]Based on Log(NTPR48)

TOTAL PROTEIN 48 HR AQUEOUS VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (mg/mg CRE)	STANDARD DEVIATION	N
0.0	0.0	0.008	0.002	6
0.0	1.0	0.008	0.001	6
0.5	0.0	0.008	0.004	6
0.5	1.0	0.008	0.003	6
1.0	0.0	0.019	0.015	6
1.0	1.0	0.008	0.003	5

[a]In the absence of TCE, 1.0 ml CHCl3/kg is significantly different than 0.0 and 0.5 ml CHCl3/kg($p<0.05$). 0.0 and 0.5 ml CHCl3/kg are not significantly different.
In the presence of TCE, there were no significant differences in the CHCl3 levels.
[b]The TCE levels are significantly different at 1.0 ml CHCl3/kg ($p=0.050$).

URINE CREATININE 6 HR

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	71		2.94	0.003
EXP	1	4140		
TCE	1	3226	7.70	0.007
CHCl3	2	2033	4.85	0.011
VEHICLE	1	1241	2.96	0.091
TCE x CHCl3	2	486	1.16	0.321
TCE x VEH	1	70	0.17	0.684
VEH x CHCl3	2	508	1.21	0.305
TCE x CHCl3 x VEH	2	15	0.04	0.964
ERROR	59	419		

URINE CREATININE 6 HR

CHCl3 (ml/kg)[a]	MEAN (mg/dl)	STD DEV	N	TCE (ml/kg)	MEAN (mg/dl)	STD DEV	N
0.0	74.2	21.5	24	0.0	57.9	24.0	36
0.5	55.8	18.4	24	1.0	71.3	21.4	36
1.0	63.7	27.4	24				

[a] 0.5 ml CHCl3/kg is significantly lower than 0.0 ml CHCl3/kg ($p<0.05$).
No other differences were significant.

URINE CREATININE 12 HR AQUEOUS VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	35		10.47	<0.001
EXP	1	GET		
CHCl3	2	0.84	12.74	<0.001
TCE	1	0.10	1.46	0.237
CHCl3 x TCE	2	1.17	17.68	<0.001
ERROR	29	0.07		

[a]Based on Log(CRE12)

URINE CREATININE 12 HR AQUEOUS VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (mg/dl)	STANDARD DEVIATION	N
0.0	0.0	115.8	9.2	6
0.0	1.0	54.4	17.5	6
0.5	0.0	45.1	15.0	6
0.5	1.0	67.7	21.3	6
1.0	0.0	45.1	6.7	6
1.0	1.0	48.9	6.4	6

[a]In the absence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly lower than 0.0 ml CHCl3/kg($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.
In the presence of TCE, there were no significant differences in the CHCl3 levels.
[b]The TCE levels are significantly different at 0.0 ml CHCl3/kg $p<0.001$).

URINE CREATININE 12 HR OIL VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	35		2.46	0.048
EXP	1	0.12		
CHCl3	2	1.66	6.97	0.003
TCE	1	<0.01	0.01	0.908
CHCl3 x TCE	2	0.03	0.14	0.869
ERROR	29	0.24		

[a]Based on Log(CRE12)

URINE CREATININE 12 HR OIL VEHICLE

CHCl3 (ml/kg)[a]	MEAN (mg/dl)	STD DEV	N	TCE (ml/kg)	MEAN (mg/dl)	STD DEV	N
0.0	115.0	27.9	12	0.0	79.6	36.7	18
0.5	67.5	34.6	12	1.0	86.0	42.4	18
1.0	65.9	35.4	12				

[a]0.5 and 1.0 ml CHCl3/kg are significantly lower than 0.0 ml CHCl3/kg ($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.

URINE CREATININE 24 HR

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	71		6.14	<0.001
EXP	1	111		
TCE	1	1106	2.53	0.117
CHCl3	2	13660	31.20	<0.001
VEHICLE	1	250	0.57	0.453
TCE x CHCl3	2	1118	2.55	0.086
TCE x VEH	1	81	0.19	0.669
VEH x CHCl3	2	203	0.46	0.631
TCE x CHCl3 x VEH	2	363	0.83	0.441
ERROR	59	438		

URINE CREATININE 24 HR

CHCl3 (ml/kg)[a]	MEAN (mg/dl)	STD DEV	N	TCE (ml/kg)	MEAN (mg/dl)	STD DEV	N
0.0	78.8	19.4	24	0.0	55.2	30.3	36
0.5	36.3	23.5	24	1.0	47.3	26.7	36
1.0	38.7	20.2	24				

[a] 0.5 and 1.0 ml CHCl3/kg are significantly lower than 0.0 ml CHCl3/kg ($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.

URINE CREATININE 36 HR

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	71		9.73	<0.001
EXP	1	10658		
TCE	1	806	1.31	0.256
CHCl3	2	13592	22.03	<0.001
VEHICLE	1	21736	35.39	<0.001
TCE x CHCl3	2	1381	2.25	0.114
TCE x VEH	1	5083	8.28	0.056
VEH x CHCl3	2	1609	2.62	0.081
TCE x CHCl3 x VEH	2	205	0.34	0.717
ERROR	59	614		

URINE CREATININE 36 HR

CHCl3 (ml/kg)[a]	MEAN (mg/dl)	STD DEV	N	TCE (ml/kg)	MEAN (mg/dl)	STD DEV	N
0.0	117.7	30.1	24	0.0	87.3	35.1	36
0.5	73.2	32.0	24	1.0	94.0	42.8	36
1.0	81.1	39.8	24				

[a] 0.5 and 1.0 ml CHCl3/kg are significantly lower than 0.0 ml CHCl3/kg ($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.

URINE CREATININE 48 HR

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	69		4.46	<0.001
EXP	1	3353		
TCE	1	1342	1.85	0.179
CHCl3	2	7396	10.17	<0.001
VEHICLE	1	14057	19.33	<0.001
TCE x CHCl3	2	497	0.68	0.509
TCE x VEH	1	1	0.00	0.964
VEH x CHCl3	2	1258	1.73	0.186
TCE x CHCl3 x VEH	2	868	1.19	0.311
ERROR	57	727		

URINE CREATININE 48 HR

CHCl3 (ml/kg)[a]	MEAN (mg/dl)	STD DEV	N	TCE (ml/kg)	MEAN (mg/dl)	STD DEV	N
0.0	96.8	23.0	24	0.0	73.7	34.2	35
0.5	62.4	28.0	24	1.0	81.7	34.1	35
1.0	73.7	41.1	22				

[a] 0.5 and 1.0 ml CHCl3/kg are significantly different than 0.0 ml CHCl3/kg ($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.

UREA NITROGEN 6 HR

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	71		2.37	0.014
EXP	1	115.58		
TCE	1	55.06	1.22	0.274
CHCl3	2	179.58	3.98	0.024
VEHICLE	1	210.72	4.67	0.035
TCE x CHCl3	2	96.42	2.14	0.127
TCE x VEH	1	170.84	3.78	0.057
VEH x CHCl3	2	45.05	1.00	0.375
TCE x CHCl3 x VEH	2	45.01	1.00	0.375
ERROR	59	45.14		

UREA NITROGEN 6 HR

CHCl3 (ml/kg)[a]	MEAN (mg/ mg UCRE)	STD DEV	N	TCE (ml/kg)	MEAN (mg/mg UCRE)	STD DEV	N
0.0	29.6	8.2	24	0.0	33.5	8.1	36
0.5	33.4	6.3	24	1.0	31.7	6.7	36
1.0	34.9	7.0	24				

[a]1.0 ml CHCl3/kg is significantly higher than 0.0 ml CHCl3/kg ($p<0.05$). No other differences were significant.

UREA NITROGEN 12 HR AQUEOUS VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	34		8.66	<0.001
EXP	1			
CHCl3	2	0.57	15.94	<0.001
TCE	1	0.29	8.14	0.008
CHCl3 x TCE	2	0.11	2.95	0.069
ERROR	28	0.04		

[a]Based on Log(NUN12)

UREA NITROGEN 12HR AQUEOUS VEHICLE

CHCl3 (ml/kg)[a]	MEAN (mg/mg UCRE)	STD DEV	N	TCE (ml/kg)	MEAN (mg/mg UCRE)	STD DEV	N
0.0	30.5	13.2	12	0.0	31.7	8.1	17
0.5	32.3	4.3	12	1.0	39.3	12.4	18
1.0	45.0	8.0	11				

[a]1.0 ml CHCl3/kg is.significantly higher than 0.0 and 0.5 ml CHCl3/kg ($p<0.05$). 0.0 and 0.5 ml CHCl3/kg are not significantly different.

UREA NITROGEN 12HR OIL VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	35		7.76	<0.001
EXP	1	<0.01		
CHCl3	2	0.24	11.59	<0.001
TCE	1	0.36	17.23	<0.001
CHCl3 x TCE	2	0.06	3.08	0.061
ERROR	29	0.02		

[a]Based on Log(NUN12)

UREA NITROGEN 12 HR OIL VEHICLE

CHCl3 (ml/kg)[a]	MEAN (mg/mg UCRE)	STD DEV	N	TCE (ml/kg)	MEAN (mg/mg UCRE)	STD DEV	N
0.0	20.9	1.5	12	0.0	27.5	6.1	18
0.5	25.6	6.5	12	1.0	22.2	3.3	18
1.0	28.0	4.9	12				

[a] 0.5 and 1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg ($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.

UREA NITROGEN 24 HR AQUEOUS VEHICLE

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	35		5.43	0.001
EXP	1	262.91		
CHCl3	2	303.85	10.65	<0.001
TCE	1	41.45	1.46	0.236
CHCl3 x TCE	2	8.45	0.30	0.746
ERROR	29	28.52		

UREA NITROGEN 24 HR AQUEOUS VEHICLE

CHCl3 (ml/kg)[a]	MEAN (mg/mg UCRE)	STD DEV	N	TCE (ml/kg)	MEAN (mg/mg UCRE)	STD DEV	N
0.0	35.7	6.1	12	0.0	34.2	6.9	18
0.5	30.0	4.5	12	1.0	36.3	7.3	18
1.0	40.1	6.9	12				

[a]0.5 ml CHCl3/kg is significantly lower than 0.0 and 1.0 ml CHCl3/kg ($p<0.05$). 0.0 and 1.0 ml CHCl3/kg are not significantly different..

UREA NITROGEN 24 HR OIL VEHICLE

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	35		5.54	0.001
EXP	1	337.86		
CHCl3	2	98.92	5.45	0.010
TCE	1	22.09	1.22	0.279
CHCl3 x TCE	2	23.12	1.27	0.295
ERROR	29			

UREA NITROGEN 24 HR OIL VEHICLE

CHCl3 (ml/kg)[a]	MEAN (mg/mg UCRE)	STD DEV	N	TCE (ml/kg)	MEAN (mg/mg UCRE)	STD DEV	N
0.0	26.8	3.5	12	0.0	29.8	7.0	18
0.5	28.0	4.2	12	1.0	28.2	4.1	18
1.0	32.2	7.4	12				

[a]1.0 ml CHCl3/kg is significantly higher than 0.0 and 0.5 ml CHCl3/kg ($p<0.05$). 0.0 and 0.5 ml CHCl3/kg are not significantly different.

UREA NITROGEN 36 HR AQUEOUS VEHICLE

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	35		8.18	<0.001
EXP	1	214.98		
CHCl3	2	122.51	7.86	0.002
TCE	1	28.44	1.83	0.187
CHCl3 x TCE	2	138.05	8.86	0.001
ERROR	29	15.58		

UREA NITROGEN 36 HR AQUEOUS VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (mg/mg UCRE)	STANDARD DEVIATION	N
0.0	0.0	25.33	5.70	6
0.0	1.0	28.08	3.13	6
0.5	0.0	28.97	3.82	6
0.5	1.0	30.50	3.54	6
1.0	0.0	37.87	5.42	6
1.0	1.0	28.29	5.88	6

[a]In the absence of TCE, 1.0 ml CHCl3/kg is significantly higher than 0.0 and 0.5 ml CHCl3/kg($p<0.05$). 0.0 and 0.5 ml CHCl3/kg are not significantly different.
In the presence of TCE, there were no significant differences in the CHCl3 levels.
[b]The TCE levels are significantly different at 1.0 ml CHCl3/kg ($p=0.007$).

UREA NITROGEN 36 HR OIL VEHICLE

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	35		7.12	<0.001
EXP	1	102.99		
CHCl3	2	399.59	14.62	<0.001
TCE	1	76.94	2.82	0.104
CHCl3 x TCE	2	93.83	3.44	0.046
ERROR	29	27.31		

UREA NITROGEN 36 HR OIL VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN (mg/mg UCRE)	STANDARD DEVIATION	N
0.0	0.0	22.2	3.3	6
0.0	1.0	25.5	2.3	6
0.5	0.0	25.9	2.6	6
0.5	1.0	34.2	4.6	6
1.0	0.0	36.8	11.4	6
1.0	1.0	34.0	3.0	6

[a] In the absence of TCE, 1.0 ml/kg CHCl3 is significantly higher than 0.0 and 0.5 ml/kg CHCl3.($p<0.05$). 0.5 and 0.0 are not significantly different. In the presence of TCE, 0.5 and 1.0 ml/kg CHCl3 are significantly higher than 0.0 ml/kg CHCl3.($p<0.05$). 0.5 and 1.0 ml/kg CHCl3 are not significantly different.

[b] CHCl3 and TCE interact significantly at 0.5 ml/kg CHCl3 ($p=0.003$).

pH 6 HR AQUEOUS VEHICLE

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	35		6.60	<0.001
EXP	1	<0.01		
CHCl3	2	1.27	4.59	0.019
TCE	1	6.19	22.32	<0.001
CHCl3 x TCE	2	1.21	4.36	0.022
ERROR	29	0.28		

pH 6 HR AQUEOUS VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN	STANDARD DEVIATION	N
0.0	0.0	8.34	0.34	6
0.0	1.0	6.78	0.63	6
0.5	0.0	7.25	0.41	6
0.5	1.0	6.82	0.47	6
1.0	0.0	7.21	0.49	6
1.0	1.0	6.71	0.68	6

[a]In the absence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly lower than 0.0 ml CHCl3/kg($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different .
In the presence of TCE, there were no significant differences in the CHCl3 levels.
[b]The TCE levels are significantly different at 0.0 ml CHCl3/kg ($p=0.001$).

pH 6HR OIL VEHICLE

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	35		5.44	<0.001
EXP	1	GET		
CHCl3	2	4.44	15.15	<0.001
TCE	1	0.21	0.70	0.409
CHCl3 x TCE	2	0.24	0.82	0.452
ERROR	29	0.29		

pH 6HR OIL VEHICLE

CHCl3 (ml/kg)[a]	MEAN	STD DEV	N	TCE (ml/kg)	MEAN	STD DEV	N
0.0	7.88	0.57	12	0.0	7.26	0.70	18
0.5	6.89	0.47	12	1.0	7.11	0.75	18
1.0	6.77	0.54	12				

[a] 0.5 and 1.0 ml CHCl3/kg are significantly lower than 0.0 ml CHCl3/kg ($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.

pH 24 HR OIL VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	35		9.55	<0.001
EXP	1	0.001		
CHCl3	2	0.064	22.48	<0.001
TCE	1	0.004	1.24	0.274
CHCl3 x TCE	2	0.016	5.51	0.009
ERROR	29			

[a]Based on LOG(pH24)

pH 24 HR OIL VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN	STANDARD DEVIATION	N
0.0	0.0	7.16	0.47	6
0.0	1.0	6.79	0.33	6
0.5	0.0	5.97	0.22	6
0.5	1.0	6.55	0.28	6
1.0	0.0	6.01	0.32	6
1.0	1.0	6.12	0.39	6

[a]In the absence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly lower than 0.0 ml CHCl3/kg($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.
In the presence of TCE, 1.0 ml CHCl3/kg is significantly lower than 0.0 and 0.5 ml CHCl3/kg ($p<0.05$). 0.0 and 0.5 ml CHCl3/kg are not significantly different.
[b]The TCE levels are significantly different at 0.5 ml CHCl3/kg ($p=0.004$).

pH 24 HR AQUEOUS VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	35		24.32	<0.001
EXP	1	0.025		
CHCl3	2	0.160	55.62	<0.001
TCE	1	0.002	0.67	0.421
CHCl3 x TCE	2	0.037	12.70	<0.001
ERROR	29	0.003		

[a]Based on LOG(pH24)

pH 24 HR AQUEOUS VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN	STANDARD DEVIATION	N
0.0	0.0	8.36	0.18	6
0.0	1.0	7.33	0.86	6
0.5	0.0	6.37	0.31	6
0.5	1.0	6.46	0.20	6
1.0	0.0	6.12	0.09	6
1.0	1.0	6.62	0.34	6

[a]In the absence of TCE, 1.0 ml CHCl3/kg is significantly lower than 0.5 ml CHCl3/kg ($p<0.05$), 0.5 ml CHCl3/kg is significantly lower than 0.0 ml CHCl3/kg ($p<0.05$) and 1.0 ml CHCl3/kg is significantly lower than 0.0 ml CHCl3/kg ($p<0.05$).
In the presence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly lower than 0.0 ml CHCl3/kg($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.
[b]The TCE levels are significantly different at 0.0 ml CHCl3/kg ($p=0.008$) and 1.0 ml CHCl3/kg ($p=0.003$).

pH 36 HR[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	71		21.39	<0.001
EXP	1	2.22		
TCE	1	0.01	0.14	0.705
CHCl3	2	6.62	100.28	<0.001
VEHICLE	1	0.60	9.08	0.004
TCE x CHCl3	2	0.26	3.87	0.026
TCE x VEH	1	0.09	1.37	0.247
VEH x CHCl3	2	0.03	0.48	0.624
TCE x CHCl3 x VEH	2	0.10	1.57	0.216
ERROR	59			

[a]Based on LOG(pH36)

pH 36 HR

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN	STANDARD DEVIATION	N
0.0	0.0	6.99	0.29	12
0.0	1.0	6.76	0.52	12
0.5	0.0	5.96	0.17	12
0.5	1.0	6.50	0.23	12
1.0	0.0	5.89	0.20	12
1.0	1.0	6.07	0.41	12

[a]In the absence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly lower than 0.0 ml CHCl3/kg($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different. In the presence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly lower than 0.0 ml CHCl3/kg. 0.5 and 1.0 ml CHCl3/kg are not significantly different.
b The TCE levels do not interact.

pH 48 HR[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	69		19.97	<0.001
EXP	1	0.43		
TCE	1	1.01	4.82	0.032
CHCl3	2	16.96	81.37	<0.001
VEHICLE	1	1.88	9.05	0.004
TCE x CHCl3	2	1.87	8.98	<0.001
TCE x VEH	1	0.09	0.47	0.495
VEH x CHCl3	2	3.21	15.42	<0.001
TCE x CHCl3 x VEH	2	1.30	6.24	0.004
ERROR		0.21		

[a]Based on LOG(pH48)

pH 48 HR

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN	STANDARD DEVIATION	N
0.0	0.0	8.31	0.18	12
0.0	1.0	7.89	0.65	12
0.5	0.0	6.47	0.61	12
0.5	1.0	7.03	0.75	12
1.0	0.0	6.23	0.38	11
1.0	1.0	6.86	0.79	11

[a]In the absence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly lower than 0.0 ml CHCl3/kg($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different. In the presence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly lower than 0.0 ml CHCl3/kg($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.

[b]The TCE levels are significantly different at 0.5 ml CHCl3/kg ($p=0.036$) and 1.0 ml CHCl3/kg ($p=0.032$).

VOLUME 6 HR OIL VEHICLE

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	35		5.66	<0.001
EXP	1	10.13		
CHCl3	2	5.50	7.79	0.002
TCE	1	1.56	2.21	0.148
CHCl3 x TCE	2	0.65	0.92	0.411
ERROR	29	0.71		

VOLUME 6 HR OIL VEHICLE

CHCl3 (ml/kg)[a]	MEAN (ml)	STD DEV	N	TCE (ml/kg)	MEAN (ml)	STD DEV	N
0.0	2.00	0.82	12	0.0	3.00	1.21	18
0.5	3.03	1.15	12	1.0	2.56	2.56	18
1.0	3.28	1.03	12				

[a] 0.5 and 1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg ($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.

VOLUME 12 HR[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	71		1.92	0.050
EXP	1	0.011		0.859
TCE	1	0.065	0.18	0.670
CHCl3	2	2.610	7.33	0.001
VEHICLE	1	0.331	0.93	0.339
TCE x CHCl3	2	0.588	1.65	0.200
TCE x VEH	1	0.004	0.01	0.914
VEH x CHCl3	2	0.157	0.44	0.645
TCE x CHCl3 x VEH	2	0.540	1.52	0.227
ERROR	59	0.355		

[a]Based on LOG(VOL12)

VOLUME 12 HR

CHCl3 (ml/kg)[a]	MEAN (ml)	STD DEV	N	TCE (ml/kg)	MEAN (ml)	STD DEV	N
0.0	2.34	0.98	24	0.0	3.58	2.05	36
0.5	4.90	3.24	24	1.0	4.12	3.29	36
1.0	4.29	2.81	24				

[a]0.5 and 1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg ($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.

VOLUME 24 HR[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	71		2.77	0.005
EXP	1	1.254		
TCE	1	0.087	0.26	0.615
CHCl3	2	3.47	10.23	<0.001
VEHICLE	1	0.116	0.34	0.560
TCE x CHCl3	2	0.099	0.29	0.748
TCE x VEH	1	0.276	0.81	0.371
VEH x CHCl3	2	0.262	0.77	0.466
TCE x CHCl3 x VEH	2	0.948	2.79	0.069
ERROR	59	0.340		

[a]Based on LOG(VOL24)

VOLUME 24 HR

CHCl3 (ml/kg)[a]	MEAN (ml)	STD DEV	N	TCE (ml/kg)	MEAN (ml)	STD DEV	N
0.0	4.75	1.29	24	0.0	7.89	5.22	36
0.5	12.71	8.99	24	1.0	9.44	8.51	36
1.0	8.54	6.16	24				

[a]0.5 and 1.0 ml CHCl3/kg are significantly higher than 0.0 ml CHCl3/kg ($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.

VOLUME 48 HR AQUEOUS VEHICLE

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	34		4.30	0.003
EXP	1	0.25		
CHCl3	2	35.01	8.83	0.001
TCE	1	7.11	1.79	0.191
CHCl3 x TCE	2	12.47	3.14	0.058
ERROR	28	3.97		

VOLUME 48 HR AQUEOUS VEHICLE

CHCl3 (ml/kg)[a]	MEAN (ml)	STD DEV	N	TCE (ml/kg)	MEAN (ml)	STD DEV	N
0.0	4.45	1.79	11	0.0	5.55	3.03	17
0.5	6.55	2.60	12	1.0	6.21	3.35	18
1.0	3.17	1.83	12				

[a] 0.5 ml CHCl3/kg is significantly higher than 0.0 and 1.0 ml CHCl3/kg ($p<0.05$). 0.0 and 1.0 ml CHCl3/kg are not significantly different.

OSMOLALITY 12 HR AQUEOUS VEHICLE

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	35		8.55	<0.001
EXP	1	666400		
CHCl3	2	627187	8.06	0.002
TCE	1	10133	0.13	0.721
CHCl3 x TCE	2	1030542	13.24	<0.001
ERROR	29	77855		

[a]Based on Log( )

OSMOLALITY 12 HR AQUEOUS VEHICLE

CHCl3 (ml/kg)[a]	TCE (ml/kg)[b]	MEAN	STANDARD DEVIATION	N
0.0	0.0	1873	163	6
0.0	1.0	1167	461	6
0.5	0.0	893	268	6
0.5	1.0	1262	418	6
1.0	0.0	1081	224	6
1.0	1.0	1318	220	6

[a]In the absence of TCE, 0.5 and 1.0 ml CHCl3/kg are significantly lower than 0.0 ml CHCl3/kg($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.
In the presence of TCE, there were no significant differences in the CHCl3 levels.
[b]The TCE levels are significantly different at 0.0 ml CHCl3/kg ($p=0.007$) and 1.0 ml CHCl3/kg ($p=0.016$).

OSMOLALITY 24 HR

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	71		7.84	<0.001
EXP	1	479383		
TCE	1	160083	1.07	0.304
CHCl3	2	6049454	40.58	<0.001
VEHICLE	1	170236	1.14	0.290
TCE x CHCl3	2	127456	0.86	0.431
TCE x VEH	1	23148	0.16	0.695
VEH x CHCl3	2	128934	0.86	0.426
TCE x CHCl3 x VEH	2	285177	1.91	0.157
ERROR	59	149057		

OSMOLALITY 24 HR

CHCl3 (ml/kg)[a]	MEAN	STD DEV	N	TCE (ml/kg)	MEAN	STD DEV	N
0.0	1617	366	24	0.0	1094	585	36
0.5	674	398	24	1.0	999	552	36
1.0	848	416	24				

[a] 0.5 and 1.0 ml CHCl3/kg are significantly lower than 0.0 ml CHCl3/kg ($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.

OSMOLALITY 36 HR

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	71		8.32	<0.001
EXP	1	1669878		
TCE	1	32555	0.25	0.616
CHCl3	2	3147160	24.53	<0.001
VEHICLE	1	3574692	27.87	<0.001
TCE x CHCl3	2	292976	2.28	0.111
TCE x VEH	1	66	0.00	0.982
VEH x CHCl3	2	270051	2.11	0.131
TCE x CHCl3 x VEH	2	56532	0.44	0.646
ERROR	59	128212		

OSMOLALITY 36 HR

CHCl3 (ml/kg)[a]	MEAN	STD DEV	N	TCE (ml/kg)	MEAN	STD DEV	N
0.0	1840	363	24	0.0	1459	597	36
0.5	1136	419	24	1.0	1417	473	36
1.0	1338	552	24				

[a] 0.5 and 1.0 ml CHCl3/kg are significantly lower than 0.0 ml CHCl3/kg ($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.

OSMOLALITY 48 HR AQUEOUS VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	34		3.04	0.020
EXP	1	0.60		
CHCl3	2	0.50	4.64	0.018
TCE	1	0.01	0.05	0.819
CHCl3 x TCE	2	0.16	1.45	0.252
ERROR	29	get		

[a]Based on Log(OSMO48)

OSMOLALITY 48 HR AQUEOUS VEHICLE

CHCl3 (ml/kg)[a]	MEAN	STD DEV	N	TCE (ml/kg)	MEAN	STD DEV	N
0.0	2116	200	12	0.0	1815	534	17
0.5	1685	468	12	1.0	1775	556	18
1.0	1562	708	11				

[a]0.5 and 1.0 ml CHCl3/kg are significantly lower than 0.0 ml CHCl3/kg ($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.

OSMOLALITY 48 HR OIL VEHICLE[a]

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	32		7.75	<0.001
EXP	1	0.13		
CHCl3	2	3.06	18.74	<0.001
TCE	1	0.54	3.28	0.082
CHCl3 x TCE	2	0.52	3.21	0.057
ERROR	26	0.16		

[a]Based on Log(OSMO48)

OSMOLALITY 48 HR OIL VEHICLE

CHCl3 (ml/kg)[a]	MEAN	STD DEV	N	TCE (ml/kg)	MEAN	STD DEV	N
0.0	1711	547	12	0.0	1008	713	16
0.5	839	473	11	1.0	1171	590	17
1.0	627	209	10				

[a] 0.5 and 1.0 ml CHCl3/kg are significantly lower than 0.0 ml CHCl3/kg ($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.

[a] 0.5 and 1.0 ml CHCl3/kg are significantly lower than 0.0 ml CHCl3/kg ($p<0.05$). 0.5 and 1.0 ml CHCl3/kg are not significantly different.

Oil vs Aqueous Comparison of ALT and BAC levels caused by CHCl3 dosages.

SERUM ALT 48 HOURS[a] CHCl3 =1.0 ml/kg

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	22	-	22.56	<0.001
VEHICLE	1	40.407	22.56	<0.001
ERROR	21	1.791	-	-

[a]Based on LOG(ALT)

VEHICLE	MEAN[a] (IU/L)	STD DEV	N
OIL	3803	3007	6
AQUEOUS	102	68	5

[a] 1.0 ml CHCl3/kg in oil is significantly greater than 1.0 ml CHCl3/kg in aqeous vehicle ($p<0.05$).

SERUM ALT 24 HOURS[a] CHCl3 =1.0 ml/kg

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	18	-	5.69	0.029
VEHICLE	1	2.098	5.69	0.029
ERROR	17	0.369	-	-

[a]Based on LOG(ALT)

VEHICLE	MEAN[a] (IU/L)	STD DEV	N
OIL	284	160.0	6
AQUEOUS	124	39.6	3

[a] 1.0 ml CHCl3/kg in oil is significantly greater than 1.0 ml CHCl3/kg in aqeous vehicle ($p=0.03$).

SERUM BAC 48 HOURS[a] CHCl3 =1.0 ml/kg

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	21	-	22.72	<0.001
VEHICLE	1	30.839	22.72	<0.001
ERROR	20	1.357	-	-

[a]Based on LOG(BAC)

VEHICLE	MEAN[a] (uM/L)	STD DEV	N
OIL	148	49	6
AQUEOUS	52	26.7	5

[a] 1.0 ml CHCl3/kg in oil is significantly greater than 1.0 ml CHCl3/kg in aqeous vehicle ($p<0.001$).

SERUM ALT 48 HOURS[a] CHCl3 =0.5 ml/kg

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	23	-	26.32	<0.001
VEHICLE	1	30.839	26.32	<0.001
ERROR	22	2.481	-	-

[a]Based on LOG(ALT)

VEHICLE	MEAN[a] (IU/L)	STD DEV	N
OIL	6962	1692	6
AQUEOUS	46	13	6

[a] 0.5 ml CHCl3/kg in oil is significantly greater than 0.5 ml CHCl3/kg in aqeous vehicle ($p<0.05$).

SERUM BAC 48 HOURS[a] CHCl3 =0.5 ml/kg

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	21	-	19.38	<0.001
VEHICLE	1	34.65	19.38	<0.001
ERROR	20	1.788	-	-

[a]Based on LOG(BAC)

VEHICLE	MEAN[a] (uM/L)	STD DEV	N
OIL	154	59	6
AQUEOUS	25	13	6

[a] 0.5 ml CHCl3/kg in oil is significantly greater than 0.5 ml CHCl3/kg in aqeous vehicle ($p<0.05$).

SERUM BAC 24 HOURS[a] CHCl3 =0.5 ml/kg

SOURCE	DF	MEAN SQUARE	F-VALUE	P-VALUE
OVERALL	14	-	4.84	0.047
VEHICLE	1	3.984	4.84	0.047
ERROR	13	0.824	-	-

[a]Based on LOG(BAC)

VEHICLE	MEAN[a] (uM/L)	STD DEV	N
OIL	296	228	4
AQUEOUS	46	18	4

[a] 0.5ml CHCl3/kg in oil is significantly greater than 0.5 ml CHCl3/kg in aqeous vehicle ($p<0.05$).