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# THE FAILURE OF MONOCHLOROACETIC ACID AND TRICHLOROACETIC ACID ADMINISTERED IN THE DRINKING WATER TO PRODUCE LIVER CANCER IN MALE F344/N RATS.

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*DeAngelo, et al., 1997*

## ABSTRACT

The chlorinated acetic acids, monochloroacetic (MCA) and trichloroacetic (TCA) acids are found as chlorine disinfection by-products in finished drinking water supplies. TCA has been demonstrated to be a mouse liver carcinogen. A chronic study in which male Fischer 344/N rats were exposed for 104 weeks to TCA and MCA in the drinking water is described. Animals, 28 day old, were exposed to 0.05, 0.5 or 2 g/l MCA, or 0.05, 0.5, or 5 g/l TCA. The 2.0 g/l MCA was lowered in stages to 1 g/l when the animals began to exhibit signs of toxicity. A time-weighted mean daily MCA concentration (MDC) of 1.1 g/l was calculated over the 104 week exposure period. Time-weighted mean daily doses (MDD) based upon measured water consumption were 3.5, 26.1, and 59.9 mg/kg/day for 0.05, 0.5 and 1.1 g/l MCA respectively; TCA MDD were 3.6, 32.5, and 363.8 mg/kg/day. Non-neoplastic hepatic changes were for the most part spontaneous and age-related. No evidence of hepatic neoplasia was found at any of the MCA or TCA doses. The incidence of neoplastic lesions at other sites was not enhanced over that in the control group. Drinking water concentrations of $\geq$0.5 g/l MCA produced a moderate to severe toxicity as reflected by a depressed water consumption and growth rate. A No Observed Effects Level (NOEL) for carcinogenicity of 0.5 g/l (26.1 mg/kg/day) MCA was calculated. TCA at drinking water levels as high as 5 g/l produced only minimal toxicity and growth inhibition and provided a NOEL of 364 mg/kg/day. Our results demonstrate that under the conditions of this bioassay, MCA and TCA were not tumorigenic in the male F344/N rat.

*DeAngelo, et al., 1997*

## INTRODUCTION

The chloroacetic acids are important industrial chemicals and environmental contaminants. Large populations of humans are chronically exposed to them due to their occurrence in finished drinking water as by-products of the chlorination disinfection process (Christian *et al.*,1983; Uden and Miller, 1983; Coleman *et al.*, 1984; Krasner *et al.*, 1989). Uden and Miller (1983) reported concentrations ranging from 34 to 160 µg/l for dichloroacetic acid (DCA) and trichloroacetic (TCA) acid. Krasner and coworkers (1989) found a median concentration of 19 µg/l for total haloacetic acids in 35 water utilities including 1 µg/l of monochloroacetic acid, (MCA). TCA and DCA have been identified in the stomach contents of experimental animals following the intubation of water containing sodium hypochlorite (Mink *et al.*,1983).

Human exposure can also occur as the result of commercial and therapeutic uses of the chloroacetic acids in addition to their generation during drinking water disinfection (Grosselin *et al.*, 1984; Stacpoole *et al.*, 1988) . The chloroacetic acids are metabolites of several high volume commercially used chlorinated alkanes and alkenes (Yllner, 1971a and 1971b; Rannug et al., 1976; Reichert *et al.*,1979; Liebler et al., 1985). TCA is a major metabolite of trichloroethylene and tetrachloroethylene, two of the most widely distributed environmental contaminants (Coleman *et al.*, 1976; Uden and Miller, 1983).

With such widespread environmental presence and substantial medical and industrial use, the chloroacetic acids hold a potential for substantial chronic exposure of

human populations. This potential for human exposure has prompted us to undertake studies to characterize the toxicity of MCA and TCA.

TCA administered in drinking water increased the incidence of liver cancer in B6C3F1 mice (Herren-Freund *et al.*, 1987; Bull *et al.*, 1990; DeAngelo *et al.*,1991). MCA administered by aqueous gavage was determined to be without carcinogenic activity in F344/N rats and B6C3F$_1$ mice (NTP, 1992). The mechanism by which TCA induced liver neoplasia in the mouse has been a matter of considerable interest since the chloroacetic acids have been found to be inactive or only weakly mutagenic *in vivo* and *in vitro* test systems (Herbert *et al.*, 1980; Rapson *et al.*, 1980; NTP, 1990; Chang *et al.*, 1992; Harrington-Brock *et al.*, 1992; DeMarini *et al.*, 1994). DeAngelo *et al.*, (1989) reported that TCA, but not MCA, induced hepatic peroxisome proliferation (PP) in mice and rats. They found a dose related increase in PP in the mouse and an increased liver PP in the rat only at the highest dose tested (39 mM; 5 g/l). Based upon these results it was suggested the rat should be less sensitive to tumor induction than the mouse. The studies reported here were undertaken to the determine the potential for MCA and TCA administered in the drinking water to induce neoplasia in the male F344/N rat using a modification of the standard carcinogenesis bioassay methodology.

*DeAngelo, et al., 1997*

## METHODS

### Chemicals

Monochloroacetic acid (CAS 79-11-8) and trichloroacetic acid (CAS 76-03-9) were purchased from Sigma Chemical Company (St. Louis, MO). Analysis of purity was performed by gas-liquid capillary chromatography and mass spectrometry and was determined to be ≥99% pure with no detectable impurities observed. The free acids were dissolved in distilled water to produce nominal (target) concentrations of 0.05, 0.5, and 2.5 g/l (MCA) and 0.05, 0.5 and 5 g/l (TCA); the pH of the solutions was adjusted to 6.9 - 7.1 by the addition of an appropriate volume of 10 N sodium hydroxide. Freshly prepared solutions were administered in brown glass water bottles fitted with Teflon stoppers and stainless-steel, double-balled sipper tubes. The drinking water bottles were changed every 5 - 7 days. The stabilities of MCA and DCA solutions over these time periods were demonstrated by gas-liquid capillary chromatography.

The drinking water solutions (except the 0.05 g/l which was prepared by diluting the 0.5 g/l solution 1:10) were sampled throughout the study to determine actual MCA and DCA concentrations. The solutions were pipetted into 7 ml liquid scintillation mini-vials which were then tightly capped with a Teflon lined urea cap. The vials were stored at 5 °C until analysis by UV absorption at 331 nm against standard concentrations set by gas-liquid capillary chromatography. The targeted and measured MCA and TCA concentrations are shown in Tables 1 and 4.

*DeAngelo, et al., 1997*

**Animals and Animal Husbandry**

Weanling male Fischer 344 rats confirmed free of viral antibodies, bacteria and parasitic infections were obtained from Charles River Laboratories (Portage, MI) and held for 1 week in quarantine. The animals were 28 - 30 days of age at the beginning of treatment with mean initial body weights ranging from 56 - 59 g. The animals were maintained at 20 - 22 °C and 40 - 60% humidity on a 12-hour light-dark cycle. They were housed two per cage, and provided Purina Rodent Laboratory Chow and distilled water *ad libitum*. Cageside observations were conducted daily for physiological and behavioral responses and overt signs of toxicity. Mortality and morbidity checks were made twice daily. Body weights and water consumption were measured at the start of exposure, twice monthly for the first 2 months and then monthly afterwards. Physical examinations were conducted bi-weekly to detect any abnormal changes of the skin, eyes, or systemic organ systems. Aspects of these studies were conducted in compliance with the guidelines of the American Association for Accreditation of Laboratory Animal Care.

**Animal Dosing**

The study was conducted at EPA's Andrew W. Bridenbach Laboratory, Cincinnati, OH. Animals were assigned to each of 8 groups: 2 g/l sodium chloride (control), 0.05 and 0.5 g/l MCA and TCA, 2.0 g/l MCA, or 5.0 g/l TCA dissolved in distilled water. The drinking water containing the NaCl was approximately isomolar (32 mM) to the neutralized 5.0 g/l TCA (31 mM) with respect to $Na^+$. The 2.5 g/l MCA concentration was sequentially lowered to 1.5 g/l at 8 weeks and to 1 g/l at 24 weeks

due to a severe inhibition of body weight gain. The animals were dosed for 104 weeks.

**Necropsy and Pathologic Examination**

Groups of animals were sacrificed at 15, 30, 45, 60 or 104 weeks. At the interim sacrifices, body, liver, kidneys, spleen, urinary bladder and testes were examined for gross lesions including discoloration, surface irregularities, nodular changes and/or tumor masses. Blocks of tissue for each organ (2 from each liver lobe) were preserved in 10% neutral buffered formalin for 24 hr and then stored in 70% ethanol. The formalin-fixed tissues were trimmed, processed, embedded in paraffin, and sectioned; slides were prepared and stained with hematoxylin and eosin. The portions liver and kidneys remaining were placed in foil packs, quick frozen in liquid nitrogen and stored at -70°C.

At the terminal sacrifices a complete rodent necropsy was performed. All surfaces and orifices, the carcass, the external surface of the brain, cervical tissues, internal organs, and the cranial, thoracic, abdominal, and pelvic cavities were examined for gross lesions, including discolorations, surface irregularities, nodular changes, and masses. All gross lesions and representative tissue samples were collected from the brain, sciatic nerve, salivary gland, pancreas, pituitary, adrenals, thymus, thyroid, parathyroids, trachea, esophagus, lungs, liver, spleen, skeletal muscle, tongue, heart and aorta, stomach, duodenum, jejunum, ileum, colon, cecum, rectum, kidneys, urinary bladder, prostate, seminal vesicles, testes, preputial gland, mammary gland, femur, nasal cavity, larynx, skin, mesenteric and mandibular lymph nodes.

*DeAngelo, et al., 1997*

The liver, kidneys, spleen and testes and all excised lesions from all animals were examined microscopically by a board-certified veterinary pathologist at interim and final sacrifices. In addition, a complete pathologic examination was performed on all tissues from all animals in the high-dose MCA and TCA treatment groups at 104 weeks. Gross observations were correlated to the microscopic diagnoses for each animal. Data were tabulated according to individual animal and summarized by group. Labcat® histopathology software (Princeton, NJ) was used for data management.

**Analysis of Liver Neoplasia**

The prevalence and multiplicity of hepatocellular carcinoma (HC) and hepatocellular adenoma (HA) are reported both individually and summed as total liver neoplasia. Tumor prevalence was calculated as the percent of the animals with tumors compared to the number of animals examined. Tumor multiplicity was calculated by dividing the total number of lesions by the number of animals examined. In addition, hepatocellular hyperplasia (hyperplastic nodules, HN) was also analyzed and summed with the neoplastic lesions as a measure of total hepatic proliferative lesions.

**Serum Enzyme Analysis.**

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined in the sera from animals exposed to the chloroacids for 104 weeks. AST and ALT were measured using assay procedures provided with quantitative diagnostic kits (Sigma Chemical Company, St. Louis, MO).

**Cyanide-insensitive Palmitoyl CoA Oxidase Analysis (PCO)**

Portions of the frozen livers were homogenized (1:10 w/v) in a buffer containing

0.25 M sucrose, 0.05 M sodium EDTA, and 0.02 M Tris-HCl, pH 7.4. The homogenates were centrifuged at 800 X G for 5 minutes, the fatty layers removed by aspiration, and the extracts were stored at -70°C until assayed. Protein concentrations were measured according to the method of Lowry *et al.*, (1951). Previous work (DeAngelo *et al., 1989)* has shown that enzyme activities in frozen extracts did not differ significantly from the activities in liver extracts not frozen prior to assay. Cyanide-insensitive palmitoyl coenzyme A (PCO) activity was measured according to the method of Osumi and Hashimoto (1978).

**Measurements of Hepatocyte Proliferation**

Five days prior to each scheduled sacrifice, Alzet model 2001 osmotic pumps (Alza Corporation, Palo Alto, CA) containing 200 µl $^{3}$H-thymidine (62-64 Ci/mmole, ICN Radiochemicals, Costa Mesa, CA) were implanted subcutaneously. Autoradiography using paraffin embedded sections from the left liver lobe was performed according to the procedure of Leblond and Percival (1948) as modified by Gride (1968). The slides were coated with Kodak NTB 3 emulsion and stored in a desiccator for 5-8 weeks at 4°C. After developing, the slides were counter stained with hematoxylin. The numbers of hepatocyte nuclei with a grain count greater than 6 were scored in 1000 cells using random numbers to choose the setting of the mechanical stage for field selection. The labeling index (LI) was calculated by dividing the number of hepatocyte nuclei in S-phase by the total number of hepatocyte nuclei scored.

*DeAngelo, et al., 1997*

**Statistical Evaluation**

The SAS v5.18 (SAS Institute Inc., 1989, 1990) software system was used for all analyses. Supplementary references for some analyses are cited below. The principal analysis goals were the assessment of (1) comparisons of responses between each dose group and control and (2) trends of response with dose. The methods used in these assessments depended upon the type of response being considered. Analyses were conducted separately for each sacrifice interval. The following response variables were treated as continuous: body weight, organ weight, relative (or corrected) weights, labeling index, PCO, and water consumption. Tumor (or lesion) prevalence and counts as well as time-to-death were analyzed using special techniques as outlined below.

The continuous variables were analyzed using a one-way analysis of variance (Winer, 1971). Detection of some overall effect of treatment groups was followed by pairwise comparisons to controls using appropriate contrasts. If either the homoscedasticity assumption (Levene's test) or the assumption of normal distribution (Shapiro-Wilk test) was violated then a non-parametric analysis (viz. an ANOVA on the ranks of the data) was performed, followed by non-parametric (Wilcoxon rank sum) pairwise comparisons with controls. Tests for trend with dose were performed using contrasts in the mean responses.

For liver tumor (or lesion) prevalence, overall differences among treatments and for comparisons with controls, respectively, the likelihood ratio $X^2$ test (Fienberg, 1980) and Fisher's Exact test were used to test at each sacrifice time. Similar comparisons involving the counts of tumors (or lesions) per liver were performed using log-rank tests

(Tarone and Ware, 1977). Trends (with dose) of tumor prevalence were evaluated using an extension of the Fisher-Irwin Test. Trends (with dose) of tumor counts were evaluated using a log-rank monotone trend test. Statistical tests of differences between tumors and/or lesion prevalence or multiplicity were one-sided. Survival curves were determined using the product limit estimates of Kaplan-Meier and tests for equality of survival curves across strata (i.e. groups) were performed using the log-rank test. *P*-values $\leq 0.05$ were considered significant.

*DeAngelo, et al., 1997*

# RESULTS

## Monochloroacetic Acid

### Concentration, Water Consumption, Dose Determination and Survival

The targeted and measured MCA drinking water concentrations are shown in Table 1. The time-weighted mean MCA concentration (MDC) of the 2.0 g/l dose group (1.1 g/l over the course of the study), the mean measured MCA concentrations, and the time-weighted water consumptions yielded time-weighted mean daily doses (MDD) of 3.5 mg/kg/day, 26.1 mg/kg/day and 59.9 mg/kg/day for groups exposed to 0.05, 0.5 and 1.1 g/l MCA respectively. There were no significant differences in animal survival between the control and treatment groups (Table 1).

### Body and Organ Weights

The initial mean body weights (56 - 59 g) of animals assigned to each MCA treatment group did not differ from one another (data not shown). Figure 1 shows that the growth rates of the animals exposed to MCA were significantly depressed; the final mean body weight for 0.5 g/l MCA were 13% and for 1.1 g/l MCA were 38% lower than the control value(413 $\pm$ 9 g) . The final weights of animals exposed to 0.05 g/l MCA did not differ from the control value.

No significant differences in the absolute and relative (percent of body weight) weights of the liver, kidney, and testis were noted for the animals treated with 0.05 g/l MCA (Table 2). Absolute and relative spleen weights were significantly increased in this group. Treatment with 0.5 g/l significantly depressed whole and relative liver and

absolute kidney weights and increased the relative testes weight when compared to the control group. Exposure to 1.1 g/l MCA significantly depressed both the absolute and relative liver weights, the absolute and relative spleen weights, whole kidney weight and increased the relative kidney weight. These results demonstrate the relatively high degree of toxicity of MCA.

**Histopathologic Findings**

*Liver.* No dose-related significant increases in the prevalence of neoplasms (HA and HC) or HN above the control values were observed in any of the MCA exposure groups. (Table 3). Animals exposed to 0.05 g/l MCA exhibited prevalences of 8.0% (HA), 0 (HC), and 0 (HN); 0.5 g/l had prevalences of 0 (HA), 0 (HC), and 8.7% (HN) while those exposed to 5 g/l had prevalences of 4.0% (HA), 0 (HC) and 0 (HN). Combining the lesions as neoplasia (HA and HC) or as total proliferative lesions (HA, HC and HN) did not yield any significant differences from the control values.

The multiplicity of hepatocellular hyperplastic and neoplastic lesions for any treatment group was not altered above the control value for HN, HA or HC; 0.05 g/l MCA exhibited multiplicities of 0.12 $\pm$ 0.09 (HA), 0 (HC), and 0 (HN); 0.5 g/l had 0 (HA), 0 (HC), and 0.09 $\pm$ 0.06 (HN); and 1.1g/l had a prevalence of 0.04 $\pm$ 0.04 (HA), 0 (HC) and 0 (HN) lesions/animal. No hepatocellular tumors were found at any of the interim sacrifice periods.

No treatment-related liver pathology was observed throughout the study in the animals exposed to MCA. The slight increase in chronic inflammation observed in the 1.1 g/l MCA exposure group and the decreased incidence and severity of cytoplasmic

vacuolization and altered cellular foci (eosinophilic and clear cell ) were not consistent with a direct hepatotoxic effect (data not shown).

*Non-hepatic Tissues.* All of the observed neoplastic lesions would be considered spontaneous for F-344 male rats at 24 months of age (data not shown). None of the neoplastic lesions exceeded the percent incidence when compared to a historical control data base (Haseman *et al.,* 1984; NIEHS, 1995). Testicular interstitial cell tumors were present in 96%, 91% and 84% of the animals exposed to 0.05, 0.5 and 1.1 g/l MCA respectively and in 96%, 90% and 91% of the animals exposed to 0.05, 0.5 and 5 g/l TCA respectively vs 96% for the control group. The incidence of mononuclear cell leukemia was 24% for the control animals and 48%, 17% and 4% ($p \leq 0.05$) of the animals exposed to 0.05 g/l and 0.5 g/l and 1.1 g/l MCA respectively and 21%, 35% and 14% of the animals exposed to 0.05 g/l and 0.5 g/l and 5 g/l TCA respectively. No kidney neoplasms were noted in the control or any of the MCA and TCA exposure groups.

Common age-related spontaneous changes were present in many of the other tissues examined from animals exposed to MCA and TCA at the interim and final sacrifices. The incidences of these changes didn't exceed historical control data for the F344/N rat. The exceptions were an increased incidence of myocardial degeneration and chronic/active inflammation of the nasal cavities of animals exposed to 1.1 g/l MCA examined at 104 weeks but not at the earlier sacrifice periods or in the lower dose groups.

*DeAngelo, et al., 1997*

**Serum Enzyme Analysis**

MCA treatment for 104 weeks did not alter the serum levels of either AST or ALT(Figure 3a). AST concentrations ± SEM were 133 ± 8, 149 ± 11, and 170 ± 19 IU/L for 0.05, 0.5, and 1.1 g/l MCA respectively vs 166 ±18 for the control value . ALT concentrations were 61 ± 5, 73 ± 4, and 78 ± 7 IU/L for 0.05, 0.5, and 1.1 g/l MCA respectively vs 66 ±7 for the control value.

**Peroxisome Proliferation**

Hepatocyte peroxisome proliferation (PP) was assessed by measuring the peroxisome marker enzyme, cyanide insensitive palmitoyl coA oxidase (PCO) at 15, 30, 45, 60 and 104 weeks (data not shown). MCA-induced PCO activity alterations were not detected at any time period.

**Hepatocyte Proliferation**

Autoradiographic studies using $^{3}$H-thymidine were conducted to determine alterations in cell proliferation. No changes from the control values of LI were demonstrated at any time period or MCA dose levels examined (data not shown).

**Trichloroacetic Acid**

**Concentration, Water Consumption, Dose Determination and Survival**

The targeted and measured TCA drinking water concentrations, the time-weighted water consumptions, and the time-weighted mean TCA daily doses (MDD: 3.6, 32.5 and 364 mg/kg/day) are shown in Table 4. There were no significant

differences in the water consumption or animal survival between the control and treatment groups.

**Body and Organ Weights**

The initial mean body weights (57 - 59 g) of animals assigned to each treatment group did not differ from one another (data not shown). Figure 2 shows that the growth rate of the animals exposed to 5 g/l TCA was depressed to 89.3% of the control value. The mean terminal body weights of animals exposed to 0.5 g/l and 0.05 g/l TCA did not differ significantly from the control value.

No significant differences from the control values in the absolute and relative (percent of body weight) weights of the liver, kidney, spleen and testis were observed with the one exception of the lower absolute liver weight of the animals exposed to 5 g/l (Table 5).

**Histopathologic Findings**

*Liver.* No hepatocellular tumors were found in the control or treatment groups at any of the scheduled sacrifices prior to 104 weeks. No dose-related significant increase in the prevalence of neoplasms (HA and HC) or HN above the control values were observed in any of the TCA exposure groups (Table 6). Animals exposed to 0.05 g/l TCA exhibited prevalences of 4.2% (HA), 0 (HC), and 4.2% (HN); 0.5 g/l had prevalences of 15% (HA), 0 (HC), and 10% (HN); 5 g/l had prevalences of 4.6% (HA), 4. 6% (HC) and 0 (HN). Combining the lesions as neoplasia (HA and HC) or as total proliferative lesions (HA, HC and HN) did not yield any significant differences from the control values.

*DeAngelo, et al., 1997*

The multiplicity of hepatocellular hyperplastic and neoplastic lesions were not altered above the control value for HN, HA and HC. Animals exposed to 0.05 g/l TCA exhibited multiplicities of 0.08 ± 0.08 HA, 0 HC, and 0.04 ± 0.04 HN; 0.5 g/l had 0.15 ± 0.08 HA, 0 HC, and 0.10 ± 0.07 HN; 5 g/l had multiplicities of 0.05 ± 0.05 HA, 0.05 ± 0.05 HC and 0 HN lesions/animal. One hepatocellular adenoma was found in the 5 g/l treatment group at 60 weeks.

Non-neoplastic hepatic alterations were for the most part spontaneous and age-related in the control and all treatment groups. A minimal to mild treatment-related increase in hepatic cytoplasmic vacuolization was observed in the animals exposed to 0.05 g/l or 0.5 g/l TCA but was absent in the 5 g/l TCA group. A mild increase in the severity of hepatocellular necrosis was observed only in the high dose animals (data not shown).

*Non-hepatic Tissues.* See the results under Monochloroacetic Acid.

**Serum Enzyme Analysis**

Serum AST levels were significantly decreased below the control value in the 0.5 g/l TCA group (Figure 3). AST concentrations in the 0.05 g/l and 5 g/l TCA treatment groups were not significantly different from the control. Serum levels of ALT were increased by 5 g/l TCA. ALT levels were not affected by 0.05 and 0.5 g/l TCA treatment. The increased serum ALT and AST levels reflected the mild hepatocellular necrosis observed in the high dose animals.

**Peroxisome Proliferation**

*DeAngelo, et al., 1997*

PCO activity in the livers of animals exposed only to 5 g/l TCA was increased approximately 2-fold above the control values during the study at all times (Table 7). The 0.05 and 0.5 g/l treatments did not alter PCO activity.

**Hepatocyte Proliferation**

Autoradiographic studies using $^{3}$H-thymidine were conducted to determine TCA-induced alterations in hepatocyte proliferation in animals exposed to TCA. No significant differences from the control LI were observed at any of the sacrifice periods (data not shown).

## DISCUSSION

This study demonstrates that MCA and TCA do not induce cancer when administered for two years in the drinking water of male F344/N rats. MCA is a relatively toxic haloacetic acid with a LD 50 of 108 mg/kg and median time to death (LT) at a LD 90 dose of 130 min (Hayes *et al.*, 1973). Like any two-carbon acetate, MCA enters the Krebs cycle where it is converted to chlorocitrate. Chlorocitrate cannot be converted further, and blocks the cycle by inhibiting the aconitase system and therefore acetate oxidation (Fuhrman *et al.*, 1955; Gosselin *et al.*, 1984). MCA reduced the sulfhydryl concentrations probably by reacting with the sulfhydryl moiety in enzymes and other compounds (Yllner, 1971c; Hayes *et al.*, 1973; Dierczk, 1984). Daniel and his coworkers (1991) reported a lowest observed adverse effect level of 15 mg/kg for a 90-day oral exposure (gavage, 5/week) to MCA. Their conclusion was based upon altered blood serum chemistries and significant trends in the incidence of microscopic lesions in the male Sprague-Dawley rat.

MCA did not increase the incidence of cancer in male and female F344/N rats or B6C3F1 mice when administered by gavage 5 days per week for 104 weeks (NTP, 1990). The high dose group was given 30 mg/kg, which was similar to the calculated MDD (26 mg/kg/day) of animals drinking 0.5 g/l. In our study, the animals exposed to 1.1 g/l MCA (MDD, 55.5 mg/kg/day) exhibited a severe depression of body weight gain, liver and spleen weights which could modify a tumorigenic response (Warner *et al.*, 1995). The 24% incidence of mononuclear cell leukemia observed in the control animals was reduced to 4% in the animals exposed to 1.1 g/l MCA. The inhibition of

body weight gain and pathologic alterations at the intermediate MCA concentration, 0.5 g/l, were mild (13% reduction in body weight) so it is reasonable to calculate a NOEL for MCA administered over 2 years in the drinking water to the male F344/N rat at 26 mg/kg/day.

TCA has been reported to induce liver cancer in the male B6C3F1 mouse (Herren-Freund *et al.*, 1987; DeAngelo and Daniel, 1990; Bull *et al.*, 1990). Herren-Freund and co-workers (1987) first reported the hepatocarcinogenicity of 5 g/l TCA in the male B6C3F1 mouse without an initiating dose of a genotoxic carcinogen. Bull *et al.* (1990) found that following 52 weeks of exposure, 45% and 79% of male mice drinking 1 g/l and 2 g/l TCA respectively exhibited hepatocellular hyperplastic or neoplastic lesions; no female mice displayed these lesions. Since the histopathology of the livers from the female mice was similar to that observed in the male mice, it is possible that neoplasia would be observed if the treatment were carried out to longer time periods. DeAngelo and Daniel (1990) reported that male mice exposed for 60 weeks to 0.5, and 5 g/l TCA (MDD, 69, and 590 mg/kg/day) in their drinking water showed a 42% and 93 % incidence of liver neoplasia respectively that was significantly different from the control value of 13%. Female mice exposed to 4.5 g/l TCA for 104 weeks also developed liver neoplasia that was significantly higher in incidence than that noted in the control animals (unpublished observations).

The present study demonstrated the failure of TCA to increase hepatocellular cancer in the male rat at doses of 3.6, 33 and 364 mg/kg/day. There was a tendency for increased nonmalignant lesion in the 0.5 g/l dose group but these values were not

statistically significant when compared to the control values. For all dosed groups at all time periods examined only one carcinoma was seen (high dose group). Systematic toxicity appeared to be minimal even at the high dose since there was little or no effect on body or organ weights. With the exception of mild cytoplasmic vacuolization observed with the lower doses and a mild inflammation and necrosis in the high dose group (reflected by an elevated ALT level), hepatic pathology was for the most part spontaneous and age-related. Unlike the mouse, TCA did not induce hepatomegaly as demonstrated by the inability of TCA to increase either the absolute or relative liver weights and the lack of histopathologic evidence for cytomegaly which is very prominent in the mouse (DeAngelo *et al.*, 1991). Several subchronic studies also found TCA administered to rats in drinking water to be relatively weak when compared to the other chlorinated acetic acids and produced little or no toxicity even at the highest concentration tested (Davis, 1990; Mather *et al.*, 1990; Bhat *et al.*, 1991).

TCA was inactive when tested in the prophage-induction assay in *E. Coli* and the *Salmonella* TA100 mutation assay (DeMarini *et al.*, 1994) or in L5178Y/TK$^{+/-}$-3.7.2C mouse lymphoma cells (Harrington-Brock *et al.*, 1992) confirming most early studies which found TCA either not active or only weakly genotoxic (Herbert *et al.*, 1980; Rapson *et al.*, 1980; Styles *et al.*, 1991; Chang *et al.*, 1992). A study in which the potential for TCA to initiate or promote enzyme altered foci in rat liver found no evidence of initiating activity (Parnell *et al.*, 1988). In this same study, TCA did significantly increase the number and size of enzyme altered foci in livers 3 - 12 months following initiation with diethylnitrosamine. Promotion regimes of 0.05, 0.5 and 5 g/l

*DeAngelo, et al., 1997*

TCA in the drinking water produced a weak response when compared to phenobarbital and did not yield a dose-related increase in either the number or size of the altered foci. Promotion was also not related to the ability of TCA to induce hepatic peroxisome proliferation.

Interestingly, 5 g/l TCA did increase PCO activity, a marker of hepatic peroxisome proliferation, approximately 2-fold throughout the 2-year exposure period. The observation that several peroxisome proliferator chemicals increased the incidence of liver cancer in rodents gave rise to the suggestion that these agents might constitute a unique class of chemical carcinogens (Reddy *et al.*, 1980). It was suggested that the heightened production and subsequent metabolism of hydrogen peroxide and other reactive oxygen metabolites resulting from the activities of peroxisomal enzymes initiate DNA strand breakage, cross linkage, and ameliorative repair processes which produce mutations and chromosomal aberrations (Cadet and Teoule, 1978). TCA is a relatively weak inducer of hepatic peroxisome proliferation in several rat strains (DeAngelo *et al.*, 1989) after a short exposure period, an observation that was confirmed in this chronic study. It is possible that the extent of peroxisome induction was insufficient to exceed the hepatocyte's capacity to scavenge reactive oxygen species and protect against oxidative damage. Unlike in the rat, TCA is a potent inducer of hepatic peroxisome proliferation in mice (DeAngelo *et al.*, 1989) which was capable of producing oxidative stress and hepatocarcinogenesis in this species (Bull *et al.*, 1990; DeAngelo and Daniel, 1990). Larson and Bull (1992) demonstrated the failure of TCA to induce thiobarbituric acid-reactive substances (TBARS), a marker of oxidative damage in rat

liver, which would support a conclusion that the degree of peroxisome induction in this species is insufficient to cause any meaningful degree of oxidative damage.

In conclusion, TCA was not carcinogenic to the male F344/N rat when administered chronically in the drinking water at concentrations up to 5 g/l. A NOEL of 33 mg/kg/day for chronic toxicity based upon the decreased body weight in the highest dose group; A NOEL of 363 mg/kg/day for carcinogenicity was determined. Under the same conditions MCA failed to induce neoplasia at drinking water concentrations up to 1.1 g/l giving a NOEL of 59.9 mg/kg/day for carcinogenicity. The reason for the decrease in mononuclear cell leukemia incidence at the highest concentration is unclear, but is likely related to the severe growth retardation that occurred with that treatment (Warner et al., 1995). At the lowest MDD tested, spleen weights were increased over the control values so a NOEL for chronic toxicity was not determined.

*DeAngelo, et al., 1997*

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This manuscript has been reviewed by the Environmental Protection Agency and approved for publication. Mention of trade names or commercial products does not constitute endorsements or recommendations for use.

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*DeAngelo, et al., 1997*

## LIST OF FIGURES

**Figure 1**. Body weights of rats exposed to MCA for 104 weeks. The 2 g/l MCA was lowered to 1.5 g/l at 8 weeks (↓) and 1 g/l at 24 weeks (↓). Statistically significant at (*) Statistically significant (*) at $p \leq 0.05$.

**Figure 2**. Body weights of rats exposed to TCA for 104 weeks. Statistically significant (*) at $p \leq 0.05$.

**Figure 3**. Serum AST and ALT levels in rats exposed to MCA (3a) and TCA (3b); Statistically significant (*) at $p \leq 0.05$.

TABLE 1

Dosing and Survival Data for Rats Exposed to MCA

| Target Concentration | Control | 0.05 g/l MCA | 0.5 g/l MCA | 2.0 g/l MCA[a] |
|---|---|---|---|---|
| Measured Concentration<br>Number of Measurements | ----- | -----[b] | 0.47 ± 0.12[c]<br>177 | 1.1 ± 0.3<br>176 |
| Water Consumption[d]<br>(ml/kg bw /day) | 76.9 ± 5.5 | 70.5 ± 5.2 | 55.6 ± 1.7[e] | 55.5 ± 4.0[e] |
| Mean Daily Dose[d]<br>(mg/kg bw/day) | ----- | 3.5 | 26.1 | 59.9 |
| Number of Animals Started on Study | 50 | 50 | 50 | 60 |
| Number of Animals At Interim Sacrifices | 21 | 18 | 18 | 21 |
| Unscheduled Deaths | 6 | 7 | 9 | 14 |
| Number of Animals at Final Sacrifice | 23 | 24 | 23 | 25 |

[a]Concentration lowered to 1.5 g/l at 8 weeks and 1 g/l at 24 weeks.
[b]Drinking water solutions prepared by diluting 0.5 g/l 1:10 were not analyzed.
[c]Mean ± SD
[d]Time weighted mean ± SD
[e]Statistically significant ($p \leq 0.05$) when compared to the control group.

TABLE 2

Organ Weights for Rats Exposed to MCA

| Treatment Group<br>Number of Animals | Control<br>23 | 0.05 g/l MCA<br>24 | 0.5 g/l MCA<br>23 | 1.1 g/l MCA[a]<br>25 |
|---|---|---|---|---|
| Liver Weight (g)<br>Percent of Body Weight | 17.6 ± 0.6[b]<br>4.35 ± 0.21 | 17.9 ± 0.6<br>4.56 ± 0.18 | 12.6 ± 0.2[c]<br>3.51 ± 0.2[c] | 8.2 ± 0.4[c]<br>3.22 ± 0.04[c] |
| Kidney Weight (g)<br>Percent of Body Weight | 3.85 ± 0.11<br>0.89 ± 0.04 | 3.49 ± 0.09<br>0.88 ± 0.02 | 3.01± 0.05[c]<br>0.84 ± 0.01 | 2.35 ± 0.06[c]<br>0.93 ± 0.02 |
| Spleen Weight (g)<br>Percent of Body Weight | 3.25 ± 0.72<br>0.85 ± 0.20 | 5.67 ± 1.39[c]<br>1.53 ± 0.40[c] | 1.91 ± 0.26<br>0.53 ± 0.08 | 0.82 ± 0.08[c]<br>0.31 ± 0.02 |
| Testes Weight (g)<br>Percent of Body Weight | 5.77 ± 0.57<br>1.39 ± 0.12 | 5.55 ± 0.63<br>1.38 ± 0.15 | 6.52 ± 0.46<br>1.82 ± 0.13[c] | 4.50 ± 0.32<br>1.74 ± 0.10[c] |

[a]Mean daily concentration.
[b]Mean ± SEM.
[c]Statistically significant ($p \leq 0.05$) when compared to the control group.

TABLE 3
Prevalence and Multiplicity of Hepatocellular Lesions

| Treatment Group<br>Number Examined | Control<br>23 | 0.05 g/l MCA<br>25 | 0.5 g/l MCA<br>23 | 1.1 g/l MCA[a]<br>25 |
|---|---|---|---|---|
| Hyperplastic Nodule | 4.4[b]<br>0.04 ± 0.04[c] | 0<br>0 | 8.7<br>0.09 ± 0.06 | 0<br>0 |
| Adenoma | 4.4<br>0.04 ± 0.04 | 8.0<br>0.12 ± 0.09 | 0<br>0 | 4.0<br>0.04 ± 0.04 |
| Carcinoma | 0<br>0 | 0<br>0 | 0<br>0 | 0<br>0 |
| Neoplasia[d] | 4.4<br>0.04 ± 0.04 | 8.0<br>0.12 ± 0.09 | 0<br>0 | 4.0<br>0.04 ± 0.04 |
| Proliferative Lesions[e] | 8.7<br>0.09 ± 0.06 | 8.0<br>0.12 ± 0.09 | 8.7<br>0.09 ± 0.06 | 4.0<br>0.04 ± 0.04 |

[a]Mean daily concentration.
[b]Prevalence (percent of animals with at least 1 lesion).
[c]Multiplicity (number of lesions/animal).
[d]Adenomas and carcinomas
[e]Adenomas, carcinomas and hyperplastic nodules.

TABLE 4

Dosing and Survival Data for Rats Exposed to TCA

| Target Concentration | Control | 0.05 g/l TCA | 0.5 g/l TCA | 5.0 g/l TCA |
|---|---|---|---|---|
| Measured Concentration<br>Number of Measurements | ---- | --[a] | 0.46 ± 0.10[b]<br>177 | 4.89 ± 0.36<br>176 |
| Water Consumption[c] (ml/kg/day) | 76.9 ± 5.5 | 71.3 ± 4.7 | 70.6 ± 2.0 | 74.2 ± 4.6 |
| Mean Daily Dose[c] (mg/kg/day) | ---- | 3.6 | 32.5 | 363.8 |
| Number of Animals Started on Study | 50 | 50 | 50 | 50 |
| Number of Animals at Interim Sacrifices | 21 | 18 | 18 | 21 |
| Unscheduled Deaths | 6 | 8 | 13 | 7 |
| Number of Animals at Final Sacrifice | 23 | 24 | 19 | 22 |

[a]Drinking water solutions prepared by diluting 0.5 g/l 1:10 were not analyzed
[b]Mean ± SD
[c]Time weighted mean ± SD.

TABLE 5

Organ Weights for Rats Exposed to TCA

| Treatment Group<br>Number of Animals | 2.0 g/l NaCl<br>23[a] | 0.05 g/l TCA<br>24 | 0.5 g/l TCA<br>19 | 5 g/l TCA<br>22 |
|---|---|---|---|---|
| Liver Weight (g)<br>Percent of Body Weight | 17.6 ± 0.5[a]<br>4.35 ± 0.21 | 16.4 ± 0.7<br>4.21 ± 0.12 | 16.6 ± 0.8<br>4.29 ± 0.15 | 15.8 ± 0.3[b]<br>4.31 ± 0.11 |
| Kidney Weight (g)<br>Percent of Body Weight | 3.85 ± 0.11<br>0.89 ± 0.04 | 3.47 ± 0.12<br>0.91 ± 0.04 | 3.55 ± 0.12<br>0.93 ± 0.03 | 3.40 ± 0.07<br>0.93 ± 0.02 |
| Spleen Weight (g)<br>Percent of Body Weight | 3.25 ± 0.72<br>0.85 ± 0.20 | 2.86 ± 0.72<br>0.72 ± 0.17 | 2.49 ± 0.36<br>0.68 ± 0.13 | 2.44 ± 0.56<br>0.72 ± 0.19 |
| Testes Weight (g)<br>Percent of Body Weight | 5.77 ± 0.57<br>1.39 ± 0.12 | 6.21 ± 0.50<br>1.59 ± 0.12 | 5.83 ± 0.58<br>1.48 ± 0.14 | 5.22 ± 0.42<br>1.40 ± 0.11 |

[a]Mean ± SEM.

[b]Statistically significant ($p \leq 0.05$) when compared to the control group.

TABLE 6
Prevalence and Multiplicity of Hepatocellular Lesions

| Treatment Group<br>Number Examined | Control<br>23[a] | 0.05 g/l TCA<br>24 | 0.5 g/l TCA<br>20 | 5.0 g/l TCA<br>22 |
|---|---|---|---|---|
| Hyperplastic Nodule | 4.4[b]<br>0.04 ± 0.04[c] | 4.2<br>0.04 ± 0.04 | 10.0<br>0.10 ± 0.07 | 0<br>0 |
| Adenoma | 4.4<br>0.04 ± 0.04 | 4.2<br>0.08 ± 0.08 | 15.0<br>0.15 ± 0.08 | 4.6<br>0.05 ± 0.05 |
| Carcinoma | 0<br>0 | 0<br>0 | 0<br>0 | 4.6<br>0.05 ± 0.05 |
| Neoplasia[d] | 4.4<br>0.04 ± 0.04 | 4.2<br>0.08 ± 0.08 | 15.0<br>0.15 ± 0.08 | 4.6<br>0.09 ± 0.09 |
| Proliferative Lesions[e] | 8.7<br>0.09 ± 0.06 | 8.3<br>0.13 ± 0.09 | 25.0<br>0.25 ± 0.10 | 4.6<br>0.09 ± 0.09 |

[a]Number of animals surviving 80 - 104 weeks.
[b]Prevalence (percent of animals with at least 1 lesion).
[c]Multiplicity (number of lesions/animal, Mean ± SEM).
[d]Adenomas and Carcinomas
[e]Adenomas, carcinomas, and hyperplastic nodules

TABLE 7

Palmitoyl CoA Activity[a]

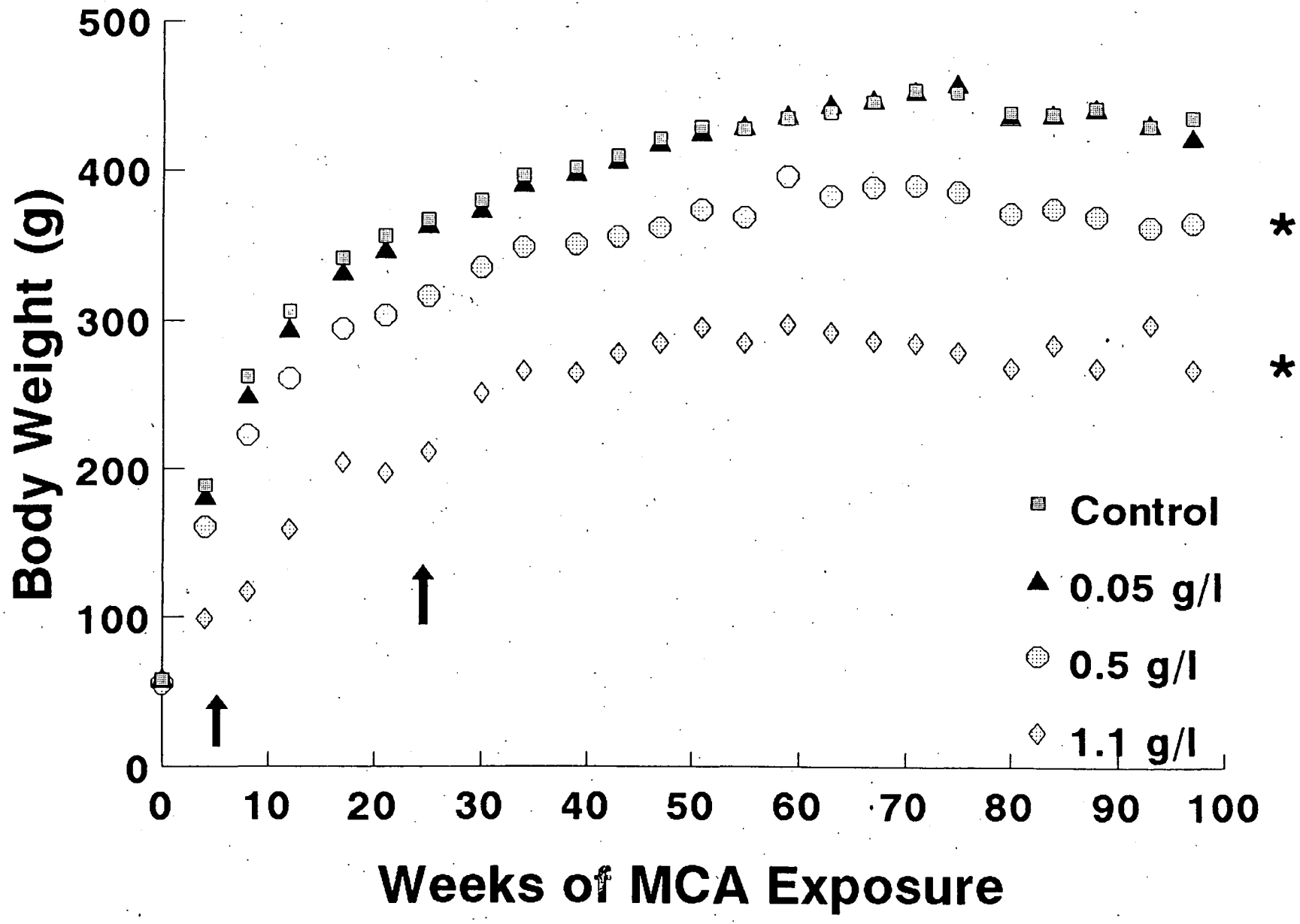
| Weeks of Exposure | Control | 0.05 g/l TCA | 0.5 g/l TCA | 5.0 g/l TCA |
|---|---|---|---|---|
| 15 | 2.42 ± 0.16[b] N = 6 | 1.10 ± 0.39 N = 6 | 2.48 ± 0.40 N = 6 | 5.14 ± 0.22[d] N = 6 |
| 30 | 2.30 ± 0.23 N = 6 | 2.40 ± 0.08 N = 6 | 2.38 ± 0.10 N = 6 | 3.81 ± 0.53[d] N = 6 |
| 45 | 3.08 ± 0.26 N = 6 | 2.96 ± 0.29 N = 6 | 3.44 ± 0.17 N = 6 | 5.74 ± 0.54[d] N = 6 |
| 60 | 3.14 ± 0.37 N = 3 | ND | ND | 6.22 ± 0.61[d] N = 3 |
| 104 | 1.35 ± 0.25 N = 6 | 1.42 ± 0.17 N = 6 | 2.09 ± 0.34 N = 6 | 5.46 ± 0.79[d] N = 6 |

[a]nmoles NAD reduced/minute/mg protein

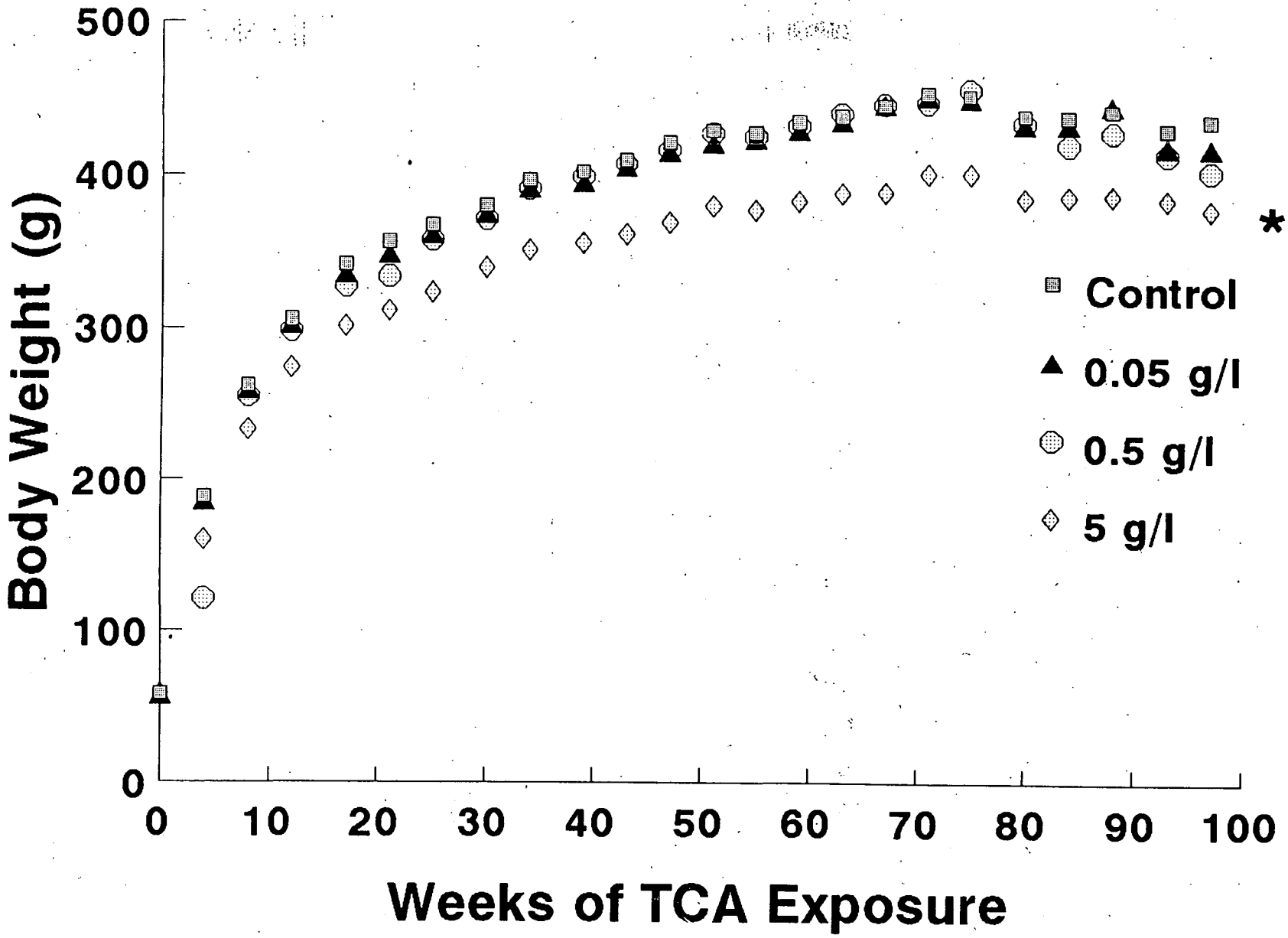
[b]Mean ± SEM

[c]Not done.

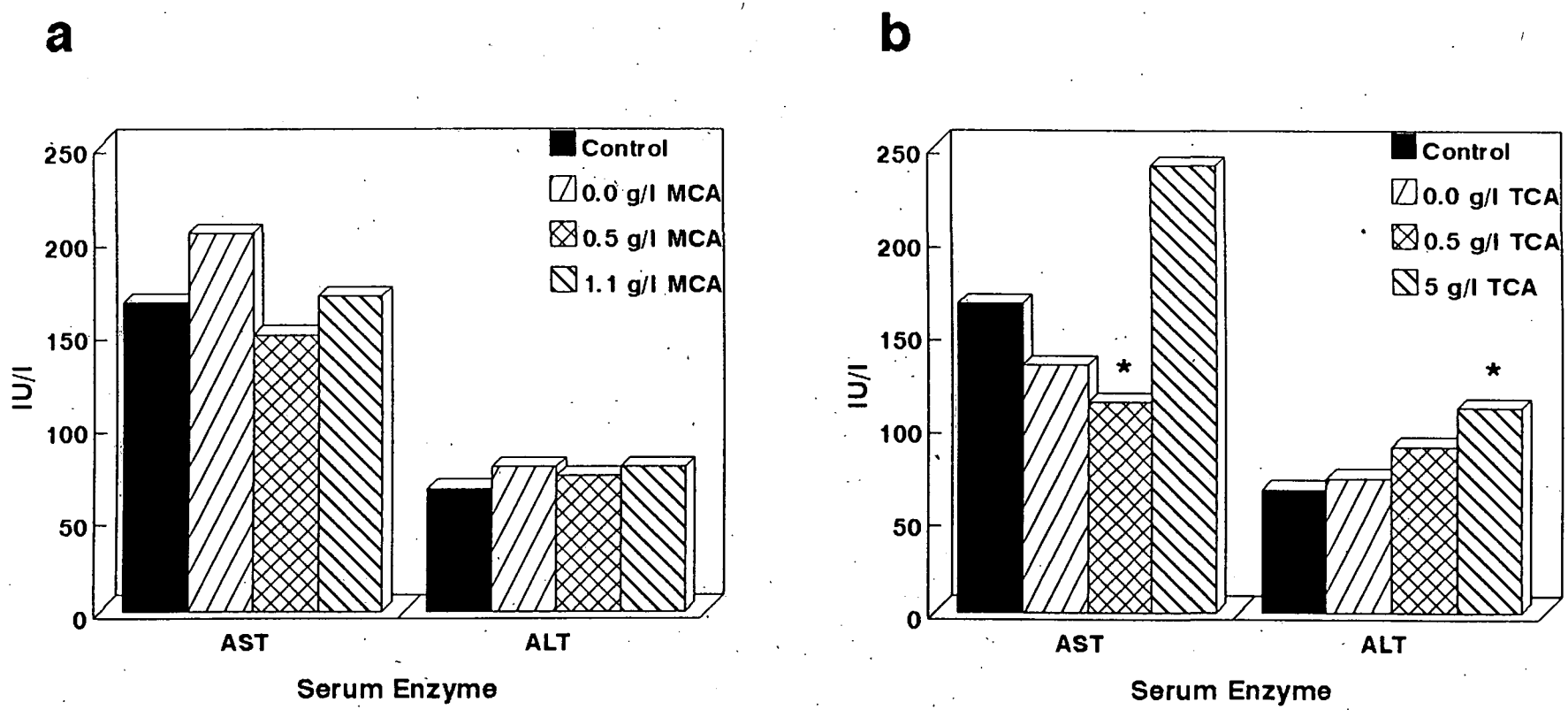
[d]Statistically significant ($p \leq 0.05$) when compared to the control group.




DeAngelo et al., 1997
Figure 1




DeAngelo et al., 1997
Figure 2




**DeAngelo et al., 1997**

**Figure3**