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## Susceptibility of Male and Female Mice to the Nephrotoxic and Hepatotoxic Properties of Chlorinated Hydrocarbons.* (31952)

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It is well known that a number of chlorinated hydrocarbons cause severe injury to the liver and kidneys. Eschenbrenner(1) reported that after administration of chloroform, renal necrosis occurred in male, but not in female mice. This sex difference has been confirmed (2,3). Culliform and Hewitt(4) have reported that female mice become fully susceptible to necrosis after treatment with androgens, and that the susceptibility of male mice to kidney necrosis is removed by castration and adrenalectomy. Sex differences in the morphology of the mouse kidney have been reported by Crabtree(5), who showed that the parietal layer of most of the Bowman's capsules in female mice is composed entirely of squamous cells, while in most of the capsules in male mice it is composed partly or entirely of cuboidal cells similar to those of the proximal convoluted tubules.

While marked differences exist between male and female mice to the susceptibility of kidney necrosis produced by chloroform, it is generally thought that no difference exists in the susceptibility to liver damage(1,4). However, recently Meshorer and Benhar(6) reported that they observed a difference in susceptibility to liver damage by carbon tetrachloride in male and female mice.

The purpose of the present work is 1) to determine if hydrocarbons, other than chloroform, also show sex differences in kidney damage, and 2) to determine if a sex difference also exists in the hepatotoxic response to carbon tetrachloride.

*Methods.* Male Swiss-Webster mice were randomized 10 per cage and used throughout. Two different weights of mice were used, 25-35 g and 35-45 g. The smaller mice were used except where stated otherwise.

Analytical grades of the following hydrocarbons were employed: chloroform; carbon tetrachloride; and 1,1,2-trichloroethane. All agents were administered intraperitoneally and were made up in corn oil to deliver the proper dosage in a final volume of 0.01 ml/g.

*Lethality.* For the 24-hour $LD_{50}$ determinations 4 or 5 groups of mice, 10 per group, were injected with a single dose of the hydrocarbon, and the number of deaths recorded at the end of 24 hours. The median lethal dose ($LD_{50}$) was then calculated for each hydrocarbon.

*Kidney function.* A urine collection unit for the kidney function phase was used as previously described by Plaa and Larson(7). Urine was collected and tested directly without ad-

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TABLE I. Summary of Acute Lethal ($LD_{50}$) and PSP Excretion ($ED_{50}$) in Male and Female Mice.

| | | |
|---|---|---|
| Chloroform | | |
| Acute $LD_{50}$ | ♂ | 1.2 (1.0 -1.3 )*ml/kg |
| | ♀ | 1.3 (1.1 -1.5 ) " |
| PSP $ED_{50}$ | ♂ | .078( .069- .088) " |
| | ♀ | † |
| 1,1,2-Trichloroethane | | |
| Acute $LD_{50}$ | ♂ | .35 (.28-.44) ml/kg |
| | ♀ | .40 (.33-.48) " |
| PSP $ED_{50}$ | ♂ | .17 (.13-.21) " |
| | ♀ | † |

* Number in parentheses represents 95% fiducial limits.

† No decrease in PSP excretion was observed, even at near-lethal doses.

sorption onto filter paper (as originally described).

Phenolsulfonephthalein (PSP) was administered intravenously into the tail vein (0.01 ml/g) of each mouse yielding a final dose of 1 mg/kg. Each animal was then hydrated with tap water by gavage (0.05 ml/g) and placed on the urine collection unit for 2 hours. At the end of the collection period, the PSP content was determined as previously described(8).

*Liver function.* Liver function was assessed by the serum glutamic-pyruvic transaminase (SGPT) determination of Reitman and Frankel(9), as adapted for the mouse(8).

*Statistics.* The method of Litchfield and Wilcoxon(10) was used to determine the 24-hour $LD_{50}$ values, and the $ED_{50}$ values for organ dysfunction as measured by SGPT elevation and PSP excretion. The $ED_{50}$ values were calculated after the functional data were transformed to all-or-none responses.

*Results.* In earlier studies(7,8) it had been shown that both chloroform and 1,1,2-trichloroethane exhibit nephrotoxic properties in male mice. Kidney studies were carried out with 1,1,2-trichloroethane to determine if female mice are resistant to the nephrotoxic properties of 1,1,2-trichloroethane, as has been reported with chloroform(1-4). In addition, the acute lethal potencies were examined in both species. Table I shows the results of this study. The mice injected with lethal doses of these two hydrocarbons became anesthetized and died within a few hours. As Table I shows, no sex difference was exhibited in the acute $LD_{50}$ for either chloroform or 1,1,2-trichloroethane.

With 75 control mice it was found that 42 ± (SD) 10% of the administered dose of PSP was excreted in 2 hours. Therefore, an excretion of 22% (mean — 2 SD) or less constituted a significant delay in excretion and, thus, renal dysfunction. This value was used as the cut-off for obtaining quantal data for calculating the PSP excretion $ED_{50}$. By these criteria the $ED_{50}$ for producing kidney dysfunction in male mice with chloroform was 0.078 ml/kg. This dose is about 15 times lower than the dose needed to produce death. However, in female mice, kidney dysfunction could not be produced even at lethal doses of chloroform. With 1,1,2-trichloroethane, the kidney dysfunction $ED_{50}$ in male mice was 0.17 ml/kg, this dose being about one-half the dose needed to produce death. In female mice, no decrease in PSP excretion could be demonstrated after 1,1,2-trichloroethane, even with lethal doses.

Since it has recently been reported(6) that a difference seems to exist in the susceptibility of male and female mice to the hepatotoxicity of carbon tetrachloride, the following experiments were performed to verify this difference. The data are summarized in Table II. The Table also contains the $LD_{50}$ values for $CCl_4$ in male and female mice. No sex difference in the acute $LD_{50}$ values was seen.

With 77 control mice, it was found that the SGPT activity was 28 ± (SD) 11 units. Thus, a value of 50 units (mean + 2 SD) was chosen as the upper limit of the normal range; any value greater than this was considered significant and indicative of liver dysfunction. This value was used as the cut-off for obtaining quantal data for calculating the elevation of SGPT activity $ED_{50}$. As is seen in Table II, no significant difference was ex-

TABLE II. Summary of Acute $CCl_4$ Lethal ($LD_{50}$) and SGPT Activity ($ED_{50}$) Values in 25-35 g Male and Female Mice.

| | | |
|---|---|---|
| Acute $LD_{50}$ | ♂ | 2.6 (2.3-3.0)*ml/kg |
| | ♀ | 2.8 (2.5-3.2) " |
| SGPT $ED_{50}$ | ♂ | .01 (.007-.015) ml/kg |
| | ♀ | .009 (.006-.014) " |

* Number in parentheses represents 95% fiducial limits.

TABLE III. Summary of Acute $CCl_4$ Lethal ($LD_{50}$) and SGPT Activity ($ED_{50}$) Values in 35-45 g Male and Female Mice.

| | | |
|---|---|---|
| Acute $LD_{50}$ | ♂ | 2.8 (2.5-3.1)*ml/kg |
| | ♀ | 2.8 (2.5-3.2) " |
| SGPT $ED_{50}$ | ♂ | .007 (.005-.010) ml/kg |
| | ♀ | .01 (.006-.016) " |

* Number in parentheses represents 95% fiducial limits.

hibited in the dosage needed to produce liver dysfunction in male and female mice. It should be noted that the dosage needed to produce elevated SGPT in both sexes is about 300 times lower than the dosage needed to produce death.

Meshorer and Benhar(6) considered that increased susceptibility of male mice to $CCl_4$ might be due to a higher fat content. They postulated that the high solubility of $CCl_4$ in fat might cause its accumulation in the fatty tissues of males, whereas in breeding females and young animals, which usually are devoid of excessive body fat, $CCl_4$ would not accumulate. Therefore, an experiment was conducted using 12-week-old mice. These mice weighed from 35-45 g, the females weighing about 5 g less than the males. The results of this study are shown in Table III. Even with these larger mice, no difference could be demonstrated between male and female mice with regard to the acute $LD_{50}$ and SGPT elevation $ED_{50}$ values for carbon tetrachloride.

*Discussion.* The results obtained with chloroform and 1,1,2-trichloroethane show that as far as kidney injury is concerned a sex difference in susceptibility does exist. While previous investigators(1-4) employed morphologic changes for their observations, the present study has made use of a function test. Although the previous studies used only chloroform, our results with 1,1,2-trichloroethane indicate that other nephrotoxic halogenated hydrocarbons probably affect only male mice. Similar results have also been reported for mercury(11), thus suggesting that male mice generally seem to be more susceptible to nephrotoxic agents. Whether or not this difference in susceptibility is related to the sex differences that have been reported in kidney cell structure(5,12) are a matter of conjecture.

With chloroform and 1,1,2-trichloroethane there was no sex difference in the 24-hour $LD_{50}$ values. This indicates that nephrotoxicity *per se* makes only a small contribution to lethality during this time period, thus strengthening an earlier hypothesis(8) that the 24-hour $LD_{50}$ is largely a measure of the deep narcosis caused by these hydrocarbons.

The results obtained with carbon tetrachloride, in young and older mice, indicate that there is no sex difference in the susceptibility of this mouse strain to the hepatotoxic properties of this agent. This conclusion is in conflict with that of Meshorer and Benhar(6), but an examination of their experimental data indicates that perhaps their conclusion regarding the existence of a sex difference was premature. These investigators studied an accidental exposure situation in which male mice showed increased mortality. The cause of death was never really established; pathologic changes were seen in the lungs and heart, as well as in the liver. Their experimental intoxication study with carbon tetrachloride failed to show any sex difference, in that the pathological changes were similar in males and females 24 and 72 hours after exposure to carbon tetrachloride. It was only 14 days later that a change could be seen with the male mice revealing liver fibrosis. It is questionable that this latter finding constitutes a difference in susceptibility. In view of these results, it is felt that there is no evidence for stating that a sex difference exists as far as the susceptibility to the hepatotoxic properties of carbon tetrachloride is concerned. It should be pointed out that others(1,4) have failed to find a sex difference in the hepatotoxic response to chloroform. However, it has been reported(13) that male rats exhibit a higher incidence of cirrhosis than do females after chronic exposure to carbon tetrachloride.

*Summary.* Male and female mice were tested for their susceptibility to the nephrotoxic properties of chloroform and 1,1,2-trichloroethane. Renal dysfunction, as determined by phenolsulfonephthalein excretion was demonstrated in male mice with chloroform at 0.078 ml/kg and 0.17 ml/kg for 1,1,2-trichloroethane. Renal dysfunction could not

be produced in female mice with chloroform and 1,1,2-trichloroethane, even at lethal doses. Male and female mice were also tested for their susceptibility to the hepatotoxic properties of carbon tetrachloride. Liver dysfunction, as determined by elevated serum glutamic-pyruvic transaminase activity, could be demonstrated in both male and female mice at 0.009 ml/kg. No sex difference was observed in the 24-hour $LD_{50}$ values for the three agents tested. This indicates that with these agents nephrotoxicity is not an important contributing factor in the deaths occurring during the first 24 hours.

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## Hemagglutination with Aldehyde-Fixed Erythrocytes for Assay of Antigens and Antibodies.* (31953)

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Passive hemagglutination methods have proved very useful for the assay of small amounts of antigen and antibody(1). Fresh or formalinized erythrocytes have been conjugated to proteins by the tannic acid or bis-diazotized benzidine and other procedures. Formalin-fixed, antigen-conjugated cells have been especially used because they can be frozen for long periods of time without loss of sensitivity to agglutination by specific antibody(2). Often, however, such fixed and sensitized erythrocytes tended to clump upon freezing and thawing, making them unsuitable for the assay of antibody. It, therefore, seemed desirable to develop other procedures for preservation and sensitization of sheep erythrocytes. Glutaraldehyde fixation of erythrocytes was investigated and found to be superior to formalin fixation of red blood cells. Conditions for the conjugation of the glutaraldehyde-preserved red blood cells to proteins by the tannic acid and bis-diazotized methods were compared and the stability of these preparations ascertained. The application of these preparations to the detection of antibody to human serum albumin and to bovine beta lactoglobulin is described.

*Materials and methods. Antigens and antisera.* Bovine beta lactoglobulin (BLG) and human serum albumin (HSA) were purchased from Pentex Laboratories, Kankakee, Ill.

Antisera were prepared in 6-lb male or female white New Zealand rabbits. Rabbits

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