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Absorption, Distribution, and Excretion of ^{14}C-Trihalomethanes in Mice and Rats

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Chloroform and other trihalomethanes have been shown to originate from reactions between chlorine and naturally-occurring organic precursors in water (Rook 1977). Data from a previous survey study (Symons et al. 1975) show total trihalomethanes values ranged from 0.052-0.120 mg/L in over 100 cities. Positive correlations with THM levels were observed by Cantor et al. (1978) for several cancers including bladder, brain, non-Hodgkins lymphoma and kidney cancers in an epidemiological study. Chloroform (TCM) has been shown, at high dose levels, to increase the tumor incidence in mice and rats (NCI 1976).

Studies by Tardiff (1976) demonstrated chloroform was not mutagenic in the Ames bioassay using Salmonella typhimurium strains TA100 and TA1535. Bromodichloromethane, dibromochloromethane and bromoform demonstrated a dose-related mutagenic response. Differences in biological responses between mice and rats have been attributed to differences in their relative rates of TCM metabolism (Reitz et al. 1978). Several predictive studies (Brown 1974; NCI 1976) estimate that the mouse metabolizes TCM at a significantly different rate than the rat. This study was initiated to determine the absorption, distribution and excretion characteristics of four trihalomethanes (TCM, TBM, DBCM and BDCM) using the carbon 14 labeled compounds under identical experimental conditions in both the mouse and rat.

MATERIALS AND METHODS

Commercially synthesized ^{14}C-THM were diluted in corn oil to an activity of 100 mg/kg (16μCi/kg) for rats and 150 mg/kg (32μCi/kg)for mice. This level of activity was required to generate sufficient disintegrations (DPMs) for analysis. Male Sprague-Dawley rats weighing ~250 g and male B6C3F1 mice ~20 g each were used throughout these studies. All animals were fasted 16 hours overnight, then the compound under investigation was administered by intragastric intubation.

The rats and mice (5/cage) were housed in glass Roth-type metabolism chambers designed for the separate collection of urine, feces and expired air. Room air was drawn by vacuum at

500 mL/min through the chambers that were situated inside a California fume hood. The air leaving the chamber passed through the series of two traps, xylene/2-methoxyethanol and ethanolamine/2-methoxyethanol, to collect the expired (^{14}C)-trihalomethane and (^{14}C)-carbon dioxide, respectively. A circulating water bath cooled the urine to 4°C in the collection vessel.

There were two phases of each experimentation with durations of 8 or 48 hours (rat) and 8 or 36 hours (mice). Each of the four compounds were studied in series using 6 rats or 20 mice for each study group.

The first phase included the collection of samples to monitor the expired air and the urine of the animal for 8 hours after which time the animals were sacrificed. Urine samples were collected at 2, 4 and 8 hours. Expired air samples were monitored at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7 and 8 hours.

The second experimentation phase had a duration of 36 or 48 hours. In this segment, urine was collected at intervals of 2, 4, 8, 16, 24, 36 and 48 hours after dosing. The test animals were administered feed and water ad libitum after the initial 8 hours. After the 36- or 48-hour period, the animals were removed from the chambers and necropsies were performed to compare organ and tissue residuals with those of the 8-hour study.

Immediately following removal from the cage, the animals received an intraperitoneal dose of pentobarbital (50 mg/mℓ) at 1 mℓ/kg of body weight. Under heavy sedation, the peritoneal cavity was opened and, with a syringe, as much blood was withdrawn from the inferior vena cava as possible until collapse occurred. Three mℓ of blood were used for liquid scintillation counting and the remaining portion was sealed and placed in the ultra-freezer (-70°C) for later analysis. Also removed from the rodents were the urinary bladder, the brain, both kidneys, the entire liver, both lungs, ~1.0 g of skeletal muscle, the pancreas, the stomach (no contents), and the thymus. Preliminary studies indicated that these were the only organs with a sufficient degree of radioactivity above the background level to warrant investigation.

RESULTS AND DISCUSSION

The data collected in these studies point out differences of distribution and elimination in respect to intercompound/interspecies comparisons. An attempt will be made to point out the major trends in a summary manner, concerning organ distribution, respiratory elimination and urinary excretion.

The total radioactivity for sampled organs ranged from 3-6% of the total dose in the rats versus 5-14% for the mice (Table 1). The actual data showed ~4-5% of the total ^{14}C-THM in all organs for the mice except in the extrapolated total blood

volume in the TBM and TCM exposed mice, which contained almost 10% of the total dose. The stomach without contents, non-perfused liver, and kidneys in both rodent species were the organs of highest residual radioactivity levels (blood in TBM and TCM also).

The urine in both species contained <5% of total radiolabel at 8 hours post-intubation and <10% of the total radiolabel at 36-48 hours for all THMs. Also in both species the rate of radiolabel excreted in the urine was as follows: TCM>TBM>BDCM>DBCM

Table 1. Total Percentage of Administered Activity Recovered Eight Hours Following a Single Peroral Dose in Sprague-Dawley Rat[a] and B6C3F1 Mice[b]

	% CO_2 Expired	% Unmet. Expired	% ^{14}C Urine	% ^{14}C Total Organ	Total ^{14}C Recovery
TCM					
Rat	6.5	64.8	2.6	3.6	78.2
Mouse	49.55	26.05	4.91	13.46	94.47
BDCM					
Rat	14.2	41.7	1.4	3.3	62.7
Mouse	81.20	7.18	2.17	3.18	92.71
DBCM					
Rat	18.2	48.1	1.1	1.4	70.3
Mouse	71.58	12.31	1.90	5.02	91.63
TBM					
Rat	4.3	66.9	2.2	2.1	78.9
Mouse	39.68	5.70	4.62	12.18	62.23

[a]Average of no less than 6 animals per group (dose = 100 mg/kg)
[b]Average of 4 groups with 5 animals per group (dose = 150 mg/kg)

The majority of all compounds in both rats and mice was eliminated through the lungs in the expired air within 8 hours. The mice eliminated 40-81% of the total ^{14}C-THM as $^{14}CO_2$ and ~5-26% as the unmetabolized parent compound (Figures 1 and 2). The rats eliminated 4-18% of the total ^{14}C-THM dose as $^{14}CO_2$ and ~41-67% as the unmetabolized parent THM (Figures 3 and 4). The half-life of the THMs in rats was 0.8 hour for TBM, 1.2 hours for DBCM, 1.5 hours for BDCM, and 2 hours for TCM. In the mice the half-life of the THMs was 8 hours for TBM, 2.5 hours for DBCM and BDCM, and 2 hours for TCM.

The intent of this study was to compare the pharmacokinetics of selected brominated and chlorinated trihalomethanes whose presence in our environment has stimulated concern for human health.

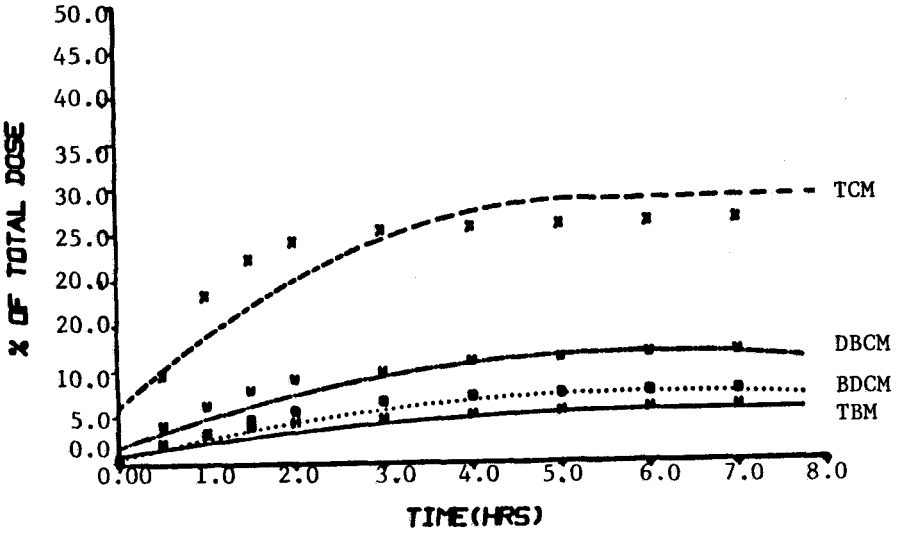

Figure 1. Cumulative Percent of Total ^{14}C-THM Expired by Mice

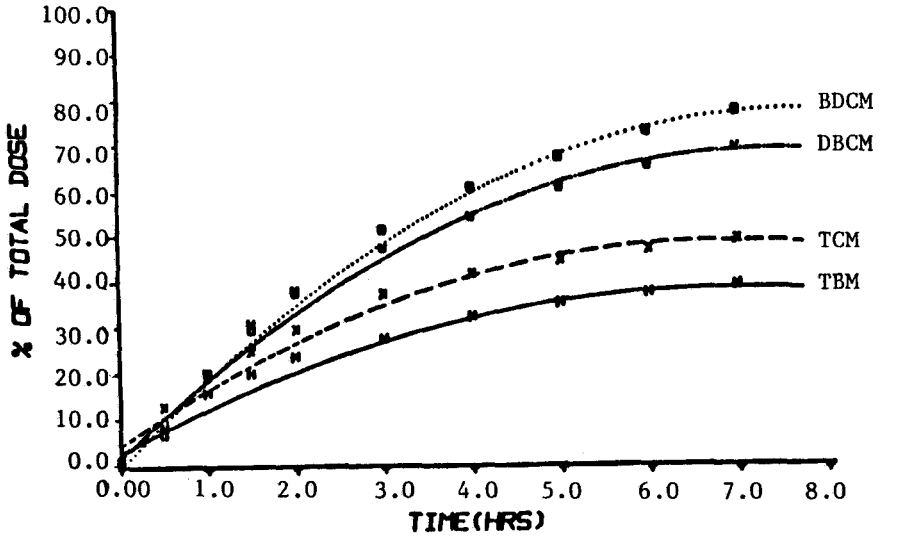

Figure 2. Cumulative Percent of $^{14}CO_2$ Expired by Mice

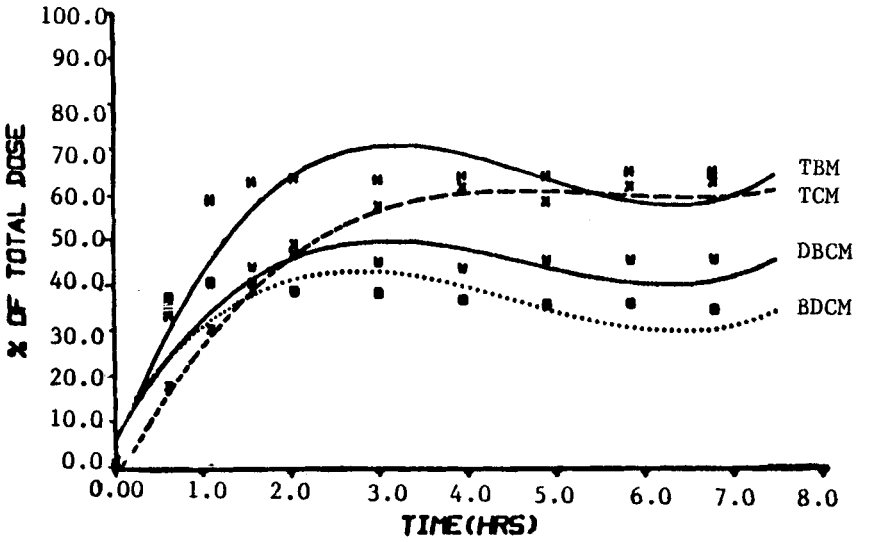

Figure 3. Cumulative Percent of Total ^{14}C-THM Expired by Rats

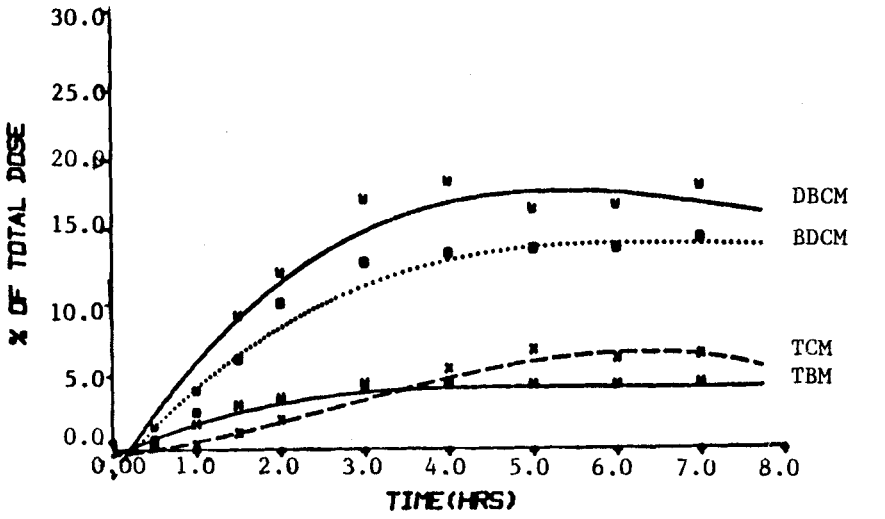

Figure 4. Cumulative Percent of $^{14}CO_2$ Expired by Rats

The literature describes extensive work concerning the uptake and elimination of TCM. It was reasoned that the TCM data obtained could serve as a basis for comparison when evaluating the other THMs.

Areas of concern are the rate of pulmonary expiration, as unaltered parent compound and in the metabolized form, the rate of urinary excretion, and the tissue residuals.

The percent of the dose that was exhaled varied considerably for each compound. Fry et al. (1972) has shown that pulmonary excretions of TCM and its carbon dioxide metabolite account for a majority of a single oral dose. The results of these studies show similar recoveries for TCM and the other haloforms under consideration.

It has been demonstrated (Hammer 1978) that there is a high degree of correlation between the partition coefficient and the volume of distribution of a particular xenobiotic which in the case of these compounds would follow the halide order. In this study (volume of distribution -- TBM>BDCM>TCM>DBCM), no similar trends were recognized.

The data clearly illustrate that each of these four compounds are acted upon by the body in a unique manner. BDCM and DBCM exhibit limited metabolic activation, which was shown by recovery of a higher percentage of the dose as parent compound. A greater amount of ^{14}-C activity is found in the blood following TBM dosing than from any of the other three. This is consistent with work by Anders et al. (1978) in which TBM was shown to elevate carboxyhemoglobin levels considerably higher than the other THMs. TCM is found in relatively large concentrations in the urine, which indicates that it undergoes conjugation more extensively than do the other compounds.

It is obvious from the data that the mouse metabolizes these compounds to a much greater extent than the rat. In fact, the differences range from 4- to 9-fold (Table 1).

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