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METABOLISM OF [^{14}C]CARBON TETRACHLORIDE TO EXHALED, EXCRETED AND BOUND METABOLITES

DOSE–RESPONSE, TIME–COURSE AND PHARMACOKINETICS

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Abstract—Fasted male rats were given six doses of $^{14}CCl_4$ ranging from non-hepatotoxic (0.1 mmole/kg) to severely hepatotoxic (26 mmoles/kg). Time–course and pharmacokinetics of CCl_4, $^{14}CO_2$ and $CHCl_3$ elimination by exhalation were monitored by measuring amounts recovered in breath during discrete 15-min intervals for 8–12 hr. Amounts of ^{14}C-labeled metabolite recovered bound to liver macromolecules at 24 hr and excreted in urine or feces for 24 hr were also determined. Comparison pharmacokinetic studies were done with $^{14}CHCl_3$ and $Na_2{}^{14}CO_3$. After all doses of $^{14}CCl_4$, the major metabolite was CO_2, twenty to thirty times less metabolite was recovered bound to liver macromolecules, and intermediate amounts of metabolite were excreted in urine and feces. $CHCl_3$ was the least abundant metabolite at low CCl_4 doses, but the second most abundant at high doses. Stronger associations were found between the magnitude of liver injury at 24 hr (quantitated as serum glutamate-pyruvate transaminase activity) and the extent or rate of CCl_4 metabolism by pathways leading to CO_2 and $CHCl_3$ than by pathways leading to ^{14}C-metabolites bound in liver or excreted in urine. Time–course and pharmacokinetic data indicated that a major pathway of CCl_4 metabolism leading to CO_2 became impaired within 2 hr after administration of hepatotoxic doses of CCl_4.

Over the past 30 years many investigators have attempted to clarify the mechanism of CCl_4 hepatotoxicity by examining its biologic fate. *In vivo* studies demonstrated that CCl_4 is converted to multiple metabolites including CO_2, $CHCl_3$, CCl_3CCl_3, soluble compounds excreted in urine, feces and bile and metabolites covalently bound to cell macromolecules of liver and other tissues [1–6]. Most *in vivo* studies of CCl_4 metabolism monitored only one or two of these metabolites at a limited number of time points. Therefore, information is lacking on the relative amounts of the various metabolites produced and on their relative rates of formation.

In vitro studies have provided convincing evidence that CCl_4 is metabolized to several kinds of reactive, potentially injurious intermediates—a trichloromethyl free radical intermediate ($^{\bullet}CCl_3$), phosgene ($O{=}CCl_2$), and an electrophilic chlorine species [7–10]. Chemical studies indicate that a reactive trichloromethyl peroxy radical ($^{\bullet}OOCCl_3$) could be formed by the direct reaction of either $^{\bullet}CCl_3$ with O_2 or CCl_4 with superoxide anion ($O_2^{\bullet-}$) [11, 12]. In addition, the potential formation of a carbene ($:CCl_2$) intermediate at low O_2 concentrations has been proposed [13, 14]. What remains uncertain are the contributions of these multiple intermediates to the *in vivo* pathways and products of CCl_4 metabolism and to its hepatotoxicity.

In vivo studies of the relationship between the hepatotoxicity of CCl_4 and its metabolism to specific types of products have not been concordant. Qualitative relationships between the extent of CCl_4 metabolized to $^{14}CO_2$ and the magnitude of liver injury were found by some investigators [15–17] but not by others [18–20]. When the relationship between the extent of CCl_4 metabolized to $CHCl_3$ and liver injury was examined, qualitative relationships were found under certain conditions which modulate injury [21–24] but not under others [22, 25]. Some have noted qualitative relationships between the extent of metabolite bound to liver macromolecules and liver injury [17, 25, 26], while others have not [27–29]. Relationships between urinary, fecal and biliary metabolites and liver injury have not been examined. The lack of concordance between studies could be due to variations in CCl_4 dose, conditions used to produce multiple degrees of liver injury, nutritional state of the animal, and times at which metabolite amounts were evaluated. Little is known about either the dose–response or time–course of CCl_4 metabolism.

The objective of this *in vivo* study was to obtain some of the missing information on the relationship between the biologic fate and hepatotoxicity of CCl_4. Exhalation of CCl_4 and $CHCl_3$ by fasted male rats was monitored by a sensitive electron capture detector which allowed direct analysis of expired air samples without the trapping systems used by others [22, 24, 30]. This sensitive detection system allowed use of a broad range of CCl_4 doses including the frequently used, severely hepatotoxic 26 mmoles/kg dose (2.5 ml/kg) and a small, non-injurious 0.1 mmole/kg dose, one-tenth that used in prior stud-

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ies of exhaled CCl_4 metabolites [22, 24, 30]. Other types of exhaled, bound and excreted metabolites were monitored in the same animals by tracing ^{14}C-label derived from $^{14}CCl_4$.

Extent of CCl_4 metabolism was determined by measuring the amounts of unchanged CCl_4, $^{14}CO_2$ metabolite, and $CHCl_3$ metabolite exhaled for 8–12 hr, of ^{14}C-metabolite excreted in urine and feces for 24 hr, and of ^{14}C-metabolite bound to liver macromolecules at 24 hr. The time–course of CCl_4 metabolism by pathways leading to CO_2 and $CHCl_3$ was examined by monitoring the exhalation of CCl_4, $^{14}CO_2$ and $CHCl_3$ during multiple discrete intervals between 0 and 12 hr and by analyzing the pharmacokinetics of the exhalation of these compounds. Since analysis of the pharmacokinetics of metabolites which are produced by parallel and crossover pathways is complex [31], comparison time–course studies were done on the exhalation of $^{14}CO_2$ after $Na_2{}^{14}CO_3$, and of $CHCl_3$ and $^{14}CO_2$ metabolite after $^{14}CHCl_3$. The degree of liver injury produced by the graduated doses of CCl_4 was quantitated by measuring serum activities of liver-derived enzymes. Relationships between the biologic fate and hepatotoxicity of CCl_4 were evaluated by comparing the relative strengths of the associations between the magnitude of liver injury in individual animals and the amounts or rates of $^{14}CO_2$ or $CHCl_3$ exhaled, ^{14}C-metabolite excreted in urine, and ^{14}C-metabolite bound in liver.

MATERIALS AND METHODS

Chemicals. CCl_4, $CHCl_3$, and CH_2Cl_2 of the highest available purity (99+ Mol% grade) were purchased from the Fisher Scientific Co., Pittsburgh, PA. CH_3Cl of 99+% purity was obtained from Matheson Gas, Pasadena, TX. $^{14}CCl_4$, $^{14}CHCl_3$ and $Na_2{}^{14}CO_3$ of a minimum 99% purity were purchased from the New England Nuclear Corp., Boston, MA. Fluids for radioactivity measurement were obtained from New England Nuclear (Oxysorb CO_2 absorber, Hyamine, Aquafluor, Aquasol), Fisher (Scintiverse) and Packard, Downers Grove, IL (Carbosorb, Permafluor V).

Animals. Male Sprague–Dawley derived Charles River C.-D. rats were obtained from Charles River Laboratories, Wilmington, MA. The rats were housed in wire bottom plastic cages suspended over absorbent paper, provided with Purina Rat Chow (Ralston Purina, Columbus, OH) and tap water *ad lib.*, and acclimated in an automatically regulated 12 hr light–12 hr dark cycle animal room for at least 1 week prior to use.

Volatile sample collection and analysis. Expired air of animals was collected in sealed 1 liter all glass and metal metabolism chambers during multiple discrete intervals (usually 15 min). Samples (1 ml) of the metabolism chamber atmosphere of glass were automatically analyzed with a specially designed system consisting of a microprocessor-sequenced gas sampling and valving system (Valco, Houston, TX), a stainless steel and Teflon circulating pump (Air Dimension, Inc., Kulpsville, PA) with a pumping rate of 0.8 to 1.0 l/min, and a 1.0-ml sampling loop. Total volume of the sampling system exclusive of the metabolism chamber was 124 ml. Prior to sample collection, the animal metabolism chamber atmosphere was circulated through the valving system and the sampling loop for 2 min to ensure adequate mixing. The coefficient of variation of replicate analyses of chamber atmosphere after a 2-min circulation was less than 2%.

The sampling system automatically injected a 1-ml air sample into a Perkin Elmer 910 gas chromatograph interfaced with a Hewlett Packard 3352-C minicomputer for data reduction. A 2.5 m × 0.32 cm stainless steel column packed with 80–100 mesh Poropak-P (Analabs, Inc., North Haven, CT) was used isothermally at 130° with nitrogen at 30 ml/min as carrier gas.

CO_2 which emerged from the column at 0.6 min was collected by diverting the column effluent from the detector during min 1 of chromatography and trapping the eluted CO_2 in Oxysorb CO_2 absorber; trapping efficiency was >99%. $^{14}CO_2$ was measured by scintillation counting. CCl_4, $CHCl_3$, CH_2Cl_2 and CH_3Cl emerged from the column between 4 and 10 min and were measured by a ^{63}Ni electron capture detector set at 225°.

The electron capture detector was calibrated by injecting air samples containing known concentrations of chlorocarbons. Stability of the calibration was verified daily and, when indicated, the detector was recalibrated. Detection limits were 0.3 pmole/l air for CH_2Cl_2, 0.08 pmole/l air for $CHCl_3$ and 0.03 pmole/l air for CCl_4. The amounts of $CHCl_3$ and CH_2Cl_2 present in the CCl_4 and $^{14}CCl_4$ administered to rats were below detection limits under the conditions used for volatile metabolite analysis.

Detection limits for $^{14}CO_2$ depended upon the specific activity of the compound administered, but were sufficient to quantitatively determine chloromethane derived $^{14}CO_2$ in effluent air for at least 8 hr after each dose of $^{14}CCl_4$, and for 6–8 hr after each dose of $^{14}CHCl_3$.

Measured amounts of $^{14}CO_2$ and chlorocarbons recovered at each time point were multiplied by the metabolism chamber volume in order to determine the total amount of each compound exhaled during the collection periods. These amounts were corrected by body weight and time, and then uniformly expressed as moles compound per hr per kg rat and plotted. Total exhalation per 24 hr was determined by connecting all points on the time curve and measuring the area under the curve with a Digitizing Electronic Planimeter (Numonics Corp., North Wales, PA). The measured amounts of exhaled compounds underestimate the actual amounts exhaled by the rats during the collection periods since the rats re-inhale and retain some fraction of the compounds exhaled into the closed system. No attempt was made to correct for this underestimation.

Non-volatile label analysis. Livers were homogenized in 0.25 M sucrose and extracted twice with 0.3 M perchloric acid. The precipitated lipids, proteins, and nucleic acids—termed the "macromolecular" fraction—were solubilized in 0.3 N KOH at 80°. For counting, liver homogenate was solubilized in Hyamine at 50° and suspended in Aquafluor; the acid soluble fraction was suspended in Aquafluor; the solubilized macromolecular frac-

tion was suspended in Scintiverse; and samples of urine were suspended in Aquasol. Radioactivity in these fractions was determined by liquid scintillation counting with quench correction. Weighed samples of thoroughly mixed feces were burned in a Packard Sample Oxidizer. The liberated $^{14}CO_2$ was trapped in Carbosorb and counted after the addition of Permafluor V.

Experimental protocol for $^{14}CCl_4$ experiments. Twenty-four rats weighing 200–250 g were fasted for 17–18 hr beginning at 5:00 p.m. The next morning CCl_4 (containing 10–80 μCi $^{14}CCl_4$/rat) was administered between 9:00 and 10:00 a.m. by gavage in 2.5 ml mineral oil/kg at doses of 0.1, 0.3, 2, 4, 10 and 26 mmoles CCl_4/kg. Each dose of $^{14}CCl_4$ was given to four animals. Four additional control rats were given only the mineral oil vehicle.

Immediately after $^{14}CCl_4$ administration, each rat was placed in a glass and metal metabolism chamber. The chamber was sealed and a sample of chamber air was withdrawn after 15 min for analysis of CCl_4, $CHCl_3$, CH_2Cl_2, CH_3Cl and $^{14}CO_2$ contents. The chamber was then promptly opened and the animal removed and placed in a plastic metabolism cage. Urine and feces, which accumulated in the glass metabolism chamber, were collected, and the chamber was rinsed with distilled water and dried with a jet of hot air.

Thirty minutes after $^{14}CCl_4$ administration, the rat was replaced in the glass metabolism chamber for a second 15-min period, and a sample of chamber air was analyzed for chlorocarbons and $^{14}CO_2$ exhaled during that time. The entire process of measuring compounds exhaled during a 15-min period was repeated at half-hour intervals for the first 8 hr after CCl_4 administration and at 9, 12 and 24 hr thereafter. Reddrop *et al.* [30] found a similar schedule useful for monitoring $CHCl_3$ exhalation by rats given CCl_4. Samples of ambient air were routinely taken before and at 2-hr intervals throughout the day of each experiment in order to verify that ambient chlorocarbon concentrations would not interfere with the analyses.

Animals were kept in plastic metabolism cages at all times when not in glass metabolism chambers. Water was provided to animals in the plastic cages at all times, and food was provided at 5:00 p.m. on the day of the experiment. Urine and feces were collected for 24 hr.

At 24 hr the animals were killed by decapitation. Blood samples were collected from the trunk. The liver was removed and weighed, and a cross section was fixed in 4% neutral buffered formaldehyde, embedded in paraffin, sectioned, and stained with hematoxylin and eosin by standard histologic techniques. The remainder of the liver and the urine and feces which had been collected for 24 hr after $^{14}CCl_4$ administration were stored at −20° until analyzed for ^{14}C-content.

Hepatic injury was assessed by observation of the tissue sections and by measurement of serum glutamate-oxalacetate (SGOT) and glutamate-pyruvate transaminase (SGPT) activities with reagent kits from Worthington Diagnostics, Freehold, NJ.

Experimental protocol for comparison of $^{14}CHCl_3$ and $Na_2{}^{14}CO_3$ experiments. Eleven animals weighing 200–250 g were fasted for 17–18 hr beginning at 5:00 p.m. $CHCl_3$ (containing 10–20 μCi $^{14}CHCl_3$/rat) was given in 2.5 ml mineral oil/kg at doses of 0.1 and 0.3 mmole/kg. $Na_2{}^{14}CO_3$ (containing 10 μCi $^{14}CO_2$) was given in 2.5 ml of 0.02 N NaOH/kg at a dose of 0.005 mmole/kg. Each dose of $CHCl_3$ was given to four rats. $Na_2{}^{14}CO_3$ was given to three rats.

Subsequent procedures were similar to those in CCl_4 experiments except that the times of sampling chamber air in the glass metabolism cages differed slightly (see Results).

Pharmacokinetic analysis. Appropriate pharmacokinetic models describing the time–course exhalation-rates of CCl_4, CO_2 and $CHCl_3$ were constructed as sets of integrated equations utilizing as constants the amounts of the compounds ultimately exhaled and utilizing as parameters the absorption and elimination rate constants. Exhalation-rate time-profiles for each compound were evaluated with a one- and, when possible, a two-compartment model for orally administered compounds [32]. For the two-compartment model, additional parameters were the intercompartment transfer rate constants and the distribution rate constant. The absorption, distribution and elimination-rate constants were estimated using the nonlinear least squares program (NONLIN) [33] assuming elimination from the central compartment. Initial estimates were obtained by graphical exponential curve stripping.

Due to variations between individual rats given the same dose level, and occasionally within individual rats, the rate constants were derived as single values using the combined data from each group of rats rather than as the mean ± S.E. from the individual animals.

Statistical analysis. The relationships between parameters of injury and metabolism were examined by single and multiple linear regression analysis.

RESULTS

Effects of CCl_4 dose on the extent of CCl_4 metabolism to exhaled, soluble and bound metabolites. After $^{14}CCl_4$ administration, the only exhaled metabolites detected were $^{14}CO_2$ and $CHCl_3$. As anticipated from the studies of Butler [2] and Paul and Rubinstein [34], neither CH_2Cl_2 nor CH_3Cl was detected in any animal given CCl_4. Considerable amounts of ^{14}C-label were recovered in feces and urine as reported by McCollister *et al.* [1]. Repeated extraction and drying of minced feces with unlabeled CCl_4 did not decrease the recovery of ^{14}C. McCollister *et al.* [1] previously reported that most of the ^{14}C-label excreted in urine of animals given $^{14}CCl_4$ consisted of unknown metabolites with only a small percentage recovered as urea. Repeated extraction and drying of homogenized liver with unlabeled CCl_4 did not decrease the recovery of ^{14}C. The acid precipitable "macromolecular fraction" of liver contained 64–95% of the ^{14}C-labeled metabolite recovered in that organ at 24 hr. Therefore, the ^{14}C-label recovered in feces, urine and liver at 24 hr was attributed to metabolite, and not to unchanged $^{14}CCl_4$.

Figure 1 shows the effects of the six $^{14}CCl_4$ doses on the total amounts of unchanged CCl_4 exhaled

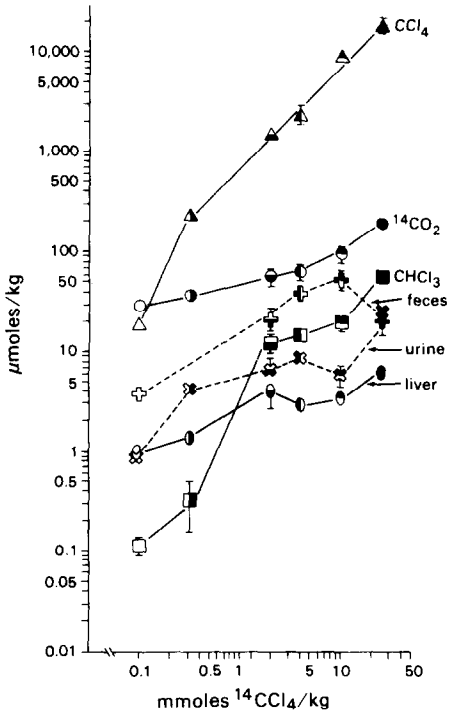

Fig. 1. Effect of CCl_4 dose on the total amounts of unchanged CCl_4 exhaled, $^{14}CO_2$ metabolite exhaled, $CHCl_3$ metabolite exhaled, ^{14}C-label excreted in feces or in urine, and ^{14}C-label in liver cell macromolecules. Fasted male rats were given $^{14}CCl_4$ by gavage. Amounts of CCl_4 metabolites recovered in exhaled breath, urine or feces during the first 24 hr and in liver at 24 hr were monitored as described in Materials and Methods. Values are the mean of four animals per group. Where error bars are not shown, the S.E. lies within the symbol.

during the first 24 hr and on the total amounts of metabolites exhaled, bound and excreted by the twenty-four animals in this study. $^{14}CO_2$ was the major metabolite at all dose levels. Approximately twenty to thirty times less ^{14}C-label was recovered bound to liver macromolecules at 24 hr. Intermediate amounts of ^{14}C-label were recovered in 24 hr urine and feces. $CHCl_3$ was the least abundant metabolite at the lowest doses but the second most abundant metabolite at the highest CCl_4 dose.

Over the 260-fold increase in CCl_4 dose from 0.1 to 26 mmoles/kg, the amounts of unchanged CCl_4 recovered increased approximately 1000-fold and $CHCl_3$ metabolite increased by a factor of 500. In contrast, the amounts of $^{14}CO_2$ increased only by a factor of 6.8, and amounts of ^{14}C-bound to liver macromolecules by 6.5. ^{14}C-Metabolites in urine and in feces increased more erratically by factors of 28 and 14 respectively. Two abrupt increases in recovery occurred, a 12-fold increase in unchanged CCl_4 between the 0.1 and 0.3 mmole CCl_4 doses and a 38-fold increase in $CHCl_3$ metabolite between the 0.3 and 2 mmole CCl_4 doses.

As the dose of CCl_4 increased, the fraction of the CCl_4 dose recovered as metabolite decreased. For example, recoveries of ^{14}C-metabolite in urine and liver decreased from 1% of the given dose at the lowest CCl_4 dose to 0.1% at the highest CCl_4 dose. Therefore, the effects of CCl_4 dose on the relative proportions of the various metabolites were analyzed by determining the proportions of each metabolite relative to the total amount of metabolite recovered (Table 1). The most striking change in relative proportions was the more than 10-fold increase in $CHCl_3$ recovered between the 0.3 and 2 mmole doses of CCl_4 which was accompanied by a corresponding decrease in the relative proportion of total metabolite recovered as $^{14}CO_2$. The proportions of CCl_4 metabolite recovered in liver, urine, and feces were not consistently altered by increases in CCl_4 dose. In fact, the proportions of CCl_4 metabolite recovered bound in liver and excreted in urine were similar, although erratic, across the entire CCl_4 dose range. Surprisingly, at all doses except the highest, the proportion of metabolite excreted in feces was second only to that recovered as $^{14}CO_2$. Part of the variability in ^{14}C-excretion in feces may be due to slow clearance of metabolite by this route [1].

Comparison recovery studies with $Na_2{}^{14}CO_3$ *and* $^{14}CHCl_3$. Table 2 compares the recoveries of parent compounds and metabolites in exhaled breath after $Na_2{}^{14}CO_3$, $^{14}CHCl_3$ and $^{14}CCl_4$. More than 95% of the given $Na_2{}^{14}CO_3$ was recovered as exhaled $^{14}CO_2$ within 4 hr. This high recovery demonstrates the efficiency of our system for monitoring exhaled

Table 1. Effect of CCl_4 dose on relative proportions of exhaled, bound and excreted metabolites recovered 0–24 hr after $^{14}CCl_4$ administration*

$^{14}CCl_4$ dose (mmoles/kg)	Percentage of total metabolite recovered as				
	$^{14}CO_2$	$CHCl_3$	^{14}C-Liver	^{14}C-Urine	^{14}C-Feces
0.1	83	0.3	2.9	2.7	11
0.3	(86)	(0.8)	(3.2)	(9.7)	
2	55	13	4.3	5.5	22
4	50	11	2.3	5.7	30
10	54	11	1.9	3.2	30
26	63	19	2.1	8.7	7

* Fasted male rats were given $^{14}CCl_4$ by gavage. Amounts of $^{14}CCl_4$ metabolites recovered in exhaled breath, urine and feces for 0–24 hr and in liver at 24 hr were monitored as described in Materials and Methods. Values in parentheses were calculated without data on label in feces and are the percentages of total exhaled, liver and urinary metabolites. Values are the mean of four animals.

Table 2. Comparison of the recoveries of exhaled parent compounds and exhaled metabolites from animals given $Na_2{}^{14}CO_3$, $^{14}CHCl_3$ or $^{14}CCl_4$*

Parent compound	Dose (mmoles/kg)	Percentage of dose exhaled as CO_2	$CHCl_3$	CCl_4
$Na_2{}^{14}CO_3$	0.005	95		
$^{14}CHCl_3$	0.1	67	5.0	
$^{14}CHCl_3$	0.3	68	12	
$^{14}CCl_4$	0.1	28	0.11	19
$^{14}CCl_4$	0.3	12	0.11	77
$^{14}CCl_4$	2	2.7	0.65	74
$^{14}CCl_4$	4	1.6	0.38	76
$^{14}CCl_4$	10	1.0	0.20	89
$^{14}CCl_4$	26	0.7	0.22	71

* Fasted male rats were given $Na_2{}^{14}CO_3$, $^{14}CHCl_3$ or $^{14}CCl_4$ by gavage. Amounts of parent compounds and metabolites recovered in exhaled breath during discrete intervals between 0 and 24 hr were monitored as described in Materials and Methods. Values are the mean of three animals given $Na_2{}^{14}CO_3$ and four animals given $^{14}CHCl_3$ or $^{14}CCl_4$.

metabolites. The bulk of both the 0.1 and 0.3 mmole doses of $^{14}CHCl_3$ was also quickly recovered in breath with two-thirds recovered as exhaled $^{14}CO_2$ metabolite and less than one-eighth as unchanged parent $CHCl_3$. Less than half of the lowest $^{14}CCl_4$ dose was recovered in the breath, one-quarter as CO_2 and one-fifth as CCl_4. In contrast, 70–90% of the five larger $^{14}CCl_4$ doses were recovered as unmetabolized CCl_4.

Considerably more $^{14}CO_2$ metabolite was recovered after both the 0.1 and 0.3 mmole $CHCl_3$ doses (67 and 203 μmoles $^{14}CO_2$/kg respectively) than after the equimolar 0.1 and 0.3 mmole CCl_4 doses (28 and 37 μmoles $^{14}CO_2$/kg respectively). In fact, the amount of $^{14}CO_2$ metabolite recovered after the 0.3 mmole $^{14}CHCl_3$ dose was similar to that recovered after the largest 26 mmole CCl_4 dose given (Fig. 1). The amounts of ^{14}C-metabolite recovered

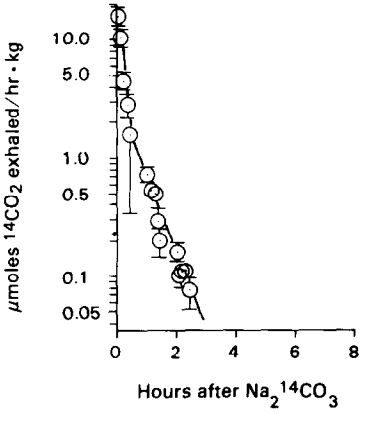

Fig. 2. Time-course of $^{14}CO_2$ exhalation. Fasted male rats were given 0.005 mmole $Na_2{}^{14}CO_3$/kg by gavage. Values are the mean ± S.E. of three animals. Where S.E. bars are not shown, the S.E. lies within the symbol.

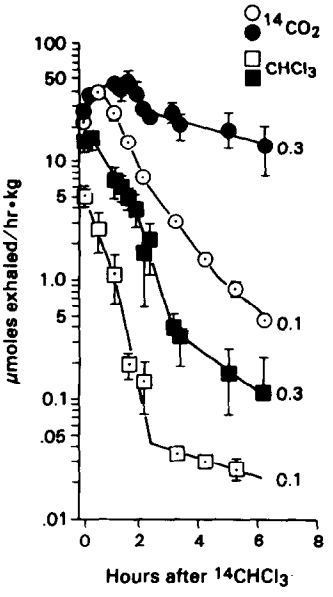

Fig. 3. Effect of $^{14}CHCl_3$ dose on the time-course of unchanged $CHCl_3$ exhalation (squares) and $^{14}CO_2$ metabolite exhalation (circles). Fasted male rats were given 0.1 (open symbols) or 0.3 (closed symbols) mmole $^{14}CHCl_3$/kg by gavage. Amounts of $CHCl_3$ and $^{14}CO_2$ exhaled were monitored. Values are the mean ± S.E. of four animals. Where S.E. bars are not shown, the S.E. lies within the symbol.

bound to liver macromolecules at 24 hr from animals given 0.1 and 0.3 mmole $CHCl_3$ were almost the same (1.1 ± 0.1 and 0.9 ± 0.1 μmole/kg respectively) and were very close to the amounts recovered bound to the livers of animals given equimolar doses of $^{14}CCl_4$ (Fig. 1).

Comparison exhalation time-course. Figures 2, 3 and 4 show the exhalation-rate time-profiles of parent compounds and metabolites after 0.005 mmole $Na_2{}^{14}CO_3$/kg, after 0.1 and 0.3 mmole $^{14}CHCl_3$/kg, and after 0.1, 0.3, 4 and 26 mmoles $^{14}CCl_4$/kg. Exhalation patterns after the two other doses of CCl_4 examined (2 and 10 mmoles/kg) were intermediate and were excluded in order to simplify Fig. 4. Peak exhalation-rates and times of peak exhalation-rate for the parent compounds and metabolites are listed in Table 3 in order to facilitate comparisons between compounds and doses.

Inspection of the exhalation-rate time-profiles for the parent compounds in Figs 2, 3 and 4 shows that rates of $^{14}CO_2$ exhalation decreased more slowly for the chlorocarbons than for $^{14}CO_2$ from $Na_2{}^{14}CO_3$. This is consistent with more prolonged retention and continuing metabolism of the lipid soluble halocarbons. Animals exhaled CO_2 rapidly after administration of $Na_2{}^{14}CO_3$ (Fig. 2) with the peak rate of exhalation occurring during the first 6 min. Animals given 0.1 or 0.3 mmole $^{14}CHCl_3$/kg exhaled the parent compound by roughly parallel profiles with the peak rates of exhalation occurring during the first half hour. At each time point, the rate of $CHCl_3$ exhalation after the larger dose was approximately triple that of the lower dose, and thus roughly proportional to the difference in the two $CHCl_3$ doses.

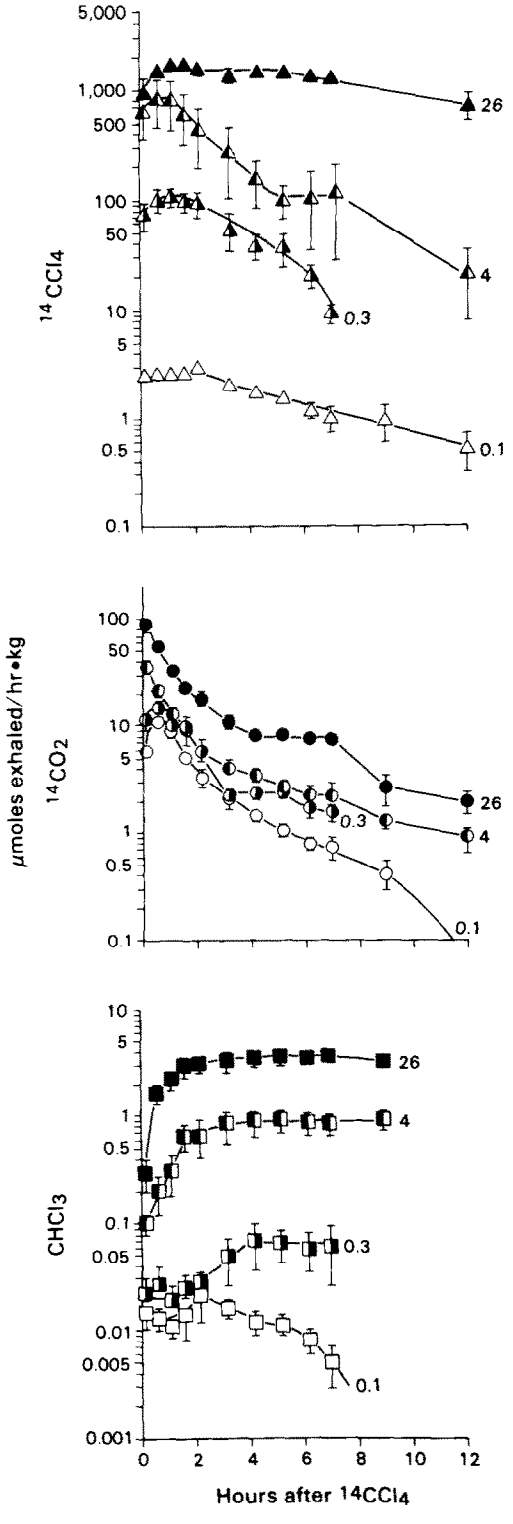

Fig. 4. Effect of $^{14}CCl_4$ dose on the time-course of unchanged CCl_4 exhalation (top panel), of $^{14}CO_2$ metabolite exhalation (center panel), and of $CHCl_3$ metabolite exhalation (bottom panel). CCl_4 dose in mmoles/kg is indicated by numbers to the right of each curve. Fasted male rats were given 0.1 to 26 mmoles $^{14}CCl_4$/kg by gavage. Amounts of CCl_4, $CHCl_3$ and $^{14}CO_2$ exhaled were monitored. Values are the mean ± S.E. of four animals. Where S.E. bars are not shown, the S.E. lies within the symbol.

Dose-dependent increases in the rates of parent compound exhalation after CCl_4 are evident at each time point in Fig. 4 (top panel). The differences in CCl_4 exhalation-rates between the two lowest CCl_4 doses exceed that of the 3-fold difference in dose, and undoubtedly are due to the more extensive metabolism of the 0.1 mmole CCl_4 dose (see Table 1). The two largest CCl_4 doses were exhaled at nearly similar rates during the first 4 hr after CCl_4. The magnitudes of the dose-dependent increases can readily be appreciated by comparison of the peak CCl_4 exhalation-rates after the six CCl_4 doses in Table 3. Animals given the lowest CCl_4 dose exhaled the parent compound at peak rate for almost 2 hr, the exhalation-rate thereafter gradually declining. Exhalation of the parent compound at peak rates persisted for 4 hr after the 10 mmole dose and for 6 hr after the 26 mmole dose. This protracted exhalation of CCl_4 at peak rate is consistent with its rapid accumulation in and slow release from body fat due to the high lipid:water partition coefficient of this compound [4].

Comparison of the metabolite exhalation-rate time-profiles after $CHCl_3$ (Fig. 3) and after CCl_4 (center and bottom panels of Fig. 4) indicates a general pattern of dose-dependent increases at each time point. The rates of CO_2 metabolite exhalation after $CHCl_3$ exceed the rates of the parent compound exhalation at every time point. However, only during the first 2 hr after the lowest CCl_4 dose did the exhalation-rates of its CO_2 metabolite exceed those of the parent.

Although the exhalation curves for parent compound after 0.1 and 0.3 mmole $CHCl_3$ were roughly parallel, exhalation curves for the CO_2 metabolite were not (Fig. 3). Animals given the larger $^{14}CHCl_3$ dose exhaled $^{14}CO_2$ metabolite at a peak rate which occurred later and persisted longer, but was only 25% greater than that after the smaller $^{14}CHCl_3$ dose.

The shapes of the CO_2 and $CHCl_3$ metabolite exhalation curves after CCl_4 were distinctively different (Fig. 4). CO_2 metabolite was exhaled at much greater rates than $CHCl_3$. After all but the lowest CCl_4 doses, the rates of $CHCl_3$ exhalation increased during the first 2–4 hr after CCl_4 and then stabilized at a peak rate for 3–7 hr. As the dose of CCl_4 increased above 2 mmoles, the time to attain peak $CHCl_3$ exhalation decreased and the duration of peak exhalation lengthened (Table 3). The parent dose-dependent increases in peak rates of $CHCl_3$ and CO_2 metabolite exhalation were proportional to, or less than, the increases in CCl_4 dose except for the 13-fold increase in peak $CHCl_3$ exhalation-rate which occurred between 0.3 and 2 mmoles CCl_4. Peak exhalation-rates of CO_2 metabolite after the lowest CCl_4 doses occurred after a 30-min lag, whereas after the four larger CCl_4 doses peak exhalation-rates of this metabolite occurred during the earliest time point sampled. CO_2 metabolite curves after the higher CCl_4 doses were roughly parallel with initial abrupt declines followed at times beyond 3 hr by several hours of exhalation at a nearly constant "stabilized" rate.

Comparison of the CO_2 metabolite curves after $^{14}CHCl_3$ with those after the equimolar $^{14}CCl_4$ doses

Table 3. Comparison of the rates and times of peak exhalation of parent compounds and metabolites by animals given $Na_2{}^{14}CO_3$, $^{14}CHCl_3$ or $^{14}CCl_4$*

Parent compound	Dose (mmoles/kg)	Peak exhalation rate (μmoles/hr·kg)			Time of peak exhalation rate (hr)		
		CO_2	$CHCl_3$	CCl_4	CO_2	$CHCl_3$	CCl_4
$Na_2{}^{14}CO_3$	0.005	16			0–0.05		
$^{14}CHCl_3$	0.1	37	6.0		0.5–0.75	0–0.25	
$^{14}CHCl_3$	0.3	46	15.2		1.0–1.75	0–0.5	
$^{14}CCl_4$	0.1	11	0.02	2.6	0.5–0.75	2–2.25	0–2.25
$^{14}CCl_4$	0.3	15	0.06	102	0.5–0.75	4–7	0.5–2.25
$^{14}CCl_4$	2	18	0.78	545	0–0.75	4–9	0.5–2.25
$^{14}CCl_4$	4	37	0.90	845	0–0.25	4–9	0.5–1.5
$^{14}CCl_4$	10	62	1.32	1250	0–0.25	3–9	0.5–4
$^{14}CCl_4$	26	88	3.40	1550	0–0.25	2–9	0.5–6

* Fasted male rats were given $Na_2{}^{14}CO_3$, $^{14}CHCl_3$ or $^{14}CCl_4$ by gavage. Amounts of parent compounds and metabolites recovered in exhaled breath during discrete intervals between 0 and 24 hr were monitored as described in Materials and Methods. Values are the mean of three animals given $Na_2{}^{14}CO_3$ and four animals given $^{14}CHCl_3$ or $^{14}CCl_4$.

reveals some similarities between the 0.1 mmole doses and marked differences between the 0.3 mmole doses. Times of peak CO_2 exhalation-rate were similar in onset and duration after the 0.1 mmole doses of both chlorocarbons; whereas after the 0.3 mmole doses, the time of peak exhalation of $^{14}CO_2$ after $^{14}CCl_4$ occurred sooner, was shorter in duration, and the exhalation profile declined more quickly than that following $^{14}CHCl_3$. Peak $^{14}CO_2$ exhalation-rates were 3-fold lower after the equimolar $^{14}CCl_4$ doses.

Assumptions of pharmacokinetics analysis. Processes assumed to occur prior to the exhalation of CCl_4, $CHCl_3$ and CO_2 as parents or metabolites are outlined in the schematization presented in Fig. 5. Pharmacokinetic analyses of the exhalation data for these compounds were based on the following six assumptions.

First, parent compounds were assumed to be completely absorbed from the gut into the blood, to be distributed quickly into the rapidly perfused tissues (RPT), and to be distributed more slowly into the poorly perfused tissues (PPT). After release from the rapidly perfused tissues and slower release from the poorly perfused tissues, the parents are eliminated from the blood by exhalation through the lungs. Absorption and elimination were assumed to be predominantly unidirectional.

Second, metabolism of $CHCl_3$ to CO_2, and of CCl_4 to $CHCl_3$ and CO_2, was assumed to occur by non-reversible processes in the rapidly perfused tissues, chiefly liver. Once formed these metabolites were assumed to be released from liver to blood, distributed to other tissues, and eliminated through lungs. CCl_4 conversion to CO_2 is shown in Fig. 5 to occur by two parallel pathways, one of which produces $CHCl_3$ as a relatively stable intermediate.

Third, amounts of compounds exhaled and recovered in the metabolism chamber atmosphere during discrete intervals after parent compound administration were assumed to be proportional to the amounts of the compounds in blood and other tissues during this interval. Experimental evidence supports the soundness of this assumption. Whitelaw *et al.* [35] found that measurement of $^{14}CO_2$ entry rates into expired air provided an excellent estimate of total CO_2 production. Chiou [36] found a linear relationship between the rate of $CHCl_3$ exhalation and its concentration in blood. Reddrop *et al.* [30] concluded that regular sampling of expired air from animals given CCl_4 permitted reasonably accurate prediction of the blood CCl_4 concentrations as well as of blood concentrations of its $CHCl_3$ metabolite.

Fourth, distributional equilibriums between blood and other tissues are assumed to make the relative concentrations in the different body spaces proportional. This has been demonstrated for CCl_4 and $CHCl_3$. Reddrop *et al.* [23] and Garner and McLean [15] consistently found that CCl_4 concentrations in liver were six times higher than CCl_4 concentrations in blood under a variety of conditions. CCl_4 concentrations in fat have been found eight to twelve times higher than in liver [4]. Thus, at equilibrium, ratios of the distribution in blood:liver:fat would approximate 1:6:60. An extensive and detailed study by Withey and Collins [37] of $CHCl_3$ distribution in tissues of rats given $CHCl_3$ showed that the rates of $CHCl_3$ elimination from all tissues, except the perirenal fat, were not very different from the rates of $CHCl_3$ elimination from blood. This finding indicates that most of the major organs together with the blood constitute a central pharmacokinetic compartment for $CHCl_3$. Because of such distributional equilibriums, and the difficulties involved in detecting the distributive phase without many early time points [32], blood and rapidly perfused tissues were assumed to belong to a single central compartment.

Fifth, differences between the exhalation-rates of parent and metabolite or of different metabolites at any one time cannot be assumed to represent relative differences in the body burdens of the compounds at any one time. For example, higher rates of CO_2 metabolite exhalation than of $CHCl_3$ parent at early times after $CHCl_3$ administration do not necessarily mean that the amounts of metabolite in the body were greater than the amounts of parent at these

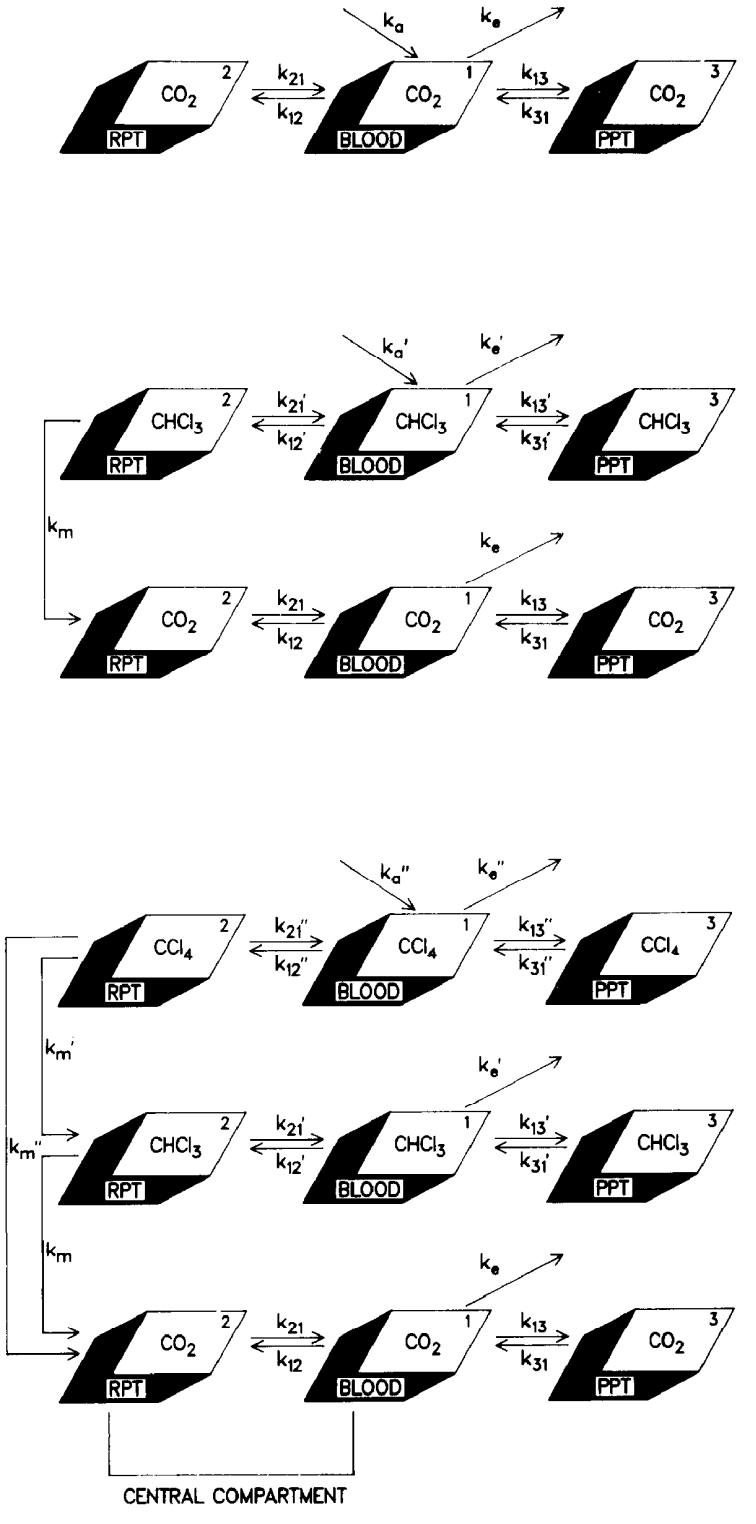

Fig. 5. Schematization of processes assumed to occur between the oral administration of the parent compounds CO_2 (after Na_2CO_3), $CHCl_3$ and CCl_4 and the exhalation of the parents and metabolites. Rate constants for compound absorption, distribution between the blood and rapidly perfused tissues (RPT), distribution between the blood and poorly perfused tissues (PPT), and elimination by exhalation are indicated, respectively, by k_a, k_{21} and k_{12}, k_{31} and k_{13}, and k_e. Single and double primes on these rate constants for absorption, distribution and elimination indicate processes involving $CHCl_3$ and CCl_4 respectively. Rate constants for the metabolism of $CHCl_3$ to CO_2, CCl_4 to $CHCl_3$, and CCl_4 to CO_2 are indicated by k_m, k_m', and k_m'' respectively.

Table 4. Comparison of the apparent absorption, distribution and elimination by exhalation of parent compounds and exhaled metabolites of animals given $Na_2{}^{14}CO_3$, $^{14}CHCl_3$ or $^{14}CCl_4$*

Parent compound	Dose (mmoles/kg)	Parent half-times (hr) of			CO_2 metabolite half-times (hr) of elimination	$CHCl_3$ metabolite half-times (hr) of elimination
		Absorption	Distribution	Elimination		
$Na_2{}^{14}CO_3$	0.005	0.04	0.15	0.43		
$^{14}CHCl_3$	0.1	0.08	0.29	3.83	2.1	
$^{14}CHCl_3$	0.3	0.13	0.41	2.27	5.6	
$^{14}CCl_4$	0.1	0.08		3.28	1.4	1.8
$^{14}CCl_4$	0.3	0.14		1.31	3.0	20.9
$^{14}CCl_4$	2	0.08		1.72	2.5	a†
$^{14}CCl_4$	4	0.09		1.80	3.9	a†
$^{14}CCl_4$	10	0.10		3.49	2.1	27.5
$^{14}CCl_4$	24	0.16		6.34	2.8	21.9

* Pharmacokinetic parameters were determined by computer fitting of exhalation data. Parent data from animals given $Na_2{}^{14}CO_3$ and $CHCl_3$ were analyzed using a two-compartment model, while that from CCl_4 were analyzed using a one-compartment model as detailed in the text. Metabolite apparent half-times were obtained from the terminal three time points as detailed in the text.

† Ascending slope.

time points. $^{14}CO_2$ undoubtedly has a smaller volume of distribution and is more quickly eliminated than $CHCl_3$ which partitions into body fat [38].

Sixth, amounts of CO_2 and $CHCl_3$ metabolite exhaled during discrete intervals were assumed to be representative of their rates of formation. This is a tenuous assumption for $CHCl_3$, since this metabolite can be sequentially metabolized to a secondary metabolite without ever leaving the liver and is also efficiently partitioned into body fat.

Pharmacokinetic analysis. A two-compartment model was used to analyze parent compound data after $Na_2{}^{14}CO_3$ and $^{14}CHCl_3$ while a one-compartment model was used to analyze parent compound data after $^{14}CCl_4$. The CO_2 exhalation-rate time-profile after $Na_2{}^{14}CO_3$ (Fig. 2) has two linear segments and had an excellent fit with the two-compartment model. The $CHCl_3$ exhalation profiles after the two $CHCl_3$ doses both have at least two linear segments in the post absorptive phase (Fig. 3) and satisfactorily fit the two-compartment model. The CCl_4 exhalation curves after the six CCl_4 doses have a predominantly linear decline in the post absorptive phase (Fig. 4) and fit the one-compartment models better than more complex multicompartment models.

The compartment models used can be readily understood by reference to the schematizations in Fig. 5. One-compartment model analysis would represent a collapse of the RPT, blood, and PPT into a single compartment. Two-compartment analysis would maintain a distinction between a "peripheral" PPT compartment and a "central compartment" consisting of RPT plus blood. We realize that some parts of our exhalation-rate data are inadequate for a rigorous pharmacokinetic analysis. If there were more time points between 8 and 24 hr, the apparent half-times of elimination of parent and metabolite after the larger CCl_4 doses could have been determined with more accuracy. Therefore, this data must be evaluated cautiously.

Table 4 summarizes the apparent absorption, distribution and elimination half-times determined for the parent compounds after $Na_2{}^{14}CO_3$, $^{14}CHCl_3$ and $^{14}CCl_4$. The relative lengths of the half-times for the three phases indicate that the compounds were more rapidly absorbed than distributed, and more rapidly distributed than eliminated. Apparent half-times for $^{14}CO_2$ parent absorption and elimination were markedly briefer than those for $CHCl_3$ and CCl_4. With the exception of the lowest CCl_4 dose, there was a consistent trend for increasing apparent half-time of CCl_4 parent elimination with increasing CCl_4 dose. The half-times for the absorption and elimination of CCl_4 are similar to those recently reported by Siegers *et al.* [39].

Also summarized in Table 4 are the apparent half-times of elimination for CO_2 metabolite after

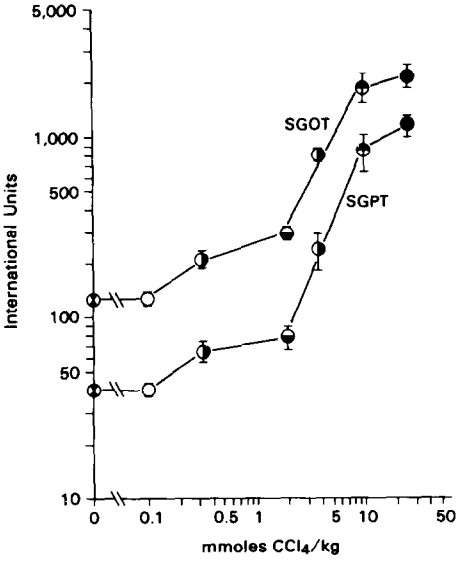

Fig. 6. Effect of CCl_4 dose on SGPT and SGOT activities at 24 hr. Fasted male rates were given $^{14}CCl_4$ (0.1 to 26 mmoles/kg) in mineral oil by gavage or mineral oil only and killed at 24 hr. Values are means ± S.E. of four rats per group. Where error bars are not shown, the S.E. lies within the symbol.

Table 5. Correlations between the magnitude of liver injury after $^{14}CCl_4$ and parameters of the extent or rate of CCl_4 metabolism*

Metabolism parameter	R^2†
Amount of exhaled $^{14}CO_2$	0.703
Amount of exhaled $CHCl_3$	0.655
Amount of ^{14}C bound in liver at 24 hr	0.546
Amount of ^{14}C excreted in 24-hr urine	0.475
Total amount of exhaled $^{14}CO_2$ and $CHCl_3$, ^{14}C bound in liver and ^{14}C excreted in urine	0.730
Rate of $^{14}CO_2$ exhalation during first hour	0.880
Rate of $CHCl_3$ exhalation during first hour	0.593
Rate of $^{14}CO_2$ exhalation during fourth to seventh hour	0.655
Rate of $CHCl_3$ exhalation during fourth to seventh hour	0.666
Rate of $^{14}CO_2$ plus $CHCl_3$ exhalation during fourth to seventh hour	0.686

* Fasted male rats were given 0.1 to 26 mmoles $^{14}CCl_4$/kg by gavage. Amounts and rates of CCl_4 metabolism between 0 and 24 hr were determined as described in Materials and Methods. Magnitude of liver injury was assessed by measurement of SGPT activities at 24 hr. Correlations were made using data on SGPT activities and amounts or rates of metabolite recovery from individual rats. N = 24.

† R^2 values represent proportion of variance in SGPT values associated with the indicated parameter of the extent or rate of CCl_4 metabolism.

$^{14}CHCl_3$ and $^{14}CCl_4$ and for $CHCl_3$ metabolite after $^{14}CCl_4$. After the larger $CHCl_3$ dose, the apparent half-time for CO_2 metabolite elimination was more than twice as long as that after the smaller $CHCl_3$ dose. Similarly, the apparent CO_2 metabolite half-time after the 0.3 and 0.1 mmole CCl_4 doses also shows a dose-dependent lengthening of the apparent half-time of CO_2 metabolite elimination. Further increases in CCl_4 dose above 0.3 mmole were not associated with consistent lengthening of the apparent CO_2 metabolite half-time. Apparent half-times for $CHCl_3$ metabolite elimination, after all except the lowest CCl_4 dose, were more than 20 hr and could not be assessed after two of the CCl_4 doses because the slopes of the terminal points were ascending.

Effects of $CHCl_3$ *and* CCl_4 *doses on hepatotoxicity.* Effects of the six CCl_4 doses on the activities of liver derived transaminases in serum (SGOT and SGPT) at 24 hr are shown in Fig. 6. The lowest CCl_4 dose did not elevate the SGOT or SGPT activities above those of control fasted rats. The five larger CCl_4 doses caused elevations in SGPT and SGOT activities which increased at first gradually, then more steeply with increasing dose.

Histologic sections from livers of rats given 0.1 mmole CCl_4/kg revealed no detectable cell necrosis at 24 hr. The magnitude of liver injury in the livers of animals given the five large doses of CCl_4 corresponded qualitatively to the increases in serum transaminase activities.

Neither 0.1 nor 0.3 mmole $CHCl_3$/kg produced liver injury.

Correlations between injury and metabolism. Relationships between the metabolism and hepatotoxicity of CCl_4 were evaluated by analyzing the strengths of the associations between the magnitude of liver injury in individual animals given CCl_4 and selected parameters of the extent or rate of CCl_4 metabolism to specific products by the individual animals. Strengths of the associations were determined by estimating the proportion of variance (R^2) in SGPT which could be attributed to its linear regression on the metabolite amounts or elimination rates. The rate correlations were done using data from the first hour and the fourth to seventh hour, since these time points usually incorporated either the peak rates or the prolonged "stabilized" rates (see Fig. 4 and Table 3).

A very strong correlation of 0.88 was found between SGPT and the rates of $^{14}CO_2$ metabolite exhalation during the first hour (Table 5). Moderately strong correlations ($R^2 > 0.6$) with SGPT were also found for the total amounts of $^{14}CO_2$ or $CHCl_3$ exhaled, and the rates of $^{14}CO_2$ or $CHCl_3$ exhalation during the fourth to seventh hour. Weaker correlations ($R^2 < 0.6$) were found for amounts of metabolite bound in liver at 24 hr and amounts of metabolite excreted in 24 hr urine.

DISCUSSION

This study demonstrates for the first time that the capacity of rats to metabolize CCl_4 to CO_2 is diminished within 2 hr after administration of hepatotoxic doses of CCl_4. Pathways of CCl_4 metabolism leading to CO_2 and $CHCl_3$ metabolite formation appear to be more relevant to the hepatotoxicity of CCl_4 than the pathways leading to urinary or covalently bound metabolites based on the results of this study with six doses of CCl_4 and those of a related study [40] in which isopropanol pretreatment was used to enhance the degree of liver injury after CCl_4. However, these findings concerning the total magnitude of covalent binding at 24 hr do not exclude the possibility that covalent binding of reactive metabolites of CCl_4 to specific hepatocyte macromolecules could be critical to the development of cell injury. Several studies [41–45] have demonstrated that the covalent binding of CCl_4 to lipids, proteins and nucleotides occurs in a non-random manner.

A major change in the overall extent of CCl_4 metabolism occurred as the dose was increased from 0.1 to 0.3 mmole/kg. The fraction of dose recovered as CCl_4 increased from 1/5 to 4/5 total dose (Table 2) and the peak exhalation-rate of CCl_4 increased 40-fold (Table 3). Efficient first pass removal of CCl_4 from the portal vein by the liver could appreciably diminish the amount of CCl_4 available for pulmonary clearance at the lowest dose and may account for part of the discrepancy between the peak rates of CCl_4 exhalation after the 0.1 and 0.3 mmole doses. An alternative explanation for this discrepancy is that the capacity of the liver to metabolize CCl_4 becomes impaired at an early time after absorption of the injurious 0.3 mmole dose, but not after the non-injurious 0.1 mmole dose.

Total amounts and rates of exhalation of both CO_2 and $CHCl_3$ metabolite increased as the dose of CCl_4 increased. However, exhalation-rate, time-profiles

of these two metabolites were dissimilar. Peak exhalation-rates of CO_2, the major metabolite of CCl_4, were far greater in magnitude, occurred at an earlier time, and were briefer in duration. Increases in the CCl_4 dose above 0.3 mmole/kg were associated with exhalation of $CHCl_3$ at peak rate after a shorter lag time and for a longer duration. Also the proportion of total metabolite recovered as $CHCl_3$ increased by more than 10-fold as the dose of CCl_4 increased from 0.3 to 2.0 mmoles/kg. This increase in proportion of metabolite recovered as $CHCl_3$ was associated with a decrease in the proportion recovered as $^{14}CO_2$. Substantial changes in the proportions of other bound or excreted metabolites were not observed.

The extent of $^{14}CO_2$ formation is rapidly reflected by changes in the rates of $^{14}CO_2$ exhalation since CO_2 is quickly cleared by exhalation. Thus, the slowly declining $^{14}CO_2$ metabolite profiles in the animals given the lowest dose of $^{14}CCl_4$ (Fig. 4) or the two doses of $^{14}CHCl_3$ (Fig. 3) are indicative of continued formation of $^{14}CO_2$ at rates that gradually diminish as body burdens of the parent compound decline. When there is continued formation of a metabolite at rates which gradually diminish, increases in the dose of parent compound result in increased apparent elimination half-times for the metabolite. For example, Rose *et al.* [46] reported that increases in the dose of prednisone were associated with longer apparent plasma elimination half-times of a metabolite or prednisone. Our pharmacokinetic analysis of CO_2 metabolite after 0.1 and 0.3 mmole $CHCl_3$ (see Table 4) shows that the larger $CHCl_3$ dose had a more than 2-fold longer apparent half-time of CO_2 metabolite elimination. In contrast, increases in the dose of CCl_4 from the non-injurious 0.1 mmole dose to injurious doses of 0.3 mmole and higher were associated with the following observations: (1) abrupt declines in CO_2 metabolite exhalation profiles within 2 hr after CCl_4 administration despite the continued exhalation of parent compound at peak rates during this time (Fig. 4 top and center); and (2) a lack of consistent increases in the elimination half-time of CO_2 metabolite after the higher CCl_4 doses (Table 4). These observations indicate that a major pathway of CCl_4 metabolism leading to CO_2 became impaired within 2 hr after administration of a hepatotoxic dose of CCl_4.

At times after 3 hr, animals given 0.3 mmole or larger injurious doses of CCl_4 exhaled CO_2 for a protracted period at nearly "stable" rates approximately ten times less than the peak rate (see Fig. 4). This "stable" part of the CO_2 metabolite exhalation profile was of greater magnitude and nearly parallel to the $CHCl_3$ metabolite exhalation profile. Since $CHCl_3$ is extensively metabolized to CO_2, a "source" for the CO_2 exhaled during this latter time could be the $CHCl_3$ metabolite formed. Based on the data from animals given $^{14}CHCl_3$ as parent (see Table 2), it is possible to estimate that less than 10% of the $CHCl_3$ metabolite actually formed from CCl_4 is exhaled unchanged, and that at least six times more is eliminated as CO_2. In fact, animals given 0.3 mmole CCl_4/kg exhaled CO_2 metabolite during the later relatively "stable" period (i.e. 4–7 hr after CCl_4) at an average rate more than thirty times that of $CHCl_3$ metabolite, whereas animals given 2 mmole or higher doses of CCl_4 exhaled CO_2 metabolite during the latter "stable" period at average rates only two to five times higher than those of $CHCl_3$ metabolite. Thus, oxidation of an intermediate $CHCl_3$ metabolite could more than account for all the CO_2 formed from the larger CCl_4 doses at times after 3 hr.

CCl_4 is metabolized predominately in the liver [34, 47, 48] by an NADPH-cytochrome P-450-dependent process [49–51]. Decreases in the liver NADPH and cytochrome P-450 are, therefore, probable causes of the observed changes in CCl_4 metabolism. The decrease in liver NADPH following CCl_4 is rapid and dose dependent [52, 53]. The decrease in liver cytochrome P-450 is rapid and selective as indicated by non-uniform loss of cytochrome P-450-dependent activities and isozymes [54].

Selective losses of specific cytochrome P-450 isozymes which catalyze the metabolism of CCl_4 to $CHCl_3$ and to CO_2 by parallel pathways (k''_m, k'_m and k_m in Fig. 5) could explain the observed dose- and time-dependent changes in CCl_4 metabolism. The rapid decline in CO_2 metabolite formation could be due to preferential destruction of a cytochrome P-450 isozyme which catalyzes CCl_4 metabolism to CO_2 (i.e. k''_m). This would be consistent with the studies of Noguchi *et al.* [55, 56] which demonstrated selective early loss of a cytochrome P-450 isozyme specifically required for activation of CCl_4 to a free radical. The greater and sustained levels of $CHCl_3$ metabolite exhalation at later times may be due to several factors including: sparing of a cytochrome P-450 isozyme which readily releases $^{\bullet}CCl_3$ for $^{\bullet}H$ abstraction and $CHCl_3$ formation (i.e. k'_m); liver hypoxia; and/or selective destruction of a cytochrome P-450 isozyme which catalyzes the metabolism of $CHCl_3$ to CO_2 (i.e. k'_m). The last factor could explain the disproportionate increase in proportion of $CHCl_3$ metabolite versus CO_2 metabolite after CCl_4 doses of 2 mmoles and larger (see Table 2).

Gillette *et al.* [57] suggested that the destruction of cytochrome P-450 by CCl_4 could "self-limit" the toxic effects of the compound. This is an intriguing suggestion especially since the rate of CO_2 exhalation during the first hour after CCl_4 administration was the parameter of CCl_4 metabolism which correlated most strongly ($R^2 = 0.88$) with the magnitude of liver injury in this study. If the major pathway of CO_2 formation during this early time involves the reactive intermediate $^{\bullet}OOCCl_3$, then this strong correlation is compatible with the proposal of Packer *et al.* [11] that $^{\bullet}OOCCl_3$ could be the major toxic intermediate of CCl_4.

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