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INTERACTIONS OF CARBON TETRACHLORIDE AND PROMETHAZINE IN THE RAT—II

ELIMINATION OF CARBON TETRACHLORIDE AND CHLOROFORM IN EXPIRED AIR AS INDICATIONS OF THEIR METABOLISM IN THE INTACT ANIMAL

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Abstract—Two methods are described for studying the kinetics of excretion and the metabolism of CCl_4 in rats. The elimination of CCl_4, and of the trace amounts of its metabolite $CHCl_3$ in the expired air of rats intoxicated with CCl_4 have been measured (i) in samples of air taken from a passive breathing chamber and using gas chromatography with a flame ionisation detector; (ii) in samples of air taken from a flow-through breathing chamber and using gas chromatography with an electron capture detector. In the first six hours following a dose of CCl_4 (13 mmoles/kg body wt) approx. 60% of the dose is expired unchanged and only 0.15% of the dose appears in expired air as $CHCl_3$. Simultaneous administration of Promethazine (78 μmoles/kg body wt) with the CCl_4 reduced the total expiry of CCl_4 and $CHCl_3$ during the first 6 hr of poisoning by 35% and 50% respectively. Promethazine also significantly increased the breathing rate of rats. Relationships between the expiry of CCl_4 and $CHCl_3$ as measured by the flow-through method (and the concentration of CCl_4 in the passive breathing chamber) with the concentrations of CCl_4 and $CHCl_3$ obtained in a previous study (accompanying manuscript) are discussed in terms of the use of expiry data for monitoring metabolism *in vivo*. The sensitivity of the flow-through method also permits studies with low doses of CCl_4 (e.g. 1.3 mmoles/kg body wt) thereby minimising secondary factors. The effects of induction of liver components with phenobarbital and of damage caused by a prior large dose of CCl_4 on the production and expiry of $CHCl_3$ have been studied with the low dose of CCl_4. The results show that the method can be used for rapid screening of gross effects of potential protective agents on the expiry kinetics and metabolism *in vivo* of CCl_4.

Although the detailed mechanisms by which carbon tetrachloride and other halogeno-methanes produce fatty degeneration and centrilobular necrosis of the liver are still a subject for debate, it is generally accepted that metabolism ('activation') of the compound is the essential starting point for significant hepatotoxic action. In the case of CCl_4, for example, evidence (background references in [1–4]) points to the trichloromethyl and trichloromethyl peroxy [5] radicals as important toxic intermediates.

Investigations of the quantitative aspects of the metabolism of CCl_4 in intact animals began with the studies of Butler [6] and Paul and Rubenstein [7] who presented evidence for the presence of the metabolic products $CHCl_3$ and $[^{14}C]$-CO_2(from $[^{14}C]$-CCl_4) in the expired air of dogs and rats. Relatively few additional experiments on the metabolism of CCl_4 *in vivo* have been reported since those early studies by Butler and by Paul and Rubenstein: Fowler [8] and Bini *et al.* [9] have identified $CHCl_3$ and hexachloroethane in rabbit and rat tissues after administration of CCl_4, and have thereby provided additional indirect evidence for the activation *in vivo* of CCl_4 to $CCl_3^{\cdot}$. In contrast to the rather limited quantitative data on the metabolism of CCl_4 *in vivo*, there are many reports showing that CCl_4 is metabolised *in vitro* to $CHCl_3$, phosgene and CO_2 (for general refs see [10–14]). Such studies have demonstrated that the conversion of CCl_4 to $CHCl_3$ is increased by anaerobic conditions, and have pointed to an important role for cytochrome P-450 in the overall mechanism of CCl_4, at least under conditions *in vitro*.

To provide additional data on the expiry and metabolism of CCl_4 *in vivo* we decided to develop a simple non-invasive monitoring system that takes advantage of 3 aspects: (i) there is a rapid and extensive elimination of CCl_4 (and of the ^{14}C-label from $[^{14}C]$-CCl_4) in expired air [7, 15]; (ii) the existence of trace amounts of $CHCl_3$ in expired air from rats dosed with CCl_4, and which can be used as an indicator of the metabolism of CCl_4 [6, 8, 9]; (iii) the gas-chromatographic properties of CCl_4 and $CHCl_3$ allow their accurate determination in low concentrations [8, 9, 16].

The objectives set for this study were firstly to measure the elimination rate of CCl_4 in the expired air of rats, and the associated content of $CHCl_3$ as a measure of the enzyme activation of CCl_4. Secondly, to study the effects of Promethazine on the contents of CCl_4 and $CHCl_3$ in expired air. Promethazine has some hepato-protective action when administered with CCl_4 [17, 18] and this section of the study was aimed at assessing the experimental procedure developed in this study for use as a screening method for potential protective substances.

Thirdly, to study the effects of changing the content of cytochrome P-450 on the conversion of CCl_4 to $CHCl_3$ in order to gain information on the importance of the cytochrome P-450 content on this process *in vivo*.

The results presented here supplement the data on tissue concentrations of CCl_4 and $CHCl_3$, and the effects of Promethazine on such tissue concentrations, and which are given in the accompanying paper [19].

MATERIALS AND METHODS

Chemicals. CCl_4 (for spectroscopy) and $CHCl_3$, diethyl ether and toluene (A.R.) from E. Merck, Darmstadt and propyl iodide (A.R.) from Fluka AG, Switzerland were used throughout the experiments.

Promethazine hydrochloride was a gift of SPECIA, Paris.

Analysis. CCl_4 and $CHCl_3$ in expired air were determined by GC by either direct injection of air samples using a flame ionization detector (FID) [16] or by analysis of bubble trap solutions of toluene using an electron capture detector (ECD) [16].

Treatment of animals. Young, adult, male Tif RAI f(SPF) rats (CIBA-GEIGY) fed on a standard diet (NAFAG 890 pellets), maintained in a climate controlled room with a 12 hr light/dark cycle and weighing approx. 200 g were fasted for 15 hr in cages with stainless steel grids above the cage floor. Tap water was available *ad libitum* throughout the fasting and post-administration periods.

The rats were weighed, etherized and administered a 13.0 or 1.3 mmoles (1.25 or 0.125 ml)/kg dose of CCl_4 as a solution in light liquid paraffin by intragastric cannula (i.g.) and, simultaneously, an intraperitoneal (i.p.) injection of either 78 μmoles Promethazine/kg (as a freshly prepared 1% (w/v) aqueous solution) or an equivalent volume of physiological saline.

Where necessary, rats were administered sodium phenobarbital 80 mg/kg/day as a 3.2% (w/v) aqueous solution by gavage for 4 days, prior to fasting and CCl_4 administration, to induce cytochrome P450.

All doses were administered mid-morning (between 08:30 and 11:30) in volumes equivalent to 0.5 ml mixture/200 g body weight.

FID analysis of expired air using a passive breathing chamber. The FID head-space analysis method previously [16] is suitable for direct injection of large volumes (up to 2 ml) of expired air into the chromatograph.

Expiration of CCl_4 was monitored by placing a CCl_4-treated rat in a sealed breathing chamber of the type described by Lindstrom and Anders [20].

Several trials with CCl_4-treated rats (13.0 mmoles/kg, i.g.) using either pure O_2, air or O_2 enriched-air mixtures in the 'air-supply' reservoir resulted in the death of the animals 90–120 min after the chamber was sealed. In each case, death was preceded by a period of extremely rapid, shallow breathing, presumably due to the effects of re-inspired CCl_4. This problem was resolved by using a strict schedule for alternate, 15 min chamber and recovery (open cage) periods by means of which the expiration of CCl_4 from two rats may be determined concurrently:

0 min: Rat A dosed, recovers in open cage;
15 min: Rat B dosed, recovers in open cage;
Breathing chamber and O_2 reservoir flushed with O_2 from cylinder;
16 min: Rat A placed in breathing chamber, chamber sealed;
29 min: Chamber air sampled;
Rat A returned to open cage;
30 min: Breathing chamber and O_2 reservoir flushed with O_2;
31 min: Rat B placed in breathing chamber, etc.

Background from the unocccupied system, probably due to CCl_4 glasswall adsorption and impregnation of the absorbant granules, was measured throughout the experimental period and found to be low and reasonably constant (the mean background was approx. 15% of experimentally observed concentrations). Using CCl_4 vapour samples of known concentration, losses between chamber samples and GC injection (average time 3 min) were also estimated and found to be consistently 20 per cent (recover 79.8 ± 2.7%). All values recorded experimentally were therefore corrected accordingly:

$$\text{(Signal response) } corr. = \frac{\text{(Signal response} - \text{mean background)}}{0.8}$$

As expired CCl_4 is re-inspired by the rat during the chamber sampling period, it is not possible to determine total expired CCl_4 using this method. Application of the equation

$$CCl_4 \text{ chamber content} = \frac{\text{Chamber volume}}{\text{Sample volume}} \times CCl_4 \text{ sample concn}$$

indicates that only extremely small percentages of the administered CCl_4 dose are recovered. Expiry data were therefore expressed as relative chamber CCl_4 concentrations (electronic integration of GC peak area was performed and the results are given as relative units corrected for losses as outlined above).

ECD analysis of expired air using a flow-through breathing chamber. A flow-through breathing chamber of the type described by Paul and Rubenstein [7] and commonly used for expired $^{14}CO_2$ measurement in this laboratory was converted and optimized for expired CCl_4 and $CHCl_3$ determination. The apparatus was constructed entirely of glass and included ground-glass ball and socket connections for all pieces.

Air was drawn through two drying columns, a rat chamber and 3 glass tube bubble traps, each trap containing 70 ml of toluene maintained at 0° by ice-filled Dewar flasks. The two drying columns contained silica gel and Sikkon blue respectively. The second column also contained a small amount of soda asbestos for partial removal of CO_2. The rat chamber used was a modified desiccator with a central metal grid, inlets for water, food and a thermometer and outlets for the collection of urine and faeces.

The principal development problem concerned the bubble traps. At room temperature, carry-over of

toluene from one tube to the next was extensive. Using ice-cold toluene, this phenomenon was reduced but still significant. Although air-flow rates of approx. 1 litre/min had been used routinely, we established that both CCl_4-treated and untreated rats displayed no air-seeking behaviour during long periods at flow-rate as low as 250 ml/min. Toluene carry-over at 0° became negligible when the sampling (bubbling) period was reduced to 15 min at this flow-rate.

We therefore decided to determine rate of expiry, rather than attempt to collect all CCl_4 and $CHCl_3$ expired. This method requires measurement of CCl_4 and $CHCl_3$ expired during a short, defined period (15 min) at regular intervals (hourly). Total expiry may then be calculated by integration of the resultant expiry rate *versus* time graph. Air was directed through the toluene traps during the sampling period and through an open by-pass at all other times.

Prior to ECD analysis, an internal standard (propyl iodide, PI) was added to the collected toluene samples (to give a final concentration of 1.0 μmole/l which were further diluted with PI 0.1 nl/ml in toluene as required. Regression equations of calibration graphs of CCl_4 and $CHCl_3$ in toluene solutions over the range 50–1000 nmoles/l were used for calculations of the concentration [16].

Contamination of toluene in the traps by residues from the previous collection period was eliminated by rinsing with toluene after sampling and ensuring that each tube and glass sieve was thoroughly dry before refilling.

To avoid having to determine samples from each of the three toluene traps routinely, we carried out a detailed analysis of the results obtained from six complete experiments.

Following CCl_4 administration (13.0 mmoles/kg, i.g.), expired air from a rat was passed through the traps for exactly 15 min at hourly intervals for 6 hr. Samples of each toluene solution were then analysed by ECD for both CCl_4 and $CHCl_3$ and the absolute amounts (concn × trap volume) expressed as a percentage of the total CCl_4 and $CHCl_3$ recovered (for details see [21]). The percentage recoveries of CCl_4 and $CHCl_3$ (mean ±S.D. of six experiments) in trap 1 compared to traps 1, 2 and 3 together were 97.91 ± 0.29 and 97.07 ± 1.65 per cent respectively. In view of these high recoveries in the first trap, it was decided to routinely analyse samples from trap 1 only and to apply correction factors of 100/97.91 and 100/97.07 for the CCl_4 and $CHCl_3$ results respectively. These correction factors were checked by an identical 6 hr experiment one month after they were first established: calculated totals for expired CCl_4 and $CHCl_3$ were 100.0 and 100.1% of the measured total amounts respectively.

Blank runs conducted intermittently over a period of two months ($n = 13$) showed backgrounds of 1.5 ± 1.25 (S.D.) μg $CHCl_3$ and 12.8 ± 15.04 μg CCl_4 per min test period. Relative to the average volumes collected during expired air experiments using CCl_4 doses of 13.0 mmoles/kg, this background assumed significant proportions only for $CHCl_3$ resulting in an over-estimation of approx. 10 per cent. However, as neither the qualitative nor quantitative aspects of the present study were significantly influenced by this inconsistent background, no correction was applied to the expired $CHCl_3$ values obtained in experiments with 13 mmoles CCl_4/kg body wt). Some experiments *in vivo* used small doses of CCl_4 (1.3 mmoles/kg body wt) and for these studies we paid extra attention to cleaning procedures of the equipment and to solvent vapour levels in the environment. As a consequence, the system background levels were considerably reduced. Pre-experiment backgrounds were lowered to 1.13 ± 1.03 (S.D.) μg CCl_4 and 0.34 ± 0.21 μg $CHCl_3$ in regular testing ($n = 21$) over a 6-week period i.e. approx. 0.2 per cent (CCl_4) and 6.0 per cent ($CHCl_3$) of the average expiry rates recorded following a low dose of CCl_4. All measurements made during the low-dose experiments were corrected for daily background. The satisfactory turn-over of air in the breathing chamber of the apparatus was also demonstrated. The expiry of CCl_4 and $CHCl_3$ from a CCl_4-treated rat was measured for 15 min as usual. The rat was quickly removed, the chamber re-sealed and, after exactly 15 min of continued air-flow, the background of the empty chamber measured for a further 15 min. The backgrounds of CCl_4 and $CHCl_3$ so obtained were only 1.8 ± 1.4 per cent and 9.9 ± 5.8 per cent respectively (means ±S.D., $n = 21$) of the expiry rates recorded 15 min previously.

To ensure that contribution to $CHCl_3$ levels did not arise through reduction of CCl_4 by the system itself, large amounts (up to 15 mg) of CCl_4 alone were injected into the breathing chamber and, following recovery in the toluene traps, analysed for $CHCl_3$ content. In three separate tests, less than 0.01 per cent of the injected amount of CCl_4 was recovered as $CHCl_3$.

To determine absolute recovery of the trapping and analysis systems the apparatus was operated as described above and known amounts of CCl_4 and $CHCl_3$ injected through an inlet port onto a watch glass placed on the metal grid of the breathing chamber. Following a standard 15 min collection period, the toluene traps were sampled and total CCl_4 and $CHCl_3$ determined against PI (1.0 μM). Recoveries of 100.4 ± 4.7 (mean ±S.D.) per cent and 110.7 ± 10.9 per cent for CCl_4 and $CHCl_3$ respectively demonstrate the efficiency of this trapping method. The slight over-estimation of $CHCl_3$ has been discussed above.

RESULTS

FID analysis of expired air using a passive breathing chamber

The mean values obtained from the expiry data from 5–7 rats are shown in Fig. 1(a) in the form of expired CCl_4 concentrations vs time in the presence and absence of Promethazine. These expiry data closely parallel those of mean CCl_4 blood concentration : time curves reported elsewhere [19]. Experimental results from the individual animals were evaluated for maximum chamber concentration (C_{max}), time of C_{max}(t_{max}) and area under the concentration : time curve (AUC). Table 1 shows that the mean C_{max} values were almost identical for the control and Promethazine groups. However, in the PM-treated rats t_{max} was delayed significantly (more

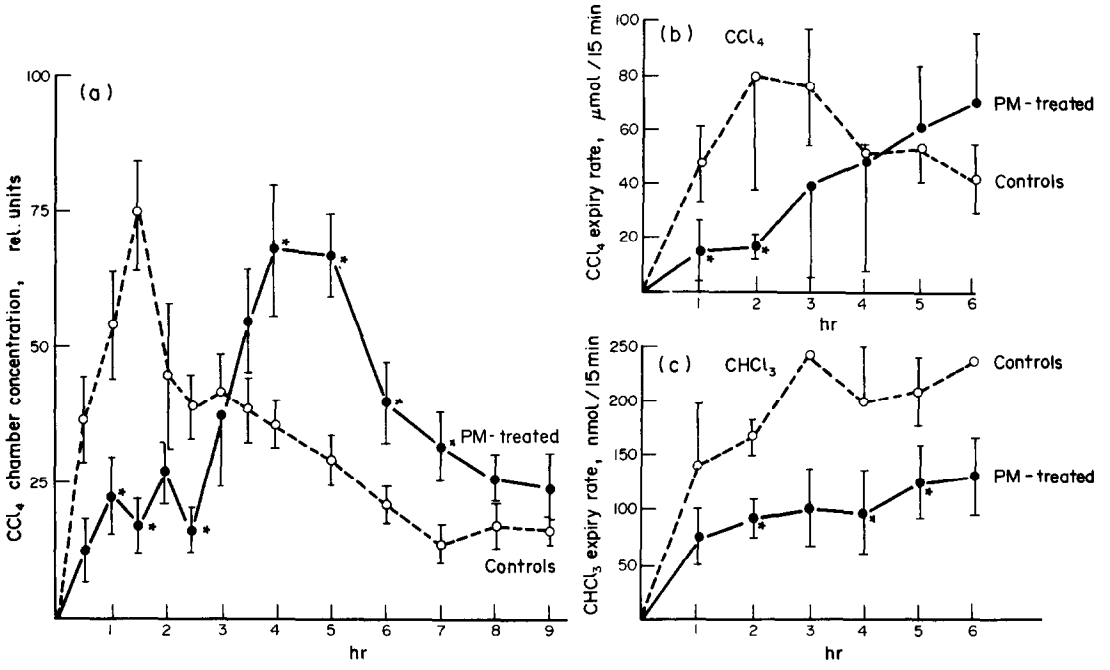

Fig. 1. Expiry of CCl_4 and $CHCl_3$ from rats in (a) a passive breathing chamber and (b) and (c) a flow-through system. The rats were treated with 13 mmoles CCl_4/kg body wt i.g. and, where required, Promethazine (78 μmoles/kg body wt i.p.). The expired air samples were analysed by a FID-technique in (a), and by an ECD-technique in (b) and (c). The numbers of rats used were 5–7 in (a) and 4 in (b) and (c). Mean values are given ±S.D. The asterisk (*) indicates $P < 0.05$ for the difference between control and treated groups. For further details see the text.

than 2 hr) and total expiration of CCl_4 (as assessed by AUC) was slightly greater than in controls. It must be emphasised that the use of 'relative units' does not imply inaccurate estimations but refers to the impossibility of obtaining total excretion data in a re-cycling situation. With this system $CHCl_3$ was detected only occasionally and then only in amounts bordering on the sensitivity limit of GC methods.

ECD analysis of expired air using a flow-through chamber

The effects of PM on the expiry of CCl_4 and $CHCl_3$ in rats treated with CCl_4 13.0 mmoles/kg i.g. were investigated using the standard analytical and correction procedures described in methods. The resultant expiry rate data (expired CCl_4, $CHCl_3$/15 min) presented in Fig. 1(b) and 1(c) display the same time-profiles as the concentrations of $CHCl_3$ and CCl_4 in blood reported earlier [19]. Cumulative expiry curves (Fig. 2) may be obtained by integration of these rate curves using the trapezoid rule approximation for the expiry concentration: time curves from individual rats.

It is of interest to note that PM significantly increased the breathing rate of rats whether they were in open, individual cages (Fig. 3) or in the sealed breathing chamber (data not shown, see ref. 21). The chamber itself had little effect on the breathing rates recorded: statistically significantly higher rates (compared with open cages) occurring in only

Table 1. Analysis of CCl_4 in expired air from rats in a passive breathing chamber, and using a flame ionisation detector technique*

Treatment	C_{max}	t_{max}	AUC
Saline (7)	8122 ± 1192	95 ± 8	22640 ± 3505
Promethazine (6)	8071 ± 957	230 ± 20*	23750 ± 2960

* The expiration of CCl_4 from male rats following a dose of 13 mmoles CCl_4/kg body wt and concomitant administration of Promethazine (78 μmoles/kg body wt) or an equivalent volume of physiological saline, as measured by the concentration of CCl_4 in the chamber. Mean values are given ±S.E.M. with the numbers of estimations in parenthesis. The results for C_{max} and AUC are in relative units; for definition of 'relative units' see the text. C_{max} and t_{max} are the maximum concentrations measured, and the time of maximum concentration respectively. The area under the chamber concentration-time graphs was measured for the period 0–6 hr after dosing and is shown as AUC. The asterisk shows a significant difference of t_{max} values with $P < 0.001$.

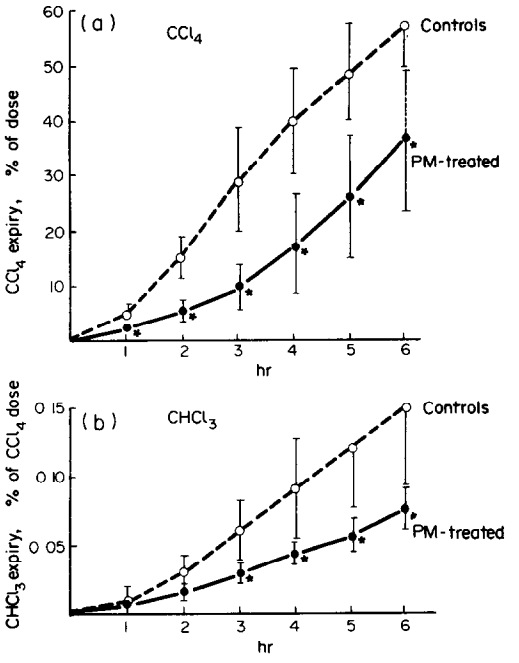

Fig. 2. Cumulative expiry of CCl_4 and $CHCl_3$ from rats in a flow-through chamber. The results are calculated from data in Fig. 1(b) and (c) using the trapezoid rule approximation. Mean values are given ±S.D.; four rats were used for each datum point. Except for the 1 and 2-hr points for the expiry of $CHCl_3$ all other points were significantly different from the controls ($P < 0.05$). For other details see the text.

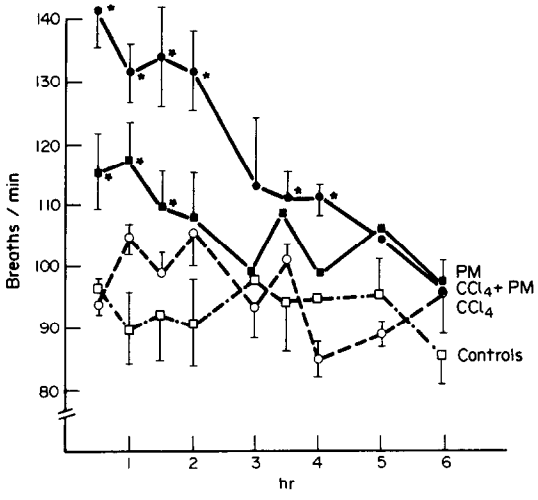

Fig. 3. Breathing rates of fasted, male rats held in open, individual cages following administration of: (i) CCl_4 13.0 mmoles/kg i.g. + Promethazine 78 μmoles/kg i.p. (●);(ii) liquid paraffin i.g. + Promethazine 78 μmoles/kg i.p. (■); (iii) CCl_4 13.0 mmoles/kg i.g. + physiological saline i.p. (○); (iv) liquid paraffin i.g. + physiological saline i.p. (=controls) (□). Ambient temperature: 24°; for further details see the text. Mean values are given ±S.E.M. (n = 5). *$P < 0.05$ (vs controls).

2 of 13 chamber periods of the controls (7 and 9 hr) and 5 of 13 periods of the PM-treated rats (2, 3, 3.5, 4 and 9 hr). However, no correlation between breathing rate and CCl_4 expiry (as measured by CCl_4 chamber concentration) is apparent, again probably because of the effect of reinhalation of CCl_4 on the final chamber concentration.

Expiry of CCl_4 *and* $CHCl_3$ *under various experimental conditions*

The expiry of CCl_4 and $CHCl_3$ was measured in various models, all employing a 1.3 mmoles/kg i.g. dose of CCl_4 (i.e. one-tenth of the dose normally used) and equivalent to only 25 μl CCl_4/200 g rat. Phenobarbital-induction or CCl_4-damage (by pre-treatment with CCl_4 13.0 mmoles/kg p.o. at −24 hr) affected $CHCl_3$ expiry in a clear-cut and predictable way (Fig. 4); variation of the measured expiry rates was small. A further test of the low dose model was obtained by monitoring expiry in rats administered 1.3 mmoles CCl_4/kg i.g. daily for 3 days. As with CCl_4 pre-treated rats above, 'foot-point' measurements were taken immediately prior to dosing to assess 'carry-over' from the dose administered 24 hr previously. Surprisingly, no significant change in $CHCl_3$ expiry could be detected over the 3-day treat-

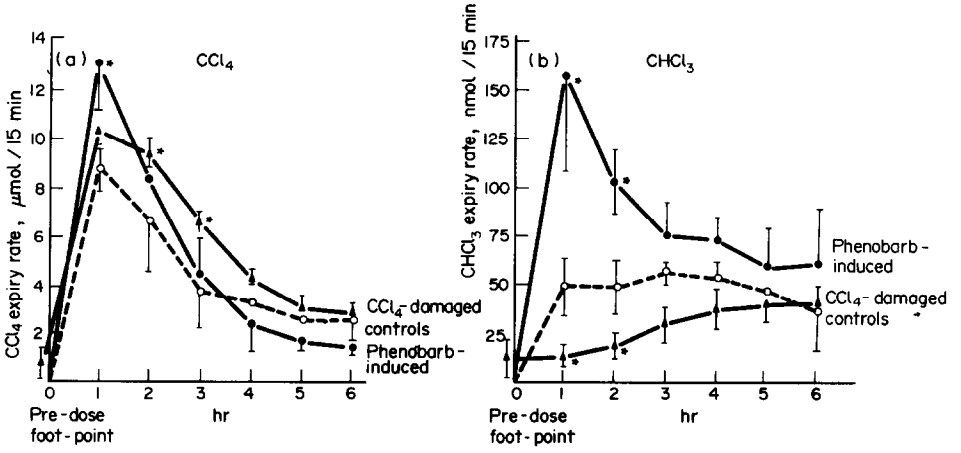

Fig. 4. Rate of expiry of CCl_4 and $CHCl_3$ following administration of CCl_4 1.3 mmoles/kg i.g. to (○) untreated controls; (●) phenobarbital-induced (80 mg/kg/day for 4 days); or (▲) CCl_4-damaged (13.0 mmoles/kg i.g. at −24 hr) fasted, 200 g, male rats. For other details see the text. Mean values are given ±S.D. (n = 4). *$P < 0.01$ (vs controls).

ment period. However, CCl_4 expiry rates increased moderately after the second and third CCl_4 doses (a similar response to that shown after high dose CCl_4 pre-treatment, Fig. 4(A)).

DISCUSSION

Regular sampling of expired air from CCl_4-treated rats to obtain expiry rate data, using either method described above, permits reasonably accurate predictions of the corresponding blood concentration: time profiles and relative C_{max}, t_{max} and AUC values for CCl_4 in blood as given in the accompanying paper [19]. Similar data on $CHCl_3$ can be obtained from use of the ECD-system.

With the FID system, a relationship exists between the blood concentrations of CCl_4 [19] and relative chamber concentrations produced by expired air; $r = 0.62$ ($P < 0.05$). This occurs despite the fact that in the PM-treated rats the t_{max} values for mean blood and chamber concentrations do not coincide. Weighting the recorded chamber concentrations for variations in breathing rate does not influence this correlation.

With the ECD system, the computed correlation between the blood concentrations of CCl_4 and $CHCl_3$ [19] and expiry rates is 0.79 for CCl_4 ($P < 0.01$) and non-significant for $CHCl_3$. Nevertheless, the expiry rate $CHCl_3$ curves display both the marked plateau effects also present in the blood concentration data and the reduced presence of $CHCl_3$ in PM-treated rats (blood AUCs reduced by 5–25% [19]).

The death of rats held in the passive breathing chamber for more than 90 min cannot be adequately explained but is presumably due to the various effects of inhalation of air containing high concentrations of CCl_4. In addition, the strain of rats used in these studies appears to be especially sensitive to CCl_4 [19].

The stimulatory effect of PM on the breathing rate of rats is surprising as the high dose administered (78 μmoles/kg) generally produces mild sedation and occasionally a trance-like state. PM, an anti-histaminic, is known to be present in the lungs in high concentrations in the first hours after administration [22]. However, in male mice equivalent doses of PM significantly depress respiratory frequency [23].

The cumulative expiry curves (Fig. 2) illustrate the strong effect of PM on the elimination of both CCl_4 and $CHCl_3$. Expiry of unchanged CCl_4 was extensive: approx. 60 per cent of the administered dose being eliminated in the first 6 hr. This result is in general agreement with the results of Paul and Rubenstein [7] who recovered approx. 50 per cent in 6 hr. $CHCl_3$ production as assessed by cumulative expiry is extremely low but appears to be constant. The absolute recovery of only about 0.1 per cent of the administered dose of CCl_4 as $CHCl_3$ is also in general agreement with the original work of Butler [6] who found $CHCl_3$:CCl_4 ratios of 1:1000–1:4000 in the expired air of dogs.

The small proportion of CCl_4 converted to $CHCl_3$ is not the only pathway of metabolism of CCl_4. Formation of the carbene–P450 complex [13] is thought to proceed to carbon monoxide, and the rapid reaction of $CCl_3^{\cdot}$ with O_2 [5] to yield $CCl_3O_2^{\cdot}$ would produce phosgene and CO_2 [24]. The expiry of [^{14}C]-CO_2 from [^{14}C]-CCl_4 is about 0.4 per cent of the administered dose over 24 hr in the rat [15]. Evidence for the production of phosgene from CCl_4 has recently been provided [12].

The sensitivity of the system in differentiating between rats with various metabolic capacity (as assessed by $CHCl_3$ production) was adequately demonstrated using a very low dose of CCl_4 (Fig. 4(B)). Here the control group again shows the marked plateau effect in $CHCl_3$ expiry observed in the high-dose treatment group (Fig. 1(C)) and throughout the $CHCl_3$ blood concentration studies referred to above.

The systems described here and in the accompanying paper offer satisfactory approaches to rapid screening of the gross effects of protective (and other) agents on the absorption, distribution, metabolism and elimination of CCl_4 (and probably other halogeno-methanes eliminated largely in expired air). One special advantage of the systems is that, following administration, the animals are subjected to no further trauma due to handling and/or sampling. In the case of the flow-through breathing chamber, the animals are not touched at all following treatment. Of greatest significance, however, is the fact that the ECG/flow-through system is sufficiently powerful to permit study of CCl_4 metabolism (as $CHCl_3$ production) by monitoring the rate and extent of CCl_4 and $CHCl_3$ expiry in individual animals administered such low doses of CCl_4 that the influence of secondary effects on the hepatoxicity model are minimal.

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REFERENCES

1. T. F. Slater, in *Free Radical Mechanisms in Tissue Injury*, pp. 283. Pion Ltd., London (1972).
2. R. O. Recknagel and E. A. Glende Jnr., *C.R.C Crit. Rev. Tox.* **2**, 263 (1973).
3. M. I. Diaz Gomez, J. A. Castro, E. C. de Ferreyra, N. D'Acosta and C. R. de Castro, *Toxic. Appl. Pharmac.* **25**, 534 (1973).
4. E. Burdino, E. Gravela, G. Ugazio, V. Vannini and A. Calligaro, *Agents Act.* **4**, 244 (1973).
5. J. E. Packer, T. F. Slater and R. L. Willson, *Life Sci.* **23**, 2617 (1978).
6. T. C. Butler, *J. Pharmac. exp. Ther.* **134**, 311 (1961).
7. B. B. Paul and D. Rubenstein, *J. Pharmac. exp. Ther.* **141**, 141 (1963).
8. J. S. L. Fowler, *Br. J. Pharmac.* **37**, 733 (1969).
9. A. Bini, G. Vecchi, V. Vannini and C. Cessi, *Pharmac. Res. Commun.* **7**, 143, (1975).
10. H. Uehleke, K. H. Hellmer and S. Tabarelli, *Xenobiotica* **3**, 1 (1973).
11. D. Rubenstein and L. Kanics, *Can. J. Biochem.* **42**, 1577 (1964).
12. H. Shah, S. P. Hartman and S. Weinhouse, *Cancer Res.* **39**, 3942 (1979).
13. C. R. Wolf, D. Mansuy, W. Nastainczyk, G. Deutschmann and V. Ullrich, *Molec. Pharmac.* **13**, 698 (1977).
14. R. O. Recknagel, E. A. Glende Jnr. and A. M. Hruzkewycz, in *Biological Reactive Intermediates, Ist Int. Symp.* (Eds D. J. Jollow, J. J. Kocsis, R. Snyder

and H. Vainio), pp. 417–428. Plenum Press, New York (1977).
15. A. A. Seawright and A. E. M. McLean, *Biochem. J.* **105**, 1055 (1967).
16. C. J. Reddrop, W. Reiss and T. F. Slater, *J. Chromat.* **193**, 71 (1980).
17. K. R. Rees, K. P. Sinha and W. G. Spector, *J. Path. Bact.* **81**, 107 (1961).
18. T. F. Slater and B. C. Sawyer, *Biochem. J.* **111**, 317 (1969).
19. C. J. Reddrop, W. Resiss and T. F. Slater, *Biochem. Pharmac.* **30**, 1443 (1981).
20. T. D. Lindstrom and M. W. Anders, *Biochem. Pharmac.* **27**, 563 (1978).
21. C. J. Reddrop, Ph.D. thesis, Brunel University, Uxbridge, Middx. U.K. (1980).
22. E. Hansson and G. G. Schmiterlöw, *Archs int. pharmacodyn.* **131**, 309 (1961).
23. H. A. O. Shater and B. J. Pleuvry, *J. Pharm. Pharmac.* **29**, 612 (1977).
24. T. F. Slater, in *Biological Reactive Intermediates, 2nd Int. Symp.* (Ed. R. Snyder). Surrey, Plenum Press (1981).