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Gas uptake studies infer metabolism from the disappearance of chemical from a recirculated atmosphere. To insure the validity of this technique, we studied the metabolism of several brominated hydrocarbons in rats both by gas uptake and by direct measurement of liberated inorganic bromide. Chemicals used were vinyl bromide (VB), halothane (HAL), bromochloromethane (BCM), and methyl bromide (MB). Gas uptake was determined by previously published methods, and plasma bromide levels were measured by an ion-specific electrode. Based on bromide release, VB metabolism had two distinct saturable

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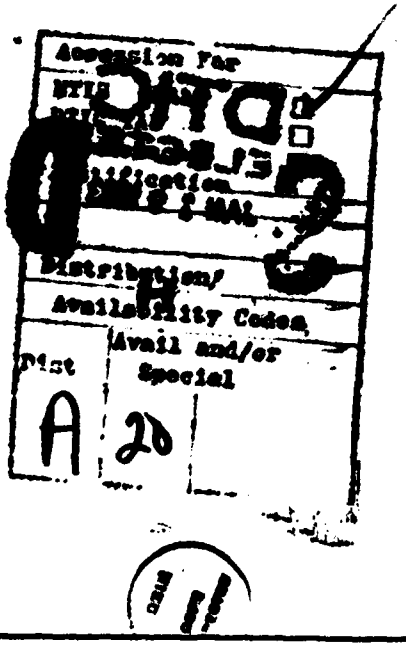
components, HAL metabolism had one saturable process, BCM metabolism was of mixed form having both first-order and saturable components, and MB metabolism was first order. Gas uptake results were similar, except only one saturable process was observed for VB, and HAL had contributions from both saturable and first-order component. First-order rate constants for MB and BCM from bromide release studies were, respectively, 0.32 and 0.085/kg/hr; from gas uptake they were 0.55 and 0.106/kg/hr. The first-order rate constant for HAL by gas uptake was 0.058/kg/hr. For VS, HAL, and BCM, bromide release studies gave estimates for K_m of 33, 73, and 79 ppm, respectively. Estimates of V_{max} were 2.3, 9.7, and 10.0 mg of parent compound metabolized/kg/hr. From gas uptake, K_m were 18.81, and 91 ppm, while the estimates of V_{max} were 2.4, 10,0, and 10.5 mg/kg/hr. With these four chemicals, gas uptake studies provided reliable estimates of the kinetic constants of metabolism.



TOXICOLOGY AND APPLIED PHARMACOLOGY 66, 55–68 (1982)

Metabolism of Inhaled Brominated Hydrocarbons: Validation of Gas Uptake Results by Determination of a Stable Metabolite[1]

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Metabolism of Inhaled Brominated Hydrocarbons: Validation of Gas Uptake Results by Determination of a Stable Metabolite. GARGAS, M. L., AND ANDERSEN, M. E. (1982). *Toxicol. Appl. Pharmacol.* **66**, 55–68. Gas uptake studies infer metabolism from the disappearance of chemical from a recirculated atmosphere. To insure the validity of this technique, we studied the metabolism of several brominated hydrocarbons in rats both by gas uptake and by direct measurement of liberated inorganic bromide. Chemicals used were: vinyl bromide (VB), halothane (HAL), bromochloromethane (BCM), and methyl bromide (MB). Gas uptake was determined by previously published methods, and plasma bromide levels were measured by an ion-specific electrode. Based on bromide release, VB metabolism had two distinct saturable components, HAL metabolism had one saturable process, BCM metabolism was of mixed form having both first-order and saturable components, and MB metabolism was first order. Gas uptake results were similar, except only one saturable process was observed for VB, and HAL had contributions from both saturable and first-order component. First-order rate constants for MB and BCM from bromide release studies were, respectively, 0.32 and 0.085/kg/hr; from gas uptake they were 0.55 and 0.106/kg/hr. The first-order rate constant for HAL by gas uptake was 0.058/kg/hr. For VB, HAL, and BCM, bromide release studies gave estimates for K_m of 33, 73, and 79 ppm, respectively. Estimates of V_{max} were 2.3, 9.7, and 10.0 mg of parent compound metabolized/kg/hr. From gas uptake, K_m were 18, 81, and 91 ppm, while the estimates of V_{max} were 2.4, 10,0, and 10.5 mg/kg/hr. With these four chemicals, gas uptake studies provided reliable estimates of the kinetic constants of metabolism.

In recent years a fairly convenient technique—referred to as gas uptake by the present authors—has been developed for assessing the kinetic constants of metabolism of inhaled gases and vapors *in vivo* (Hefner *et al.*, 1975; Bolt *et al.*, 1977; Filser and Bolt, 1979; Andersen *et al.*, 1979, 1980). In gas uptake studies animals are exposed to a test chemical in a closed atmosphere inhalation chamber. The rate of loss of chemical from

[1] Naval Medical Research and Development Command, Research Task No. MF65572001.4014. The opinions and assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large. The experiments conducted herein were conducted according to the principles set forth in the current edition of the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council. Portions of this work were presented at the 19th Annual Meeting of the Society of Toxicology, 9–13 March 1980, Washington, D.C., and at the 10th Annual Conference on Environmental Toxicology, 13–15 November 1979, Wright–Patterson Air Force Base, Ohio (see Gargas and Andersen, 1979). Reprints of this article are designated by the Air Force Aerospace Medical Research Laboratory as AFAMRL-TR-80-151.

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the chamber is determined at various initial concentrations, and a curve is constructed relating the rate of disappearance to the chamber concentration. Disappearance from the atmosphere is equivalent to uptake by the test animals, which is the rationale for the terminology, gas uptake. *In vivo* kinetic constants are derived by mathematical analysis of the uptake curve. Despite the simplicity of this approach for studying metabolism, the technique is indirect. Metabolism is inferred from the dependence of rate of uptake on inhaled concentration, and metabolites are not measured directly.

Total metabolism can be estimated by exposing animals to a radiolabeled test chemical and conducting a total inventory of the radioactivity (Watanabe *et al.*, 1978; McKenna *et al.*, 1978). This process is a tedious, technically demanding procedure. With vinyl chloride monomer (VCM) and 1,1-dichloroethylene (1,1-DCE), kinetic constants determined by total inventory agree remarkably well with those determined by gas uptake (Andersen, 1981a). A simple, alternative method exists for assessing total metabolism in those limited cases where the initial step in biotransformation yields a product resistant to further metabolism. Bromide ion is released in the initial bioactivation step of the metabolism of a variety of brominated hydrocarbons (Van Stee, 1976; Van Stee *et al.*, 1977). It is retained in extracellular fluid (Woodbury, 1966), is stable to further biotransformation, and is very slowly excreted (Holaday, 1977). In addition, bromide is easily analyzed by ion-specific electrodes.

It was our intention in this study to compare kinetic constants for brominated hydrocarbons determined by gas uptake with those determined by direct measurement of liberated bromide ion. This comparison was regarded as a test of the reliability of gas uptake techniques for measuring inhalant metabolism. Four brominated chemicals were selected—vinyl bromide, halothane, bromochloromethane, and methyl bromide. These chemicals had been previously studied by gas uptake methods (Andersen *et al.*, 1980), and their rate of uptake versus concentration curves were known to have significant individual differences.

METHODS

Animals

For gas uptake and bromide production studies and for determination of the half-life of inorganic bromide, male Fischer (F-344) rats (Charles River Breeding Lab., Kingston, N.Y.) weighing between 200 and 300 g were used. During inhalation exposures, rats did not have access to food or water. In gas uptake studies rats were returned to commercial rat chow (Purina Rat Chow) and water *ad libitum* immediately after exposure. For plasma bromide determinations rats were killed immediately after exposure for the collection of plasma. In studies for determination of the half-life of plasma bromide, rats were returned to commercial rat chow and water *ad libitum* immediately following dosing.

Chemicals

The chemicals used were vinyl bromide (VB, bromoethylene),[3] halothane (HAL, 2-bromo-2-chloro-1,1,1-trifluoroethane),[4] bromochloromethane (BCM),[5] and methyl bromide (MB).[3] Vinyl bromide and methyl bromide were obtained from the manufacturer in pressurized cylinders. Halothane and bromochloromethane were obtained as liquids.

Exposure Systems

For gas uptake work, exposures were conducted in a 31-liter battery jar chamber modified from the original apparatus described by Leach (1963). The closed, recirculating atmosphere chamber design and operation have been previously described (Andersen *et al.*, 1979; 1980; Gargas and Andersen, 1979). Atmosphere samples were analyzed with a gas chromatograph equipped with a hydrogen flame ionization detector and an automatic gas sampling valve. A 30-ft, 1/8th in. stainless steel column with Dexsil 300 GC as the stationary phase[6] was used for all chemicals. Injection temperature was 250°C, flame ionization detector temperature was 300°C, car-

[3] Matheson, Dayton, Ohio

[4] Halocarbon Laboratories, Inc., Hackensack, N.J.

[5] Dow Chemical Co., Midla d, Mich.

[6] Analabs "Hi Plates," high-efficiency packed column, Analabs, Inc., New Haven, Conn.

rier gas (N_2) flow was 33 ml/min, oven temperatures for VB, HAL, BCM, and MB were 70, 85, 90, and 85°C, respectively. Under these conditions, the respective retention times were 4.4, 5.5, 5.7, and 4.1 min.

In gas uptake exposures, measured amounts of liquid or gas were injected into the closed recirculating system. Samples were taken 5 min after injection and every 10 min thereafter for the duration of the exposures, which lasted 175 to 205 min. Prior to each animal exposure, the loss of chemical from an unoccupied chamber was calculated. As reported previously (Gargas and Andersen, 1979), this nonspecific loss was adequately represented by a single exponential whose rate constant was essentially independent of chamber concentration. With animal exposures, the observed data were corrected by subtracting the contribution of the nonspecific loss rate from the observed rate. Corrected curves for VB, HAL, and BCM were biphasic, containing a rapid equilibrium phase complete in 70 to 110 min, and a slow metabolism phase that was nearly linear after this time. Corrected curves for MB were also biphasic, but the equilibrium phase was complete in 25 to 35 min. Rates of metabolism were calculated from the slower phase and expressed both as parts per million per kilogram body weight per hour and milligrams per kilogram per hour.

For plasma bromide studies, constant concentration exposures were carried out in a 31-liter battery jar chamber (Leach, 1963). For HAL and BCM, a 9.5 liter stainless-steel sampling cylinder, fitted with valves on both input and output sides, was charged with 100 ml of the liquid sample and pressurized with N_2 to 490 KPa. With VB and MB, cylinders supplied by the manufacturer were used. In all cases, contaminant-containing gas was bled from the cylinders through a regulator; flow was monitored and adjusted with a flowmeter; and the gas was mixed with chamber input air (9 liter/min) supplied by a diaphragm pump. On the effluent side of the chamber, a second diaphragm pump was used to maintain a slightly negative chamber pressure (from 1 to 2 in. of H_2O). During animal exposures, atmospheric samples were collected every 15 to 30 min and analyzed by gas chromatography as previously described. For VB, HAL, BCM, and MB, exposures were carried out at various concentrations for 6, 4, 4, and 2 h, respectively. The exposure durations were chosen to provide a convenient amount of bromide at termination and were based on rates of metabolism determined by gas uptake techniques.

Plasma Preparation and Bromide Determinations

Immediately after constant concentration exposures, test and control rats were killed by ip injection of sodium pentobarbital, the abdomen was rapidly opened, and the rats were exsanguinated from the portal vein into a heparinized syringe. Plasma was collected after two separate 10-min centrifugations at 4000 rpm in a Sorvall GLC-1 centrifuge.[7] Bromide determinations were performed on a bromide-specific electrode, reference electrode, and Ionalyzer.[8] The bromide procedure followed methods supplied by the manufacturer with slight modifications. These included a 1:10 dilution of the plasma with 10% trichloroacetic acid to produce a protein free filtrate and subsequent 1:10 dilution with deionized H_2O to obtain sufficient volume for measurement. Standard solutions used in the procedure were appropriately balanced with 10% trichloroacetic acid and physiological saline before analyses. This procedure compensated for the slight interference which would otherwise have been expected from endogenous chloride and exogenous trichloroacetate. Measured bromide levels are mean group values (n = three to six rats/group), and the rate is expressed as millimoles plasma inorganic bromide produced per liter per hour of exposure minus the corresponding mean control values (ΔBr^- as mM). To compare this value with the results obtained by gas uptake, the overall rate of metabolism was multiplied by the volume of distribution (0.26 liters/kg) of bromide in the rat (Woodbury, 1966) and the molecular weight of the compound (mg/mmol). This calculation yields velocity in units of milligrams per kilogram per hour.

Half-life of Plasma Inorganic Bromide in the Rat

Since rates of bromide production are based on endpoint measurement of plasma bromide, the amount of bromide excreted during the time of exposure must also be considered. To do this the half-life ($t_{1/2}$) of plasma inorganic bromide was determined in the male Fischer-344 rat.

Seven groups, each containing three rats, were dosed ip with 2.6 mmol of sodium bromide/kg. These rats were killed at 2, 4, 8, 24, 48, 72, and 168 hr. Plasma samples were obtained and analyzed for plasma bromide. The results, expressed as ΔBr^- (mM), were plotted on a semilogarithmic scale versus time. The $t_{1/2}$ was 67 ± 9 hr (2.8 ± 0.4 days), which is in agreement with an estimate of 2.9 days reported by Gehring and Young (1977) in a study of the pharmacokinetics of 2,2-dibromo-3-nitrilopropionamide.

In determining rates of bromide production, it was assumed that blood concentrations of these vapors (and active site concentrations at metabolizing organs) reached steady-state levels very rapidly with respect to duration of exposure and that the rate of metabolism was essentially constant during the exposure and was given by k_m times the steady-state blood concentration of vapor, C_2. By reference to Fig. 1, the concentration of bromide at the termination of an exposure is

[7] DuPont Co., Instrument Products Division, Newtown, Conn.

[8] Orion Research, Inc., Cambridge, Mass.

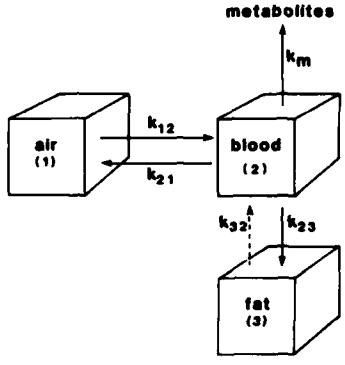

FIG. 1. Schematic of a three-compartment pharmacokinetic model of inhalant metabolism. The compartments are chamber air, the central blood compartment, and the deep, nonmetabolizing, fat compartment. (The dashed line represents the negligible contribution of k_{32} as compared to the other rate constants.)

$$C_{\text{observed}} = k_m C_2 t, \tag{1}$$

where t is the duration of the exposure. A more thorough description would take into consideration the amount of bromide lost by excretion during exposure and would have had the relationship

$$C_{\text{calculated}} = \frac{k_m C_2 (1 - e^{-kt})}{k}, \tag{2}$$

where k is the first-order rate constant for elimination of bromide from the rat. This is the equation for constant input and first-order excretion (Goldstein *et al.*, 1974).

The percentage error due to the assumption that no excretion has occurred is approximated by

$$\% \text{ error} = \left[1 - \frac{C_{\text{calculated}}}{C_{\text{observed}}}\right] \times 100$$

$$= \left[1 - \frac{(1 - e^{-kt})}{kt}\right] \times 100. \tag{3}$$

It depends on the first-order rate constant and is independent of $k_m C_2$. Since exposures with VB were the longest (6 hr), the percentage error associated with this chemical was the largest. But even for VB the calculated percentage error in concentration of inorganic bromide is only expected to be 3.0%. Since the predicted error is small, no attempt was made to include these corrections in the rate determinations.

Kinetic Constants

A simplified compartmental analysis of gas uptake (Fig. 1) was previously used to obtain a rate equation for disappearance of chemical from the gas phase (Andersen *et al.*, 1980). The rate equation derived referred to a condition where there was no net change in the amount of chemical in the central compartment (2), and the fat compartment (3) was storing chemical but releasing negligible amounts back to the central compartment. Filser and Bolt (1981) have provided a more detailed pharmacokinetic analysis of the gas uptake system in which test animals were regarded as a one-compartment system. In their approach, the concentration in the central compartment is rightly recognized as declining throughout the experimental period. With the general approach of Filser and Bolt (1981), a clearance equation can be derived for the two-compartment model. The applicable relationships are

$$\frac{-V_1 dC_1}{dt} = Cl_{12}C_1 - Cl_{21}C_2, \tag{4}$$

$$\frac{+V_2 dC_2}{dt}$$

$$= Cl_{12}C_1 - (Cl_{21} + Cl_m + Cl_{23})C_2 + Cl_{32}C_3. \tag{5}$$

With the assumption that $Cl_{32}C_3$ is negligible, these equations can be combined to yield

$$\frac{-V_1 dC_1}{dt} = \frac{V_2(dC_2/dt)Cl_{21} + (Cl_{23} + Cl_m)Cl_{12}C_1}{Cl_{21} + Cl_m + Cl_{23}}. \tag{6}$$

Here, C_x and V_x are, respectively, the concentration of chemical in compartment x and the volume of the compartment. Cl_{xy} is clearance from compartment x to compartment y, and Cl_m is metabolic clearance from the central compartment.

When clearance is primarily by exhalation ($Cl_{21} \gg Cl_m + Cl_{23}$), Eq. (4) reduces to

$$\frac{-V_1 dC_1}{dt} = \frac{V_2 dC_2}{dt} + \frac{Cl_{12}(Cl_m + Cl_{23})C_1}{Cl_{21}}. \tag{7}$$

The saturable portion under this condition will be related to metabolism and, in general, will have a Michaelis–Menten-type dependence on inhaled concentration.

Conversely, when clearance is primarily by metabolism ($Cl_m \gg Cl_{21} + Cl_{23}$), it becomes:

$$-V_1 \frac{dC_1}{dt} \simeq Cl_{12}(C_1) \tag{8}$$

In this latter case respiration, Cl_{12}, is the most important parameter in determining the rate of disappearance of gas from the chamber.

It has been pointed out that metabolic pathways of a wide variety of inhaled gases and vapors are, indeed, limited by respiration, and this includes the vapors studied here (Andersen, 1981b). This kinetic behavior is expected when the affinity of the metabolizing enzyme for substrate is high and perfusion of the metabolizing organ becomes rate limiting for metabolism *in vivo*. In this case the apparent K_m calculated for the metabolic reaction *in vivo* overestimates the actual K_m of the enzyme

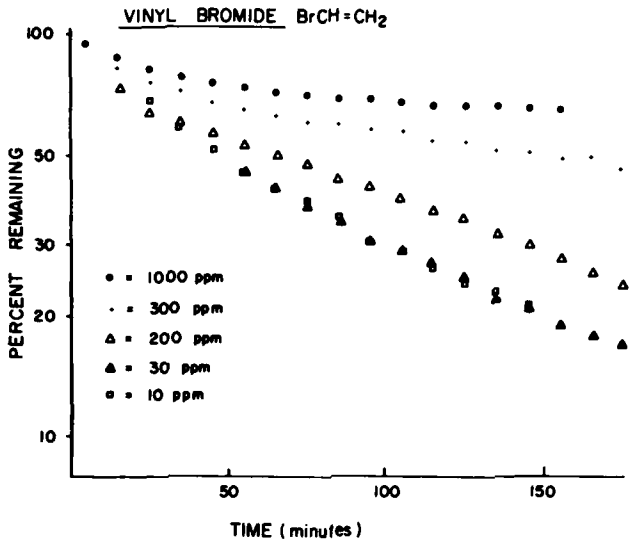

FIG. 2. Uptake of vinyl bromide by male rats at five initial concentrations. Plotted data were derived from corrected uptake curves and expressed as percentage initial concentration remaining at each sampling time. Nine rats were used in each exposure and the plot is semilogarithmic.

system. For this paper we have fitted both gas uptake and bromide production data assuming a straightforward Michaelis–Menten (M–M) dependence. The limitations of the M–M equation in this regard have been amply documented (Andersen, 1979, 1981a, 1981b), but no other simple analysis of the *in vivo* rate curves can be used at present. Our choice of the M–M model should be regarded as a convenience to allow comparison of the curves obtained by the two, independent kinetic methods. It is not meant to imply that the M–M equation is an accurate description of the shape of these rate curves or that the hybrid constant calculated is an accurate estimate of the molecular K_m of the enzyme systems. In fact, it has been found that rate curves for many inhaled vapors are accurately first order, before abruptly assuming a pseudo-zero-order dependence (Filser and Bolt, 1979). The theoretical basis for this dependence was demonstrated by Andersen (1981b).

When metabolic clearance is large with respect to exhalation, the arterial blood:inhaled air concentration ratio will be lower than that predicted by the partition coefficient. A relationship for the steady-state blood:air concentration ratio based on physiological variables has been derived (Andersen, 1981b, Eq. 9, Appendix I). This ratio was called the effective partition coefficient, N_{eff}:

$$\frac{C_{art}}{C_{inh}} = N_{eff} = \frac{\dot{V}_{alv}N}{\dot{V}_{alv} + E_t\dot{Q}_tN}. \quad (9)$$

In this equation, N is the blood:air partition coefficient, equivalent to the ratio of arterial blood concentration to end alveolar concentration, $\dot{V}_{alv}$ and $\dot{Q}_t$ are alveolar ventilation and cardiac output, respectively, and E_t is the systemic extraction ratio. This latter term is the ratio of the difference in concentration of chemical in arterial blood minus its concentration in mixed venous blood divided by the arterial concentration.

For chemicals metabolized predominantly by the liver, the maximum value of E_t is 0.25 because liver blood flow is 25% of cardiac output. This limiting behavior is observed when all the chemical presented to the liver is removed by the organ and hepatic perfusion is rate limiting for metabolism. The metabolism of each of the four chemicals used in this study appears to be limited by hepatic perfusion at low concentrations (Andersen, 1981a, 1981b). Blood:air partition coefficients (N) for the four chemicals were determined by a vial-equilibration technique (Sato and Nakajima, 1979). For VB, HAL, BCM, and MB, N values (± standard error of the mean) were 5.41 ± 0.47, 3.99 ± 0.40, 48.18 ± 2.40, and 98.60 ± 9.48, respectively (n = 4). N_{eff} values were then calculated from Eq. (9), where $\dot{V}_{alv}$ and $\dot{Q}_t$ were assumed to be equal, and E_t was set to 0.25. Calculated N_{eff} values for VB, HAL, BCM, and MB were, respectively, 2.30, 2.00, 3.69, and 3.84.

Because the volume of distribution of the gas includes both the chamber and the rats in the chamber, calculation of the maximum velocity of uptake must account for loss in both compartments (Bolt *et al.*, 1977; Filser and Bolt, 1979; 1981). Thus, in calculating V_{max} we have

$$V_{max} = \text{observed loss } [31 + N_{eff} \cdot \text{b. wt.}]/\text{b. wt.} \quad (10)$$

Observed loss is the maximum disappearance from

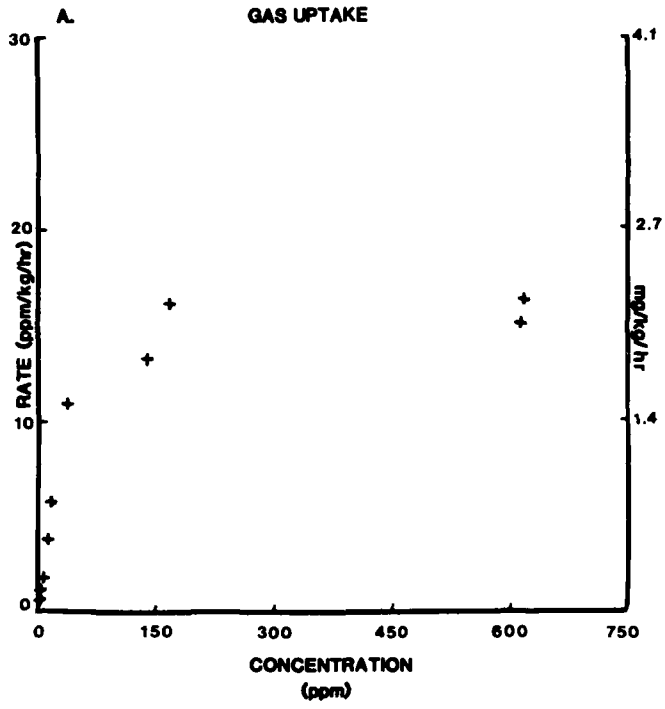

FIG. 3A. Dependence of the rate of uptake of vinyl bromide by male rats on the ambient concentration in a closed, recirculated atmosphere. The concentrations (x axis) were those at which the rate was calculated following equilibration (~70 to 110 min) and not the initial concentration of the exposure.

the chamber in milligrams per liter per hour; 31 is the chamber volume in liters; and b. wt. is the weight of rats in the chamber in kilograms. It is assumed here that each kilogram of rat occupies about 1 liter of volume. This correction is necessary since a decrease in chamber concentration is accompanied by a decrease in concentration in the tissues of the exposed animals. The (N_{eff} · b. wt.) term of this equation then in analogous to the V_2 (dC_2/dt) term of Eq. (7). After the region of perfusion limitation is exceeded, the effective partition coefficient will increase. But at the low concentrations and near the transition from first-order to zero-order behavior, the limiting value of this parameter, N_{eff}, should prevail and for this reason has been used for these present calculations. Units of V_{max} then are milligrams per kilogram per hour.

RESULTS

Vinyl Bromide

In gas uptake studies, rats in groups of nine were exposed to 10 initial concentrations of VB ranging from 10 to 1000 ppm. The rate constant of the nonspecific loss of VB from an unoccupied chamber varied between 0.074 and 0.092 hr^{-1}. Corrected curves with animals were biphasic, containing both a fast equilibrium phase and a slower metabolic phase (Fig. 2). As the concentration increased, the rate constant of the slow phase decreased. The slow phase uptake rate was plotted versus concentration (Fig. 3A), and the overall curve was represented by a saturable process consistent with a Michaelis–Menten dependence. By linearizing (r^2 = 0.996) the data with a modified Eadie–Hofstee plot (Laidler and Bunting, 1973), the maximum rate of metabolism (V_{max}) and the apparent inhalation Michaelis constant (K_m) were estimated (Table 1).

For the bromide production studies with VB, nine separate concentrations were utilized ranging from 24 to 9603 ppm. End exposure plasma inorganic bromide levels were determined, and the rates of production (ΔBr^- and mg/kg/hr) were plotted against concentration (Fig. 3B). This data set ap-

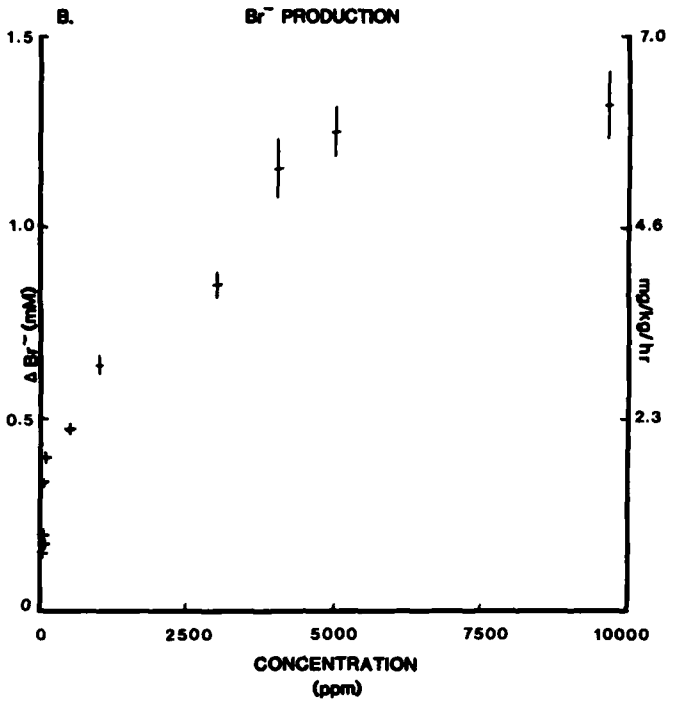

FIG. 3B. Dependence of the rate of production of plasma inorganic bromide on the ambient concentration of vinyl bromide following a 6-hr exposure. Three to six rats were used in each exposure and data are $\bar{x} \pm$ SE which varied between 1.9 and 8.0% of the means. The size of the standard error about each point is indicated by the length of the vertical line associated with the data point. The scales for the x and y axes for the plots in A and B are not identical. Different units were maintained to show the full range of concentrations used in these studies and to accentuate the differences in shape of the pairs of rate curves. This difference is also true for Figs. 5A and B. The concentrations (x axis) were the average concentrations calculated for each constant exposure.

peared to be a composite of two saturable reactions. The first reaction was saturated at concentrations well below 100 ppm. The data points for the first reaction were linearized ($r^2 = 0.998$), and the best fit constants were determined (Table 1). The kinetic constants for the second saturable component were obtained by subtracting the contribution of the first reaction from the composite curve. Corrected points were linearized ($r^2 = 0.515$), and the best fit constants were calculated (Table 1).

Halothane

For the gas uptake studies of HAL, the concentrations used ranged from 30 to 3000 ppm, and the nonspecific loss rate of HAL in an empty chamber varied between 0.087 and 0.100 hr^{-1}. The corrected curves were biphasic, and the percentage remaining plots were similar to those obtained with VB. The rate curve (Fig. 4A) was best represented as having a complex dependence, containing contributions from both a saturable and a first-order component. An unweighted least-squares line ($r^2 = 0.954$) was fitted from the points obtained above 200 ppm. By this technique, the first-order rate constant was estimated to be 0.058 kg^{-1} hr^{-1}. The contribution of this first-order process was calculated at each point and subtracted from the observed rate. The corrected rates were linearized ($r^2 = 0.972$) to estimate kinetic constants for the saturable component (Table 1). For bromide studies, rats were exposed to

TABLE 1

COMPARISON OF THE KINETIC CONSTANTS OBTAINED BY THE TWO TECHNIQUES

Chemical	Technique	K_m[a] (ppm)	V_{max}[a] (mg/kg/hr)	k'[e] (kg^{-1} hr^{-1})
Vinyl bromide[b]	Gas uptake	I— 18 II—(N/O)[c]	2.4 (N/O)	—[d]
	Bromide release	I— 33 II—11,700	2.3 9.3	—[d]
Halothane	Gas uptake	81	10.0	0.058
	Bromide release	73	9.7	(N/O)
Bromochloromethane	Gas uptake	91	10.5	0.106
	Bromide release	79	10.0	0.085
Methyl bromide	Gas uptake	—[d]	—[d]	0.55
	Bromide release	—[d]	—[d]	0.32

[a] Small differences in K_m and V_{max} values in this work and in results reported earlier (Andersen *et al.*, 1980; Gargas and Andersen, 1979) are due to two factors; incorporation of the rat volume in V_{max} determinations by gas uptake (Eq. (9)), and addition of extra data points for this study in order to better define the overall shapes of each of the rate curves.

[b] With vinyl bromide the two saturable steps observed by metabolite determination are designated I and II.

[c] (N/O) designates that this process, observed by the alternative technique, was not observed in these experiments.

[d] No kinetic process with this form was observed by either technique for this particular chemical.

[e] The observed first-order rate constant.

nine separate concentrations of HAL ranging from 25 to 1666 ppm. The rate curve (Fig. 4B) was described by a single saturable process with no apparent contribution from a first-order process (Table 1).

Bromochloromethane

For the gas uptake studies, animals were exposed to nine separate initial concentrations of BCM ranging from 100 to 10,000 ppm. The nonspecific loss rate varied between 0.030 and 0.076 hr^{-1}. The rate plot (Fig. 5A) was a composite, with contributions from a saturable and an apparently first-order process. An unweighted least-squares line ($r^2 = 0.945$) was fitted from the points obtained above 500 ppm, giving a first-order rate constant of 0.106 kg^{-1} hr^{-1}. After the rate curve was corrected for the contribution of the first-order component, the kinetic constants for the saturable phase were determined (Table 1).

For bromide production, rats were exposed to seven concentrations ranging from 100 to 4844 ppm. The bromide rate plot (Fig. 5B) was also a composite of a saturable and a first-order phase. The first-order rate constant ($r^2 = 0.994$) was 0.085 kg^{-1} hr^{-1}, and the contribution of this first-order process was subtracted from the total curve to allow analysis of the saturable component (Table 1).

Methyl Bromide

For the gas uptake studies of MB, rats were exposed to initial concentrations of 100, 1000, and 3000 ppm, and the bromide production studies were conducted at 197, 496, and 924 ppm. The rate plots of MB metabolism for both methods were adequately described by a single, rapid first-order process. (The rate plot for gas uptake of MB has been published previously in Andersen *et al.*, 1980.) Nonweighted least-squares lines were

constructed through the points and gave first-order rate constants of 0.55 kg^{-1} hr^{-1} (r^2 = 0.985) and 0.32 kg^{-1} hr^{-1} (r^2 = 0.999) for gas uptake and bromide production, respectively.

DISCUSSION

On comparing the kinetic constants of metabolism obtained by these two methods, we found excellent agreement for two of the chemicals, MB and BCM (Table 1). In the concentration range examined, the *in vivo* metabolism of inhaled MB was first order with respect to inhaled MB concentration. This observation does not necessarily indicate a nonenzymatic pathway of MB biotransformation. An enzymatically catalyzed reaction will appear first order if observations are restricted to concentrations below K_m. Of the four chemicals used, MB was the most acutely toxic. To avoid overt toxicity from either MB itself or bromide, we restricted exposure duration to 2 hr and concentrations to several thousand ppm and below. These concentrations may be well below the inhalation K_m in which case saturation of the pathway would not be expected. The low-affinity pathway observed for vinyl bromide had an inhalation K_m of greater than 5000 ppm.

Two distinct activation reactions were observed for the *in vivo* metabolism of the dihalomethane, BCM. There was a high-affinity, saturable pathway, which predominated at low concentrations, and a second pathway which appeared first order at all BCM concentrations investigated. These two processes were identified by both gas uptake and metabolite determination. Because of the different kinetic characteristics of the two pathways, their relative contribution to the total amount metabolized will vary with exposure concentration. In the region of exposure concentration where the high-affinity reaction is saturated, the proportion of metabolite formed by the first-order pathway will increase with increasing concentration.

Two independent pathways of dihalomethane metabolism have also been described *in vitro*. There is an oxidative reaction catalyzed by enzymes of the endoplasmic reticulum and a second reaction which requires glutathione (GSH) and is catalyzed by a soluble cytoplasmic enzyme, probably one of the glutathione-*S*-transferases (Anders *et al.*, 1977). The former sequence yields carbon monoxide (CO) as an end product; the latter yields carbon dioxide. Rodkey and Collison (1977) and McKenna *et al.* (1979) have presented evidence indicating that the microsomal oxidative pathway is saturated at relatively low concentrations of the dihalomethanes. On the basis of available evidence then, the high-affinity pathway appears to be that sequence which yields CO (see Andersen, 1981a). Inasmuch as elevated HbCO levels are associated with performance decrements (Stewart, 1975; Putz *et al.*, 1979) and limits of exposure to dihalomethanes, like methylene chloride, are set to control increased HbCO concentrations, knowledge of the kinetic parameters of the high-affinity pathway is essential for evaluating hazards from impaired behavior. The ability to distinguish independent bioactivation pathways is vital to hazard assessment for a variety of inhaled chemicals.

The first-order process observed for BCM metabolism is likely an enzymatic reaction being observed at concentrations below K_m. In this instance both the pathways of reactions studied *in vitro* are known to be enzymatic. The apparently first-order process is most likely the GSH pathway involving glutathione-*S*-transferase. In contrast to the oxidative pathway, this biotransformation reaction has a much lower affinity for the dihalomethane substrate.

Gas uptake methods identified two distinct kinetic processes for HAL utilization. Only one of these—the high affinity, saturable pathway—was detected by bromide determination. The slow first-order process in the gas uptake rate curve may be either a metabolic step that does not release bromide or

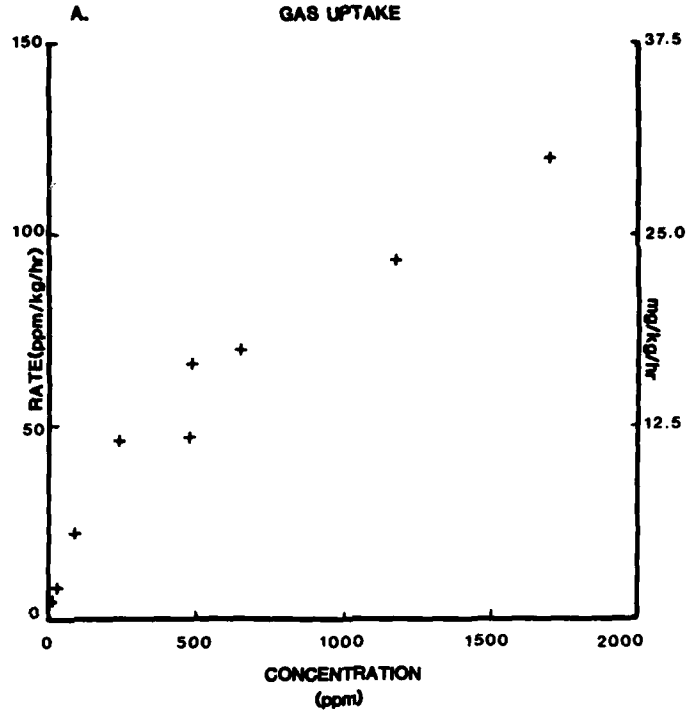

FIG. 4A. Dependence of the rate of uptake of halothane on its ambient concentration in a closed, recirculated atmosphere. The concentrations (x axis) were those at which the rate was calculated following equilibration (~70 to 110 min) and not the initial concentration of the exposure.

a nonmetabolic process indicative of continued accumulation of HAL in body tissues even after 2 hr of exposure. The former explanation is unlikely since all the major routes of HAL metabolism are associated with loss of bromide from the parent anesthetic material (Van Stee, 1976; Holaday, 1977).

The rate of bromide release from HAL decreased at very high concentrations, i.e., near 1500 ppm (Fig. 4B). Rats at these higher HAL exposure concentrations were visibly effected: they were noticeably less active and responded sluggishly to external stimuli. Decreased metabolism may be related to decreased alveolar ventilation, decreased hepatic blood flow (i.e., flow to the metabolizing organ), or some combination of the two. This phenomenon deserves more detailed study since the situation of clinical concern involves rates of HAL metabolism under anesthetic conditions.

The discrepancies between the two techniques for VB were the reverse of those observed for HAL. Endpoint bromide determinations indicated two processes, both of which were saturable. Gas uptake accurately predicted only the high-affinity pathway, but yielded no indication of a second, low-affinity process. The difficulty encountered here was the requirement to measure loss of very small amounts of VB from the gas phase at relatively high-exposure concentrations. (The estimated V_{max} of the low-affinity process is 9.3 mg/kg/hr.) Measurements of low rates of uptake at high concentrations are especially difficult when the system used, as is true with ours, has an appreciable nonspecific chamber loss rate. When the rate constant for nonspecific loss is substantial with respect to that

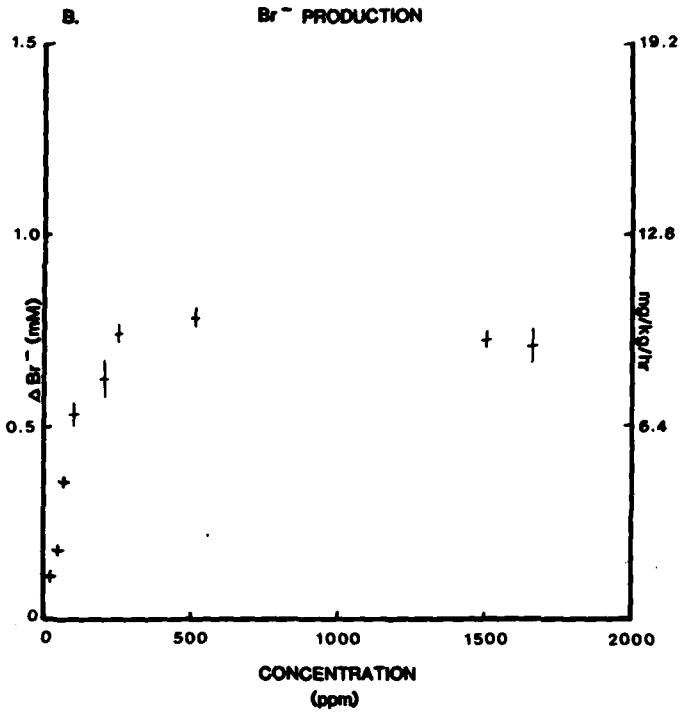

FIG. 4B. Dependence of the rate of production of plasma inorganic bromide on the ambient concentration of halothane following a 4-hr exposure. Data are mean values of plasma bromide. Standard errors of the mean varied between 2.6 and 11.2%. The concentrations (*x* axis) were the average concentrations calculated for each constant exposure.

for metabolism, it becomes difficult to distinguish the two. In this way low-affinity, low-capacity pathways will be difficult to evaluate by gas uptake techniques. Gas uptake methods work best for high-affinity pathways with a high V_{max}, and moderately well for high-affinity/low-capacity and low-affinity/high-capacity pathways. Other workers have used gas uptake systems without recirculation (Bolt *et al.*, 1977; Filser and Bolt, 1979, 1981). These designs have carbon dioxide and water adsorbents in the chamber and rely on manual sampling of the atmosphere. They have a lower intrinsic loss rate and, therefore, a better sensitivity for low-capacity, low-affinity pathways. Use of these systems might allow examination of the second VB pathway by gas uptake.

As with BCM, it is likely that the two pathways of VB metabolism *in vivo* are related to oxidative (high affinity) and conjugative (low affinity) reactions. Our estimate of the K_m of the high-affinity pathway agrees well with the estimate of the *in vitro* K_m of VB metabolism by a microsomal system (Bolt *et al.*, 1978). Preliminary evidence indicates that the yield of the low-affinity reaction *in vivo* can be diminished by pretreating rats with reagents that deplete hepatic GSH (unpublished data). The potential importance of these two pathways in the acute and chronic toxicity of VB in experimental animals has been summarized (Andersen, 1981a).

The good agreement between kinetic constants determined by these two methods implies that systemic metabolism is responsible for the vast majority of uptake from the closed chamber. In terms of metabolism of

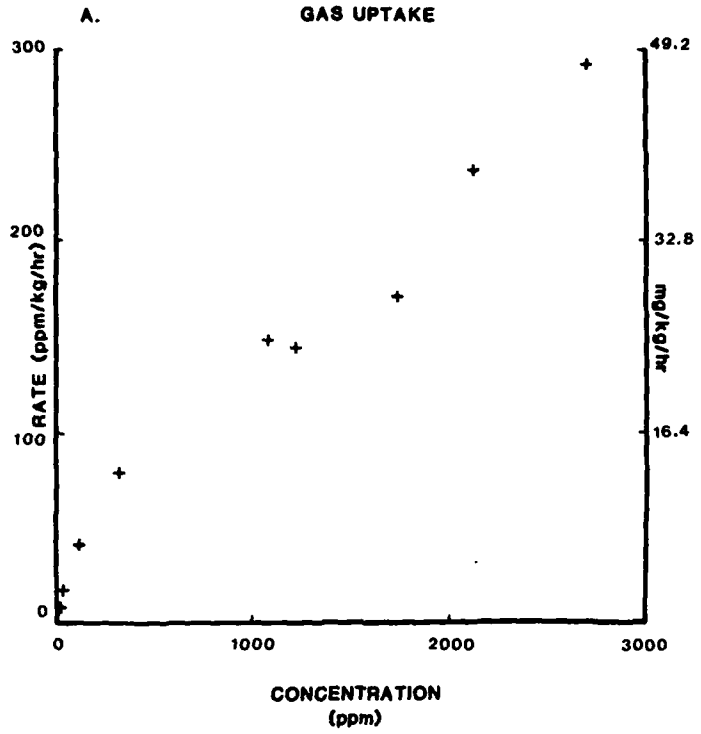

FIG. 5A. Dependence of the rate of uptake of bromochloromethane on its ambient concentration in a closed, recirculated atmosphere. The concentrations (x axis) were those at which the rate was calculated following equilibration (~70 to 110 min) and not the initial concentration of the exposure.

these four brominated hydrocarbons, this finding means that bromide release must either be involved in the rate-limiting step for vapor uptake or in a rapid step following that which is rate limiting. Only with HAL does the possibility exist that there is a metabolic reaction with significant capacity which is not associated with release of inorganic bromide. The physiologic basis of the continued uptake of HAL at higher concentrations, i.e., metabolism or tissue storage, could be evaluated in gas uptake experiments by exposing animals for various times before estimating rates of uptake. Finally, the close agreement of V_{max} values indicates that the choice of a limiting value for N_{eff} was probably valid. Nonetheless, future experiments in this area could be greatly facilitated by direct determination of vapor concentration in blood at selected points during the gas uptake studies. This information would enable unequivocal determination of effective partition coefficients for these calculations.

In summary, we have evaluated the *in vivo* metabolism of four brominated hydrocarbons by two distinct experimental methods. The kinetic constants for metabolism determined by the two methods agreed very well. The two instances of disagreement, with HAL and VB, illuminated the essential differences between the two methods and caution against overzealous interpretation of low-affinity constants derived by gas uptake. They may be due to fat storage and not metabolism. When convenient techniques exist for endpoint metabolite analysis, they are naturally preferred and produce unambigous definition of kinetic constants. The issues

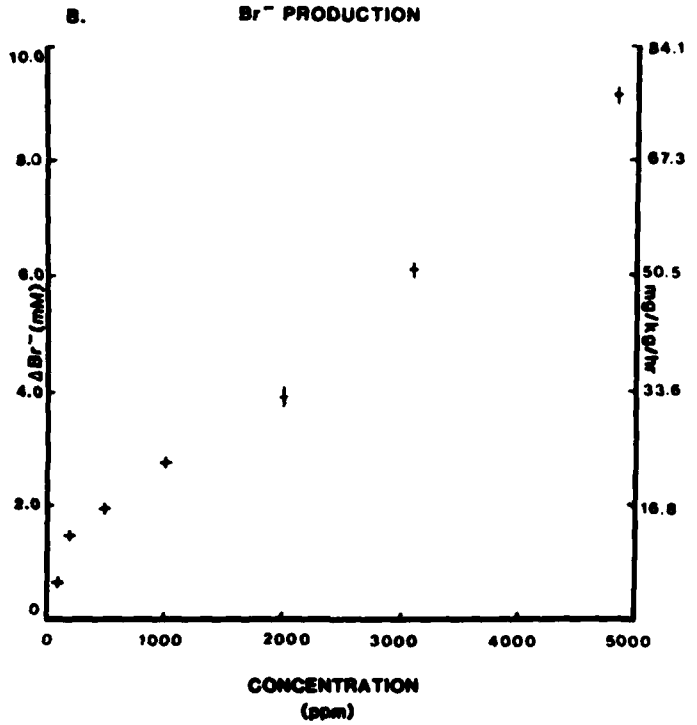

FIG. 5B. Dependence of the rate of production of plasma inorganic bromide on the ambient concentration of bromochloromethane following a 4-hr exposure. Data are mean values of plasma bromide. Standard errors of the mean varied between 1.5 and 8.4%. The concentrations (x axis) were the average concentrations calculated for each constant exposure.

here were to show that gas uptake reflects metabolism and to validate the gas uptake technique.

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