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Uptake and Distribution of ^{14}C during and following Exposure to [^{14}C]Methyl Isocyanate

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Uptake and Distribution of ^{14}C during and following Exposure to [^{14}C]Methyl Isocyanate. FERGUSON, J. S., KENNEDY, A. L., STOCK, M. F., BROWN, W. E., AND ALARIE, Y. *Toxicol. Appl. Pharmacol.* **94**, 104–117. Guinea pigs were exposed to [^{14}C]methyl isocyanate ($^{14}CH_3$-NCO, ^{14}C MIC) for periods of 1 to 6 hr at concentrations of 0.5 to 15 ppm. Arterial blood samples taken during exposure revealed immediate and rapid uptake of ^{14}C. Clearance of ^{14}C was then gradual over a period of 3 days. Similarly ^{14}C was present in urine and bile immediately following exposure, and clearance paralleled that observed in blood. Guinea pigs fitted with a tracheal cannula and exposed while under anesthesia showed a reduced ^{14}C uptake in blood indicating that most of the ^{14}C MIC uptake in normal guinea pigs occurred from retention of this agent in the upper respiratory tract passages. In exposed guinea pigs ^{14}C was distributed to all examined tissues. In pregnant female mice similarly exposed to ^{14}C MIC, ^{14}C was observed in all tissues examined following exposure including the uterus, placenta, and fetus. While the form of ^{14}C distributed in blood and tissues has not yet been identified, these findings may help to explain the toxicity of MIC or MIC reaction products on organs other than the respiratory tract, as noted by several investigators. © 1988 Academic Press, Inc.

Following the incident which occurred in Bhopal, India on December 3, 1984, several investigators have studied the toxicity of the major chemical released at that site, methyl isocyanate (MIC). The early findings revealed the potency of this substance as an irritant to the respiratory tract (Ferguson *et al.*, 1986; Nemery *et al.*, 1985, Alarie *et al.*, 1987; James *et al.*, 1987). However, following a single exposure to MIC, toxicity was observed in bone marrow cells (Conner *et al.*, 1987) and an adverse effect on fertility of both male and female mice was demonstrated (Varma *et al.*, 1987). Although the possibility exists that these effects were induced as a result of the general stress induced following irritation of the respiratory tract, it is equally possible that MIC was systemically absorbed and distributed as MIC or in a irreversibly conjugated form. Recent experiments with another isocyanate, toluene diisocyanate, have shown that this chemical is circulating in blood following inhalation exposure, irreversibly conjugated to a protein of 77 kDa (Hill, 1986). Since isocyanates also react reversibly with SH groups in proteins (Brown *et al.*, 1987), it is further possible that following absorption in blood the methyl isocyanate group attached to plasma proteins can be transferred to proteins at target cells far removed from the lungs. Therefore, we have undertaken a series of experiments to analyze the uptake and *in vivo* fate of inhaled ^{14}C MIC prior to determination of the conjugates or metabolites circulating in blood following exposure to this chemical.

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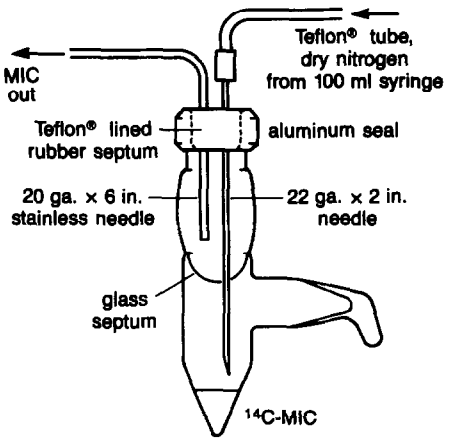

FIG. 1. Vial containing ^{14}C MIC or MIC used to generate the desired exposure concentration. The glass septum was broken prior to experiments by the stainless-steel needle and dry nitrogen was used to push out MIC vapor via a second needle. This vial was connected to the system shown in Fig. 2.

MATERIALS AND METHODS

Chemicals

Methyl isocyanate (MIC) was purchased in 25-g bottles (Lot 3317AL) from the Aldrich Chemical Company (Milwaukee, WI) and was used as received. ^{14}C MIC (8.78 mCi/mmol) was synthesized by New England Nuclear (Boston, MA) with the ^{14}C label on the methyl group ($^{14}CH_3NCO$). Gas chromatographic analysis provided by NEN showed the radiochemical to be 99% pure. It was shipped in a glass vial (see Fig. 1) within a few days of synthesis and used on the day it was received. "Cold" MIC was also received from the same source in similar glass vials and was used to set up the proper parameters for exposures using the system described in Fig. 2 prior to the ^{14}C MIC experiments.

Animals

Male, English short-haired guinea pigs weighing 300–350 g were obtained from Hilltop Lab Animals (Scottdale, PA). They were held in an animal room for at least 7 days prior to exposure with food and water provided *ad libitum* and on a 12-hr light/dark cycle. Just prior to exposure, 0.3 ml of blood was withdrawn from cannulated animals via the carotid artery and from noncannulated animals via the nail bed. Blood samples were placed in Vacutainer tubes (BD) with buffered sodium citrate and stored at 4°C.

Male and female SPF Swiss-Webster mice weighing 20–25 g were purchased from Hilltop Lab Animals and also kept in an animal room with food and water provided *ad libitum* and on a 12-hr light/dark cycle. Day 0 of gestation in females was determined by the presence of vaginal plugs in the morning following a night of cohabitation with male mice. Body weight gains and abdominal palpitation were used to confirm the pregnancies of vaginal plug-positive animals. Pregnant females were exposed to ^{14}C MIC at Days 8–10 of gestation.

Carotid Artery Cannulation

Several guinea pigs were exposed (see below) following implantation of a carotid cannula which permitted blood samples to be obtained during and following exposure. These were prepared as follows. A 26-cm length of PE 10 Intramedic polyethlene tubing was used. The PE tubing was passed through a 1-mm hole of a plastic hub (the removable piece from a new plastic syringe) and one end was attached by a 30-gauge needle to a 1-ml syringe containing heparinized (1 unit/ml) Lactated Ringers solution (Abbott Laboratories). This length permitted the cannula to be fixed on the neck of the animal and to pass through a port of the exposure chamber (see Fig. 2) for blood sampling during exposure. The cannula was implanted following anesthesia with injection of both Acepromazine maleate (10 mg/ml, Aveco Co., Fort Dodge, IA) and Ketamine HCl (100 mg/ml, Parke Davis, Morris Plains, NJ) at a dose of 0.08 ml/100 g of body weight given sc. An sc injection of 2% Lidocaine HCl (20 mg/ml, Astra Pharmaceutical, Worcester, MA) was given in both the nape and the ventral midline of the neck to supplement the general anesthetics. Approximately 0.1 ml was used at each site. The animals were then shaved around the front and back areas of the neck. A 10-mm incision was made on the dorsal side of the animal near the left nape area, and a 15-mm incision was made in the ventral midline of the neck. The unattached end of the PE tubing, filled with Lactated Ringers solution, was then pulled under the skin from the dorsal side to the ventral side and looped to the right midline to prevent blocking by an unusual angle. The right common carotid artery was then located and lifted by a 20-mm length of paper support covered with latex dental dam. Two 4-0 silk threads (120 mm in length) were also placed under the artery; one above and one below the support. The top silk thread was loosely tied around the PE tubing and the artery, anchoring the PE tubing to prevent it from sliding inside the neck. A minute incision was then made with the tip of a fine pair of scissors and the PE tubing was pushed through this opening into the artery. A small drop of Superglue was then placed on the artery just behind the cannula and the tubing was pressed against the outside of the artery; this closed the incision and glued the cannula to the artery. Lactated Ringers solution was then injected to push the blood into the artery and to check the patency of the cannula. The second thread was then loosely tied around the tubing and the artery near the

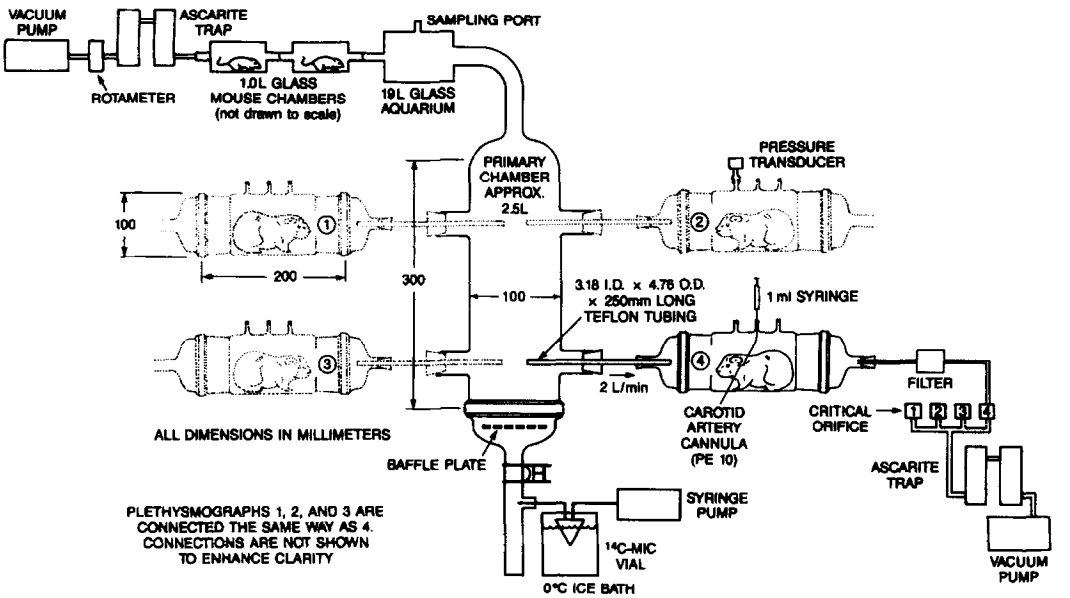

FIG. 2. System for exposure of guinea pigs and mice to ^{14}C MIC. For analysis of exposure concentrations samples were taken in succession from the guinea pig exposure chambers as well as from the mice exposure chambers when used. Modified from Alarie *et al.* (1987).

incision to hold the two together but not tight enough to obstruct blood from flowing past the cannula. The paper–latex support was then removed and the cannula loop was pushed inside the neck pocket. The ventral incision was sutured and the animal turned over. The plastic hub was glued (Superglue) under the skin of the nape to prevent crimping of the cannula as the animals moved in their cage. Following this procedure it was ascertained that the cannula was working properly, the syringe was removed, and the end of the tubing was blocked with clay. The tubing was then looped and taped near the plastic hub so that the animal could not pull it. Each animal was placed in a separate cage and used for exposure 1 or 2 days following the procedure.

Tracheostomy, Arterial and Venous Cannulas

This procedure was used with only two guinea pigs and both were exposed while still anesthetized. Following anesthesia, as given above, an incision was made in the ventral midline of the neck. The trachea was isolated and an incision made. A 35-mm length of PE 350 tubing was inserted and anchored. The right common carotid artery was then located and fitted with a PE 10 tubing as described above but without passing it first through the dorsal side. The right exterior jugular vein was also cannulated in a similar manner using PE-50 tubing and inserting it to reach the superior vena cava to obtain mixed venous blood. The cannula was attached to a 23-gauge needle and a 1-ml syringe. Both arterial and venous cannulas were passed through a plastic syringe hub which was then glued (Superglue) to the adjacent skin. The tracheal cannula was then slightly elevated with a small piece of gauze to place it out of the animal fur. Thus fitted and still under anesthesia, each animal was placed on its back in a glass chamber (see Fig. 2) and the exposure to ^{14}C MIC was carried out for 1 hr while the animal remained anesthetized.

Exposure System

Four series of experiments were performed using an exposure system similar to that described by Alarie *et al.* (1987) with modifications as shown in Fig. 2. For each series the system was designed to maximize the use of available ^{14}C MIC and to accommodate the number of animals involved. Except for chamber configurations, the exposure system was kept uniform throughout. The designated chambers were placed in line between the outlet of the central chamber and the Ascarite traps which were used to retain all of the MIC vapor passing through the system. To generate the ^{14}C MIC vapor, a 100-ml glass syringe filled with dry nitrogen was mounted on a Harvard apparatus infusion pump and connected to the ^{14}C MIC vial maintained in an ice bath as shown in Figs. 1 and 2. To initiate the experiments, the glass septum of the vial was broken with a sturdy needle following piercing of the rubber septum. A 22 gauge × 50 mm steel needle was passed through the Teflon-coated rubber septum and attached to the 100-ml syringe via a 130-mm Teflon

TABLE I

SERIES I: EXPOSURES OF GUINEA PIGS WITH OR WITHOUT CAROTID ARTERY CANNULA AND MICE TO $^{14}CH_3NCO$ FOR 1 hr

Animal no.	Cannulated (C) or noncannulated (NC)	Times (from start of exposure) at which blood samples were taken during 1-hr exposure to ^{14}C MIC (hr)	Average exposure concentration (ppm)	Time (from start of exposure) of euthanasia and terminal blood sample (hr)
Male guinea pigs				
89	C	0 0.48	5.02	2.75
91	C	0 0.33 1.28	5.02	18.00[a]
92	C	0 0.15 0.82 1.17 1.93 3.05 4.48 5.00 5.57	5.02	5.57
94	C	0 0.10 0.65 1.07 1.52 2.57 3.60	5.02	3.62
97	NC	0	2.10	1.68[b]
98	NC	0	2.10	48.0
99	NC	0	2.10	2.17
100	NC	0	2.10	1.72
101	NC	0	2.10	168.00
Swiss-Webster mice				
Male				
0 1	NC	No. blood samples were taken prior to or during the exposure	2.10	1.50
0 2	NC		2.10	1.75
0 3	NC		2.10	2.00
0 4	NC		2.10	2.25
0 5	NC		2.10	23.00
0 6	NC		2.10	23.25
0 7	NC		2.10	23.50
0 8	NC		2.10	23.75
Pregnant females				
0 1	NC		2.10	2.50
0 2	NC		2.10	2.75
0 3[c]	NC		2.10	3.00
0 4	NC		2.10	24.00
0 5	NC		2.10	24.25
0 6	NC		2.10	24.50
Althymic nude mice				
0 1	NC		2.10	1.17
0 2	NC		2.10	1.33

[a] Animal died 18 hr postexposure at which time blood was obtained and autopsy performed.
[b] Whole animal was frozen in liquid nitrogen, no blood was taken.
[c] Found nonpregnant at autopsy.

tube for delivery of dry nitrogen to the vial. Delivery rates of dry nitrogen varied between 0.028 and 14 ml/min depending on the exposure condition desired. A 20 gauge × 150 mm steel needle also penetrated the septum to deliver the MIC vapor to the exposure system. Cold MIC was used to determine the syringe pump settings and exhaust airflow rates required to provide the target concentration prior to animal exposure. Gas chromatographic

TABLE 2

SERIES II AND III: EXPOSURES OF GUINEA PIGS FITTED WITH CAROTID ARTERY CANNULA TO $^{14}CH_3NCO$ FOR 1, 2, or 6 hr

Animal no.	Times (from start of exposure) at which blood samples were taken (hr)	Average exposure concentration (ppm)	Duration of exposure (hr)	Time (from start of exposure) of euthanasia and terminal blood sample (hr)
	Series II			
Male guinea pigs				
105[b]	0 0.05 0.73 1.97 4.53 5.93	0.47	6	70.00
106[b]	0 0.17 0.83 2.17 3.43 4.98	0.47	6	29.60
107[a]	0 0.27 1.00 2.22 3.22 4.22 5.22	0.47	1	5.75
108[a]	0	0.47	1	1.30
109[b]	0 0.32 1.22 2.43	0.47	6	6.28
111[b]	0	0.47	6	7.23
112[b]	0 0.50 1.45 2.73 3.97	0.47	6	11.30
113[b]	0 0.67 1.72 2.98 5.50	0.47	6	21.73
	Series III			
114[a]	$t = 0$ 0.05 0.17 0.33 0.67 1.0 1.5 1.97 2.50 2.98 3.15	15.2	1	4.58
115[c]	$t = 0$ 0.10 0.25 0.50 1.25	15.2	2	2.37

[a] Animals exposed to ^{14}C MIC vapor for 1 hr.
[b] Animals exposed to ^{14}C MIC vapor for 6 hr.
[c] Animals exposed to ^{14}C MIC vapor for 2 hr.

analysis of MIC and ^{14}C MIC to determine chamber concentrations was conducted as described previously (Ferguson *et al.*, 1986). Air samples were taken from the glass chambers at 1-min intervals using a 1-ml gas-tight syringe for injection into the GC. Alternate chambers were sequentially sampled to monitor exposure concentrations. For all series of experiments, the individual guinea pig chambers were ventilated at a rate of 1.8 liter/min and the airflow in the primary chamber varied between 4 and 54 liters/min depending upon the concentration required. As an additional quantitation of the actual chamber concentrations, air samples were withdrawn and trapped in a nitroreagent (*p*-nitrobenzyl-*N*-*n*-propylamine hydrochloride) trapping solution. Using a separate pump, a 3-liter air sample was withdrawn into two serial impingers at a rate of 1 liter/min. Each impinger was filled with 10 ml of the trapping solution which was prepared following the procedure of Schroeder and Moore (1985). Immediately following air sample collection, a 100-μl aliquot of the solution from each impinger was taken and placed in Scintiverse Bio HP cocktail for liquid scintillation analysis.

Exposure Series

Series I. For this series, four cannulated guinea pigs placed in individual glass chambers and five noncannulated guinea pigs placed in a glass aquarium (Fig. 2) were used. In addition, six pregnant female Swiss-Webster mice and two female nude mice were placed in the first 1-liter glass chamber while eight male Swiss-Webster mice of the same strain were exposed in the second 1-liter glass chamber. The exposure lasted 1 hr. The concentration as determined by gas chromatography averaged 5.02 ppm (n = 30, SD = 1.25) during the first 50 min, declining slowly to zero from min 50 to min 60 as the labeled MIC in the first vial was exhausted. The exposure concentration in the aquarium and the mice exposure chambers averaged 2.1 ppm (n = 30, SD = 0.63). Arterial blood samples were collected during the exposure from alternate cannulated animals. Collection for this series began with a 3-min time point and continued at approximately 10-min intervals. Blood samples were placed directly into Vacutainer tubes and were treated identically to the preexposure samples. The details of blood collection and times at which euthanasia was performed are given in Table 1.

Series II. For this series the exposure system shown in Fig. 2 was altered by removing the 19-liter glass aquarium and the mice exposure chambers. Also, two additional individual guinea pig exposure chambers were attached to each sidearm of the primary chamber. This enabled exposure of eight guinea pigs fitted with a carotid

TABLE 3

SERIES IV: EXPOSURES OF ANESTHETIZED GUINEA PIGS FITTED WITH A TRACHEAL CANNULA AND CANNULAS FOR ARTERIAL AND VENOUS BLOOD SAMPLES: EXPOSURE TO $^{14}CH_3NCO$ WAS FOR 1 hr

Animal no.	Times (from start of exposure) at which blood samples were taken (hr)												Average exposure concentration (ppm)	Duration of exposure (hr)	Times (from start of exposure) of euthanasia and terminal blood sample (hr)
Male guinea pigs															
120[a]	0	0.05	0.17	0.25	0.48	0.73	1.0	1.18	1.33	1.57	1.82	2.08	2.58	3.23	3.05	1	3.23
121[b]	0	0.12	0.17	0.35	0.50	0.73	1.0	1.73	2.0						0.55	1	3.08

[a] Arterial and venous blood samples taken simultaneously.

[b] No samples could be obtained from the venous cannula, only arterial blood was taken.

artery cannula. Air samples were taken as in Series I and the exposure concentration averaged 0.47 ppm (n = 180, SD = 0.19). After 1 hr of exposure, two exposure chambers were disconnected and euthanasia of one animal was obtained with 2 ml of sodium pentobarbital (50 mg/ml). Hourly blood samples were obtained from the second animal up to 4 hr postexposure prior to euthanasia at 4.5 hr postexposure. The remaining six guinea pigs were exposed for another 5 hr. Arterial blood samples (0.3 ml) collected every 10 min from alternate guinea pigs during the first hour of exposure were then taken every 15 min during the next 2 hr of exposure and every 30 min during the last 3 hr of exposure. Blood samples were then collected periodically until euthanasia, obtained at various postexposure periods as given in Table 2.

Series III. For this series, only two guinea pigs, both fitted with a carotid artery cannula, were exposed for 1 or 2 hr by the attachment of two individual glass exposure chambers to the primary chamber shown in Fig. 2. Air samples were taken to ascertain the exposure concentration which averaged 15.2 ppm (n = 115, SD = 1.90). Arterial blood samples (0.3 ml) were collected once every 5 min (from alternate animals) during the first 20 min and once every 10 min thereafter until 1 hr. Times of euthanasia and further blood sampling following exposure are given in Table 2.

Series IV. Two guinea pigs prepared with tracheal cannula and arterial and venous cannulas as given above were exposed while still under anesthesia in individual glass chambers attached to the primary chamber as given in Fig. 2. The duration of exposure was 1 hr in each case. The first animal was exposed to an average of 3.1 ppm (n = 60, SD = 0.41), and the second animal was exposed to an average of 0.55 ppm (n = 60, SD = 0.16). In the second animal, venous blood could not be collected; only arterial blood was obtained. For the first animal simultaneous arterial and venous blood samples (0.3 ml) were obtained every 10 to 15 min. Further details are given in Table 3.

Analysis of ^{14}C Uptake into the Bloodstream

Aliquots of 100 μl were taken from each blood sample and these were suspended in Scintiverse Bio HP and counted. An additional 100 μl was removed and placed in a glass vial for complete solubilization. To each of the vials, 1.2 ml of NCS tissue solubilizer (Amersham) was added and the suspension was heated at 50°C for 20 min. Twenty percent benzoyl peroxide decolorizing solution (0.2 ml) was mixed in the vial and incubated at 50° for an additional 30 min. After cooling to room temperature, organic scintillant (toluene, 2,5-diphenyloxazole, and 1,4-bis 2-(5-phenyloxazolyl) benzene) was added to bring the final volume in the vial to 20 ml. The samples were then counted and total radioactivity was calculated on a cpm per milliliter basis. The remaining portion of whole blood was spun at 478g for 15 min. Plasma was separated

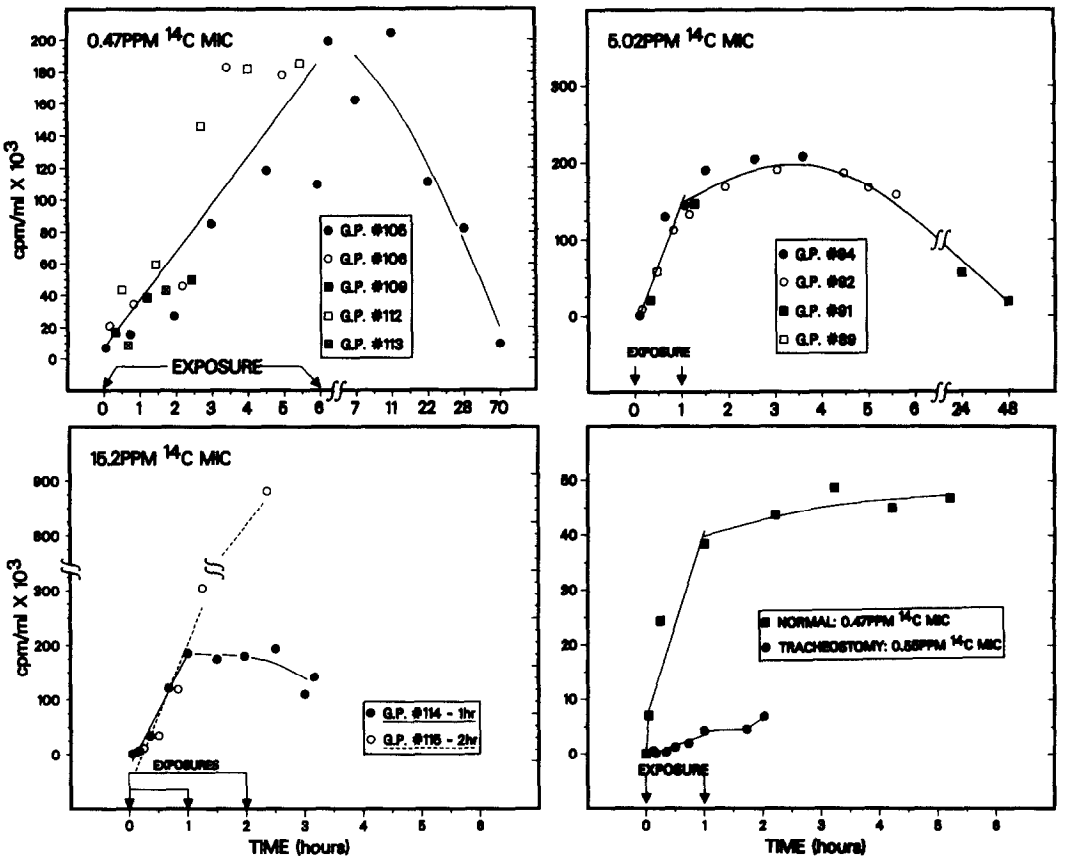

FIG. 3. ^{14}C uptake in blood during and following exposure of guinea pigs at different concentrations of ^{14}C MIC. The data points during each exposure were used to calculate the uptake using linear least-squares regression analysis. The uptake equations are given in Table 8. The bottom right panel illustrates the difference in uptake between a normal guinea pig and a guinea pig fitted with a tracheal cannula exposed to comparable ^{14}C MIC concentrations.

from cellular components and was stored at −20°C. Cellular pellets were stored at 4°C to avoid freeze fracturing.

Collection of Terminal Blood, Body Fluids, and Tissue Samples

For every series, each animal was terminated at a designated postexposure time as shown in Tables 1 to 3. Once removed from the chamber, the animal was injected with 2 ml sodium pentobarbital (50 mg/ml). The animal was then dipped in warm soapy water to decrease the amount of radioactive fur contamination. Terminal blood samples were collected via cardiac punctures and the blood was immediately mixed in a Vacutainer tube with anticoagulant and treated identically to those previously described. Four of the exposed animals (two from Series I and two from Series II) were taken for lung lavage with a total of 30 ml of Hanks' Balanced Buffered Salt Solution (GIBCO) which was delivered intratracheally as 10-ml washes. Bile was collected by puncturing the gall bladder with a 22-gauge needle attached to a syringe and similarly, urine samples were collected directly from the bladder. All body fluids were stored at −20°C after the removal of cellular portions by low speed centrifugation. Each major organ was dissected from all of the exposed animals as well as four control animals. For unpaired organs equal sections were taken; one was immediately frozen in liquid nitrogen and stored at −70°C for biochemical analysis and the other portion was immersed in 10% buffered formalin for histological studies. Paired organs were separated and treated similarly.

Creatinine Assay for Urine Standardization

Two 10-μl aliquots of each urine sample were diluted to 100 μl with HPLC grade water and the amount of cre-

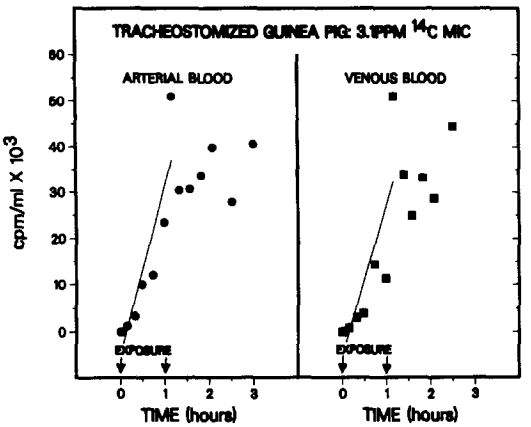

FIG. 4. ^{14}C uptake in arterial or venous blood during and following exposure to ^{14}C MIC. The data points during exposure were used to calculate the uptake using linear least-squares regression analysis. The uptake equations are given in Table 8. ^{14}C in venous blood was always lower than in arterial blood (samples taken simultaneously) during exposure.

atinine was quantitated with a standard creatinine assay kit (Sigma). Experimental values were evaluated vs a standard calibration curve.

Tissue Solubilization

A representative fragment was cut from each major organ and weighed. The fragment was transferred to a glass scintillation vial. NCS solubilizer (Amersham) was added to a volume six times the total sample weight and was incubated at 50°C for 24 hr. Samples were cooled and acidified to pH 7 with glacial acetic acid. In some samples decolorization with benzoyl peroxide was necessary. Organic scintillant was added (see above) and the samples were counted.

RESULTS

Four separate exposure series were completed with various concentrations of ^{14}C MIC. Tables 1 to 3 summarize each experiment and the individual animals involved.

Determination of Specific Activity in Exposure Chamber

Samples of exposure atmospheres were taken, as described under Methods, for the analysis of the specific activity in the chambers during exposure Series I, II, and III. Using the cpms recovered and the original specific activity of the synthesized compound (8.78 mCi/mmol Series I, 8.0 mCi/mmol Series II and III), the concentration of the compound was determined in parts per million (ppm). The results of these calculations and the comparison with the GC analysis values for the same sampling times revealed a difference of 10% or less between exposure concentrations on the basis of cpm vs the GC values. Therefore exposure was only to MIC and not to possible products of MIC.

TABLE 4

RADIOACTIVITY OF BODY FLUIDS IN GUINEA PIGS AFTER 1 hr EXPOSURE TO 5 ppm $^{14}CH_3NCO$ (SERIES 1)

Animal no.	Time[a] (hr)	Blood (cpm/ml)	Bile (cpm/ml)	Urine (cpm/mg creatinine)	Urine (cpm/ml)
100	1.72	202,040	—[b]	4,573	256,072
99	2.17	133,912	—[b]	4,871	155,858
89	2.75	237,597	32,121	12,389	1,635,332
94	3.62	320,041	735,720	9,711	971,120
92	5.57	244,302	521,566	6,871	43,884
91	18	55,486	173,526	—[b]	—[b]
98	48	17,944	23,996	—[b]	—[b]
101	168	10,517	35,332	130	9,718

[a] Time of euthanasia given from the beginning of the 1-hr exposure.
[b] Insufficient amount for analysis.

TABLE 5

RADIACTIVITY OF BODY FLUIDS IN GUINEA PIGS AFTER 1 hr EXPOSURE TO 0.47 ppm $^{14}CH_3NCO$ (SERIES II)

Animal no.	Time[a] (hr)	Blood (cpm/ml)	Bile (cpm/ml)	Urine (cpm/mg creatinine)	Urine (cpm/ml)
108	1.3	89,375	12,760	2,287	99,411
107	5.75	24,571	3,012	749	10,694

[a] Time of euthanasia given from the beginning of the 1-hr exposure.

Uptake of ^{14}C in the Bloodstream

Two separate aliquots of each blood sample were taken and one was analyzed directly in Scintiverse while the other aliquot was digested before scintillation analysis. Both treatments yielded the same general profile of radioactive uptake, but in all cases the values for digested samples were higher on a cpm per milliliter basis. This result suggests that the cellular component contributed to the increase and all the results presented here are the digested sample values.

The uptake and clearance of ^{14}C in blood during and following exposures to ^{14}MIC for all exposure series are shown in Figs. 3 and 4 and Tables 4–7. For all exposures ^{14}C was detected in arterial or venous blood within minutes. For each series of experiment, the data points for ^{14}C in blood, during exposure, were used for regression analysis using the linear least-squares method (Ostle, 1963). The regression line is presented for each series of experiment (Figs. 3 and 4) and the calculated uptake equations for each are given in Table 8. The clearance rate of ^{14}C from blood could not be calculated as for the uptake rate because of fewer data points available. However the data shown in Figs. 3 and 4 and Tables 4–7 indicate that most of the ^{14}C was cleared within 72 hr.

Uptake of ^{14}C in Other Body Fluids

The results for ^{14}C in urine and bile obtained following exposure for each series of experiments are presented in Tables 4–7. The data indicate that large amounts of ^{14}C were present in both bile and urine, with counts diminishing more rapidly from urine than from bile.

Distribution of ^{14}C in Tissues of Exposed Animals

^{14}C was detected in all examined tissues of mice and the results are shown in Figs. 5 and

TABLE 6

RADIOACTIVITY OF BODY FLUIDS IN GUINEA PIGS AFTER 6 hr EXPOSURE TO 0.47 ppm $^{14}CH_3NCO$ (SERIES II)

Animal no.	Time[a] (hr)	Blood (cpm/ml)	Bile (cpm/ml)	Urine (cpm/mg creatinine)	Urine (cpm/ml)
109	6.28	199,049	39,184	15,788	1,562,226
111	7.23	161,978	39,137	13,775	715,672
112	11.3	204,137	35,766	5,475	263,030
113	21.87	110,909	16,274	3,094	48,081
106	27.6	81,842	9,288	2,348	95,206
105	70	9,401	2,788	596	33,491

[a] Time of euthanasia given from the beginning of the 6-hr exposure.

TABLE 7

RADIOACTIVITY OF BODY FLUIDS IN GUINEA PIGS AFTER EXPOSURE TO 15.2 ppm $^{14}CH_3NCO$ (SERIES III)

Animal no.	Time[a] (hr)	Duration of exposure (hr)	Blood (cpm/ml)	Bile (cpm/ml)	Urine (cpm/mg creatinine)	Urine (cpm/ml)
115	2.36	2	881,508	25,456	5967	216,665
114	4.58	1	300,699	36,936	7304	286,913

[a] Time of euthanasia given from the beginning of the 1-hr exposure.

6. The high counts and variation obtained in the uteri and fetuses of female mice at 24 hr postexposure occurred because of a high value in a single dam. Upon euthanasia it was discovered that the gestational age of the fetuses of this mouse was 12–14 days instead of 8–10 days. The increased vascularization of the uterus and development of the placenta at this stage of pregnancy may account for the higher counts obtained.

DISCUSSION

Several investigators have published data on uptake, distribution or retention of a variety of reactive gases such as formaldehyde (Heck *et al.*, 1985, 1983), phosgene (Slade *et al.*, 1983), methylphosphonic difluoride (Dahl and Bechtold, 1985), sulfur dioxide (Balchum *et al.*, 1959, 1960; Frank *et al.*, 1967, 1969), ruthenium tetroxide vapor (Snipes, 1981), hydrogen fluoride (Morris and Frank, 1982), toluene diisocyanate (Hill, 1986), ozone (Wiester *et al.*, 1987), and nitric oxide (Yoshida and Kasema 1987; Oda *et al.*, 1975). Of these studies, uptake in arterial blood during exposure was obtained only for sulfur dioxide (Balchum *et al.*, 1960; Frank *et al.*, 1967) which was reported to appear in blood (measured as ^{35}S) within 5 min of exposure and for nitrogen dioxide which also appeared in blood (measured as ^{13}N) within minutes from the start of exposure (Goldstein *et al.*, 1977).

In our study with ^{14}MIC, ^{14}C was detected in blood, arterial, and venous, within minutes from the start of exposure. A linear uptake was observed for all series including the tracheostomy experiments. These findings are similar to those reported with NO_2 (Goldstein *et al.*, 1977) and SO_2 (Balchum *et al.*, 1960; Frank *et al.*, 1967). Some uptake of ^{14}C following exposure occurred in some experiments. This may indicate that ^{14}C MIC was conjugated to proteins or peptides and thus, these accumulated conjugates continued to be cleared into blood after exposure was terminated. Again, this is similar to the findings reported for NO_2 (Goldstein *et al.*, 1977).

It is of interest to note that uptake into blood was not constant for the different exposure concentrations of 0.47, 5.0, and 15 ppm as shown in Table 8. Since MIC is a potent sensory and pulmonary irritant which changed the breathing pattern of the animals (Ferguson *et al.*, 1986; Alarie *et al.*, 1987, and see Table 8) this may be a contributing factor. However, what may be more important is that nasal secretions were seen to increase and are quite noticeable in animals exposed to 5 ppm and above. This was also observed in rats, but in this species a blood-tinged frothy discharge was also seen (Gupta *et al.*, 1987). This factor, and most probably an increase in tracheal and bronchial secretions as well, would favor trapping of MIC, preventing its absorption into blood from the surfaces of the respiratory tract and favoring clearance of MIC trapped in those secretions via the oral route.

Another possibility is that with higher exposure concentrations, despite increase in se-

TABLE 8

LINEAR LEAST-SQUARES REGRESSION ANALYSIS FOR ^{14}C UPTAKE IN CAROTID ARTERIAL BLOOD DURING EXPOSURE TO $^{14}CH_3NCO$

Series	Exposure concentration (ppm)	Exposure duration (hr)	Data presented in Fig.	Number of blood samples taken	Number of animals sampled	Uptake in blood equation	r value	Slope different from zero[a]	Uptake in blood: cpm ($\times 10^3$)/ml/min/ppm exposure concentration	% Change from control values induced by exposure to those concentrations of MIC for the following variables[d]		
										V_T	f	$V_T \cdot f$
II	0.47	1	3	4	1	$Y(X) = 6 + 34.5X$	0.92	—[b]	1.4	+6.6	−22.5	−17.4
II	0.47	6	3	22	5	$Y(X) = 7.1 + 29.9X$	0.86	yes	1.3	+6.6	−22.5	−17.4
I	5.02	1	3	8	4	$Y(X) = -10 + 154.7X$	0.96	yes	0.48	−4.3	−26.1	−29.3
III	15.2	1	3	6	1	$Y(X) = 14.9 + 195.4X$	0.98	yes	0.23	+30.8	−24.6	−0.8
III	15.2	2	3	6	1	$Y(X) = 37.0 + 236.6X$	0.95	yes	0.22	+30.8	−24.6	−0.8
IV[c] Arterial	3.1	1	4	8	1	$Y(X) = -4.7 + 36X$	0.90	yes	0.17	—	—	—
Venous	3.1	1	4	8	1	$Y(X) = -4.9 + 32X$	0.81	yes	0.15	—	—	—
IV[c]	0.55	1	3	7	1	$Y(X) = 0.4 + 4.0X$	0.93	yes	0.12	—	—	—

[a] From analysis of variance.
[b] Too few blood samples taken.
[c] Animals fitted with tracheal cannula and exposed under anesthesia.
[d] From prior experiments (Alarie *et al.*, 1987), V_T, tidal volume; f, respiratory frequency.

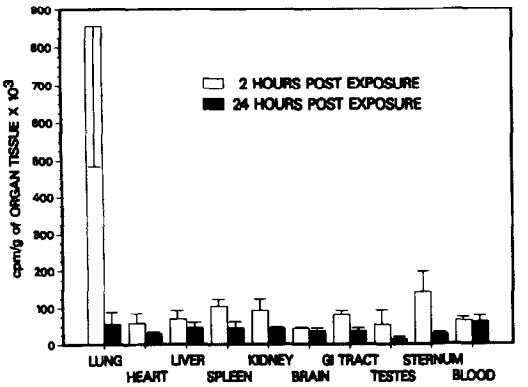

FIG. 5. Distribution of ^{14}C in the organs and blood of male Swiss-Webster mice at 2 and 24 hr following a 6-hr exposure to 2.10 ppm ^{14}MIC vapor (Series I). The bar graphs represent the mean cpm per gram of wet tissue with standard deviations for $n = 4$ mice at each time point.

cretions, the upper respiratory tract may have been saturated with MIC. This would favor penetration into the deeper regions of the respiratory tract where less retention and absorption occurred. However, because of the high reactivity of MIC with water and proteins, this is less likely. However, actual measurements are needed to resolve this issue.

Nevertheless, a large amount of ^{14}C found in blood must have come from absorption on the nasal mucosa and upper respiratory tract since the uptake in blood in animals exposed via a tracheal cannula was much lower than in normal animals exposed at the same concentration. MIC has been shown to cause extensive damage at the nasal level (Uraih *et al.*, 1987) and thus retention, followed by absorption, at this site is expected.

These findings are similar to the results obtained for HF, another highly reactive chemical retained by the upper respiratory tract in rats. Morris and Frank (1982) concluded that HF deposited in the upper respiratory tract was of sufficient magnitude to account for plasma fluoride levels in rats after exposure to HF.

Anesthesia of those animals fitted with a tracheal cannula resulted in 50–55% decrease in their normal respiratory frequency, but no change in their tidal volume, and thus must have contributed to the lower uptake in blood. However, this effect is too small to explain a factor of 10 found in blood uptake of ^{14}C between the two sets of animals. In contrast to the normal animals, the uptake rate in animals fitted with a tracheal cannula was similar at the two concentrations used, 0.5 and 3 ppm, thus reinforcing our above suggestion that uptake in blood from the nasal mucosa was probably greatly influenced by the increase in nasal secretions. This effectively decreased the amount reaching the blood when uptake was expressed in cpm per milliliter per minute per ppm exposure concentration (Table 8). In order to resolve this issue, accurate measurements of nasal secretions would be needed. Also, measurements of nasal retention and fraction of MIC deposited (or retained) with each breath are needed. This is possible using techniques described by Morris and Cavanagh (1986) and Wiester *et al.* (1987) such that uptake in blood could then be correlated with fractions retained with each breath. Nevertheless, our study shows that rapid uptake of ^{14}C occurred in blood upon exposure to ^{14}MIC and that this uptake was much higher in normal animals than in those breathing via a tracheal

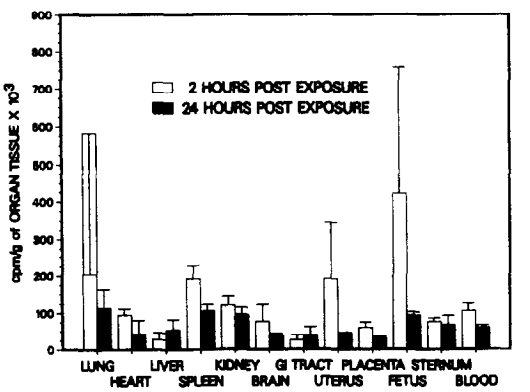

FIG. 6. Distribution of ^{14}C in the organs and blood of pregnant female Swiss-Webster mice at 2 and 24 hr following a 6-hr exposure 2.10 ppm ^{14}MIC vapor (Series I). The bar graphs represent the mean cpm per gram of wet tissue with standard deviations for $n = 3$ mice at each time point. Values for the fetuses and placentas represent the mean counts for $n = 31$ and $n = 36$ at 2 and 24 hr postexposure, respectively.

cannula. In the animal exposed via tracheal cannula and with simultaneous sampling of arterial and venous blood, cpm in arterial blood was always higher than in venous blood, thus showing a net uptake in arterial blood from the lungs.

This raises a question about the form of ^{14}C found in blood. Due to the high reactivity of isocyanates with SH, NH_2, OH, and COOH groups (Brown *et al.*, 1987) it is likely that most of the ^{14}C found in blood was not ^{14}C MIC. The conjugated form of MIC in blood would be important in terms of explaining systemic toxicity found on a variety of target organs other than the lungs (Conner *et al.*, 1987; Varma *et al.*, 1987; Kligerman *et al.*, 1987; Tice *et al.*, 1987). For example, Bruggeman *et al.* (1986) have shown that in the case of allyl isothiocyanate conjugation to glutathione is rapid and reversible. Thus glutathione could act to transport and release MIC to a variety of target cells since the reaction of isocyanate with SH groups is also reversible (Brown *et al.*, 1987). The amount of glutathione recovered from lung lavage has been shown to be very large (Cantin *et al.*, 1987) and thus it, along with other potential plasma conjugates, should be primary targets of future investigations.

The exposure system presented here permitted us to work with a highly toxic and reactive vapor and work with a small amount of expensive radiolabel. In comparison to the large volume of data on uptake in blood of nonreactive vapors (for examples see Fiserova-Bergerova, 1983) we have few examples of uptake in blood of reactive vapors, as noted above. A most interesting finding with MIC was that uptake cannot be predicted by using a single exposure concentration because of its irritating action on the respiratory tract and because of the important role played by the upper respiratory tract. Because of these factors we are unable to arrive at general rules of exposure concentration–uptake in blood relationships as determined for nonreactive vapors (Fiserova-Bergerova, 1983). The role played by the upper respiratory tract in the uptake of MIC will also create more difficulties in extrapolation of results to humans breathing via the mouth. Nevertheless, a better understanding of uptake of reactive vapors in blood is needed and can be gained with the system described.

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REFERENCES

ALARIE, Y., FERGUSON, J. S., STOCK, M. F., WEYEL, D. A., AND SCHAPER, M. (1987). Sensory and pulmonary irritation of methyl isocyanate in mice and pulmonary irritation and possible cyanide-like effects of methyl isocyanate in guinea pigs. *Environ. Health Perspect.* **72,** 159–167.

BALCHUM, O. J., DYBICKI, J., AND MENEELY, G. R. (1959). Absorption and distribution of $^{35}SO_2$ inhaled through the nose and mouth by dogs. *J. Appl. Physiol.* **197,** 1317–1321.

BALCHUM, O. J., DYBICKI, J., AND MENEELY, G. R. (1960). Pulmonary resistance and compliance with concurrent radioactive sulfur distribution in dogs breathing $^{35}SO_2$. *J. Appl. Physiol.* **15,** 62–66.

BROWN, W. E., GREEN, A. H., CEDEL, T. E., AND CAIRNS, J. (1987). Biochemistry of portein–isocyanate interactions: A comparison of the effects of aryl vs alkyl isocyanates. *Environ. Health Perspect.* **72,** 5–12.

BRUGGEMAN, I. M., TEMMINK, J. H. M., AND VAN BLADEREN, J. P. (1986). Glutathione and cysteine mediated cytotoxicity of allyl and benzyl isothiocyante. *Toxicol. Appl. Pharmacol.* **83,** 349–359.

CANTIN, A. M., NORTH, S. L., HUBBARD, R. C., AND CRYSTAL, R. G. (1987). Effect of methyl isocyanate on the eyes of Fischer 344 rats. *Environ. Health Perspect.* **72,** 105–108.

CONNER, M. K., RUBINSON, H. F., FERGUSON, J. S., STOCK, M. F., AND ALARIE, Y. (1987). Evaluation of sister chromatid exchange and cytotoxicity in murine tissues *in vivo* and lymphocytes *in vitro* following methyl isocyanate exposure. *Environ. Health Perspect.* **72,** 177–182.

DAHL, A. R., AND BECHTOLD, W. E. (1985). Deposition and clearance of a water-reactive vapor, methylphos-

phonic difluoride (difluoro), inhaled by rats. *Toxicol. Appl. Pharmacol.* **81,** 58–66.

FERGUSON, J. S., SCHAPER, M., STOCK, M. F., WEYEL, D. A., AND ALARIE, Y. (1986). Sensory and pulmonary irritation with exposure to methyl isocyanate. *Toxicol. Appl. Pharmacol.* **82,** 329–335.

FISEROVA-BERGEROVA, V. (1983). *Modeling of Inhalation Exposure to Vapors; Uptake, Distribution, and Elimination,* Vol. I–II. CRC Press, Boca Raton, FL.

FRANK, N. R., YODER, R. E., BRAIN, J. E., AND YOKOAMA, E. (1969). SO_2 (^{35}S Labeled) absorption by the nose and mouth under conditions of varying concentration and flow. *Arch. Environ. Health* **18,** 315–322.

FRANK, N. R., YODER, R. E., YOKOYAMA, E., AND SPEIZER, F. E. (1967). The diffusion of $^{35}SO_2$. *Health Phys.* **13,** 31–38.

GOLDSTEIN, E., PEEK, N. F., PARKS, N. J., HIRES, H. H., STEFFEY, E. P., AND TARKINGTON, B. (1977). Fate and distribution of inhaled nitrogen dioxide in rhesus monkeys. *Amer. Rev. Resp. Dis.* **115,** 403–412.

GUPTA, B. N., STEFANSKI, S. A., BUCHER, J. R., AND HALL, L. B. (1987). Effect of methyl isocyanate on the eyes of Fischer 344 rats. *Environ. Health Perspect.* **72,** 105–108.

HECK, H. A., CASANOVA-SCHMITZ, M., DODD, P. B., SCHACTER, E. M., WITEK, T. J., AND TOSUN, T. (1985). Formaldehyde (CH_2O) concentrations in the blood of humans and Fischer-344 rats exposed to CH_2O under controlled conditions. *Amer. Ind. Hyg. Assoc. J.* **46,** 1–3.

HECK, H. A., CHIN, T. Y., AND SCHMITZ, M. C. (1983). Distribution of ^{14}C formaldehyde in rats after inhalation exposure. In *Formaldehyde Toxicity* (J. E. Gibson, Ed.), pp. 26–36. Hemisphere, Washington, DC.

HILL, B. L. (1986). *Biochemical Studies on the in Vivo Target of Toluene Diisocyanate.* Ph.D. thesis, Carnegie Mellon University.

JAMES, J. T., BUETTNER, L. C., AND HSU, S. S. (1987). Sensory irritation of methyl isocyanate vapor. *J. Appl. Toxicol.* **7,** 147–148.

KLIGERMAN, A. D., CAMPBELL, J. A., EREXON, G. L., ALLEN, J. W., AND SHELBY, M. D. (1987). Sister chromatid exchange analysis in lung and peripheral blood lymphocytes of mice exposed to methyl isocyanate by inhalation. *Environ. Mutagen.* **9,** 29–36, 1987.

MORRIS, J. B., AND CAVANAGH, D. G. (1986). Deposition of ethanol and acetone vapors in the upper respiratory tract of the rat. *Fundam. Appl. Toxicol.* **6,** 78–88.

MORRIS, J. B., AND FRANK, F. A. (1982). Regional deposition and absorption of inhaled hydrogen fluoride in the rat. *Toxicol. Appl. Pharmacol.* **62,** 81–89.

NEMERY, B., DINSDALE, D., SPARROW, S., AND RAY, D. E. (1985). Effects of methyl isocyanate on the respiratory tract of rats. *Brit. J. Ind. Med.* **42,** 799–805.

ODA, H., KUSTUMOTO, S., AND NAKAJIMA, T. (1975). Nitrosyl-hemoglobin formation in the blood of animals exposed to nitric oxide. *Arch. Environ. Health* **30,** 453–456.

OSTLE, B. (1963). *Statistics in Research.* Iowa State Univ. Press, Ames, IA.

SCHROEDER, R., AND MOORE, K. D. (1985). *A Reverse Phase Liquid Chromatographic Determination of Hexamethylene Diisocyanate in Inhalation Chamber Atmospheres.* Mobay Toxicology Report, No. 639.

SLADE, R., GRAHAM, J. A., AND HATCH, G. E. (1983). Inhaled ^{14}C-phosgene: Species comparison and biochemical fate. *Toxicologist* **3,** 110.

STEVENS, M. A., FITZGERALD, S., MENACHE, M. G., COSTA, D. L., AND BUCHER, J. R. (1987). Functional evidence of persistent airway obstruction in rats following a two-hour inhalation exposure to methyl isocyanate. *Environ. Health Perspect.* **72,** 89–94.

TEPPER, J. S., WIESTER, M. J., COSTA, D. L., WATKINSON, W. P., AND WEBER, M. F. (1987). Cardiopulmonary effects in awake rats four and six months after exposure to methyl isocyanate. *Environ. Health Perspect.* **72,** 95–103.

TICE, R. R., LUKE, C. A., AND SHELBY, M. D. (1987). Methyl isocyanate: An evaluation of *in vivo* cytogenetic activity. *Environ. Mutagen.* **9,** 37–58.

URAIH, L. C., TALLEY, F. A., MITSUMORI, K., GUPTA, B. N., BUCHER, J. R., AND BOORMAN, G. A. (1987). Ultrastructural changes in the nasal mucosa of Fischer 344 rats and B6C3F1 mice following an acute exposure to methyl isocyanate. *Environ. Health Perspect.* **72,** 77–88.

VARMA, D. R., FERGUSON, J. S., AND ALARIE, Y. (1987). Reproductive toxicity of methyl isocyanate in mice. *J. Toxicol. Environ. Health* **21,** 265–275.

WEISTER, J. J., WILLIAMS, T. B., KING, M. E., MENACHE, M. G., AND MILLER, F. J. (1987). Ozone uptake in awake Sprague–Dawley rats. *Toxicol. Appl. Pharmacol.* **89,** 429–437.

YOSHIDA, K., AND KASEMA, K. (1987). Biotransformation of nitric oxide. *Environ. Health Perspect.* **73,** 201–206.