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The Uptake and Elimination of 1,1,1-Trichloroethane during and following Inhalation Exposures in Rats[1,2]

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The Uptake and Elimination of 1,1,1-Trichloroethane during and following Inhalation Exposures in Rats. DALLAS, C. E., RAMANATHAN, R., MURALIDHARA, S., GALLO, J. M., AND BRUCKNER, J. V. (1989). *Toxicol. Appl. Pharmacol.* **98**, 385–397. The pharmacokinetics of 1,1,1-trichloroethane (TRI) was studied in male Sprague–Dawley rats in order to characterize and quantify TRI uptake and elimination by direct measurements of the inhaled and exhaled compound. Fifty or 500 ppm TRI was inhaled for 2 hr through a one-way breathing valve by unanesthetized rats of 325–375 g. Repetitive samples of the separate inhaled and exhaled breath streams, as well as arterial blood, were collected concurrently both during and following TRI inhalation and analyzed for TRI by gas chromatography. Respiratory rates and volumes were continuously monitored during and following exposure and were used in conjunction with the pharmacokinetic data to characterize profiles of uptake and elimination. TRI was very rapidly absorbed from the lung, in that substantial levels were present in arterial blood at the first sampling time (i.e., 2 min). Blood and exhaled breath concentrations of TRI increased rapidly after the initiation of exposure, approaching but not reaching steady state during the 2-hr exposures. The blood and exhaled breath concentrations were directly proportional to the exposure concentration during the exposures. Percentage uptake of TRI decreased 30–35% during the first hour of inhalation, diminishing to approximately 45–50% by the end of the exposure. Total cumulative uptake in the 50 and 500 ppm groups over the 2-hr inhalation exposures was determined to be 6 and 48 mg/kg body wt, respectively. By the end of the exposure period, 2.1 and 20.8 mg, respectively, of inhaled TRI was eliminated from rats inhaling 50 and 500 ppm TRI. A physiological pharmacokinetic model for TRI inhalation was utilized to predict blood and exhaled breath concentrations for comparison with observed experimental values. Overall, values predicted by the physiological pharmacokinetic model for TRI levels in the blood and exhaled breath were in close agreement with measured values both during and following TRI inhalation.

1,1,1-Trichloroethane (TRI), also known as methyl chloroform, has been used in large quantities for decades in industry as a solvent and metal degreasing agent. Other applications include its use in adhesives, spot removers, aerosols, and water repellents. The toxicity of TRI is considered to be of a relatively low order of magnitude, with depression of the central nervous system (CNS) (Torkelson

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and Rowe, 1981; Kleinfeld and Feiner, 1966; Stewart, 1968) and cardiac arrhythmias (Dornette and Jones, 1960; Reinhardt *et al.*, 1973; Herd *et al.*, 1974) the major effects seen after high doses in animals and humans. Hepatic and renal toxicity have been demonstrated only after very high acute doses in animals (Plaa and Larson, 1965; Klaassen and Plaa, 1966, 1967; Gehring, 1968). Historically, human exposures to TRI have been of greatest significance in industry and other occupational settings, where exposures are primarily by inhalation. Workers are routinely exposed to TRI vapors in open or closed (i.e., recirculating) work environments. Employees may be inadvertently exposed to high concentrations when there has been a spill or equipment malfunction.

Studies of the pharmacokinetics of inhaled solvents such as TRI are playing an increasingly important role in toxicology. Knowledge of the uptake, disposition, and elimination of these chemicals is quite useful in health risk assessments. There is presently little kinetic data available involving direct measurements of TRI in laboratory animals during inhalation exposures. The fate of ^{14}C-TRI has been investigated following the termination of single 6-hr 150 or 1500 ppm inhalation exposures in rats and mice (Schumann *et al.*, 1982a). By 72 hr postexposure, 87–98% of the total recovered radioactivity was eliminated as unchanged TRI in the expired air. Respiratory elimination and metabolism of TRI remained approximately the same after TRI inhalation exposures were repeated 5 days/week for 18 months (Schumann *et al.*, 1982b). The fraction of the total inhaled dose which is eliminated during ongoing inhalation exposures, however, has not been delineated in laboratory animals. Likewise, the rate and magnitude of uptake have not been quantified over time during the course of TRI inhalation exposures in animals. It was necessary, for example, for Schumann *et al.* (1982a) to base estimates of pharmacokinetic parameters for rats on an assumed constant uptake of 60% of inhaled TRI over 6 hr of exposure.

Therefore, an objective of the current investigation was to provide accurate measurements of the respiratory uptake and elimination of TRI during inhalation exposures. Inhaled and exhaled breath concentrations were monitored at frequent intervals in rats both during and following TRI inhalation, as were the minute volume and respiratory rate. Blood levels of TRI were monitored concurrently, so systemic uptake and elimination could be correlated with the respiratory measurements. The exhaled breath and blood TRI concentrations were then utilized to assess the accuracy of values predicted by a physiologically based pharmacokinetic model for TRI inhalation.

METHODS

Animals. Adult, male Sprague–Dawley rats were obtained from Charles River Breeding Laboratories (Raleigh, NC). The animals were maintained on a constant light–dark cycle, with light from 0700 to 1900 hr and darkness from 1900 to 0700 hr. They were housed in stainless-steel cages in a ventilated animal rack. Tap water and Ralston Purina Formulab Chow were provided *ad libitum*. The rats were used after at least a 14-day acclimation period, at which time they were approximately 12 weeks old and their body weight ranged from 325 to 375 g. Solvent exposures were initiated at approximately the same time each day (1000 to 1200 hr).

Test material. 1,1,1-Trichloroethane, 98.3% minimum purity, was obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ). The purity of the chemical during the conduct of the study was verified by gas chromatography to be slightly less than 99%.

Animal preparation. An indwelling carotid arterial cannula was surgically implanted into each animal. The rats were anesthetized for the surgical procedure by im injection of 0.8 ml/kg of a mixture consisting of ketamine HCl (100 mg/ml):acepromazine maleate (10 mg/ml):xylazine HCl (20 mg/ml) in a proportion of 3:2:1 (v/v/v). The cannulated animals were maintained in a harness and pulley system that allowed relative freedom of movement in metabolism cages during a 24-hr recovery period.

Inhalation exposures. Each cannulated rat was placed into a restraining tube of the type used in nose-only inhalation exposure chambers (Battelle-Geneve, Switzer-

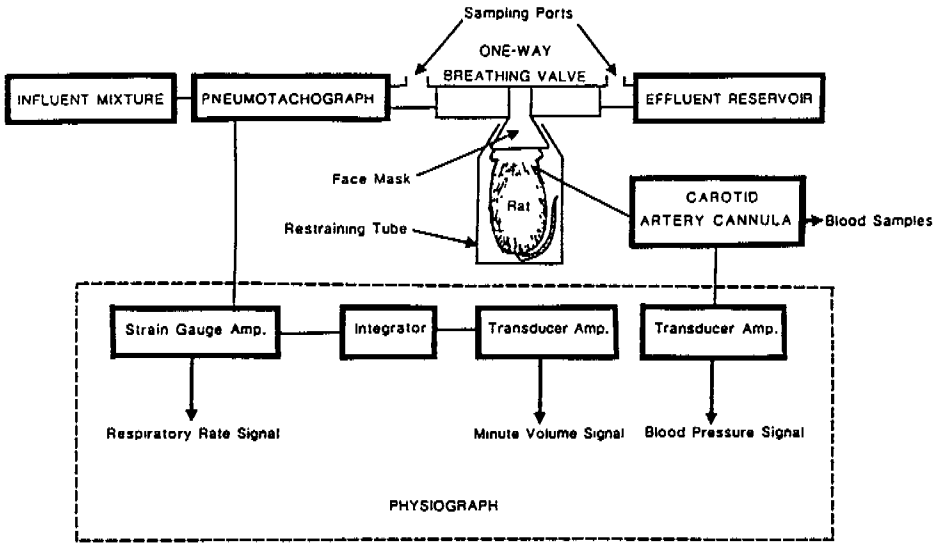

FIG. 1. Schematic diagram of the inhalation exposure system. An unanesthetized rat in the restraining tube inhaled TRI through the one-way breathing valve attached to the face mask. TRI was inhaled from the influent mixture gas sampling bag and exhaled into the effluent reservoir bag. Inhaled and exhaled breath samples were taken from their respective sampling ports and arterial blood samples from an indwelling cannula. The rate and volume of respiration were monitored on a physiograph. For sake of clarity, the breathing valve, gas sampling bags, and other components are not drawn to scale.

land). A face mask designed to fit the rat was held firmly in place on the animal's head by the use of elastic straps, which were secured to the restraining tube. A miniaturized one-way breathing valve (Hans Rudolph, Inc., St. Louis, MO) was attached to the face mask so that the valve entry port was directly adjacent to the nares of the test animal. The dead space of the valve was 0.5 ml. The valve was designed so that the negative pressure generated by the animal's inspiration pulled the inhalation diaphragm open and the exhalation diaphragm closed against its seat. Upon expiration, the positive pressure generated within the device pushed the exhalation diaphragm open and the inhalation diaphragm closed against its seat. This established separate and distinct airways for the inhaled and exhaled breath streams with no significant mixing of the inhaled and exhaled air. The use of such a device for pharmacokinetic studies of inhaled halocarbons in small animals has been described in detail (Dallas *et al.*, 1986). Inhalation and exhalation sampling ports were located immediately adjacent to the breathing valve. A known concentration of TRI was generated within a 70-liter gas sampling bag (Calibrated Instruments, Ardsley, NY) by injecting the appropriate quantity of the test chemical into the bag filled with air. Uniform dispersion of the vapor was ensured by a magnetic stirring bar within the bag. The bag was then connected in series by Teflon tubing with a pneumotachograph, a three-way connector, the breathing valve, and an empty 70-liter gas collection bag (Fig. 1). The latter bag served as a reservoir to collect exhaled gas. Thereby, a closed system was maintained to prevent release of the agent into the laboratory. TRI inhalation exposures were initiated only after stable breathing patterns were established for the cannulated animals in the system. Just before the initiation of exposure, the solvent vapor was first drawn out of the gas sampling bag by an air pump attached to the three-way connector. In this manner, the animal was assured of being subjected at the very start of the exposure to a TRI concentration equivalent to the target concentration in the bag, without significant dilution from dead space air in the system. The test animals then were subjected to 2-hr TRI inhalation exposures. During this period and for up to 4 hr afterward, inhaled and exhaled breath samples were taken from the sampling ports at approximately the same time as blood samples from the carotid artery cannula. Both air and blood samples were then analyzed for TRI content by gas chromatography.

Respiratory measurements and calculations. The respiration of each animal was continuously monitored. The respiratory monitoring was conducted according to the methods previously used in solvent exposure studies by this laboratory (Dallas *et al.*, 1983, 1986). The airflow created by the animal's inspiration was detected by a pneumotachograph located in the inhaled airstream be-

tween the influent bag and the breathing valve. The signal from the pneumotachograph and accompanying transducer was employed in recording the number of respirations per minute (f) in one channel of a physiograph. This signal was then intergrated over a 1-min interval to yield the volume of respiration per minute, or minute volume (V_E). A value for the average tidal volume (V_T) during that 1-min interval was determined by dividing V_E by f for that minute. An average value for these parameters for individual animals was obtained by averaging the measurements taken at 10-min intervals during the 2-hr exposure. The mean ± SE of these values for the 500 ppm exposure group (n = 6) were V_E = 236.3 ± 22.9 ml/min; f = 135.3 ± 6.6 breaths/min; and V_T = 1.74 ± 0.18 ml. The mean ± SE of these values for the 50 ppm exposure group (n = 6) were V_E = 252 ± 14.7 ml/min; f = 129.5 ± 13.5 breaths/min; and V_T = 1.96 ± 0.1 ml.

Since the V_E and the TRI exhaled breath concentration at each sampling point were measured, subtraction of the quantity of TRI exhaled from the amount inhaled yielded an approximation of the quantity of TRI taken up each sampling period (cumulative uptake, or Q_{upt}),

$$Q_{upt} = (C_{inh} V_E t) - (C_{exh} V_E t), \quad (1)$$

where C_{inh} is the inhaled concentration; V_E and C_{exh} are the minute volume and exhaled breath measurements, respectively, made at each time point; and t is the interval of time between sampling points (every 10 min for Q_{upt}). The successive Q_{upt} values are summed to determine cumulative uptake over the 2-hr exposure.

Determination of the cumulative elimination of TRI during inhalation exposure was made as a function of the Q_{upt} and measurements of the inhaled dose. In their calculation of exhaled breath concentration, Ramsey and Andersen (1984) assumed that alveolar respiration accounts for 70% of total respiration, with 30% of total respiration delegated to the inhaled air that does not participate in alveolar ventilation. By adding instrumental dead space of the breathing valve in the exposure system in the present study to this assumed physiological dead space, a value of 50% of total respiration was assigned to alveolar ventilation. Therefore, cumulative elimination (Q_{elim}) of TRI was estimated by

$$Q_{elim} = (C_{inh} V_{alv} t) - Q_{upt}, \quad (2)$$

where the alveolar ventilation is $V_{alv} = 0.5\ V_E$ and t is the time interval between sequential sampling of the exhaled breath. As for Q_{upt}, with sequential determination of Q_{elim} it is possible to measure the cumulative elimination of TRI during inhalation exposures. The successive elimination of TRI following exposure was calculated as $Q_{elim} = C_{exh} V_E t$.

The percentage uptake (% Upt) of the total inhaled dose at each successive time point during the inhalation exposure period was calculated as

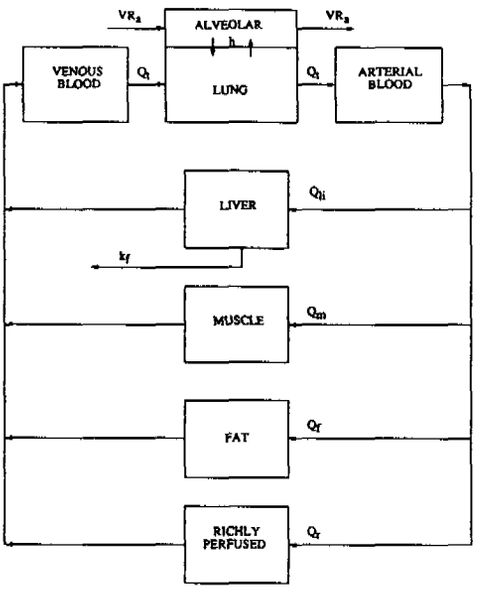

FIG. 2. Diagram of the physiologically based pharmacokinetic model used to simulate the uptake and elimination of inhaled TRI. The symbols and parameters used to describe the model are included in Table 1 and in the equations given under Methods.

$$\%\ \text{Upt} = \frac{(C_{inh} - C_{alv}) \times 100}{C_{inh}}, \quad (3)$$

where the TRI alveolar concentration is $C_{alv} = C_{art}/N$, in which C_{art} is the measured TRI arterial blood level and N is the blood:air partition coefficient for TRI.

A physiologically based pharmacokinetic (PBPK) model was used to describe the disposition of TRI in the rat (Fig. 2). It was assumed that a blood flow-limited model was adequate to characterize the tissue distribution of TRI. Compartmental volumes and organ blood flows were obtained from the literature (Gerlowski and Jain, 1983; Ramsey and Andersen, 1984) and scaled to 340 g, the mean body weight of rats used in the present study. Partition coefficients and the metabolic rate constant for TRI were taken from Gargas *et al.* (1986, 1989), except for the richly perfused tissue:blood and lung: blood partition coefficients, which were assumed to be the same as the liver:blood partition coefficient. The lung:air partition coefficient was then derived by multiplying the blood:air coefficient from Gargas *et al.* (1986) by the lung:blood coefficient. The alveolar:lung mass transfer coefficient was estimated from the value used for methylene chloride (Angelo and Pritchard, 1984). Differential mass balance equations, incorporating the parameters listed in Table 1, that described the transport

TABLE 1

PARAMETERS FOR THE PHYSIOLOGICAL PHARMACOKINETIC MODEL OF TRI IN THE RAT (340 g)

Parameter	Value
Alveolar ventilation rate (ml/min), VR_a	126 (50 ppm exposure) 118 (500 ppm exposure)
Inhaled gas concentration (μg/ml), C_{inh}	0.279 (50 ppm exposure) 2.70 (500 ppm exposure)
Blood flows (ml/min)	
Cardiac output, Q_b	106.4
Fat, Q_f	9.4
Liver, Q_l	39.8
Muscle, Q_m	12.8
Richly perfused, Q_r	44.4
Tissue volumes (ml)	
Blood, V_b	25.4
Fat, V_f	30.5
Liver, V_l	13.6
Muscle, V_m	248.0
Richly perfused, V_r	17.0
Alveolar, V_a	2.0
Lung, V_l	3.97
Partition coefficients	
Lung:air, R_a	8.6
Fat:blood, R_f	47.7
Liver:blood, R_l	1.49
Lung:blood, R_l	1.49
Muscle:blood, R_m	0.55
Richly perfused:blood, R_r	1.49
Miscellaneous constants	
Lung:alveolar mass transfer coefficient, k	500 ml/min
Metabolic rate constant, K_f	0.115 min^{-1}

of TRI in the rat were numerically integrated with the Advanced Continuous Simulation Language (ACSL) computer program (Mitchell and Gauthier, Concord, MA). The solution to the equations provided predicted TRI concentrations over time. The model-predicted cumulative uptake values were the sum of the simulated amounts of TRI in each tissue compartment in the model.

Analysis of TRI in air and blood. The concentration of TRI in the inhaled and exhaled air samples collected during and following the inhalation exposures were measured with a Tracor MT560 gas chromatograph (GC) (Tracor Instruments, Austin, TX). Analyses for the 500 ppm exposures were conducted using a flame ionization detector (FID), while the analyses for the 50 ppm exposures were conducted using an electron capture detector (ECD). In either case, air samples were procured with a gas-tight, 1-ml syringe and injected directly onto an 8 ft × ⅛ in. stainless-steel column packed with 0.1% AT 1000 on GraphPak. Standards were prepared in each of four 9-liter standard bottles with Teflon stoppers containing needles used for taking the air samples with the syringe. Operating temperatures were 150°C, injection port; 200°C, FID detector; 350°C, ECD detector; and 110°C, isothermal column operation. When using the ECD, gas flow rates of 40 ml/min were employed for nitrogen (carrier gas), with an additional makeup gas flow rate of 30 ml/min to the detector.

TRI levels in the blood were measured by GC headspace analysis. Blood samples were withdrawn from the arterial cannula via a stopcock by a 1-ml syringe. Depending on the anticipated blood concentration, between 25 and 200 μl of the blood was taken from the stopcock with an Eppendorf pipet and transferred to chilled headspace vials (Perkin–Elmer, Norwalk, CT). These vials were capped immediately with PTFE-lined butyl rubber septa and washers and tightly crimped. Each sample vial was then placed into the HS-6 autosampler unit of a SIGMA 300 gas chromatograph (Perkin–Elmer, Norwalk, CT), where it was heated to 80°C by a high-precision thermostat device. A predetermined volume of the vapor was then injected automatically into the column for analysis. Standard solutions were made and assayed by diluting calculated amounts of pure TRI in toluene, transferring to vials, and analyzing as previously described. The concentration of TRI in the blood samples was then determined from a standard curve generated from blood that was spiked with these standard solutions. The column used was an 8 ft × ⅛ in. stainless-steel column packed with FFAP Chromasorb W-AW (80–100 mesh). Operating temperatures were 200°C, injection port; 350°C, ECD detector; and 85°C, column oven. The carrier gas was 5% argon-methane, at a flow rate of 40 ml/min with a makeup gas flow rate of 20 ml/min to the detector.

RESULTS

The target concentrations for the TRI inhalation exposures were 50 and 500 ppm. The starting concentration of TRI in the bag from which the test animal inhaled the test compound was measured just prior to the initiation of each exposure. TRI bag concentrations were 515.8 ± 20.6 and 53.6 ± 2.2 ppm ($\bar{x}$ ± SE) for the 500 and 50 ppm groups, respectively. The actual concentrations inhaled by the animals were determined by measurements of air samples taken from the airway

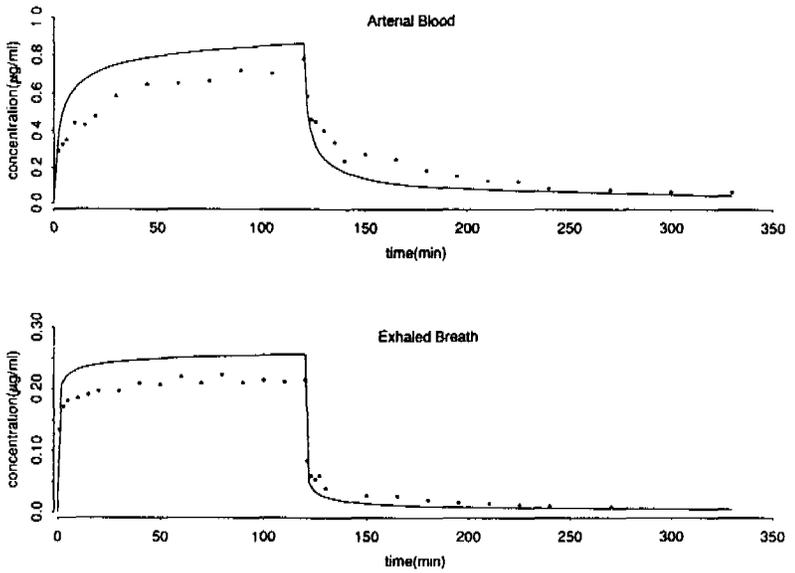

FIG. 3. Observed (●) and model-predicted (—) TRI concentrations in the blood (top graph) and exhaled breath (bottom graph) of rats during and following a 2-hr 50 ppm inhalation exposure. Each point represents the mean value for six rats.

immediately adjacent to the breathing valve. Inhaled TRI concentrations for the six rats in each group were 491.6 ± 11 ppm for the 500 ppm exposures and 51.2 ± 1.2 ppm ($\bar{x} \pm$ SE) for the 50 ppm exposures.

TRI concentrations in the blood and exhaled breath of rats during and following inhalation of TRI are shown for 50 ppm exposures in Fig. 3 and for 500 ppm exposures in Fig. 4. Concentrations of TRI in the exhaled breath generally paralleled concentrations in the arterial blood, though some differences were noted. TRI was rapidly absorbed from the lungs and readily available for distribution to tissues of the body, in that arterial blood concentrations of TRI were quite high at the first sampling time (i.e., 2 min). After an initial rapid rise, the blood levels increased steadily but did not reach steady state by the end of the 2-hr exposures. Exhaled breath levels increased even more rapidly than blood levels after the initiation of exposures, attaining near steady state within 10 to 15 min. The exhaled breath versus time curves were asymptotic, in that they gradually increased throughout the remainder of the 2-hr inhalation period. An increase in the inhaled concentration from 50 to 500 ppm produced an equivalent (i.e., 10-fold) increase in the observed blood and exhaled breath concentrations of TRI. Upon cessation of TRI inhalation, the chemical was rapidly eliminated. As can be seen in Figs. 3 and 4, TRI concentrations in the exhaled breath initially diminished more rapidly than did blood concentrations. Disappearance of TRI from the blood paralleled that in the expired air during the latter part of the postexposure period.

PBPK model-generated blood and exhaled breath concentrations of TRI are shown as solid lines in Figs. 2 and 3. Concentrations of TRI in the expired air were well simulated by the model during and following the 50 and 500 ppm exposures. Model predictions that TRI levels in the exhaled breath would quickly reach near steady state after the exposures began were consistent with the observed data, with the observed levels slightly lower

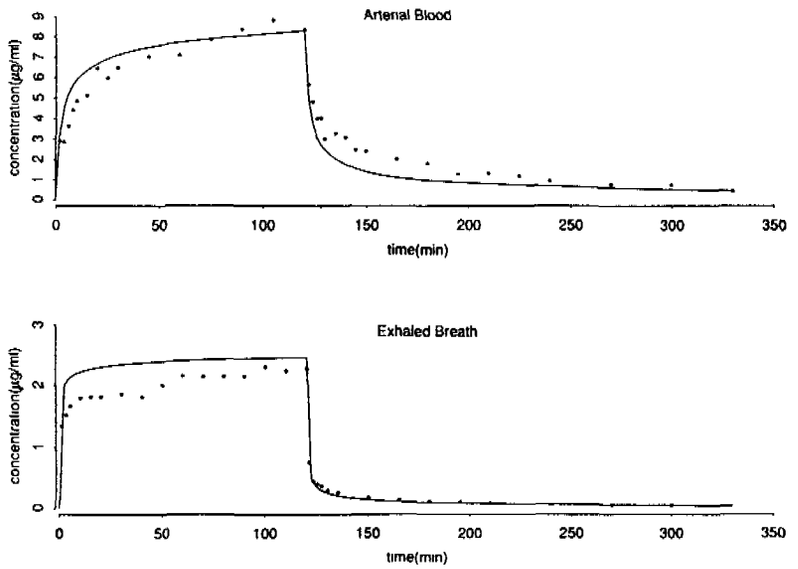

FIG. 4. Observed (●) and model-predicted (—) TRI concentrations in the blood (top graph) and exhaled breath (bottom graph) of rats during and following a 2-hr 500 ppm inhalation exposure. Each point represents the mean value for six rats.

than simulated levels over the course of the 50 and 500 ppm exposures. The model accurately predicted both rapid and slow elimination phases of expiration of TRI postexposure. When the model was used to describe the time course of TRI in the arterial blood, a relatively good fit was obtained during the 500 ppm exposure (Fig. 4). Arterial blood concentrations were overpredicted by approximately 20% during the 50 ppm exposure (Fig. 3). The model predicted a slightly more rapid decline in blood levels postexposure in both groups than was observed during the period of 130–200 min, but levels at subsequent time points were accurately predicted.

Percentage systemic uptake of TRI appeared to be both concentration- and time-dependent (Fig. 5). Although percentage uptake was quite high during the initial minutes of inhalation of 50 and 500 ppm TRI, a decrease of 30–35% occurred during the first hour. Percentage uptake diminished more slowly during the remainder of the exposure period. As can be seen in Fig. 5, the mean values after 10 min are slightly but consistently lower in the 500 ppm group.

Plots of cumulative uptake of TRI during the inhalation sessions, as calculated by Eq.

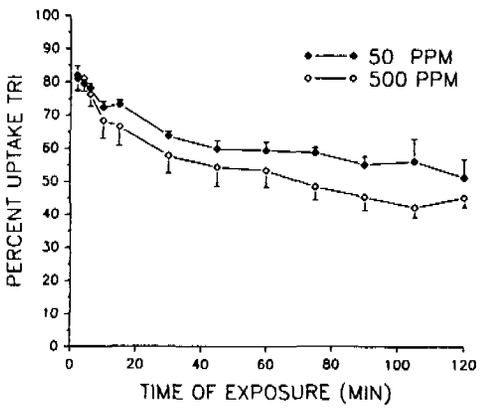

FIG. 5. Percentage uptake of TRI during inhalation exposures. Rats inhaled 50 or 500 ppm TRI for 2 hr. Each point represents the mean ± SE for six rats. The percentage uptake of the inhaled dose over time was determined after 1, 3, 5, 10, 15, and 20 min and at 10-min intervals thereafter.

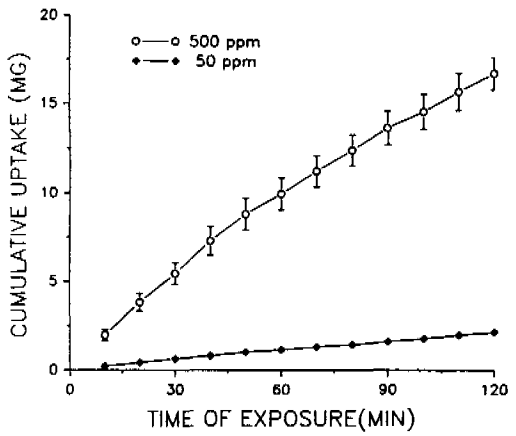

FIG. 6. Cumulative uptake of TRI during inhalation exposures. Rats inhaled 50 or 500 ppm TRI for 2 hr. The quantity of inhaled TRI retained during successive 10-min intervals was calculated on the basis of the measured minute volume and difference between inhaled and exhaled TRI concentrations. Each point represents the mean ± SE for six rats. The diminutive SE bars are omitted from the 50 ppm values for sake of clarity.

(1), are shown in Fig. 6. Cumulative uptake, as determined from direct measurements of the minute volume and TRI concentrations in the inhaled and exhaled breath, was not linear in either the 50 or 500 ppm animals. The departure from linearity was more apparent at the higher exposure level. Total cumulative uptake during the 2-hr exposures, as ascertained from the direct measurement data, was 2.2 ± 0.2 and 16.7 ± 0.9 mg ($\bar{x}$ ± SE) in the 50 and 500 ppm groups, respectively. Predicted values for uptake, derived by summating the predicted levels of TRI in the model compartments, were significantly less than these measured uptake values (i.e., after 2 hr exposure to 500 ppm TRI, predicted uptake was 50% of measured uptake).

Cumulative elimination of TRI in the exhaled breath during and following inhalation exposure is shown in Fig. 7. During TRI exposure, TRI in the pulmonary blood and TRI not absorbed from the alveolar space each contribute to the TRI eliminated in the exhaled breath. Cumulative pulmonary elimination, as determined by Eq. (2), was proportional to the inhaled concentration. By the end of the 2-hr exposure to 50 and 500 ppm TRI, 2.1 ± 0.2 and 20.8 ± 3.0 mg ($\bar{x}$ ± SE), respectively, were eliminated from the rats in the exhaled breath. Model-predicted elimination at the end of 2 hr in the 50 and 500 ppm groups was approximately 40 and 50% greater, respectively, than these measured values. Following the termination of exposure, TRI was eliminated in the exhaled breath in progressively smaller quantities, as reflected by postexposure plateaus in the elimination curves. During the 2-hr postexposure period, an additional 0.3 and 3.3 mg of TRI were eliminated from the animals in the 50 and 500 ppm exposure groups, respectively.

DISCUSSION

Pharmacokinetic studies are playing an increasingly important role in toxicology and

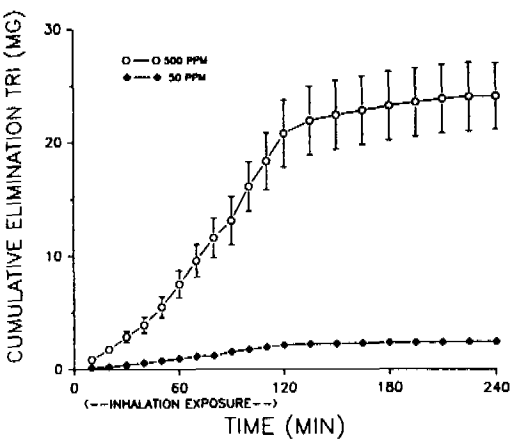

FIG. 7. Cumulative elimination of TRI during and following inhalation exposures. Rats inhaled 50 or 500 ppm TRI for 2 hr. The quantity of inhaled TRI eliminated in the breath over time was calculated using direct measurements of the minute volume and TRI concentrations in the inhaled and exhaled breath. Each point is the mean ± SE for six rats. Cumulative elimination was determined for successive 10-min intervals during the 2-hr exposure, and for successive 15-min intervals postexposure.

in health risk assessments (Clark and Smith, 1984; Clewell and Andersen, 1985; NRC, 1987). Unfortunately, there is relatively little information available on the uptake and disposition of TRI and many other VOCs during ongoing inhalation exposures. Most pharmacokinetic studies of TRI have focused on the elimination of the chemical and its metabolites following the cessation of exposures (Stewart *et al.*, 1969; Ikeda and Ohtsuji, 1972; Eben and Kimmerle, 1974; Seki *et al.*, 1975; Holmberg *et al.*, 1977; Caperos *et al.*, 1982; Schumann *et al.*, 1982a,b). Although Schumann *et al.* (1982a) utilized rats with an indwelling jugular cannula, just three blood samples were taken and analyzed for TRI content during a 6-hr inhalation session. Similarly, blood levels appear to have been taken for TRI analysis only three times from human volunteers during 6 hr of TRI inhalation (Nolan *et al.*, 1984). These few time points are not sufficient to accurately define blood concentration versus time profiles, or to recognize changes which may occur in kinetics during the course of exposures.

The monitoring of blood and breath levels of VOCs in animals has been primarily restricted to times after termination of exposures, due to problems involving restricted access to subjects in inhalation chambers and metabolism cages and technical difficulties in working with small animals. In the present study we have utilized a technique which allowed direct, simultaneous measurements of respiratory parameters, inhaled and exhaled breath concentrations of TRI, and TRI blood levels in unanesthetized rats during exposures. The separation of the inhaled and exhaled breath streams, by use of a miniaturized one-way breathing valve, facilitated accurate serial determinations of airflow and TRI concentrations in the inhaled and exhaled breath. A similar nonrebreathing valve has been used previously for assessing respiratory volumes and gas exchange (Mauderly *et al.*, 1979), though it has apparently not been used in pharmacokinetic studies. This approach allowed us to directly monitor respiratory uptake and elimination of TRI, a VOC for which pulmonary clearance is the major route of elimination. Indeed by the end of the 2-hr 50 and 500 ppm inhalation exposures, we determined that 52.5 and 56.3%, respectively, of the inhaled dose had been exhaled.

Percentage uptake of inhaled TRI was highly time-dependent. The percentage uptake of inhaled TRI has apparently not been previously determined in laboratory animals. Schumann *et al.* (1982a) assumed that 60% of inspired TRI was absorbed by rats throughout a 6-hr exposure. The rate of transfer of TRI from alveoli to blood should initially be very rapid, but become progressively slower as the chemical accumulates in the blood and tissues. This pattern was reflected by the time course of systemic uptake of TRI in the current study, where percentage uptake decreased from more than 80% at the beginning to less than 50% at the end of the 2-hr exposure. Initial uptake of inhaled TRI is governed by tissue loading and metabolism. Once the tissues have reached steady state, continued uptake will be dependent upon the rate of metabolism of the chemicals. Since TRI is very poorly metabolized by the rat and by humans (Ikeda and Ohtsuji, 1972; Schumann *et al.*, 1982a; Nolan *et al.*, 1984), percentage uptake would be expected to be very low once steady state was reached. Steady state was not reached in our study, as percentage uptake progressively decreased over the 2 hr of exposure. Monster *et al.* (1979) found that percentage uptake of inhaled TRI by humans decreased rapidly from approximately 95% at the onset to 30% at the end of 4-hr exposures. Nolan *et al.* (1984) reported that human volunteers exposed for 6 hr to 35 or 350 ppm TRI retained about 25% of the chemical to which they were exposed. The greater percentage uptake in rats than in humans is consistent with a higher TRI blood:air partition coefficient and greater cardiac output/pulmonary blood flow in rats

than in humans (Reitz *et al.*, 1988). Since systemic uptake of TRI is time-dependent, average values of percentage uptake for short intervals may be misleading and have little relevance for health risk assessments.

TRI exhibits linear kinetics over a wide dosage range. Exhaled breath levels and blood levels of TRI were directly proportional to the inhaled concentration (i.e., 50 and 500 ppm) of TRI throughout the 2-hr exposures in the current study. Similar findings were reported in humans exposed for 6 hr to 35 and 350 ppm TRI (Nolan *et al.*, 1984). Schumann *et al.* (1982a) found that the amount of TRI exhaled by rats and mice increased eight- to ninefold when the inhaled concentration of TRI was increased from 150 to 1500 ppm. These investigators also observed that blood levels, tissue levels, and body burden of ^{14}C-TRI were each proportional to exposure level in both species. Although TRI was poorly metabolized, Schumann *et al.* (1982a) demonstrated that its biotransformation by mice and rats was a dose-dependent, saturable process. Metabolic saturation in rats was believed to occur between 500 and 1500 ppm, if not near 500 ppm. Metabolic saturation, however, had little apparent effect on the overall pharmacokinetics of TRI, since biotransformation was a minor route of elimination. The kinetics of TRI is governed largely by its partition coefficients (e.g., blood:air, tissue:blood) and the physiology (e.g., respiratory rate and volume, cardiac output, tissue volumes, and blood flow rates) of the animal.

The major route of elimination of TRI in laboratory animals and in man is exhalation of the parent compound (Schumann *et al.*, 1982a; Nolan *et al.*, 1984). Nolan and his colleagues measured TRI in the exhaled breath of male human volunteers during and after 6-hr inhalation sessions. The exhaled breath levels after 1.5 hr of exposure to 35 and 350 ppm TRI were 0.14 and 1.28 μg/ml, respectively. Assuming a linear scaleup to a 50 and 500 ppm exposure, the exhaled breath levels in humans would be 0.2 and 1.83 μg/ml. These values are quite comparable to exhaled breath levels measured in the present study after 1.5 hr of exposure of rats to 50 and 500 ppm TRI (i.e., 0.21 and 2.16 μg/ml, respectively). The similarity in magnitude in exhaled breath levels of TRI between rats and man is an unexpected finding. It would be anticipated that alveolar and presumably exhaled breath concentrations of TRI would be lower in rats than in man, due to the rat's higher blood:air partition coefficient and greater percentage uptake of inhaled TRI.

The aforementioned physiological parameters and biochemical constants were used to input into a PBPK model for inhalation of TRI. Our model accurately predicted the time courses of TRI in the blood and exhaled breath of rats both during and following exposure to 50 and 500 ppm TRI. Cumulative uptake over the 2-hr exposure, however, was underpredicted by our model. The source of the discrepancy between the predicted and the measured uptake value is unclear. Revisions of the model may be warranted by findings in ongoing studies of TRI concentrations in tissues of exposed animals. These data should be useful in verifying tissue:blood partition coefficients, tissue compartments, and tissue volumes. Reitz *et al.* (1988), for example, found that most of the changes in the pharmacokinetics of TRI in older rats could be accounted for by increasing the size of the fat compartment in their PBPK model. Reitz *et al.* (1988) used their model to accurately predict blood and exhaled breath concentrations measured in humans subjected to TRI inhalation exposures. The investigators also utilized the PBPK model to predict TRI blood levels and amounts metabolized post-exposure in mice, rats, and humans, as well as to describe the kinetics of TRI in rats after iv injection, bolus gavage, and drinking water administration. Thus, it appears that PBPK models can be quite useful in predicting the time course of TRI concentrations in the

body of different species under different exposure conditions.

Major species differences have been observed in the pharmacokinetics of inhaled TRI. After 1.5 hr of exposure to 35 or 350 ppm TRI, humans had mean blood levels of 0.14 and 1.62 μg/ml, respectively (Nolan *et al.*, 1984). After being normalized for differences in inhaled concentrations, mean blood levels in rats in the present investigation were approximately 3.6-fold higher than the levels measured in humans. Schumann *et al.* (1982a) reported blood levels of TRI in rats similar to those observed in the present study (when normalized for inhaled concentration). The lower blood levels in humans are consistent with a lower TRI blood:air partition coefficient for man (2.53 versus 5.76 for rats) and the greater adipose tissue volume in man (23.1% versus 11% in rats). In a comparison with the normalized data of Schumann *et al.* (1982a), Nolan *et al.* (1984) noted that blood levels in mice and rats inhaling TRI were 17.3 and 3.5 times higher, respectively, than those measured in humans. When determining the actual inhaled dose at equivalent inhaled concentrations, one must consider the wide variation in volume of respiration and body weight between species. Assuming a 4.2 liters/min alveolar ventilation and 70 kg body wt for man (Ganong, 1979), the rats in the present study received an inhaled dose approximately six times greater than that of the humans in the study by Nolan *et al.* (1984). Nolan and his colleagues determined following a 6-hr exposure to 150 ppm TRI that mice, rats, and humans metabolized 0.16, 0.06, and 0.014 μmol/kg/ppm, respectively. Thus, mice and rats should be more susceptible than humans to TRI toxicity at equivalent inhaled concentrations, due to significantly greater systemic absorption and metabolism of the chemical.

Meaningful health risk assessments require a careful selection of the measure of dose. In the present study systemic uptake of TRI is measured directly during the initial phase of inhalation exposure, when significant loading of tissues is occurring. Once tissue loading is completed (i.e., steady state is reached), very little uptake of TRI should occur because of the poor metabolism of the chemical. Thus, the common practice of assessing dose by multiplying ventilation rate by inhaled concentration would be very misleading during prolonged exposures. A more logical measure of target organ dose or tissue exposure would be the area under the tissue concentration versus time curve. The concept and rationale for selection of appropriate target organ dose measures (i.e., tissue dosimetry) are discussed in detail by Andersen (1987). It is important that target organs and mechanisms of toxicity be elucidated, so that the agent(s) responsible for toxic effects are identified and can subsequently be quantified and correlated with the magnitude of toxicity in the target tissue(s). It is not clear for TRI whether the parent compound or its metabolites should serve as the dose measure, or surrogate. Near-lethal exposures are required for effects on most target organs. Carcinogenicity bioassays have been negative, or inconclusive. Reitz *et al.* (1988) decided to use the average concentration of TRI in the liver over a lifetime (ACL) as a dose surrogate. These investigators used a PBPK model to calculate ACLs for comparison of internal doses received by mice and rats in long-term toxicity studies versus humans drinking TRI-contaminated water. Unfortunately, there is a paucity of data on actual concentrations of TRI in the liver and other organs. Direct measurement studies are needed to generate tissue concentration versus time data sets for rigorous validation of PBPK model predictions of dose surrogates.

APPENDIX

*Mass Balance Differential Equations for Physiological Pharmacokinetic Model**

Arterial Blood

$$V_b \frac{dC_b}{dt} = Q_t\left(\frac{C_l}{R_l} - C_b\right).$$

Venous Blood

$$V_b \frac{dC_v}{dt} = Q_{li} \frac{C_{li}}{R_{li}} + Q_m \frac{C_m}{R_m} + Q_f \frac{C_f}{R_f} + Q_r \frac{C_r}{R_r} - Q_t C_v.$$

Alveolar Space

$$V_a \frac{dC_a}{dt} = VR_a C_{inh} u_1(t)^{**} - VR_a C_a + h\left(\frac{C_l}{R_a} - C_a\right).$$

Lung

$$V_l \frac{dC_l}{dt} = Q_t\left(C_v - \frac{C_l}{R_l}\right) + h\left(C_a - \frac{C_l}{R_a}\right).$$

Liver

$$V_{li} \frac{dC_{li}}{dt} = Q_{li}\left(C_b - \frac{C_{li}}{R_{li}}\right) - k_f \frac{X_{li}}{R_{li}}.$$

Muscle

$$V_m \frac{dC_m}{dt} = Q_m\left(C_b - \frac{C_m}{R_m}\right).$$

Fat

$$V_f \frac{dC_f}{dt} = Q_f\left(C_b - \frac{C_f}{R_f}\right).$$

Richly Perfused

$$V_r \frac{dC_r}{dt} = Q_r\left(C_b - \frac{C_r}{R_r}\right).$$

Note. $^*C_i$ = concentration of TRI in compartment i. See Table 1 for the definition of the other symbols. $^{**}\mu(t) = 1$ for $t \leq 120$ min and 0 for $t > 120$ min.

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REFERENCES

ANDERSEN, M. E. (1987). Tissue dosimetry in risk assessment, or what's the problem here anyway? In *Pharmacokinetics in Risk Assessment*, Vol. 8, *Drinking Water and Health*, pp. 8–26. National Academy Press, Washington, DC.

ANGELO, M. J., AND PRITCHARD, A. B. (1984). Simulations of methylene chloride pharmacokinetics using a physiologically based model. *Regul. Toxicol. Pharmacol.* **4**, 329–339.

CAPEROS, J. R., DROZ, P. O., HAKE, C. L., HUMBERT, B. E., AND JACOT-GUILLARMOD, A. (1982). 1,1,1-Trichloroethane exposure, biological monitoring by breath and urine analysis. *Int. Arch. Occup. Environ. Health* **49**, 293–303.

CLARK, B., AND SMITH, D. A. (1984). Pharmacokinetics and toxicity testing. *CRC Crit. Rev. Toxicol.* **12**, 343–385.

CLEWELL, H. J., AND ANDERSEN, M. E. (1985). Risk assessment extrapolations and physiological modeling. *Toxicol. Ind. Health* **1**, 111–131.

DALLAS, C. E., BRUCKNER, J. V., MAEDGEN, J. L., AND WEIR, F. W. (1986). A method for direct measurement of systemic uptake and elimination of volatile organics in small animals. *J. Pharmacol. Methods* **16**, 239–250.

DALLAS, C. E., WEIR, F. W., FELDMAN, S., PUTCHA, L., AND BRUCKNER, J. V. (1983). The uptake and disposition of 1,1-dichloroethylene in rats during inhalation exposure. *Toxicol. Appl. Pharmacol.* **68**, 140–151.

DORNETTE, W. H. L., AND JONES, J. P. (1960). Clinical experiences with 1,1,1-trichloroethane. A preliminary report of 50 anesthetic administrations. *Anesth. Analg.* **39**, 249–253.

EBEN, A., AND KIMMERLE, G. (1974). Metabolism, excretion and toxicology of methyl chloroform in acute and subacute exposed rats. *Arch. Toxikol.* **31**, 233–242.

GANONG, W. F. (1979). *Review of Medical Physiology*, p. 500. Lange Medical Publications, Los Altos, CA.

GARGAS, M. L., ANDERSEN, M. E., AND CLEWELL, H. J., III (1986). A physiologically based simulation approach for determining metabolic constants from gas uptake data. *Toxicol. Appl. Pharmacol.* **86**, 341–352.

GARGAS, M. L., BURGESS, R. J., VOISARD, D. E., CASON, G. H., AND ANDERSEN, M. E. (1989). Partition coefficients of low molecular weight volatile chemicals in various liquids and tissues. *Toxicol. Appl. Pharmacol.* **98,** 87–99.

GEHRING, P. J. (1968). Hepatoxic potency of various chlorinated hydrocarbon vapors relative to their narcotic and lethal potencies in mice. *Toxicol. Appl. Pharmacol.* **13,** 287–293.

GERLOWSKI, L. E., AND JAIN, R. K. (1983). Physiologically-based pharmacokinetic modeling: Principles and applications. *J. Pharm. Sci.* **72,** 1103–1127.

HERD, P. A., LIPSKY, M., AND MARTIN, H. F. (1974). Cardiovascular effects of 1,1,1-trichloroethane. *Arch. Environ. Health* **28,** 227–233.

HOLMBERG, B., JAKOBSON, I., AND SIGVARDSSON, K. (1977). A study on the distribution of methyl chloroform and n-octane in the mouse during and after inhalation. *Scand. J. Work Environ. Health* **3,** 43–52.

IKEDA, M., AND OHTSUJI, H. (1972). A comparative study of the excretion of Fujiwara reaction-positive substances in urine of humans and rodents given trichloro- or tetrachloro-derivatives of ethane and ethylene. *Brit. J. Ind. Med.* **29,** 99–104.

KLAASSEN, C. D., AND PLAA, G. L. (1966). Relative effects of various chlorinated hydrocarbons on liver and kidney function in mice. *Toxicol. Appl. Pharmacol.* **9,** 139–151.

KLAASSEN, C. D., AND PLAA, G. L. (1967). Relative effects of various chlorinated hydrocarbons on liver and kidney function in dogs. *Toxicol. Appl. Pharmacol.* **10,** 119–131.

KLEINFELD, M., AND FEINER, B. (1966). Health hazards associated with work in confined spaces. *J. Occup. Med.* **8,** 358–364.

MAUDERLY, J. L., TESAREK, J. E., SIFFORD, L. J., AND SIFFORD, L. J. (1979). Respiratory measurements of unsedated small laboratory mammals using nonrebreathing valves. *Lab Anim. Sci.* **29,** 323–329.

MONSTER, A. C., BOERSMA, G., AND STEENWEG, H. (1979). Kinetics of 1,1,1-trichloroethane in volunteers: Influence of exposure concentration and work load. *Int. Arch. Occup. Environ Health* **42,** 293–302.

MORGAN, A., BLACK, A., AND BELCHER, D. R. (1972). Studies on the absorption of halogenated hydrocarbons and their excretion in breath using 38Cl tracer techniques. *Ann. Occup. Hyg.* **15,** 273–282.

NOLAN, R. J., FRESHOUR, N. L., RICK, D. L., MCCARTY, L. P., AND SAUNDERS, J. H. (1984). Kinetics and metabolism of inhaled methyl chloroform (1,1,1-trichloroethane) in male volunteers. *Fundam. Appl. Toxicol.* **4,** 654–662.

National Research Council (NRC) (1987). *Pharmacokinetics in Risk Assessment,* Vol. 8, *Drinking Water and Health.* National Academy Press, Washington, DC.

PLAA, G. L., AND LARSON, R. E. (1965). Relative nephrotoxic properties of chlorinated methane, ethane, and ethylene derivatives in mice. *Toxicol. Appl. Pharmacol.* **7,** 37–44.

RAMSEY, J. C., AND ANDERSEN, M. E. (1984). A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. *Toxicol. Appl. Pharmacol.* **73,** 159–175.

REINHARDT, C. F., MULLIN, L. S., AND MAXFILED, M. E. (1973). Epinephrine-induced cardiac arrhythmic potential of some common industrial solvents. *J. Occup. Med.* **15,** 953–955.

REITZ, R. H., MCDOUGAL, J. N., HIMMELSTEIN, M. W., NOLAN, R. J., AND SCHUMANN, A. M. (1988). Physiologically based pharmacokinetic modeling with methylchloroform: Implications for interspecies, high dose/low dose, and dose route extrapolations. *Toxicol. Appl. Pharmacol.* **95,** 185–199.

SCHUMANN, A. M., FOX, T. R., AND WATANABE, P. G. (1982a). [^{14}C]Methyl chloroform (1,1,1-trichloroethane): Pharmacokinetics in rats and mice following inhalation exposure. *Toxicol. Appl. Pharmacol.* **62,** 390–401.

SCHUMANN, A. M., FOX, T. R., AND WATANABE, P. G. (1982b). A comparison of the fate of inhaled methyl chloroform (1,1,1-trichloroethane) following single or repeated exposure in rats and mice. *Fundam. Appl. Toxicol.* **2,** 27–32.

SEKI, Y., URASHIMA, Y., AIKAWA, H., MATSUMURA, H., ICHIKAWA, Y., HIRATSUKA, F., YOSHIOKA, Y., SHIMBO, S., AND IKEDA, M. (1975). Trichloro-compounds in the urine of humans exposed to methyl chloroform at subthreshold levels. *Int. Arch. Arbeitsmed.* **34,** 39–49.

STEWART, R. D. (1968). The toxicology of 1,1,1-trichloroethane. *Ann. Occup. Hyg.* **11,** 71–79.

STEWART, R. D., GAY, H. H., SCHAFFER, A. W., ERLEY, D. S., AND ROWE, V. K. (1969). Experimental human exposure to methyl chloroform vapor. *Arch. Environ. Health* **19,** 467–472.

TORKELSON, T. R., AND ROWE, V. K. (1981). Halogenated aliphatic hydrocarbons containing chlorine, bromine and iodine. In *Patty's Industrial Hygiene and Toxicology* (G. D. Clayton and E. Clayton, Eds.), 3rd ed., pp. 3502–3510. Wiley, New York.

VENG-PEDERSEN, P. (1984). Pulmonary absorption and excretion of compounds in the gas phase: Theoretical pharmacokinetic and toxicokinetic analysis. *J. Pharm. Sci* **73,** 1136–1141.

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Type I and Type II Pyrethroids Increase Inhibition in the Hippocampal Dentate Gyrus of the Rat[1]

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Type I and Type II Pyrethroids Increase Inhibition in the Hippocampal Dentate Gyrus of the Rat. JOY, R. M., ALBERTSON, T. E., AND RAY, D. E. (1989) *Toxicol. Appl. Pharmacol.* **98**, 398–412. Urethane-anesthetized rats were prepared for stimulation of the perforant path and for recording from the granule cell region of the hippocampal dentate gyrus. Subjects were administered varying doses of allethrin (a prototype type I pyrethroid) or deltamethrin (a prototype type II pyrethroid), and the excitability of the perforant path and granule cells was tested. Both pyrethroids produced a dose-dependent decrease in the responsiveness of granule cells, following stimulation of the perforant path, that lasted up to 100 msec. Analysis suggested that the pyrethroid-induced effects were associated with an increase in interneuronally mediated inhibition. Neither the perforant path axon or terminal nor the granule cell was affected by doses which appeared to affect interneurons. Basal excitability of the granule cells was also decreased by deltamethrin. This effect may have been secondary to an increase in tonic inhibition evoked by the same mechanisms responsible for the increase in phasic inhibition.

The synthetic pyrethroids constitute a diverse group of chemicals with important insecticidal actions. They are highly toxic to a wide spectrum of insects but possess relatively low oral toxicity to mammals. Their intravenous toxicity is much greater, however, suggesting that this selectivity is at least partly based on pharmacokinetic and metabolic factors (Vijverberg and van den Bercken, 1982). The symptoms produced in mammals vary depending upon the specific pyrethroid involved. Some produce primarily tremor associated with increased sensitivity to external stimuli (type I pyrethroids). Others produce a state more characterized by salivation and a wobbling motor dysfunction that progresses to choreoathetoid movements and convulsions (type II pyrethroids) (Verschoyle and Aldridge, 1980). Most pyrethroids can be placed into one or the other of these two categories, although examples intermediate in symptomatology are known to exist.

All of the pyrethroids that produce mammalian toxicity are known to interact with sodium channels in nervous tissue. They all prolong the sodium current evoked by depolarization of the membrane (Narahashi, 1971, 1985, 1986; Vijverberg and van den Bercken, 1982; Vijverberg and de Weille, 1985; Vijverberg *et al.*, 1986). At least part of the distinction between type I and type II pyrethroids appears to be related to the time during which the sodium channel remains in an open state. Type I pyrethroids hold the sodium channel open for relatively short periods of time (milliseconds). This effect pro-

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1,1,1-Trichloroethane Formulation: A Chronic Inhalation Toxicity and Oncogenicity Study in Fischer 344 Rats and B6C3F1 Mice

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1,1,1-Trichloroethane Formulation: A Chronic Inhalation Toxicity and Oncogenicity Study in Fischer 344 Rats and B6C3F1 Mice. QUAST, J. F., CALHOUN, L. L., AND FRAUSON, L. E. (1988). *Fundam. Appl. Toxicol.* **11**, 611–625. Groups of male and female Fischer 344 rats and B6C3F1 mice (80/sex/group) were exposed to vapor concentrations of 0, 150, 500, or 1500 ppm 1,1,1-trichloroethane formulation 6 hr/day, 5 days/week, for 2 years. Ten rats and mice/sex from each group were predesignated for interim sacrifices after 6, 12, and 18 months of exposure. Fifty rats and mice/sex/group were assigned to the study to be terminated after 24 months. Parameters measured during the study included mortality, in-life clinical signs of toxicity, hematology, urinalysis (rats only), clinical chemistry, body weight, organ weights (liver, kidneys, brain, heart, testes), gross pathology, and histopathology. Inhalation exposure of male and female Fischer 344 rats to 1500 ppm vapor of the 1,1,1-trichloroethane formulation for 2 years resulted in a significant decrease in body weights of females. In addition, very slight microscopic hepatic effects were seen in the liver of 1500 ppm-exposed male and female rats necropsied at 6, 12, and 18 months. The hepatic effects could not be discerned at 24 months due to confounding geriatric changes. In the rats exposed to 150 and 500 ppm there were no changes that were considered due to exposure to the 1,1,1-trichloroethane formulation. There were no toxic effects noted in male or female mice at any exposure concentration tested. There were no indications of an oncogenic effect in rats or mice following 2 years of exposure to this 1,1,1-trichloroethane formulation.

1,1,1-Trichloroethane (methyl chloroform) is used in formulations for cold cleaning and industrial degreasing processes as well as in aerosol applications. It is the major ingredient of CHLOROTHENE[1] solvents. Commercial formulations commonly contain as much as 5% (total volume) chemical stabilizers and traces of chlorinated aliphatic hydrocarbons.

A review of the scientific literature on the toxicity of 1,1,1-trichloroethane in rats and mice indicates that the two species respond to the solvent in comparable fashion over a similar range of exposure concentrations. The LC50 for a single 7-hr exposure of rats to 1,1,1-trichloroethane was reported to be 14,250 ppm (Adams *et al.*, 1950). Gehring (1968) found that the time to 50% mortality (LT50) for mice exposed to 13,500 ppm was approximately 6 hr. One study compared the response of rats and mice to 1,1,1-trichloroethane inhalation exposure (rats and mice, published in two parts, MacEwen and Vernot, 1974; McNutt *et al.*, 1975). Continuous exposure, 24 hr/day, of rats and mice to 0, 250, or 1000 ppm 1,1,1-trichloroethane for 14 weeks resulted in the following findings in 1000 ppm-exposed animals: increased relative liver weights in both rats and mice, increased liver triglycerides in mice, and microscopic liver changes detectable in rats and

[1] Registered trademark of The Dow Chemical Company.

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mice. At 250 ppm the findings were increased relative liver weight and minimal microscopic liver changes in the rats and minimal cytoplasmic alterations of centrilobular hepatocytes observed only by electron microscopy in the mice.

The National Cancer Institute (NCI) has reported the results of a carcinogenesis bioassay of a 1,1,1-trichloroethane formulation (containing 3% 1,4-dioxane and 2% minor impurities) in Osborne–Mendel rats and B6C3F1 mice (NCI, 1977). The test material was administered by gavage in corn oil to 50 animals/sex/species, 5 days/week for 78 weeks. Rats received doses of 750 or 1500 mg/kg/day. Dose levels for mice were initially 0, 2000, or 4000 mg/kg/day but were increased twice during the study, resulting in time-weighted average doses of 2807 and 5615 mg/kg/day. There was a moderate decrease in body weight gain observed in the high-dose male rats during the first year and in both sexes of mice throughout the study. Early mortality attributed to intercurrent murine pneumonia was observed in both sexes of rats and in female mice. All surviving rats were killed at 117 weeks of age and mice at 96 weeks of age. No neoplastic or nonneoplastic pathological findings were observed that were attributable to treatment. However, the study was ruled inadequate by NCI for assessment of carcinogenicity due to a shortened life span in rats and mice.

A chronic inhalation study of a 1,1,1-trichloroethane formulation in Sprague–Dawley rats has been conducted in this laboratory (Quast *et al.*, 1978; Rampy *et al.*, 1977). The rats were exposed to 0, 875, or 1750 ppm of the test material 6 hr/day, 5 days/week, for 12 months. The animals were then allowed to survive until 31 months. No treatment-related changes were detected except that female rats exposed to 1750 ppm showed an increased incidence of focal hepatocellular alterations. No evidence for a carcinogenic response was detected; the tumor incidence of 1,1,1-trichloroethane-exposed rats was comparable to that of controls.

A report of a long-term ingestion study of 1,1,1-trichloroethane in Sprague–Dawley rats has recently been published (Maltoni *et al.*, 1986). Forty male and forty female rats were dosed by gavage with 500 mg/kg body wt/day 1,1,1-trichloroethane in olive oil, while 50 rats of each sex served as controls and were dosed with olive oil alone. Animals were treated once daily, 4–5 days/week, for 104 weeks. After this time the surviving animals were held without treatment until death (up to 141 weeks). The authors reported an increase in the incidence of rats with leukemia, particularly immunoblastic lymphosarcomas. The authors stated that "The design and size of our experiment do not allow us to draw definite conclusions" but recommended additional testing to assess the carcinogenic potential of 1,1,1-trichloroethane.

In preparation for selecting appropriate exposure concentrations for the 2-year inhalation study herein reported, a 90-day subchronic inhalation study of the 1,1,1-trichloroethane formulation was conducted in this laboratory (Calhoun *et al.*, 1981). Groups of rats and mice exposed to 2000 ppm, 6 hr/day, 5 days/week, showed statistically significant changes in body and liver weights and minimal microscopic changes in the olfactory epithelium. Based on these findings in the subchronic inhalation study, the maximum exposure level selected for the 2-year study was 1500 ppm.

METHODS

Test Material

A production-grade 1,1,1-trichloroethane formulation, approximately 94% (by volume) 1,1,1-trichloroethane, 5% stabilizers (butylene oxide, t-amyl alcohol, methyl butynol, nitroethane, and nitromethane), and <1% minor impurities, was used for the study. Each drum of material was analyzed by gas–liquid chromatography when opened for use. Analyses of samples from all drums of test material used in the study were comparable and showed no sample degradation during the test interval.

Experimental Design

Groups of Fischer 344 rats and B6C3F1 mice were simultaneously exposed to target concentrations of 0, 150, 500, or 1500 ppm of the 1,1,1-trichloroethane formulation; these concentrations correspond to 0, 0.82, 2.73, or 8.19 mg/liter in air. Exposures were conducted 6 hr/day, 5 days/week (except holidays), for 24 months (total of 516 exposure days).

Male and female Fischer 344 rats, 4–6 weeks of age, and male and female B6C3F1 mice, 5–6 weeks of age, were purchased from Charles River Laboratories, Portage, Michigan. Fifty animals/sex/species/group were designated for 24 months of exposure. Interim sacrifices of 10 predesignated animals/sex/species/group were conducted after 6, 12, and 18 months of exposure. An additional 10 animals/sex/species/group were maintained on test approximately 16 months for pharmacokinetic assessment; results of the pharmacokinetic studies in the repeatedly exposed rats and mice have been reported by Schumann *et al.* (1982).

The test animals were assigned to treatment groups by a statistical randomization procedure. The animals were identified by a numbered metal eartag. Mice were housed singly; rats of the same sex were housed two per cage. Food (Purina Laboratory Chow, Ralston Purina Co., St. Louis, MO) was available to all animals *ad libitum* except during exposure. Water was available *ad libitum* at all times from an automatic watering system. Rats and mice exposed to the 1,1,1-trichloroethane formulation and their respective controls were maintained in the inhalation chambers throughout the study with clean air supplied during nonexposure periods.

Exposures

Exposures were conducted in walk-in chambers 8 × 8 × 8 ft (14.5 m^3), equipped with stainless-steel ceilings in the shape of a regular quadrangular pyramid and epoxy resin-coated walls and floors. The chambers were operated under dynamic airflow conditions and were maintained at a slight negative pressure relative to the air in the surrounding area. The chambers were kept on a regular 12-hr photocycle. Air supplied to the chambers was controlled by a system designed to maintain temperature at approximately 70°F and relative humidity at approximately 50%. The temperature and humidity were recorded daily during exposure.

Vapors of the 1,1,1-trichloroethane formulation were generated by metering the liquid at a calculated rate into a glass J-tube vaporization apparatus (Miller *et al.*, 1980). The air supplied to the J-tube assembly was heated to the minimum extent necessary to ensure complete vaporization of the test material (approximately 90–100°C). Vapors from the J-tube were then introduced into the chamber inlet ducts where there was further mixing and dilution to the intended target concentrations. Total airflow through the chambers was maintained at approximately 2000 liters/min.

The daily nominal concentration of the 1,1,1-trichloroethane formulation vapor in each chamber was calculated from the total amount of liquid vaporized and the total chamber airflow. The analytical concentration of 1,1,1-trichloroethane vapor in the chamber was determined by infrared spectrophotometry (Miran 1A, Foxboro Analytical, Norwalk, CT) at a wavelength of 9.2 μm. Each exposure chamber was analyzed at least once per hour during the 6-hr exposure period. The output of the infrared analyzer was fed to a microprocessor which calculated the time-weighted average (TWA) exposure concentration for each chamber.

There was close agreement between mean daily TWA analytical concentration and mean daily nominal concentration for each exposure chamber. The TWA analytical values were 151 ± 2, 502 ± 5, and 1505 ± 11 ppm for the 516 exposure days. The distribution of the 1,1,1-trichloroethane vapor within each chamber was determined to be uniform (within ±10% of the chamber TWA) prior to initiation of the study.

Clinical Determinations

Animals were observed daily for changes in appearance and demeanor. Each animal on test was checked for palpable masses prior to initiation of the study and monthly from the 12th month of exposure until termination. In-life palpable "masses" included not only possible neoplasms, but any swelling regardless of location or neoplastic character. Animals were weighed at approximately monthly intervals throughout the study.

Clinical laboratory studies were conducted for all surviving animals that were designated for the 6-, 12-, and 18-month interim sacrifices. Ten rats/sex/exposure group were used for all clinical laboratory studies at 24 months. Total red blood cells, hemoglobin concentration, packed cell volume, total white cells, and platelets were evaluated (Ortho ELT-8, Ortho Instruments, Boston, MA) for all samples except those of mice at the 6-month interval. Differential white blood cell counts (Honeywell ACS-1000, Honeywell, Inc., Denver, CO) were determined for all samples from the 12-month interval, and for control and 1500-ppm samples at the remaining intervals (except mice at 6 months). Urine samples were obtained from the same rats (not from mice) 1–2 weeks prior to each scheduled necropsy for analysis of specific gravity (T. S. Meter, American Optical Co., Keene, NH), pH, protein, glucose, ketones, bilirubin, blood, and urobilinogen (Multistix, Ames Co., Elkhart, IN). Clinical chemistry determinations (CentrifiChem System, Methods File, Union Carbide Corp., Rye, NY) on all samples included serum levels of urea nitrogen, alkaline phosphatase activity, glutamic-pyruvic trans-

TABLE 1

TISSUES COLLECTED AND PRESERVED AT NECROPSY

Adrenals	Larynx	Prostate
Aorta	Liver	Salivary glands
Bone	Lungs	Seminal vesicles
Bone marrow	Mammary gland	Skeletal muscle
Brain	Mediastinal lymph node	Skin and subcutis
Cecum	Mediastinal tissues	Small intestine (duodenum, jejunum, ileum)
Cervix	Mesenteric lymph node	Spinal cord
Coagulating glands	Mesenteric tissues	Spleen
Epididymides	Nasal tissues	Stomach
Esophagus	Oral tissues	Testes
Eyes	Ovaries	Thymus
Gallbladder (mice)	Oviducts	Thyroid gland
Heart	Pancreas	Tongue
Kidneys	Parathyroid glands	Trachea
Lacrimal/ Harderian glands	Peripheral nerve	Urinary bladder
Large intestine (colon, rectum)	Pituitary	Uterus
		Vagina

aminase activity, glucose, cholesterol, triglycerides, total protein, albumin, and globulin. Blood samples were collected from the orbital sinus of rats 1–2 weeks prior to necropsy for hematology and from severed cervical vessels at necropsy for clinical chemistry. Blood samples were collected from the orbital sinus of the mice at the time of necropsy for all determinations.

Pathology

Rats and mice submitted for necropsy were weighed, anesthetized with methoxyflurane, and bled for clinical hematology and chemistry determinations. A complete gross necropsy was performed by a veterinary pathologist, and tissues listed in Table 1 were preserved in neutral, phosphate-buffered 10% formalin. Examination of the eyes by a moist microscope slide technique under fluorescent illumination was performed. Liver, kidneys, brain, heart, and testes (males) were weighed and the organ weight:final body weight ratios were calculated. Animals that died or were submitted moribund were examined in a similar manner, except organs were not weighed and the detailed eye examinations were not conducted.

The tissues (Table 1) were processed in the standard manner, embedded in paraffin, sectioned at 5–6 μm, stained with hematoxylin and eosin, and examined microscopically by a veterinary pathologist. All tissues from control and 1500 ppm-exposed groups of rats and mice designated for 6-, 12-, 18-, and 24-month (terminal) sacrifices were examined. At the 6-, 12-, and 18-month interim sacrifices, only selected tissues from mice and livers from rats were generally examined from animals in the groups exposed to 150 and 500 ppm. For male and female rats exposed to 150 and 500 ppm and assigned to the 24-month portion of the study, histopathologic evaluation of the following tissues was performed: liver, spleen, kidneys, pituitary, adrenals, thyroid, testes, mesenteric lymph node, lung, trachea, larynx, and nasal turbinates. All tissues of male and female mice exposed to 150 or 500 ppm and assigned to the 24-month portion of the study were examined. In addition, any grossly observed lesions suggestive of a tumor were examined microscopically from all animals.

Statistics

Cumulative mortality data were tabulated by daily intervals and analyzed for overall differences by the Gehan–Wilcoxon test (Breslow, 1970), $\alpha = 0.05$. Body weight data were evaluated by Dunnett's test, $\alpha = 0.05$, two-sided (Steel and Torrie, 1960); statistical outliers were identified by the sequential procedure described by Grubbs (1969) and were excluded from further calculations.

Interim data collected through 18 months of the study, including absolute and relative organ weights, urinary specific gravity, hematology (except differential leukocyte counts), and clinical chemistry data, were statistically evaluated as follows. Statistical outliers were determined by the procedure described by Grubbs (1969) but were not excluded from calculations. The data were evaluated by Bartlett's test for equality of variances (Winer, 1971), $\alpha = 0.01$. Based on the outcome of Bartlett's test, a parametric or nonparametric analysis of variance (ANOVA) was performed. If the ANOVA was significant, it was followed by Dunnett's test (Winer, 1971) or the Wilcoxon rank-sum test (Hollander and Wolfe, 1973), $\alpha = 0.05$, two-sided, with Bonferroni's correction (Miller, 1966).

Clinical laboratory data (hematology, urine specific gravity, and clinical chemistry) and organ/body weight data obtained from the 24-month sacrifice were not statistically analyzed for differences from control because by 24 months these parameters generally reflect a variety of geriatric changes, spontaneous neoplasms, and agonal changes. Statistical evaluation of these data is of doubtful scientific merit. Descriptive statistics were determined for differential leukocyte counts at all intervals; however, no statistical analysis of these data was conducted.

For the results of the histopathologic examination of the tissues of mice scheduled for 24 months of exposure, Fisher's exact probability test (Siegel, 1956) was used as the main interpretive statistical comparison. To ensure that pairwise comparisons had not missed any observa-

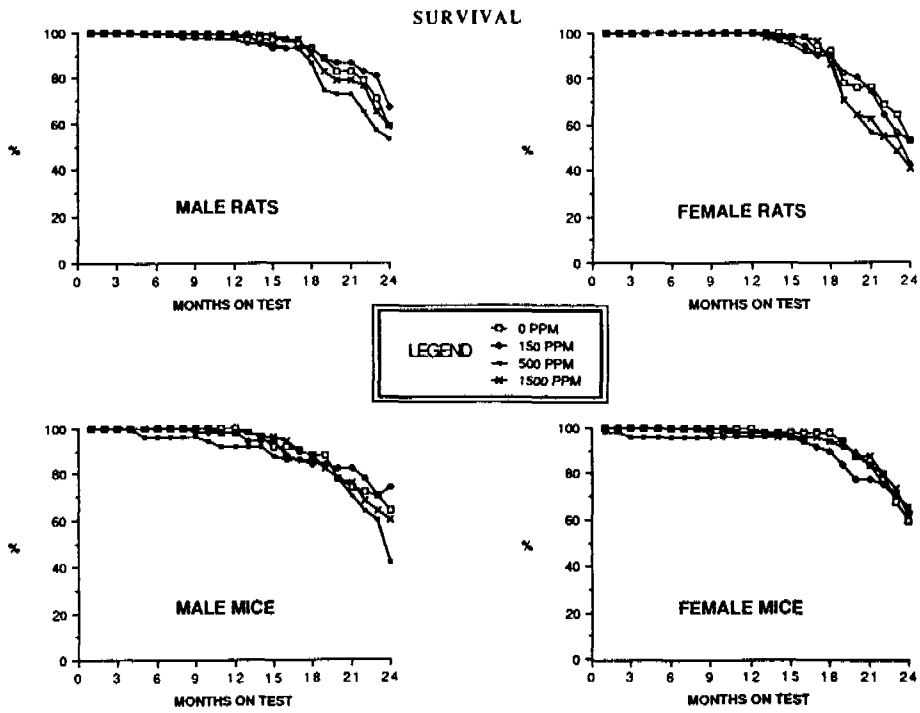

FIG. 1. Survival of male and female rats and mice exposed to vapors of a 1,1,1-trichloroethane formulation for 2 years.

tions, the Cochran–Armitage trend test (Armitage, 1971) was also applied if Fisher's test was negative. The Bonferroni correction procedure (Miller, 1966) was used when the control incidence of the lesion was at least 6%. The nominal α level in all cases was 0.05, one-sided.

For tissues intended for histopathologic examination from all rats at all dose levels scheduled for 2 years of exposure, the incidences of specific observations were first tested for deviation from linearity using ordinal spacings of the doses. If linearity was not rejected the data were then tested for a dose–response relationship using the Cochran–Armitage trend test (Armitage, 1971). If the trend was statistically significant, or if significant deviation from linearity was found, incidences for each dose were compared with that of the control using a pairwise χ^2 test with Yate's correction (Fleiss, 1981). For tissues which were evaluated from all control and high-dose-level rats, but only from selected rats from the middle- and low-dose groups, statistical analysis was limited to the pairwise comparison of control and top dose. The nominal α levels used were $\alpha = 0.01$ (χ^2 test for linearity); $\alpha = 0.02$, two-sided (trend test); and $\alpha = 0.05$, one-sided (pairwise comparison Yates χ^2 test). The trend test, rather than Fisher's method, was used as the primary statistical tool for the evaluation of histopathologic data on rats because it is a global test utilizing all the data and because of its superior operating characteristics (Haseman, 1983, 1985). The trend test is more sensitive than Fisher's test and achieves its nominal α level more uniformly (Park, 1985; Park and Kociba, 1985).

Since multiple, interrelated parameters were statistically compared in the same group of animals, the frequency of false-positive errors may be much greater than the nominal α level. Thus, in addition to statistical analyses, the final toxicologic interpretation of the data also considered factors such as dose–response relationships and whether or not the findings appeared to be plausible and consistent in light of other biologic findings.

RESULTS AND DISCUSSION

Rats

There were no statistically significant differences in survival rates of male or female exposed rats when compared with their respective controls (Fig. 1).

The body weights of female rats of both 500 ppm- and 1500 ppm-exposed groups

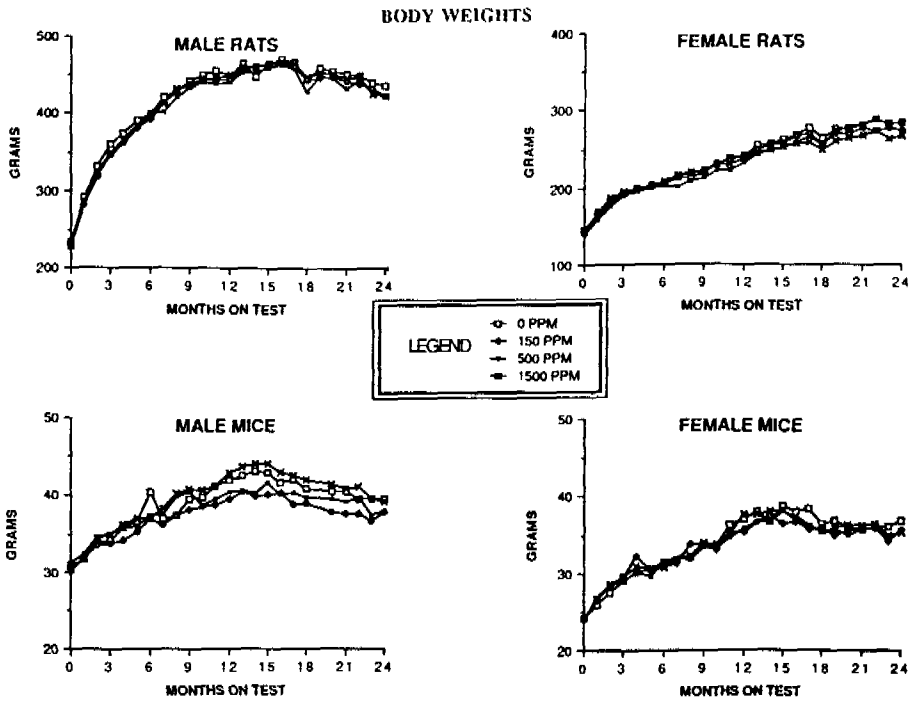

FIG. 2. Body weights of male and female rats and mice exposed to vapors of a 1,1,1-trichloroethane formulation for 2 years.

were decreased compared with the controls through much of the study (Fig. 2). The 500 ppm-exposed female rats had statistically decreased body weights from approximately the 7th month to the 18th month, and the 1500 ppm-exposed group of female rats showed a statistical decrease in weight from approximately 11 through 24 months. It should be noted that these groups weighed slightly less than their respective control groups prior to initiation of exposure. Nonetheless, the greater and more consistent decreased body weight in the 1500 ppm-exposed females appears to have been due to exposure to the test material. Other statistically significant deviations in body weight were observed, but these were interpreted to be a reflection of normal biological variability since they did not follow a pattern that suggested an exposure-related effect.

There were no unusual in-life clinical observations during the study that were considered to be exposure related.

The hematology, urinalysis, and clinical chemistry values, absolute organ weights, and relative organ weights (g/100 g body wt) for male and female rats sacrificed at the various time intervals throughout the study were unaffected by treatment.

The gross necropsy observations for all groups of rats from the predesignated interim sacrifices or from the 2-year portion of the study did not reveal an exposure-related effect.

There were no early deaths in groups of rats sacrificed at 6 and 12 months. Several rats predesignated for the 18-month sacrifice died prior to the scheduled sacrifice. Review of the gross and microscopic findings in the animals that died revealed a spectrum of normal age-related changes not considered related to exposure.

The only apparent exposure-related effect observed in rats from the 6-, 12-, and 18-month interim sacrifices was found micro-

TABLE 2

SELECTED NEOPLASMS: RATS[a]

	Males				Females			
Exposure concentration (ppm)	0	150	500	1500	0	150	500	1500
Number of rats examined	50	50	50	50	50	50	50	50
Subcutaneous tissue (number of tissues examined)	50	50	50	50	50	50	50	50
Fibroma, benign, primary	11	6	2[b]	0[b,c]	4	0	1	0
Testes (number of tissues examined)	50	50	50	50	—[d]	—	—	—
Interstitial cell tumor, unilateral, benign, primary	7	11	3	4	—	—	—	—
Interstitial cell tumor, bilateral, benign, primary	36	30	38	45[c]	—	—	—	—
Interstitial cell tumor, unilateral or bilateral	43	41	41	49	—	—	—	—
Multiple organs (number of tissues examined)	50	50	50	50	50	50	50	50
Mononuclear cell leukemia, malignant	24	29	23	20	17	25	18	11

[a] Data are the number of animals with the specified observation noted on microscopic examination of tissues.
[b] Statistically identified difference from control group, Yates χ^2 test, $\alpha = 0.05$.
[c] Linear trend by Cochran–Armitage Test, $\alpha = 0.02$ (two-sided).
[d] —, Not applicable.

scopically in the liver. An accentuation of the normal hepatic lobular pattern was observed in males and females in the 1500 ppm-exposed group. The effect on the hepatocytes consisted of altered cytoplasmic staining in the cells surrounding the central vein. Generally, the hepatocytes in the portal region appeared smaller in the exposed rats when compared with their respective controls. The very slight response in the hepatocytes surrounding the central vein was interpreted to have resulted from exposure to the test material and/or its metabolites. The minimal exposure-related effects in the liver observed at earlier necropsy times were no longer discernible after 2 years of exposure due to confounding geriatric changes.

The only neoplastic lesions that were statistically identified as different from controls were subcutaneous fibromas and *bilateral* testicular interstitial cell neoplasms in male rats (Table 2). The incidence of subcutaneous fibromas in male rats was statistically decreased compared with controls at the 1500-ppm exposure level. The total incidence of unilateral *or* bilateral benign testicular interstitial cell tumors was not statistically different from that of controls at any exposure level; however, the number of rats with *bilateral* tumors was statistically identified as increased from controls. Since the total number of male rats with interstitial cell tumors was comparable in all groups and this tumor type has such a high incidence in the Fischer 344 rat, this finding was not considered to have any toxicological significance. There was no indication of an increased incidence of mononuclear cell leukemia, or any lymphoreticular proliferative process, in rats of either sex (Tables 2 and 3). Other statistically identified observations were noted and interpreted to be reflective of normal variability in aged rats. Due to normal age-related geriatric changes there were no discernible exposure-related microscopic hepatic effects noted in the rats at the end of the 2-year study.

Mice

There were no statistically significant differences in survival rates of male or female exposed groups of mice when compared with their respective controls (Fig. 1). There was

TABLE 3

TUMOR INCIDENCE: RATS[a]

	Males				Females			
Exposure concentration (ppm)	0	150	500	1500	0	150	500	1500
Number of rats examined	50	50	50	50	50	50	50	50
Adrenals (number of tissues examined)	50	50	50	50	49	50	50	50
Adenoma, cortex, benign	1	0	0	0	0	0	1	1
Carcinoma, cortex, malignant	1	1	0	0	0	0	0	0
Ganglioneuroma, benign	0	1	0	0	0	0	0	0
Pheochromocytoma, benign	5	2	5	4	1	0	1	0
Pheochromocytoma, malignant	1	1	1	1	1	1	0	0
Brain (number of tissues examined)	50	0	1	50	50	2	0	50
Astrocytoma, malignant	1	—[b]	0	1	0	0	—	1
Cervix (number of tissues examined)	—	—	—	—	50	3	1	50
Fibroma, benign	—	—	—	—	1	0	0	1
Adenocarcinoma, malignant	—	—	—	—	0	0	1	0
Stromal sarcoma, malignant	—	—	—	—	0	2	0	0
Epididymides (number of tissues examined)	50	0	3	49	—	—	—	—
Sarcoma, undifferentiated, malignant	0	—	0	1	—	—	—	—
Jejunum (number of tissues examined)	39	2	0	41	41	0	0	37
Adenocarcinoma, malignant	1	0	—	0	0	—	—	0
Leiomyosarcoma, malignant	0	1	—	0	0	—	—	0
Kidneys (number of tissues examined)	50	50	50	50	50	50	50	50
Adenoma, tubule cell, benign	0	0	0	1	0	0	0	0
Mixed mesenchymal tumor, malignant	0	0	1	1	0	0	0	0
Liver (number of tissues examined)	50	50	50	50	50	50	50	50
Neoplastic nodule, benign	1	1	1	4	1	1	0	0
Carcinoma, hepatocellular, malignant	0	0	3	0	1	0	0	0
Lung (number of tissues examined)	50	49	50	50	50	50	50	50
Adenoma, alveolar/bronchiolar, benign	0	0	1	1	0	0	0	0
Mammary gland (number of tissues examined)	49	1	1	50	50	3	8	49
Adenoma, benign	1	0	0	0	0	1	1	1
Fibroadenoma, benign	1	0	1	3	6	0	7	4
Adenocarcinoma, malignant	0	0	0	0	1	2	1	1
Multiple organs (number of tissues examined)	50	50	50	50	50	50	50	50
Mesothelioma, malignant	2	1	3	3	0	0	0	0
Mononuclear cell leukemia, malignant	24	29	23	20	17	25	18	11
Ovaries (number of tissues examined)	—	—	—	—	47	1	2	50
Granulosa/thecal cell tumor, benign	—	—	—	—	0	0	0	2
Granulosa/thecal cell tumor, malignant	—	—	—	—	0	1	0	0
Pancreas (number of tissues examined)	50	5	6	50	48	0	0	49
Adenoma, acinar cell, benign	1	0	0	1	0	—	—	0
Adenoma, islet cell, benign	7	1	4	2	0	—	—	1
Carcinoma, islet cell, malignant	3	3	1	1	0	—	—	0
Pituitary (number of tissues examined)	47	48	50	50	49	49	48	47
Adenoma, benign	13	18	13	10	22	20	19	16
Carcinoma, malignant	2	3	2	2	2	2	0	0
Preputial/clitoral gland (number of tissues examined)	3	1	0	4	3	1	0	1
Carcinoma, malignant	1	1	—	2	1	0	—	0
Squamous cell carcinoma, malignant	0	0	—	1	0	0	—	0

[a] Data are the number of animals with the specified observation. Only tissues with a primary tumor are tabulated; other examined tissues are not shown.

[b] —, Not applicable.

TABLE 3—*Continued*

	Males				Females			
Exposure concentration (ppm)	0	150	500	1500	0	150	500	1500
Number of rats examined	50	50	50	50	50	50	50	50
Prostate (number of tissues examined)	50	0	0	50	—	—	—	—
Adenoma, benign	1	—	—	0	—	—	—	—
Salivary gland (number of tissues examined)	50	0	0	50	50	0	1	50
Fibrosarcoma, malignant	0	—	—	0	0	—	1	0
Spleen (number of tissues examined)	50	48	50	50	49	50	50	50
Histiocytic sarcoma, malignant	1	0	0	0	0	0	0	0
Subcutaneous tissue (number of tissues examined)	12	8	4	6	7	0	2	3
Fibroma, benign	11	6	2	0	4	—	1	0
Neurofibroma, benign	0	0	1	1	0	—	0	0
Trichoepithelioma, benign	0	0	0	1	0	—	0	0
Fibrosarcoma, malignant	0	0	0	0	1	—	0	0
Myxosarcoma, malignant	0	0	0	0	1	—	0	0
Osteosarcoma, malignant	1	2	0	0	0	—	0	0
Testes (number of tissues examined)	50	50	50	50	—	—	—	—
Interstitial cell tumor, benign, unilateral	7	11	3	4	—	—	—	—
Interstitial cell tumor, benign, bilateral	36	30	38	45	—	—	—	—
Thyroid (number of tissues examined)	47	44	49	45	45	49	45	48
Adenoma, "C" cell, benign	2	2	2	2	2	0	2	0
Adenoma, follicular cell, benign	1	1	0	1	1	0	0	1
Carcinoma, "C" cell, malignant	2	1	2	1	1	1	3	1
Carcinoma, follicular cell, malignant	0	0	0	0	0	0	0	1
Thymus (number of tissues examined)	38	0	1	43	43	0	0	42
Thymoma, malignant	0	—	1	0	0	—	—	0
Tongue (number of tissues examined)	50	0	1	50	50	1	0	50
Squamous cell papilloma, benign	0	—	1	0	0	1	—	0
Urinary bladder (number of tissues examined)	47	2	0	48	46	0	0	49
Transitional cell papilloma, benign	0	2	—	0	0	—	—	0
Uterus (number of tissues examined)	—	—	—	—	50	12	11	50
Adenoma, benign	—	—	—	—	0	1	0	0
Endometrial stromal polyp, benign	—	—	—	—	14	8	9	7
Leiomyoma, benign	—	—	—	—	0	0	1	0
Adenocarcinoma, malignant	—	—	—	—	0	1	0	2
Squamous cell carcinoma, malignant	—	—	—	—	0	0	1	0

a slightly greater, not statistically identified, frequency of dead or moribund male mice submitted for necropsy from the 500-ppm group during the last several months of the study.

The body weights of male and female mice of all exposed groups were comparable to those of the controls throughout the study (Fig. 2).

There were no unusual in-life clinical observations during the study that were considered to be exposure related. In all groups of male mice the most common in-life palpable "mass" noted was in the preputial area. These were not neoplasms but rather were the result of an inflammatory process involving the penile and preputial region. Some mice recovered from the inflammatory process and no gross or microscopic lesion was found. However, swelling, ulceration, paraphimosis, and urinary tract obstruction which resulted in death or a moribund condition were observed in some mice from all exposure groups. The 500-ppm group of male

TABLE 4

SELECTED NEOPLASMS: MICE[a]

	Males				Females			
Exposure concentration (ppm)	0	150	500	1500	0	150	500	1500
Number of mice examined	50	50	50	50	50	50	50	50
Lacrimal/Harderian gland(s) (number of tissues examined)	50	49	50	50	50	50	50	50
Adenoma, benign, primary	0	0	0	0	0	0	0	1
Cystadenoma, acini, benign, primary	8	7	5	4	3	1	2	6
Cystadenoma, acini, benign, primary (two)	0	1	0	0	0	0	0	0
Total number of mice with adenoma or cystadenoma	8	8	5	4	3	1	2	7[b]
Multiple organs (number of tissues examined)	50	50	50	50	50	50	50	50
Leukemia—myeloid cell—primary in bone marrow	0	0	0	1	0	0	0	0
Lymphosarcoma, localized or disseminated								
Primary in Peyer's patch	1	1	0	0	0	0	0	1
Primary in kidneys	0	0	0	0	0	0	0	1
Primary in liver	0	0	0	0	0	0	0	1
Primary in mediastinal lymph node	0	0	1	0	2	0	1	1
Primary in mesenteric lymph node	7	3	3	4	12	13	12	11
Primary in subcutaneous lymph node	0	1	0	0	0	0	0	0
Primary in miscellaneous lymph nodes	0	0	0	0	1	0	1	0
Primary in spleen	0	0	0	1	2	1	1	2
Total number of mice with lymphosarcoma of any site	8	5	4	5	17	14	15	17
Liver (number of tissues examined)	50	50	50	50	50	50	50	50
Benign, primary hepatocellular neoplasm (adenoma)	26	13[c]	19	16	10	9	5	5
Malignant, primary hepatocellular neoplasm (carcinoma)	12	10	12	12	4	1	5	2
Total with one or more primary hepatocellular neoplasms, benign or malignant	29	22	28	24	13	10	10	7

[a] Data are the number of animals with the specified observation noted on microscopic examination of tissues.
[b] Statistically identified linear trend by Cochran-Armitage test, $\alpha = 0.05$ (one-sided).
[c] Statistically identified difference from control group by Fisher's exact probability test, $\alpha = 0.05$.

mice was more frequently affected than the other groups, and this was the primary reason for the slightly higher mortality in this group during the last few months of the study.

The hematology and clinical chemistry values for male and female mice sacrificed at the various time intervals throughout the study were unaffected by treatment. The final body weights, absolute organ weights, and relative organ weights (g/100 g body wt) for male and female mice sacrificed at the various time intervals were considered to be unaffected by exposure.

The gross necropsy observations for all groups of mice from the predesignated interim sacrifices or from the 2-year portion of the study did not reveal a significant adverse effect that was considered due to exposure. Although the livers of some male mice in the 1500-ppm group at 6 months appeared grossly to have an accentuated lobular pattern, there were no findings on microscopic examination of the liver or evaluation of liver weights and clinical chemistry values that suggested an exposure-related effect. Eight male mice and three female mice from various exposure groups died prior to scheduled sacrifices at 6, 12, and 18 months. For two of the mice an apparent cause of death was not determined. The remaining mice died with inflammation and obstruction of the genitourinary tract (4), trauma (2), liver tumor (one control and one 1500 ppm male), and bilateral adrenal gland tumor (one 150 ppm male).

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TABLE 5

TUMOR INCIDENCE: MICE[a]

	Males				Females			
Exposure concentration (ppm)	0	150	500	1500	0	150	500	1500
Number of mice examined	50	50	50	50	50	50	50	50
Adrenals (number of tissues examined)	50	48	48	49	49	48	50	50
Adenoma, cortex, benign (two)	0	0	1	0	0	0	0	0
Pheochromocytoma, benign	0	0	0	0	1	0	1	0
Pheochromocytoma, malignant, no metastasis	0	0	0	0	1	0	0	0
Pheochromocytoma, malignant, metastasis	0	0	0	0	0	1	1	0
Bone (number of tissues examined)	50	49	50	50	50	50	50	50
Osteogenic sarcoma, pelvis, malignant, metastasis	0	1	0	0	0	0	0	0
Bone marrow (number of tissues examined)	50	49	50	50	50	50	50	50
Leukemia—myeloid cell, multiple organs, malignant	0	0	0	1	0	0	0	0
Mast cell tumor, multiple organs, malignant, metastasis	0	1	0	0	0	0	0	0
Brain (number of tissues examined)	50	50	50	50	50	50	50	50
Granular cell tumor, meninges, benign	1	0	0	0	0	0	0	0
Cecum (number of tissues examined)	50	50	50	50	50	50	50	50
Lymphosarcoma, Peyer's patch, malignant, metastasis	0	1	0	0	0	0	0	0
Cervix (number of tissues examined)	—[b]	—	—	—	50	50	49	50
Leiomyoma, muscularis, benign	—	—	—	—	0	1	0	0
Stromal cell sarcoma, malignant, no metastasis	—	—	—	—	0	0	0	1
Epididymides (number of tissues examined)	50	50	49	50	—	—	—	—
Adenoma, head, benign	1	0	0	0	—	—	—	—
Fibrosarcoma, tail, malignant, no metastasis	2	0	1	1	—	—	—	—
Kidneys (number of tissues examined)	50	50	50	50	50	50	50	50
Adenoma, cortex, benign	0	0	1	0	0	0	0	1
Adenocarcinoma, cortex, malignant, no metastasis	1	0	0	1	0	0	0	0
Lymphosarcoma, malignant, no metastasis	0	0	0	0	0	0	0	1
Lacrimal/Harderian gland(s) (number of tissues examined)	50	49	50	50	50	50	50	50
Adenoma, benign	0	0	0	0	0	0	0	1
Cystadenoma, acini, benign	8	7	5	4	3	1	2	6
Cystadenoma, acini, benign (two)	0	1	0	0	0	0	0	0
Large intestine (number of tissues examined)	50	50	50	50	50	50	50	50
Squamous cell carcinoma, anus–rectum, malignant, no metastasis	0	0	0	0	0	0	0	1
Liver (number of tissues examined)	50	50	50	50	50	50	50	50
Adenoma, hepatocellular, benign	20	11	16	12	8	8	4	3
Adenoma, hepatocellular, benign (two)	4	2	3	4	1	1	1	2
Adenoma, hepatocellular, benign (three)	2	0	0	0	1	0	0	0
Hemangioma, benign	0	0	1	0	0	0	0	1
Carcinoma, hepatocellular, malignant, no metastasis	8	5	5	7	3	1	1	2
Carcinoma, hepatocellular, malignant, no metastasis (two)	0	0	0	0	1	0	0	0
Carcinoma, hepatocellular, malignant, metastasis	4	6	7	5	0	0	4	0
Lymphosarcoma, malignant, no metastasis	0	0	0	0	0	0	0	1
Lungs (number of tissues examined)	50	50	50	50	50	50	50	50
Adenoma, alveoli, benign	10	7	4	3	2	3	4	6
Adenoma, alveoli, benign (two)	0	0	0	0	1	0	1	0
Adenocarcinoma, alveoli, malignant, no metastasis	2	2	0	0	1	0	0	0

[a] Data are the number of animals with the specified observation. Only tissues with a primary tumor are tabulated; other examined tissues are not shown.

[b] —, Not applicable.

TABLE 5—*Continued*

	Males				Females			
Exposure concentration (ppm)	0	150	500	1500	0	150	500	1500
Number of mice examined	50	50	50	50	50	50	50	50
Mammary gland (number of tissues examined)	0	0	0	0	45	44	48	48
Fibroadenoma, benign	—	—	—	—	2	3	1	2
Carcinoma, malignant, no metastasis	—	—	—	—	3	3	2	2
Carcinoma, malignant, no metastasis (two)	—	—	—	—	1	0	0	0
Carcinoma, malignant, metastasis	—	—	—	—	0	0	1	1
Mediastinal lymph node (number of tissues examined)	43	39	33	41	35	35	33	40
Lymphosarcoma, multiple organs, malignant, metastasis	0	0	1	0	2	0	1	1
Mesenteric lymph node (number of tissues examined)	49	50	45	49	50	49	49	50
Lymphosarcoma, malignant, no metastasis	0	0	0	0	0	0	0	1
Lymphosarcoma, multiple organs, malignant, metastasis	7	3	3	4	12	13	12	10
Multiple organs (number of tissues examined)	50	50	50	50	50	50	50	50
Lymphosarcoma, subcutaneous, malignant, no metastasis	0	1	0	0	0	0	0	0
Lymphosarcoma, multiple organs, malignant, metastasis	0	0	0	0	1	0	1	0
Reticulum cell sarcoma, lumbar, malignant, metastasis	0	0	0	0	0	1	0	0
Oral tissues (number of tissues examined)	14	16	8	8	4	11	11	9
Odontoma, benign	0	0	0	0	0	0	0	1
Squamous papilloma, hard palate, benign	0	2	0	0	0	0	0	0
Ovaries (number of tissues examined)	—	—	—	—	43	47	45	49
Granulosa—thecal cell tumor, benign	—	—	—	—	1	1	0	0
Teratoma, benign	—	—	—	—	0	0	1	0
Granulosa—thecal cell tumor, malignant, metastasis	—	—	—	—	0	0	0	1
Oviducts (number of tissues examined)	—	—	—	—	50	50	49	50
Papillary adenoma, benign	—	—	—	—	0	0	0	1
Pancreas (number of tissues examined)	50	50	48	50	50	50	50	50
Adenoma, islets, benign	0	0	0	0	2	0	0	0
Adenocarcinoma, ducts, malignant, metastasis	0	0	0	0	0	0	0	1
Pituitary (number of tissues examined)	49	46	47	44	43	45	48	48
Adenoma, anterior (pars distalis), benign	0	0	1	1	10	5	11	5
Adenoma, pars intermedia, benign	1	0	0	0	3	0	1	0
Carcinoma, anterior (pars distalis), malignant, no metastasis	0	0	0	0	1	0	0	0
Carcinoma anterior (pars distalis), malignant, metastasis	0	0	0	0	1	0	1	1
Skin and subcutis (number of tissues examined)	48	49	48	49	50	49	50	49
Hemangioma, subcutaneous, benign	0	0	0	1	0	0	1	2
Squamous papilloma, benign	0	0	0	0	1	0	0	0
Basal cell carcinoma, malignant, no metastasis	0	0	0	0	1	0	0	0
Fibrosarcoma, head, malignant, no metastasis	0	0	1	0	0	0	0	0
Fibrosarcoma, subcutaneous, malignant, no metastasis	0	0	0	0	0	0	0	1
Fibrosarcoma, pinna, malignant, no metastasis	0	0	0	0	0	1	0	0
Fibrosarcoma, pinna, malignant, metastsis	0	0	0	0	0	1	0	0
Undifferentiated sarcoma, subcutaneous, malignant, no metastasis	0	0	0	0	0	1	0	0
Small intestine (number of tissues examined)	50	50	50	50	50	50	50	50
Polyp, mucosa, benign	0	0	0	0	0	2	0	0
Adenocarcinoma, mucosa, malignant, no metastasis	0	0	0	0	0	1	0	0
Lymphosarcoma, Peyer's patch, malignant, no metastasis	1	0	0	0	0	0	0	1
Spleen (number of tissues examined)	50	50	49	50	50	50	50	50
Hemangioma, benign	0	0	1	0	1	0	0	0
Lymphosarcoma, malignant, no metastasis	0	0	0	0	0	1	1	1

TABLE 5—*Continued*

	Males				Females			
Exposure concentration (ppm)	0	150	500	1500	0	150	500	1500
Number of mice examined	50	50	50	50	50	50	50	50
Lymphosarcoma, multiple organs, malignant, metastasis	0	0	0	1	2	0	0	1
Reticulum cell sarcoma, multiple organs, malignant, metastasis	0	0	0	1	0	0	0	0
Stomach (number of tissues examined)	50	50	50	49	50	50	50	50
Papilloma, nonglandular mucosa, benign	0	0	1	0	1	1	0	1
Polypoid adenoma, glandular mucosa, benign	0	1	0	0	0	0	0	0
Squamous cell carcinoma, nonglandular mucosa, malignant, no metastasis	0	0	0	0	1	0	0	0
Thyroid gland (number of tissues examined)	48	47	49	48	49	50	50	50
Adenoma, follicle(s), benign	1	0	1	0	1	1	0	1
Tongue (number of tissues examined)	50	50	50	50	50	50	50	50
Squamous papilloma, benign	0	0	0	0	1	0	0	0
Squamous cell carcinoma, malignant, no metastasis	0	1	0	0	0	0	1	0
Uterus (number of tissues examined)	—	—	—	—	50	50	49	50
Endometrial stromal polyp, benign	—	—	—	—	2	0	2	2
Hemangioma, benign	—	—	—	—	1	1	0	0
Leiomyoma, muscularis, benign	—	—	—	—	2	0	2	1
Adenocarcinoma, malignant, no metastasis	—	—	—	—	0	1	0	0
Adenocarcinoma, malignant, metastasis	—	—	—	—	0	0	0	1
Leiomyosarcoma, muscularis, malignant, metastasis	—	—	—	—	1	0	0	0
Reticulum cell sarcoma, malignant, metastasis	—	—	—	—	0	0	1	0
Stromal cell sarcoma, malignant, no metastasis	—	—	—	—	0	1	0	0
Stromal cell sarcoma, malignant, metastasis	—	—	—	—	1	0	0	0
Vagina (number of tissues examined)	—	—	—	—	50	50	49	50
Fibrosarcoma, malignant, no metastasis	—	—	—	—	1	0	0	0

1978). There was no indication of an increased incidence of lymphoreticular proliferative processes in either Fischer 344 rats or B6C3F1 mice in this 2-year inhalation study. A previous gavage bioassay study conducted in rats also did not demonstrate an oncogenic effect with a 1,1,1-trichloroethane formulation (NCI, 1977). The present study in rats, as well as several other chronic bioassays, does not indicate an increased incidence of immunoblastic lymphosarcoma as suggested by the inconclusive bioassay conducted by Maltoni *et al.* (1986).

In conclusion, the repeated exposure of male and female Fischer 344 rats and B6C3F1 mice to 0, 150, 500, or 1500 ppm of a 1,1,1-trichloroethane formulation 5 days per week for 2 years resulted in very slight microscopic hepatic effects in rats. The hepatic effect was discernible in the rats from the 1500-ppm group sacrificed at 6, 12, and 18 months. Due to confounding geriatric changes in aged rats, the liver effects were no longer detected at the end of the 2-year study. A slight exposure-related decrease in body weight was observed in the 1500-ppm female rats. There were no changes in the rats exposed to 150 and 500 ppm, or in the mice at any exposure level, that were considered due to the 1,1,1-trichloroethane formulation. Exposure to vapors of the 1,1,1-trichloroethane formulation for 2 years did not result in an oncogenic effect in either the Fischer 344 rat or the B6C3F1 mouse.

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REFERENCES

ADAMS, E. M., SPENCER, H. C., ROWE, V. K., AND IRISH, D. D. (1950). Vapor toxicity of 1,1,1-trichloroethane (methyl chloroform) determined by experiments on laboratory animals. *Arch. Ind. Hyg. Occup Med.* **1**, 225–236.

RMITAGE, P. (1971). *Statistical Methods in Medical Research.* Wiley, New York.

BRESLOW, N. (1970). A generalized Kruskal–Wallis test for comparing K samples subject to unequal patterns of censorship. *Biometrika* 57, 579–594.

CALHOUN, L. L., QUAST, J. F., SCHUMANN, A. M., SCHUETZ, D. J., CARREON, R. E., DITTENBER, D. A., AND MCKENNA, M. J. (1981). *CHLOROTHENE VG: Preliminary Studies to Establish Exposure Concentrations for a Chronic Inhalation Study with Rats and Rats.* Unpublished Report–Manuscript in Progress, Mammalian and Environmental Toxicology Research Laboratory, Dow Chemical USA.

FLEISS, J. L. (1981). *Statistical Methods for Rates and Proportions.* Wiley, New York.

GEHRING, P. J. (1968). Hepatotoxic potency of various chlorinated hydrocarbon vapors relative to their narcotic and lethal potencies in rats. *Toxicol. Appl. Pharmacol.* **13**, 287–298.

GRUBBS, F. E. (1969). Procedures for detecting outlying observations in samples. *Technometrics* **11**(1), 1–21.

HASEMAN, J. K. (1983). A reexamination of false-positive rates for carcinogenesis studies. *Fundam. Appl. Toxicol.* 3, 334–339.

HASEMAN, J. K. (1985). *Use of Multiple Control Groups to Estimate False Positive Rates in Carcinogenicity Studies.* Paper presented at annual meeting of American Statistical Association, August 1985, Las Vegas, NV.

HOLLANDER, M., AND WOLFE, D. A. (1973). *Nonparametric Statistical Methods.* Wiley, New York.

MACEWEN, J. D., AND VERNOT, E. H. (1974). *The Biological Effect of Continuous Inhalation Exposure of 1,1,1-Trichloroethane (Methyl Chloroform) on Animals,* THRU Annual Technical Report AMRL-TR-74-78, pp. 81–90.

MALTONI, C., COTTI, G., AND PATELLA, V. (1986). Results of long-term carcinogenicity bioassays on Sprague–Dawley rats of methyl chloroform, administered by ingestion. *Acta Oncol.* **1**(2), 101–117.

MCNUTT, N. S., AMSTER, R. L., MCCONNELL, E. E., AND MORRIS, F. (1975). Hepatic lesions in rats after continuous inhalation exposure to 1,1,1-trichloroethane. *Lab. Invest.* 32, 642–654.

MILLER, R. G., JR. (1966). *Simultaneous Statistical Inference.* McGraw–Hill, New York.

MILLER, R. R., LETTS, R. L., POTTS, W. J., AND MCKENNA, M. J. (1980). Improved methodology for generating controlled test atmospheres. *Amer. Ind. Hyg. Assoc. J.* **41**, 844–846.

National Cancer Institute (NCI) (1977). *Bioassay of 1,1,1-Trichloroethane for Possible Carcinogenicity,* NCI-CG-TR-3. U.S. DHEW Public Health Service, National Institutes of Health.

PARK, C. N. (1985). *Control of False Negative and False Positive Error Rates in Chronic Bioassays.* Paper presented at annual meeting of American Statistical Association, August 1985, Las Vegas, NV.

PARK, C. N., AND KOCIBA, R. J. (1985). Statistical evaluation of long-term animal bioassays for carcinogenicity. In *Handbook for Carcinogen Testing* (H. A. Milman and E. K. Weisburger, Eds.), pp. 358–371. Noyes Publications, Park Ridge, NJ.

QUAST, J. F., RAMPY, L. W., BALMER, M. F., LEONG, B. K. J., AND GEHRING, P. J. (1978). *Toxicologic and Carcinogenic Evaluation of a 1,1,1-Trichloroethane Formulation by Chronic Inhalation in Rats.* Unpublished Report, Toxicology Research Laboratory, Dow Chemical U.S.A.

RAMPY, L. W., QUAST, J. F., LEONG, B. K. J., AND GEHRING, P. J. (1977). *Results of Long-Term Inhalation Studies on Rats of 1,1,1-Trichloroethane and Perchloroethylene Formulations.* Paper presented at First International Congress on Toxicology, Toronto, April 1977.

SCHUMANN, A. M., FOX, T. R., AND WATANABE, P. G. (1982). A comparison of the fate of inhaled methyl chloroform (1,1,1-trichloroethane) following single or repeated exposure in rats or mice. *Fundam. Appl. Toxicol.* 2, 27–32.

SHELDON, W. G., CURTIS, M., KODELL, R. L., AND WEED, L. (1983). Primary Harderian gland neoplasms in mice. *J. Natl. Cancer Inst.* **71**(1), 61–65.

SIEGEL, S. (1956). *Nonparametric Statistic for the Behavioral Sciences.* McGraw–Hill, New York.

STEEL, R. G. D., AND TORRIE, J. H. (1960). *Principles and Procedures of Statistics.* McGraw–Hill, New York.

WINER, B. J. (1971). *Statistical Principles in Experimental Design,* 2nd ed. McGraw–Hill, New York.