III. BIOLOGIC EFFECTS OF EXPOSURE

Extent of Exposure

Inhalation anesthesia was first used in 1842 [1,2]. Since then, a number of different chemical compounds have been used as inhalation anesthetic agents. Table III-1 lists the most widely used agents and the year each was introduced as an anesthetic. The more important physical and chemical properties of these anesthetic agents are presented in Table XIII-1 [3-11].

TABLE III-1

INHALATION ANESTHETIC AGENTS

Generic Name	Commercial Name	Year of Introduction
Diethyl ether	Ether	1842
Nitrous oxide	Nitrous oxide	1844
Chloroform	Chloroform	1847
Cyclopropane	Cyclopropane	1933
Trichloroethylene	Trilene	1934
Fluroxene	Fluoromar	1954
Halothane	Fluothane	1956
Methoxyflurane	Penthrane	1960
Enflurane	Ethrane	1974
Isoflurane	Forane	Investigational

Derived from references 1 and 2

Many of the earlier inhalation anesthetics, such as diethyl ether and cyclopropane, are flammable and explosive and have been largely replaced by nonexplosive, nonflammable agents, such as halothane and methoxyflurane. Halothane and nitrous oxide are currently the two most widely used inhalation anesthetic agents in the United States. Diethyl ether is still used to some extent.

It has been estimated that approximately 20 million patients are anesthetized each year with inhalation anesthetics in the 25,000 hospital operating rooms throughout the United States [1,2] and about 4.5 million patients are anesthetized by dentists [12]. In addition, dentists deliver a much larger number of analgesic sedations than anesthesias. Approximately 50,000 hospital operating room personnel are exposed daily to waste anesthetic gases in the US. This figure does not include surgeons, who usually do not operate every day. Table III-2 [12,13] lists by professional group the number of potentially exposed operating room and dental personnel in the United States in 1975.

In addition to hospital operating room and dental personnel, veterinarians and their technical assistants have the potential for exposure to waste anesthetic gases; their estimated number is also presented in Table III-2. Similarities in the practice of anesthesia in human and veterinary hospitals and clinics result in many of the same problems of exposure for both groups. Exposures among veterinarians may range from sporadic to almost continuous on a daily basis.

TABLE III-2

NUMBER OF OPERATING, DENTAL, AND VETERINARY
PERSONNEL POTENTIALLY EXPOSED TO ANESTHETIC GASES

Professional Group	Membership
Anesthesiologists (ASA)	13,700
Nurse-anesthetists (AANA)	17,546
Operating room nurses (AORN)	21,600
Operating room technicians (AORT)	12,000
Dentists and assistants (ASOS,ADA,ADAA)	100,000
Veterinarians and employees (AVMA)	50,000

Derived from reference 12 and 13

Historical Reports

Diethyl ether was first used as an inhalation anesthetic in Jefferson, Georgia, in 1841 [2,2]. Crawford Long, a general practitioner, anesthetized a patient with diethyl ether and removed a tumor from his neck. The procedure went unrecognized until several years later. William Morton used diethyl ether as an anesthetic at the Massachusetts General Hospital in 1846 to produce surgical anesthesia and is generally recognized as the one who introduced the technique.

Nitrous oxide was used by Horace Wells to alleviate the pain of dental extractions. He attempted to introduce nitrous oxide as an

inhalation anesthetic in 1844 at the Massachusetts General Hospital but failed. Despite this failure, nitrous oxide is now the inhalation anesthetic most widely used in medical and dental practice.

Chloroform was introduced as an inhalation anesthetic in 1847 by James Simpson, an English surgeon and obstetrician, who reported his discovery that same year [14,15]. He advocated the use of chloroform because it was more pleasant to inhale than diethyl ether and required only a small amount to produce narcosis. He recommended chloroform especially for the obstetrical patient. The deleterious effects of exposure to decomposing chloroform on operating room personnel was observed during the late 1880's [16].

Halothane, presently the most commonly used halogenated anesthetic agent, was introduced in 1956 [17]. It seemed to be the most promising of a number of fluorinated agents because of its volatility, non-explosiveness when mixed with oxygen, and apparent lack of any serious physiologic side effects.

Recognition by anesthesiologists of possible deleterious effects from repeated exposure to anesthetic gases during their administration to patients was the subject of a 1922 editorial [18]. It paid tribute to a noted Chicago anesthesiologist, Edward Costain, who had administered anesthetics to more than 30,000 people during 30 years of practice. Gilbert Fitzpatrick, an associate of Dr. Costain, was quoted as saying, "While we have not been able to prove it definitely, still we have much evidence to show that the administration of anesthetics, over a long period of years, produces a condition of nephritis that results fatally."

Hirsch and Kappus [19] reported, in 1929, the first quantitative study of concentrations of anesthetic gases in the air of operating rooms. They cited references in the German literature of reports from operating room personnel of headaches, fatigue, and, in older surgeons, heart complaints. Chloroform and ether were the anesthetic agents associated with these adverse effects.

The case studies on three persons from a surgical team (surgeon, surgical nurse, and anesthesiologist) who were exposed to ether vapor over a period of 4-14 years were reported in 1949 by Werthmann [20]. The exposed personnel showed signs and symptoms of general fatigue, rapid exhaustion, frequent headache, lymphocytosis, eosinophilia, and in the case of the eldest of the three, of electrocardiographic evidence of myocardial damage.

Effects on Humans

Much of the data on side effects of acute exposure have been obtained by studying patients who experienced some type of complication following clinical anesthesia. Since it is not known if acute effects will be experienced after chronic exposure, the side effects of acute exposure are reviewed here.

(a) Acute Effects on the Liver and Kidneys

A retrospective survey of the incidence of massive hepatic necrosis and death in patients after general anesthesia in 34 hospitals from 1959 to 1962 was reported by the National Academy of Sciences [21] in 1966. The main conclusion was that fatal postoperative massive hepatic necrosis was rare. It could usually be explained on the basis of circulatory shock,

sepsis, or preexisting hepatic disease. The possible rare occurrence of halothane-induced hepatic necrosis after single or multiple administrations could not be ruled out.

Other studies [21-32] of the hepatotoxicity of halothane inhalation anesthesia have been reported and are noted here but not summarized. The extent to which this might occur in workers chronically exposed to subanesthetic concentrations is unknown.

A series of reports of possible renal toxicity following methoxyflurane general anesthesia have appeared since 1966 [34-39]. The primary signs of such toxicity were increased blood urea nitrogen (BUN), increased urinary inorganic and nonvolatile organic fluoride levels, and polyuria. Five fatal cases were reported following methoxyflurane anesthesia [34,35] with the most significant post-mortem finding being oxalate crystals within the distal, cortical, and medullary kidney tubules. The authors speculated that fluoride and oxalate are both nephrotoxic and possible metabolites of methoxyflurane.

(b) Psychologic Effects

Several studies have been conducted with human volunteers to measure the effects on psychologic performance from exposure to low concentrations of trichloroethylene [40-45]. Kylin et al [40] exposed 12 subjects to trichloroethylene at 1,000 ppm for 2 hours. They concluded that exposure had an effect on the CNS based on the development of optokinetic nystagmus, but the effect was less marked than in similar tests with alcohol.

Vernon and Ferguson [41] reported the results of experimental 2-hour exposures of eight young male volunteers, aged 21-30, to trichloroethylene at concentrations of 0, 100, 300, and 1,000 ppm. On the basis of various

psychophysiologic tests, statistically significant decrements in performance were reported only at 1,000 ppm. One subject exposed at 300 ppm complained of lightheadedness and dizziness.

Stopps and McLaughlin [42] reported the results of psychophysiologic testing of one human subject exposed to trichloroethylene for 2.5-hour periods at concentrations of 100, 200, 300, and 500 ppm. Their studies indicated no significant effects on psychomotor performance at the 100-ppm level. There was a slight decline in performance at 200 ppm, which became progressively more pronounced at the 300- and 500-ppm concentrations.

Stewart et al [43,45] reported the results of a series of experimental exposures of human subjects to trichloroethylene. Time-weighted average (TWA) exposures of 265 ppm and 211 ppm were used for periods of 83 and 190 minutes, respectively. Results of psychophysiologic testing were reported normal in all subjects. In a second study [45], Stewart et al conducted a series of experimental 7-hour exposures of five human subjects to a nonfluctuating 200-ppm level of trichloroethylene on 5 consecutive days. After 30 minutes, two of the subjects complained of throat dryness and one of them of mild eye irritation. The investigators noted that the results of the performance tests were normal. One consistent response was the complaint of "feeling fatigued" on the 4th and 5th days of the exposure.

In 1971, Salvini et al [44] reported the exposure of six male volunteers to trichloroethylene at 110 ppm for two 4-hour periods separated by 1.5-hour intervals. The study showed a significant decrease in performance ability for the perception test with tachistoscopic presentation, the Wechsler Memory Scale, a complex reaction time test, and

a manual dexterity test using a crossed scheme analysis. The authors concluded that such concentrations interfered with the psychologic efficiency of the volunteers.

Stewart et al [46], under NIOSH contract, attempted to duplicate the study of Salvini et al [44] but were unable to corroborate their findings. No significant decrements in performance of the four behavioral tests were found following exposure to trichloroethylene at 110 ppm for 4 hours.

Perceptual, cognitive, and motor skills were studied by Bruce et al [47] using 40 male medical and dental students, 20-30 years old. The subjects were exposed on two occasions to 4 hours of inhalation of either air (control) or 500 ppm nitrous oxide in air with or without 15 ppm halothane. Compared with responses after breathing air, responses after exposure to nitrous oxide and halothane showed statistically significant decreases in the performance of tasks in which attention was divided between auditory and visual signals, a visual tachistoscopic test, and memory tests involving digit span and recall of word pairs. Subjects exposed to nitrous oxide alone scored significantly lower on the digit-span test only. Subsequently, Bruce and Bach [48] exposed 30 human subjects for 4 hours to nitrous oxide at 500 ppm with or without 15 ppm enflurane in air. Within 5 minutes, the subjects were given a 35-minute battery of psychologic tests. Performance of a divided-attention audiovisual task and a digit-span memory test was significantly decreased compared with control data obtained following exposure to air. A pattern-recognition task, four tests from the Wechsler Memory Scale, and five others from the Wechsler Adult Intelligence Scale were unaffected. The 30 subjects exposed at 500 ppm nitrous oxide in air scored significantly lower on the digit-span test

only. The authors concluded from both studies that trace anesthetic concentrations in amounts found in unscavenged operating rooms may interfere with optimum performance on psychologic tests measuring perceptual, cognitive, and motor skills and that if the tests had been performed while the subjects were being exposed, the performance decrements probably would have been even greater.

Further studies by Bruce and Bach [49] showed measurable decrements in performance of volunteers exposed during testing at concentrations as low as 50 ppm of nitrous oxide and 50 ppm nitrous oxide with 1 ppm halothane. A total of 100 male subjects, all between the ages of 20 and 30, were exposed to the anesthetics and each volunteer was tested twice. Visual perception, immediate memory, and a combination of perception, cognition, and motor responses required in a task of divided attention to simultaneous visual and auditory stimuli were tested. Testing began 2 hours after each subject had begun breathing the appropriate gas mixture. Exposure to the anesthetics continued throughout the entire testing period. Exposure at 50 ppm nitrous oxide with 1 ppm halothane caused performance decrements in four of the seven tests administered. Similar effects were not seen in subjects exposed to nitrous oxide at 25 ppm with 0.5 ppm halothane. Exposure at 500 ppm nitrous oxide alone caused performance decrements in six of the seven tests administered. Exposure at 50 ppm nitrous oxide resulted in performance decrements in audiovisual tasks only. A 3-minute and 7-minute audiovisual task was administered approximately 2.75 and 4 hours, respectively, after the subjects had begun breathing the gas mixture.

(c) Metabolism Studies

Kelley and Brown [50] reviewed the literature on biotransformation of trichloroethylene in man. Trichloroethylene is biotransformed into chloral hydrate, trichloroacetic acid, and trichloroethanol. Oxidation of trichloroethylene to chloral hydrate is accomplished by the liver microsomal enzymes, requiring the presence of NADPH and oxygen. Trichloroacetic acid is excreted into the urine unchanged and trichloroethanol is first conjugated with glucuronic acid and then excreted in urine.

The literature on biotransformation of diethyl ether and chloroform was reviewed by Van Poznak [51]. Both agents are biotransformed by liver microsomal enzymes. Although the metabolic pathways for degradation of diethyl ether and chloroform have not been extensively studied, Van Poznak postulated that diethyl ether may form acetaldehyde, which is subsequently reduced to ethanol or oxidized to carbon dioxide. Chloroform is known to be metabolized to carbon dioxide; however, other possible metabolites have not been determined.

Mazze and Cousins [52,53] reviewed the literature on the metabolism of methoxyflurane, enflurane, and isoflurane and on the toxicity of their metabolites. Although several pathways have been suggested for each of these agents, none has been widely accepted. High concentrations of serum and urinary inorganic fluoride and increased levels of urinary oxalic acid have been demonstrated in patients receiving methoxyflurane. Serum and urinary fluoride were increased following enflurane and isoflurane anesthesia. Carbon dioxide, dichloroacetic acid, and difluromethoxyacetic acid have also been suggested as metabolites of methoxyflurane. Mazze and

Cousins suggested that the inorganic fluoride metabolites are possibly the chief etiologic agents for methoxyflurane nephrotoxicity.

Studies of fluroxene metabolism were reviewed by Cascorbi [54]. The observed metabolites are trifluoroethanol, trifluoroacetic acid, and carbon dioxide, although the exact pathway for this metabolism is unknown. Studies using volunteers have shown that 12-15% of the administered dose is metabolized within 24 hours.

Rehder and Sessler [55], Sawyer and Eger [56], Cascorbi [57], and Van Dyke [58] have reviewed the literature concerning biotransformation of halothane. Although not well elucidated, the most widely accepted mechanism is the formation of trifluoroacetic acid with resulting urinary excretion of trifluoroacetic acid and bromide. Man is one of the species most capable of carrying out this reaction, since the rate of conversion to trifluoroacetic acid and bromide is much higher than in other species studied. These metabolities may be found in patients up to 15 days after administration of halothane. Chloride ions have also been considered a metabolite of halothane.

In 1970, Holaday et al [59] reported on the metabolism and excretion of 14-C-labeled methoxyflurane in 12 human subjects, 5 of whom were exposed to methoxyflurane through anesthetic procedures. Biodegradation of the methoxyflurane began immediately after exposure and continued for 9-12 days after which storage areas of the drug approached depletion. In addition to the exhalation of unaltered methoxyflurane, identified products of biotransformation included carbon dioxide, fluoride, dichloroacetic acid and 2,2-methoxyfluoroacetic acid.

Johnstone et al [60] noted serum bromide concentrations after halothane anesthesia in which seven healthy male volunteers received 6.6% hours (SE $\pm$ 0.5) (% concentration X hours of administration) halothane-oxygen anesthesia without surgery. Venus blood samples were obtained immediately before and after anesthesia and 1, 2, 3, 6, and 9 days after anesthesia. In addition, urine samples were taken before and immediately after anesthesia and also 1 and 2 days after anesthesia. Serum bromide and plasma and urinary fluoride analyses were performed. Serum bromide concentrations increased from 0.6 mEq/liter (SE $\pm$ 0.1) before anesthesia to 2.9 mEq/liter (SE $\pm$ 0.2) on the 2nd day after anesthesia. On the 9th day, serum bromide was still elevated to 2.5 mEq/liter (SE $\pm$ 0.1). Plasma and urinary fluoride concentrations did not increase significantly. The authors [60] stated that the concentrations of bromide observed were 50% of toxic concentrations and probably represented sedative levels which may account for some cases of postoperative psychosis and depression.

Although a dose-response relationship for halogenated anesthetic agent toxicity has not been defined, it has been suggested that increased levels of toxicity may result from repeated anesthetic administrations in humans [22-33] or from chronic low-level exposures in animals [61]. Individual variances in response might include hypersensitivity, an immune-type response, an abnormally high rate of metabolism, or an inability to excrete toxic metabolites. It appears that microsomal enzyme induction and metabolism of the anesthetic agents play a major role in their potential toxicity.

It has been shown that exposure at low levels to the halogenated anesthetic agents results in an increased metabolism rate of the agent in

man [62-66]. The possible accumulation of toxic metabolites is presumably greater in a person chronically exposed to such agents than in someone receiving a massive dose of the agents.

Cascorbi et al [62,63] observed that urinary excretion of halothane metabolites was greater when a dose of low-level radioactive tracer was injected into unanesthetized subjects than when injected into the same subjects but under anesthesia. Also, four of five anesthetists excreted more radioactivity during the first 2 hours after tracer injections than four pharmacists who had not been exposed professionally to anesthetic gases or vapors. The elevated metabolism suggested a microsomal enzyme induction caused by chronic exposure to halothane vapor.

Cohen et al [64] studied the urinary metabolites of halothane in man by utilizing 14-C-labeled halothane. The study included five individuals given tracer doses (25 microcuries) and three subjects (heart donors) given large doses (1 millicurie). Identification of three halothane metabolites, trifluroacetic acid, N-trifluoroacetyl-2-aminoethanol, and N-acetyl-S-(2-bromo-2-chloro-1,1-difluroethyl)-L-cysteine was determined by nuclear magnetic resonance and mass spectrometry. The authors concluded that the formation of these metabolites suggest the presence of reactive intermediates which could be the source of potential hepatotoxicity. In reviewing this work, Van Dyke [65] cautioned about the use of urinary metabolites as a means of determining toxic biotransformation. However, he did not rule out the significance of the findings and indicated that the work did contribute to the needed information on the biochemistry of halothane.

Cascorbi et al [66] reported on investigations of halothane metabolism in humans after an intravenous injection of 14-C-labeled halothane. Although the authors found a wide variation in the metabolism rate from person to person, the studies showed that occupational exposure to halothane vapor could cause an increased rate of halothane biotransformation in man. Controls, including identical and fraternal twins, were used for comparison with those injected. Urine excretion and breath samples were utilized to monitor metabolism.

In 1974, Evers and Racz [67] reported preliminary results of studies of blood enzymes and serum proteins in anesthesia residents. A total of 15 new residents with either minimal anesthesia exposure or none at all were followed for periods ranging from 12 to 48 months. The investigators observed increases in serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), lactic drogdrogenase (LDH), and alkaline phosphatase, which peaked between the 9th and the 15th month of training and then leveled off or returned to near normal values. Abnormal transaminase levels were not frequently found nor were there significant changes in the hematologic data. Blood albumin levels showed frequent decreases and changes in globulins (alpha 1, alpha 2, beta, and gamma), considered by the authors to be consistent with a chronic disease process, and appearing 3-6 months after the beginning of training.

Johnstone and coworkers [68] reported measurements of serum bromide concentrations in anesthetists and operating room personnel. Serum bromide concentrations were measured in 12 operating room workers (primarily anesthetists) from two hospitals where halothane was administered; operating room halothane concentrations ranged from 30 to 104 ppm. A

comparison was made with 10 healthy laboratory technicians. Serum bromide concentrations in the halothane-exposed group ranged from 0.24 to 0.97 millimole/liter and averaged 0.53 millimole/liter, whereas values for the laboratory workers ranged from 0.11 to 1.25 millimoles/liter and averaged 0.38 millimole/liter. The authors reported that the differences were not significant at the 5% level.

Epidemiologic Studies

A number of epidemiologic studies have been conducted in an attempt to identify any prominent health effects associated with chronic exposure to waste anesthetic gases. Nearly all the studies were questionnaire surveys conducted among nurses, anesthetists, and operating room technicians. Several of the surveys suffer from low response rates, lack of or poor definition of control groups, and potential biases on the part of respondents. In all of the surveys, no information was presented on the anesthetic agents used or on the environmental concentrations of the gases present. The main health effects seen with consistency from one study to another were an increased incidence of spontaneous abortions and an increased incidence of congenital malformations among the children of exposed females and wives of exposed males.

In 1966, Vaisman [69] surveyed by questionnaire 303 Russian anesthesiologists (193 men and 110 women). Ninety-eight percent reported using diethyl ether; 59%, nitrous oxide; 28%, halothane; and 21%, other agents. Scavenging of waste anesthetic gases was not practiced and concentration levels were not presented. A high incidence of headache, fatigue, irritability, nausea, and itching was reported. The authors also

noted that 18 of 31 pregnancies among anesthesiologists who were between the ages of 24 and 38 ended in spontaneous abortions. In addition, there were two premature births and one child was born with a congenital malformation. It was also reported that two of the women discontinued working in the operating room because of threatened abortion. The anesthesiologists with abnormal pregnancies had exposures of 25 hours/week or more while those with normal pregnancies did not exceed 15 hours/week.

In 1968, Bruce et al [70] reported on a retrospective cohort mortality analysis of the causes of death in anesthesiologists over the 20-year period of 1947 to 1966. The study was based on an analysis of 441 deaths among members of the American Society of Anesthesiologists (ASA). The survey revealed a trend toward higher than normal incidences of death from suicide, reticuloendothelial and lymphoid malignancies, and a low incidence of lung cancer and coronary artery disease. The 441 deaths were grouped by age at death, year of death, and cause of death according to the International Classification of Diseases, Injuries and Causes of Death, 7th Revision, into two 10-year periods, 1947-1956 and 1957-1966. Ratios of observed-to-expected deaths for white males were determined for each group using data obtained from the US Bureau of Vital Statistics, and white male policyholders with the Metropolitan Life Insurance Company as the comparison group. The comparison groups were adjusted to ASA population distributions.

A prospective study of anesthesiologist mortality for the period 1967-1971 was published by Bruce et al [71] in 1974. Their earlier finding of an apparently high death rate from malignancies of the lymphoid and reticuloendothelial tissues, which included lymphosarcoma, Hodgkin's

disease, multiple myeloma, and lymphoma, was not confirmed in the prospective study. The ASA suicide rate was found to be 3 times that of a comparable control group, which made it consistent with the finding reported in the 20-year retrospective study, which was 2.7 times as high as the control group.

Askrog and Harvald [72] reported the results of a 1970 questionnaire survey of 578 nurses in anesthesia departments and of 174 female and male anesthetists. The survey was intended to determine if long-term, low-dosage inhalation of anesthetics had a teratogenic effect. Five hundred and seventy questionnaires were usable and included information on 212 pregnancies started before and 392 started during employment in the anesthesia department. The abortion frequency was significantly higher during employment (20%) than before (10%), not only for exposed female personnel but also for the wives of anesthesiologists. Though not statistically significant, the number of male children born in all groups was decreased. The authors [72] did not find a significant difference between the frequency of congenital malformation in children conceived before or during employment.

In 1971, Cohen et al [73] presented the results of a double survey conducted among California nurses and female physicians. The first study consisted of personally interviewing 67 female operating room nurses and 92 female general duty nurses (control). The nurses were unaware of the purpose of the study in order to avoid any possible bias. The second study was a questionnaire survey in which responses were obtained from 50 female anesthesiologists and 82 female physicians in specialties other than anesthesia who were used as controls. The results from the nurses,

surveyed from 1966 to 1970, showed that 29.7% of pregnancies in operating room nurses ended in spontaneous miscarriage compared with 8.8% in the control group, which is statistically significant ($P = 0.045$). For the period 1965-1970, the anesthetists showed a 37.8% spontaneous miscarriage rate compared with 10.3% in the control group, which is statistically significant ($P = 0.0035$). Miscarriages occurred earlier in both operating room nurses and anesthetists compared with their control groups (8th versus 10th week). The anesthetic gas concentrations and the types of gases to which the study group was exposed were not reported. The operating room nurses averaged 78% full-time employment in the operating room.

A questionnaire survey of 1,241 female anesthetists and 1,678 female physicians not associated with anesthesia was reported in 1972 by Knill-Jones et al [74]. The responses of 563 married female anesthetists and 828 nonanesthetist married female physicians were usable in analysis of the survey returns. The frequency of spontaneous abortion was significantly higher (18.2%) for anesthetists working during the first and second trimester than for the control group (14.7%, $P<0.025$), but not significantly different from that of the anesthetists who were not working while pregnant (13.7%). Also, anesthetists working during pregnancy had a significantly higher frequency of congenital abnormalities in live births (6.5%) than did those not at work (2.5%), but not a significantly higher frequency than the control group (4.9%). Involuntary infertility among anesthetists (12%) was twice as frequent as in the control group. There was no significant difference between the anesthetists and the control group in sex ratio, stillbirths, neonatal deaths, or total number of children with congenital abnormalities. Types of anesthetic gases or

concentrations at which the anesthetists had been exposed were not reported.

In 1973, Corbett et al [75] reported the results of their questionnaire survey of 621 female nurse-anesthetists in Michigan, with 525 usable responses. The survey was conducted to determine whether there was a higher than expected incidence of malignancies in the group. A total of 33 malignancies were diagnosed in 31 nurse-anesthetists during a period of 1-31 years after beginning anesthesia training. Of the tumors reported, several were of unusual types, including malignant thymoma, hepatocellular carcinoma, and leiomyosarcoma of subcutaneous tissue. The incidence of malignancy in the Michigan nurse-anesthetists was compared to age-adjusted statistics from the Connecticut Tumor Registry. It was found that, excluding skin cancers, the study group had a three-fold excess in malignancies, which was statistically significant, at the 3.1% level. Types of anesthetic gases and concentrations at which the study group were exposed were not presented.

Corbett and coworkers [76] conducted a further analysis of their 1973 survey. The purpose of the study was to evaluate the incidence of birth defects among the offspring of the Michigan female nurse-anesthetists. It was found that in children whose mothers worked during pregnancy, 16.4% had birth defects, compared with an incidence of 5.7% among children whose mothers did not work during pregnancy (significant at $P<0.005$). Excluding anomalies of the skin, the total number of birth defects among offspring of the working exposed mothers was 8.8%, while the total among the nonexposed group was 3.8% (significant at $P<0.025$). Three neoplasms were reported in two children whose mothers worked during pregnancy. One child had a

neuroblastoma at birth and developed a thyroid malignancy at puberty. Another child developed a parotid tumor at the age of 22. A single case of leukemia at age 3 was reported in one of the children from the group of mothers who did not practice anesthesia during pregnancy.

American Society of Anesthesiologist (ASA) Ad Hoc Committee on the Effects of Trace Anesthetics reported the results of a national study of occupational diseases among hospital operating room personnel and dentists, including oral surgeons [12,13]. The study was conducted by mailing questionnaires to: (1) 49,585 operating room personnel in four professional societies (anesthesiologists, nurse-anesthetists, operating room nurses, and operating room technicians); (2) 4,797 general dental practitioners and 2,642 oral surgeons; and (3) 23,911 individuals in two other professional societies (pediatricians and general nurses). Data on types of anesthetic agents and concentration levels at which the study groups were exposed were not presented for either survey [12,13]. It was reported that approximately 20% of the exposed respondents from the hospital setting worked in operating rooms with waste anesthetic gas scavenging devices of unknown efficiency. The response rates were: 67% for male anesthesiologists, 75.7% for female anesthesiologists, 65.4% for male nurse-anesthetists, 59.3% for female nurse-anesthetists, 53.5% for male operating room nurses and technicians, 55.4% for female operating room nurses and technicians, 38.9% for the general dentists, and 64.5% for the oral surgeons. The general nurse and pediatrician control groups had response rates of 44.3% for male nurses, 41.8% for female nurses, 41.2% for male pediatricians, and 72.1% for female pediatricians.

The results indicated that female anesthesiologists, nurse-anesthetists and operating room nurses and technicians in the operating room-exposed group (exposure during first trimester of pregnancy and the preceding year) were subject to a statistically significant risk of spontaneous abortion, 1.3-2 times that of unexposed personnel. It was also found that there was evidence of an increased risk of congenital abnormalities among the live-born babies of exposed female respondents in the survey. An intragroup analysis of the children of the exposed nurse-anesthetists compared with the unexposed members of this group indicated an increase in congenital abnormalities of more than 60% ($P<0.01$) in the former group. The exposed female anesthesiologists showed a two-fold increase in congenital abnormalities in their children compared with the unexposed female physician anesthesiologists and female pediatricians ($P=0.13$ and 0.07, respectively). There was also an increase of 25% in the incidence of congenital abnormalities for children of the wives of exposed physician anesthesiologists ($P=0.04$).

The authors [12] reported that the collected data suggested an increased occurrence of cancer in the exposed female respondents compared with those in unexposed control groups. The increases ranged from approximately 1.3 to somewhat less than two-fold, with $P = 0.05$, 0.01, and 0.07 for the anesthesiologists, the nurse-anesthetists, and the operating room nurses and technicians, respectively. Analyses by type and location of tumor indicated that, with the exception of leukemia and lymphoma, there was no significant difference as to the location or type of malignancy. The increased incidence of cancer was not found in exposed male respondents. Hepatic disease was reported more frequently in the exposed

female respondent groups than in the unexposed controls (even after excluding serum hepatitis). The range of increase was approximately 1.3- to 2.2-fold, with P values of 0.04, <0.01, and 0.08 for the three group comparisons. A statistically significant increase in hepatic disease was reported by the exposed male anesthesiologists compared with the male pediatricians (P<0.01). The exposed female groups reported higher rates of renal disease (pyelonephritis and cystitis excluded) ranging from 1.2- to 1.4-fold in magnitude (P = 0.28, 0.01 and 0.05 for the female physicians, nurse anesthetists, and operating room nurses, respectively). No increased risk of renal disease for male physician anesthetists was observed.

Among general practitioner dentists and oral surgeons, it was reported that 20.2% and 74.8%, respectively, of the respondents had anesthetic exposures (type and levels not given) exceeding 3 hours/week. Results were analyzed according to the anesthetic exposure of the dental respondent. Respondents who reported that they worked in dental surgery with anesthetics a minimum of 3 hours/week during the calendar year preceding their spouse's pregnancy were separated from those who reported no exposure to anesthetics. Individuals with intermediate exposure (less than 3 hours/week) were not included in the analysis. In the analysis for cancer, liver, and kidney disease among the dental respondents, exposure to anesthetics was defined as at least 1 year of exposure, but not necessarily including the immediate year before onset of the disease. Respondents from both dental groups were combined into single exposed and unexposed groups since the effects of exposure to anesthetic gases proved to be similar.

For the dental portion of the study [13], the investigators found that the incidence of spontaneous abortion was increased about 78% (P<0.01)

in the wives of exposed dentists compared with the spouses of unexposed dentists. Congenital abnormality rates appeared to be slightly higher in the children of wives of exposed dentists than in the children of persons in the unexposed control group. Although the differences were not statistically significant, the children of women in the exposed group showed a 15% increase in fetal abnormalities over those found for the children of the unexposed dentists' wives. The of cancer incidence (35%) in the male respondents appeared higher in the exposed group than in the control group. The sample sizes were small and the authors concluded that the difference was not statistically significant.

The incidence of liver disease, calculated after excluding cases of serum hepatitis to eliminate possible differences in exposure to blood and blood products, increased 156% in the exposed group compared with that of the unexposed control group [13]. This difference was highly significant ($P<0.01$). There were no noteworthy differences in incidence of kidney disease between the two groups.

A questionnaire survey of members of the German Anesthesia and Reanimation Association was reported by Garstka et al [77] in 1974 to clarify whether there was an increased incidence of certain complications during pregnancy among anesthesiologists. Of the 877 questionnaires mailed, 257 replies were usable. The authors formed the following groups in evaluating the replies: Group A, pregnancies in exposed female anesthesiologists; Group B, pregnancies in women married to exposed anesthesiologists; Group C, pregnancies in exposed married anesthesiologist couples plus groups A and B; Group D, pregnancies before exposure of individuals in group C. They found a significant difference

($P < 0.05$) in abortion frequency between pregnancies in group A (17.9%) and those in group D (10.6%). They also compared the abortion frequency of the exposed female anesthesiologists (17.9%), which they noted to be relatively high, compared with national German and United States statistics (not age-adjusted) of 13.5 and 15%, respectively, but drew no conclusions. The authors stated that their study agreed with the ASA Ad Hoc Committee study [12] in that there was no increased frequency of abortion in wives of exposed anesthesiologists.

The authors [77] also reported premature births in 19.7% of the pregnancies of exposed female anesthesiologists and concluded that such a rate was high. In regard to pregnancy complications (not defined by the authors), they reported a statistically significant difference ($P<0.05$) between complications among exposed female anesthesiologists (8.0%) compared to group D (1.5%). They agreed with the ASA Ad Hoc Committee findings [12] which reported a higher malformation incidence in the children of female anesthesiologists but did not find an increase in malformations in the children of the wives of exposed anesthesiologists (group B). Garstka et al [77] noted that the anesthesiologists who had given birth to malformed babies had been exposed to halothane for an average of 20 months before the start of their pregnancies and for 6-7 months during the pregnancies in question. Data on concentration levels were not presented.

In 1972, Uhlirova and Pokorny [78] conducted a questionnaire survey of 857 workers (200 male and 657 female physicians, nurses, and technicians) in the anesthesiology and resuscitation (operating and recovery rooms) divisions in Czechoslovakia to determine occupationally

related health problems. The survey was of a preliminary nature and aimed at obtaining fundamental information. The authors [78] stated that control groups were not used but would be created for future studies. Workers were divided into those with less than 1 year of service, less than 5 years of service, and more than 5 years of service. As work experience in the field increased, the number of workers reporting problems increased. Increased incidences of headache, excessive fatigue, and allergic diseases were reported to be directly proportional to work experience in anesthesiology. An increasing trend of spontaneous abortions was reported in those with more than 5 years of service (8.1%) compared with those with less than 5 years of service (4.8%). The authors also reported an increase in parenchymal damage in the liver, in kidney dysfunction, and in hematologic disorders in the respondents commensurate with the length of service. Anesthetic agents or exposure levels were not identified.

Wyatt and Wilson [79] published the results of a limited survey to determine the sex ratio of children of anesthetists. All anesthetists of registrar grade or above in the Sheffield Hospital Region were asked to complete a postal questionnaire giving their age and sex, the date they started regular anesthetic practice, and the sex and dates of birth of their offspring. A total of 117 questionnaires (75%) were answered and of these, 21 respondents were childless and 9 were women in regular anesthesia practice. The remainder (87) were men in regular anesthesia practice whose wives had 157 live-born children. Of these children, 56.8% were females compared with 48.6% females for the Sheffield Region or England and Wales. The authors concluded that the difference was significant ($P<0.05$).

A questionnaire survey of 7,949 physicians was conducted in the United Kingdom by Knill-Jones et al [80] to determine whether there was a relationship between operating room exposures and abnormalities in the obstetric history of the respondents. The 5,507 usable replies were given by anesthetists (26%), surgeons (9%), radiologists (1%), other hospital staff (17%), nonhospital physicians (39%), and others (8%). The investigators reported there was no apparent influence of paternal exposure on the frequency of spontaneous abortion (exposed 11.1%, not exposed 10.9%). However, maternal exposure was associated with an abortion frequency of 15.5% compared with 10.9% where neither parent was exposed. The apparent effect of maternal exposure was highly significant ($P<0.01$). Exposure had no obvious effect on the average length of gestation prior to abortions: no exposure, 11.2 weeks; father exposed, 10.9 weeks; mother exposed, 11.2 weeks. Female exposure in the first pregnancy was associated with a 16.1% frequency of abortion compared with 7.7% when there was no exposure ($P<0.001$). The corresponding values for the second pregnancy were 11.2 and 9.2%, respectively, a difference which was not statistically significant.

Congenital abnormalities were described as major (life threatening, resulting in either major surgery or serious disability) and minor. Male exposure and nonexposure were associated with similar frequencies of major abnormalities (1.08% vs 1.05%). Female exposure was associated with a frequency of 1.59% for major abnormalities, although this represented only seven children. There was an increase in minor abnormalities in children of exposed males, 3.09%, compared with those of nonexposed male parents, 2.35% ($P<0.02$). Female exposure to anesthetic gases was associated with a

frequency of 3.19% in minor abnormalities in children but this represented only 14 children. The frequency of reporting of all abnormalities (including some which could not be classified as major or minor) in exposed females was 5.5% compared with 3.6% in nonexposed pregnancies ($P<0.05$). However, the abnormality rate of 4.5% associated with male exposure was significantly higher than in the nonexposed group rate of 3.6% ($P<0.05$). When either parent had been exposed during the pregnancy, there were 63 stillbirths out of a total of 6,414 births (0.98%) compared with 59 out of a total of 7,296 (0.80%) for nonexposed parents. The difference was not statistically significant.

Parental exposure had no apparent effect on the frequency of perinatal death of children (1.85% for both exposed and nonexposed groups), or on the frequency of cancer or leukemia in children (exposed, 0.20%; nonexposed, 0.26%). Among males, there were 117 (9.7%) anesthetists, 132 (10.6%) other hospital physicians, and 234 (10.8%) physicians not working in hospitals who reported involuntary infertility. There was no significant difference between the exposed and nonexposed groups of males concerning involuntary infertility.

The authors [80] also matched the pregnancies for maternal smoking habits, birth order, and maternal age at the time of birth. Of all pregnancies in which male exposure had occurred, 4,074 (69.2%) were matched successfully with pregnancies where there was no male or female exposure. For these matched groups, there was no significant difference in the frequency of spontaneous abortion between the exposed group (10.6%) and the nonexposed group (9.8%). A 4.5% frequency of all types of congenital abnormalities in the exposed group was significantly greater than the 3.2%

reported by the nonexposed group ($P<0.01$), and this was explained largely by an increase in the reporting of minor congenital abnormalities by the exposed group. Exposure had no effect on the stillbirth rate. All the pregnancies in exposed females were matched by maternal smoking habits, birth order, maternal age at the time of birth, and paternal age at the time of response (both ages within 2 years). There was a striking difference in the frequency of spontaneous abortion in the exposed group (14.9%) compared with the nonexposed group (5.5%) ($P<0.001$). The exposed group had a significantly greater frequency of all types of congenital abnormalities, attributable to increased reporting of both major and minor abnormalities. However, in this analysis, the total number of anomalies was small.

To increase the size of the control group, the authors [80] also matched each exposed pregnancy with two control groups; 73.8% of the pregnancies were matched successfully. These analyses also showed that a clear increase in the frequency of spontaneous abortion was associated with female exposure. However, there was no significant difference in the frequency of congenital abnormalities.

The authors [80] concluded: (1) matched and unmatched data showed that there was an increased frequency of congenital abnormalities in liveborn children of exposed men, but this was attributable to an increase in the frequency of minor abnormalities as the authors defined them; (2) the present data confirmed their earlier findings [74] that female exposure was associated with an increased frequency of spontaneous abortion; and (3) the results of their earlier survey [74] suggested a possible increase in the frequency of congenital abnormalities associated with maternal

exposure, but the present data did not support this, except for a possible increase in the reporting of minor congenital abnormalities. Types of anesthetic agents and exposure levels were not presented by the authors.

Animal Toxicity

(a) Acute Effects

The acute effects of exposure to chloroform and trichloroethylene were presented in the NIOSH documents Criteria for a Recommended Standard...Occupational Exposure to Chloroform [81] and Criteria for a Recommended Standard...Occupational Exposure to Trichloroethylene [82]. A number of studies have shown that chloroform causes fatty infiltration and necrosis of the liver [83-89] and fatty degeneration and necrosis of the convoluted tubules in the kidney [83,90]. Inhalation studies using trichloroethylene showed it to be less hepatotoxic than chloroform, causing mild fatty degeneration of the liver [84,91,92].

Green [93] exposed 30 Sprague-Dawley and 18 Long-Evans rats to a 70% nitrous oxide (700,000 ppm)/20% oxygen/10% nitrogen mixture or to air (control) for 8 days. White blood cell and differential counts were determined, along with analyses for RNA and DNA of bone marrow and thymus. The exposed Long-Evans rats showed a 25% decrease in white blood cell count with a predominance of lymphocytes in the differential count compared to controls. No change was seen in white blood cell or differential counts of the Sprague-Dawley rats. There was a moderate alteration in the RNA/DNA ratio in the Long-Evans rats, while only a small effect was seen in the Sprague-Dawley rats.

Hughes and Lang [94] observed hepatic necrosis in guinea pigs following repeated administration of halothane. Seventy female Dunkin-Hartley guinea pigs were randomly divided into seven groups of 10 animals each. Two groups were used as controls with one group breathing room air and the second group breathing 100% oxygen. The five experimental groups (50 animals) were anesthetized one to five times for 1 hour each using 1% (10,000 ppm) halothane with oxygen. Five guinea pigs from each group were killed immediately after the last period of anesthesia; the remaining five were killed a week later. Blood samples were measured for SGPT and SGOT, total LDH, and isoenzyme fractions.

Total leukocyte counts, differential counts, SGPT, SGOT, and total LDH showed no significant differences between any of the groups [94]. Eosinophilia or lymphocytosis were not found. Seven of the anesthetized animals showed focal hepatic lesions visible by light microscopy while no similar lesions were observed in any control animals. Both the number of animals with lesions and the severity of the lesions increased with the number of times the animals were anesthetized. Animals with one to three anesthesia administrations showed early hepatic necrosis. These lesions, which radiated from the central vein along the hepatic cord, showed swollen hepatic cells with basophilic cytoplasm and shrunken nuclei which were irregularly shaped and without nucleoli. Animals anesthetized four to five times showed more lesions, characterized by degenerating hepatocytes, mononuclear cells, and cellular debris. These lesions were located around the central vein and extended into the lobule. Larger areas of necrosis were usually seen in the midzonal regions. Electron microscopic studies not reported.

Kosek et al [95] exposed 75 male Fischer 344 rats to methoxyflurane at 0.25% (2,500 ppm) for 1.5 hours, at 0.5% (5,000 ppm) for 3 hours, and at 0.75% (7,500 ppm) for 6 hours without the usual premedicant drugs, induction agents, or nitrous oxide. The renal damage produced by methoxyflurane was proportional to the dose of anesthetic received. One to 7 days after anesthesia, rats receiving the highest dose (0.75%) had advanced changes in the convoluted tubules. Calcium oxalate crystals were frequently found in the kidney tubules, sometimes accompanied by tubular disruptions and peritubular edema. Electron microscopic examination of kidneys of all rats receiving the high dose showed severe mitochondrial swelling and destruction in most of the proximal convoluted tubules. Four to seven days after anesthesia, the rats exposed to an intermediate dose showed normal mitochondria but an apparent increase in the number and size of dense bodies. Kidney sections of rats treated with the low dose (0.25%) of methoxyflurane appeared normal at 7 days when examined with light and electron microscopy.

(b) Chronic Effects

Chenoweth et al [96] reported on the comparative chronic inhalation toxicities of methoxyflurane, halothane, and diethyl ether on rats, guinea pigs, and rabbits. The exposure concentrations were at one-tenth the average anesthetic concentrations for dogs and humans. Animal groups were exposed 7 hours/day, 5 days/week for 7 weeks to methoxyflurane at 200 ppm, to halothane at 500 ppm, to diethyl ether at 2,000 ppm or to filtered air as a control. Each animal group consisted of 20 Wistar rats, 12 guinea pigs, and 4 rabbits (species not given), all divided equally as to sex. Food and water were withdrawn from the animals during the 7-hour exposure

period. Determinations of SGPT and SGOT levels were carried out on representative groups of rats, guinea pigs, and rabbits at the termination of exposure. Gross and histopathologic examinations and organ-to-body weight ratios were determined on all animals after they were killed at the end of the experiment. P values less than 0.01 were considered by the authors to be statistically significant.

Terminal body weight data showed a significant decrease in the female rats exposed to halothane and in male guinea pigs exposed to methoxyflurane [96]. The most noteworthy change in organ-to-body weight ratios was the significant increase of liver ratios in male and female rats exposed to halothane and in female guinea pigs exposed to halothane and methoxyflurane. No significant changes were seen in SGOT and SGPT levels. Rabbits which developed pathologic changes showed elevated SGOT and SGPT levels after exposure to methoxyflurane. Several male rats exposed to methoxyflurane had a minimal amount of focal hepatic fatty infiltration. Most guinea pigs exposed to halothane and methoxyflurane had a minimal to moderate amount of central lobular fatty infiltration in the liver and several had significant fatty infiltration. Several rabbits exposed to halothane and methoxyflurane had minimal central lobular fatty infiltration in the liver, and one rabbit exposed to methoxyflurane had a minimal amount of central lobular necrosis. Diethyl ether did not cause any noteworthy hepatotoxic responses. The authors suggested that their finding of fatty infiltration in the liver following chronic exposure to subanesthetic concentrations of halothane and methoxyflurane bears on the question of chronic toxicity to operating room workers.

In 1975, Stevens et al [61] exposed groups of 16 Sprague-Dawley rats, 16 Hartley guinea pigs, and 48 ICR mice to halothane at 15, 50, 150, and 300 ppm; to isoflurane at 150, 500, and 1,500 ppm; or to diethyl ether at 1,000 and 10,000 ppm. The animals were young and in an active phase of growth. Exposures were continuous over a 5-week period, except in the 10,000-ppm ether experiment, in which the guinea pigs and mice were killed after 20 days. In the 150-ppm halothane study, 31% of the mice and 38% of the guinea pigs died before 35 days. Control groups were treated identically except for receiving the anesthetic agent.

Halothane produced a dose-related detrimental effect on weight gain in all species. The ratio of liver-to-total body weight in the halothane and ether exposures was smallest with the lowest concentrations. No significant change in liver weight was found by the investigators [61] with isoflurane. Animals exposed to isoflurane and ether showed little or no increase in lesions compared to their controls. Livers from halothane-exposed animals developed degenerative lesions which increased in frequency with an increasing dose. Degenerative lesions in the liver included granular and vacuolar degeneration, zonal centrilobular lipidosis, focal lipidosis, and focal necrosis. A probit analysis by the authors [61] suggested that a 50% incidence of lesions would occur at 140 ppm halothane in mice and at 100 ppm in rats. Table III-3 presents the number of hepatic lesions found in exposed groups compared to their controls.

TABLE III-3

DEGENERATIVE HEPATIC LESIONS FOUND AFTER CONTINUOUS SUBANESTHETIC EXPOSURE TO HALOTHANE, ISOFLURANE, AND DIETHYL ETHER*

Anesthetic Agent	Concentration, ppm	Mice Con	Mice Ex	Rats Con	Rats Ex	Guinea Pigs Con	Guinea Pigs Ex
Halothane	300	3/77	35/38	7/32	16/16	6/25	14/15
	150		12/35		12/16		16/16
	50		9/27		5/16		16/16
	15		8/37		2/16		4/16
Isoflurane	1,500	10/65	8/31	2/24	4/16	6/21	6/16
	500		2/32		0/16		3/16
	150		3/30		0/16		3/15
Diethyl ether	10,000	0/64	4/20	0/16	0/16	1/16	2/16
	1,000		0/32		0/16		2/14

*Animals with lesions/animals available for microscopic examination;
Con = Control, Ex = Exposed

Adapted from Stevens et al [61]

Chang et al [97] exposed 10 young Sprague-Dawley rats, male and female, to low levels of halothane over 4-8 weeks. Five animals were exposed to halothane at 10 ppm, 8 hours/day, 5 days/week for 8 weeks, and five animals were exposed at 500 ppm, 8 hours/day, 5 days/week for 4 weeks. A control group of six animals was exposed to room air. The animals were killed at the end of the exposure periods and cerebral cortex tissues were examined by electron microscopy. In the group exposed at 10 ppm for 8 weeks, the authors found collapse of the neuronal rough endoplasmic reticulum and reported that ribosomes were associated only with the noncollapsed portions of the membranes. Dilatations of the Golgi complex and focal cytoplasmic vacuolation were seen in some cortical neurons.

Severe dilatation and vacuolar degeneration of the Golgi complex was found in the group exposed to halothane at 500 ppm for 4 weeks [97]. Occasional membranous degeneration of the neuronal mitochondria, coagulative necrosis of the cortical neurons, and intracellular edema of the glial cells were also reported. The investigators considered halothane to be neurotoxic under the conditions of low concentrations and chronic exposure.

Chang et al [98,99] reported ultrastructural changes found in the rat kidney and liver following chronic exposure to low levels of halothane. Twenty-four Sprague-Dawley rats, of both sexes, were divided into three groups of eight animals each. Group I was exposed to halothane at 10 ppm for 8 hours/day, 5 days/week for 8 weeks. Group II was exposed at 500 ppm for 8 hours/day, 5 days/week for 4 weeks and Group III was the control. The animals were killed at the end of the exposures and kidney and liver tissues were examined by both light and electron microscopy.

Kidney tissues, under light microscopy, [98] showed cellular injury in the proximal convoluted tubules (PCT) of animals exposed at 10 ppm and more severe damage in animals exposed at 500 ppm. The control group showed no cellular damage. Ultrastructural changes in the kidneys were more prominent and more frequently observed in the animals exposed to halothane at 500 ppm. Many epithelial cells in the PCT contained an accumulation of membranous bodies presumably linked to rapid mitochondrial degeneration. The report [98] also described an increase in lysosomes in many PCT cells, frequently fused to form dense bodies. Clusters of smooth endoplasmic reticulum, areas of focal cytoplasmic degradation, and swelling of mitochondria were occasionally found in the PCT cells.

Chang et al [99] also examined liver tissues by electron microscopy from the rats exposed to halothane at 10 and 500 ppm. The animals exposed at 10 ppm showed an increase in the matrical density in mitochondria of some hepatocytes. Animals exposed at 500 ppm showed all the changes seen in those exposed at 10 ppm as well as severe dilation of the biliary canaliculi and focal cytoplasmic degradation. No remarkable pathologic or ultrastructural changes were reported in the livers of the control animals.

(c) Metabolism Studies

Many studies substantiated and described the metabolism of volatile anesthetics by liver microsomal enzymes [50-58,63,100]. If toxic metabolites of the anesthetic agents are a prerequisite to liver damage, then studies dealing with metabolism of the agents at low concentrations (occupational) are of prime interest.

Van Dyke [101] saw an enhancement in the methoxyflurane ether-cleaving and halothane and methoxyflurane dechlorination systems when male Wistar rats were pretreated with phenobarbital, a known microsomal enzyme-inducing agent. In vivo studies were conducted by exposing the pretreated rats to methoxyflurance at 300 ppm, 7 hours/day for 10 days, and in vitro studies were conducted using a mixture of rat liver microsomes in cell supernate. A significant finding reported by the author [101] was that rats exposed only to subanesthetic doses of methoxyflurane with no phenobarbital pretreatment showed an enhanced rate of metabolism of the agent. This study was the first to indicate the possibility of low level exposure to anesthetic agents as being responsible for microsomal enzyme induction and a resultant increase in metabolism of the anesthetic.

Liebman and McAllister [102] reported an increased rate of in vitro metabolism of trichloroethylene to chloral hydrate by rat liver microsomal enzyme preparations when male Holtzman rats were pretreated with trichloroethylene at 4,000 ppm for 0.5 hour/day for 4 days. A more significant increase in metabolism was seen when the rats were pretreated with trichloroethylene at 40,000 ppm, 6 hours/day for 4 days.

Linde and Berman [103] noted the ability of subanesthetic concentrations of inhalation anesthetics to stimulate drug metabolizing liver microsomal enzymes in male Sprague-Dawley rats. The extent of drug metabolism was judged by differences in hexabarbital sleeping time between exposed and control rats. Significant reductions in hexobarbital sleeping time occurred after a single 7-hour exposure to subanesthetic concentrations of diethyl ether (16,000 ppm), isopropyl ether (8,000 ppm), fluroxene (15,000 ppm), enflurane (6,000 ppm), and isoflurane (2,900 ppm). Two 7-hour/day exposures to halothane (4,000 ppm) were necessary to produce a significant reduction in sleeping time. Neither nitrous oxide nor cyclopropane caused any decrease in hexobarbital sleeping time.

In 1972, Ross and Cardell [104] reported on the ability of repeated halothane administrations to increase the capacity of hepatic microsomal enzymes to dechlorinate methoxyflurane. Male Sprague Dawley rats were exposed to halothane at 2,500 ppm in air 7 hours/day for 7 days. The animals were then killed and their livers removed for the metabolism study. Microsomes from the halothane-treated rats demonstrated approximately 2.6 times the capacity to dechlorinate methoxyflurane than microsomes from control animals.

(d) Reproductive Effects

The effects of anesthetic concentrations of inhalation anesthetics on reproduction have been reported by many authors [105-127].

Chang et al described the effects of in-utero exposure of rat fetuses to halothane [111-113]. Eight female Sprague-Dawley rats were exposed, after conception, to halothane at 10 ppm for 8 hours/day, 5 days/week throughout pregnancy. The pups were born in a halothane-free atmosphere. An equal number of pregnant female controls were used. Four randomly chosen pups from each litter were killed within 24 hours after birth, and tissue samples from the liver, kidney, and brain were examined. The investigators examined the liver tissues by electron microscopy [111]. No ultrastructural or histologic changes were seen in the control animals. However, cellular damage was seen in the livers of pups exposed to halothane. Myelin figures, large areas of focal cytoplasmic degradation, and accumulation of lipids within the hepatocytes were found. Areas of focal necrosis were reported in more than 50% of the tissue samples. The authors [111] suggested that halothane, at the levels normally associated with occupational exposure, may be hazardous to fetal development based on demonstrated hepatotoxic effects on the fetal liver.

Chang et al [112] also examined tissue samples from the renal cortex. No significant pathologic lesions were reported in the control animal kidneys when examined with light or electron microscopy. Most of the renal lesions in the halothane exposed rats were confined to the proximal convoluted tubules (PCT). The distal convoluted tubules and glomeruli were reported to appear normal. The pathologic changes reported included a flattening or absence of basal infoldings in many PCT cells, accumulation

of large lipid droplets in many PCT cells, formation of clusters of smooth endoplasmic reticulum, an increase in lysosomes, and severe swelling of some mitochondria. The authors [112] suggested that the formation of clusters of smooth endoplasmic reticulum may represent the detoxification response of the neonatal kidney.

According to Chang et al [113], no gross anomalies were observed when neonatal brain tissues were examined following fetal exposure to halothane. However, ultrastructural study of the brain tissues revealed focal weakening and disruption of the nuclear envelope of cortical neurons, neuronal vacuolation, myelin figure formation, and occasional neuronal necrosis. The postsynaptic membrane density failed to form in many synapses. The authors [113] stated that such abnormal synaptic complexes, persisting through adulthood, could contribute to behavioral changes and poorer learning abilities.

In a 1974 report, Quimby et al [119] addressed the question of whether chronic exposure to halothane at 10 ppm would produce lasting behavioral deficits and CNS damage. The investigators exposed Sprague-Dawley rats to halothane at a concentration ranging from 8 to 12 ppm for 8 hours/day, 5 days/week. Four experimental groups were treated as follows: the first group was exposed throughout early development, from conception to 60 days of age; the second group was exposed from 60 days of age through the end of the behavioral testing period (75-105 days); the third group was exposed throughout both age periods; and the fourth group was a control. All groups were tested for behavior and learning ability at 130 and 150 days of age. The results indicated that early exposure to halothane in trace amounts apparently caused permanent learning deficits (groups one and

three). Exposure to halothane only after 60 days of age produced no behavioral deficits in learning tasks (group two). With electron microscopy, cerebral cortex tissue samples from rats exposed from conception showed evidence of neuronal degeneration as well as permanent failure of formation of the synaptic web and postsynaptic membrane density in 30% of the postsynaptic membranes. Only slight neuronal damage was evident in rats exposed to halothane as adults. The authors [119] raised the question of whether or not pregnant women should avoid chronic halothane exposure even at trace levels of 10 ppm, as a precaution against possible lasting damage to the brain of the fetus.

In 1974, Schwetz et al [120] demonstrated the effects of repeated exposures to chloroform on the rat embryo and fetal development. Pregnant Sprague-Dawley rats were exposed to chloroform at 30, 100, or 300 ppm for 7 hours/day on days 6-15 of gestation. Pregnant rats exposed at 100 ppm showed a significant increased incidence of fetal abnormalities compared to controls. There were significantly increased incidences of acaudia (taillessness), imperforate anus, subcutaneous edema, missing ribs, and delayed skull ossification. Rats exposed at 30 ppm showed significant increased incidences of delayed skull ossification and wavy ribs, but no other effects compared to controls.

Fink et al [108,109] investigated the potential teratogenicity of nitrous oxide in female Sprague-Dawley rats, presumed to be in estrus by the presence of a copulatory plug and vaginal spermatozoa. The rats were exposed on day 8 of pregnancy to nitrous oxide at 50% (500,000 ppm), 21-25% oxygen, and 25-29% nitrogen for 2, 4, or 6 days, or for a single day to 70% nitrous oxide/30% oxygen within days 5-11. After exposure, the animals

were returned to a standard cage until day 20, when they were killed, the fetuses examined, and the number of implantations plus resorptions (similar to spontaneous abortion in man) determined. The most common anomalies from exposure to 50% nitrous oxide were death and resorption of embryos and abnormalities of the vertebrae and ribs. The male/female sex ratio among the surviving fetuses was significantly smaller ($P=0.023$) than those in the controls. Exposures for a single 24-hour period within day 5 through 11 indicated that a peak incidence of malformation occurred after exposure on day 9. The skeletal abnormalities were similar to, but less marked than, those seen in animals exposed for more than 1 day.

In 1968, Basford and Fink [110] reported the exposure of female Sprague-Dawley rats in estrus to halothane at 8,000 ppm in 25% oxygen for 12-hour periods at different stages of pregnancy. Nine experimental and nine control groups were used. Experimental groups were exposed on day 6, 7, 8, 9 or 10 (9 AM-9 PM) or on day 6.5, 7.5, 8.5 or 9.5 (9 PM-9 AM). On day 20, the rats were killed and the number of fetuses and resorptions noted. Incidences of skeletal malformations in fetuses near term were significantly higher ($P<0.001$) following exposure on day 8 or 9.5 of pregnancy when compared to control groups. The authors concluded that halothane appeared to be teratogenic in rats with the degree of change varying directly with concentration and duration of exposure during a critical period.

Short periods of anesthesia with halothane were found by Smith et al [121] to be teratogenic in pregnant C-57 mice. Pregnant mice (154) were anesthetized with 1 or 1.5% halothane for 3 hours on day 12, 13, 14, or 15 of gestation or, alternatively, on 3 consecutive days in the same period.

Examination of 752 live fetuses, 675 of which were cleared for skeletal examination, showed a definite increase of cleft palate, limb hematomas, and ossification defects in the limbs of exposed animals. No cleft palate and fewer than 1% of other defects were seen in 541 control fetuses, 410 of which were cleared for skeletal examination.

Bussard et al [116] studied fetal changes in hamsters anesthetized with nitrous oxide and halothane. Pregnant hamsters (54) were divided randomly into three exposure and three control groups. Each of the experimental groups was exposed to 60% nitrous oxide with 0.6% (6,000 ppm) halothane for 3 hours on the 9th, 10th, or 11th day of gestation with controls placed in identical chambers but receiving no anesthetic. Chamber oxygen was carefully maintained at 40% for both the exposed and the control groups. Compared with controls, the number of resorptions was increased ($P<0.05$) only in those anesthetized on day 11. Statistically significant decreases were seen in fetal weight for the hamsters exposed on days 10 and 11 ($P<0.001$) and in crown-rump length for day 10 ($P<0.001$) and day 11 ($P<0.02$) compared to control groups. The ratios of female fetuses-to-total surviving fetuses were similar in exposed and in control groups.

In 1975, Doenicke et al [117] published their study in which 505 pregnant female Sprague-Dawley rats were anesthetized for 6-12 hours with various mixtures of halothane, nitrous oxide, and oxygen between days 6-10 of pregnancy. A group of exposed rats was used as controls. The abortion rates are summarized in Table III-4.

TABLE III-4

ABORTION RATES IN RATS EXPOSED TO
NITROUS OXIDE AND HALOTHANE

Anesthetic Agent	Concentration Vol %	Length of Exposure, Hours	Abortion Rate %
Halothane/nitrous oxide	0.8/25	12	44
"	0.8/25	6	30
"	1.25/25	6	39
Halothane/oxygen	0.8/100	12	50
Nitrous oxide/oxygen	75/25	12	18
"	50/50	12	7.7
"	25/75	12	10
None	-	-	15
Oxygen	100	12	21

Adapted from reference 117

The authors [117] concluded that halothane demonstrated an abortive effect directly proportional to the concentration inhaled and that nitrous oxide did not have such an effect. Although this study did not clearly establish a relationship between nitrous oxide exposure concentrations and abortion rate, neither did it rule out such a relationship.

Doenicke and Wittmann [118] reported a study in which 252 pregnant Sprague-Dawley rats were exposed to various mixtures of halothane, nitrous oxide, and oxygen to identify any teratogenic effects of the anesthetic agents. Approximately 9,000 embryos were examined for vertebral and rib anomalies. The authors concluded that they could not find an association between vertebral malformations in the embryos and the concentration of halothane to which the mothers were exposed. They did state that there was a teratogenic effect proportional to the concentration of halothane regarding costal development.

In 1973, Corbett et al [122] published the results of a study in which they exposed pregnant rats (Simmonson Laboratories) to nitrous oxide in concentrations of 0 (control), 100, 1,000 and 15,000 ppm for either 8 or 24 hours/day on various days during pregnancy. The authors found that the rats exposed at 15,000 ppm and 1,000 ppm for 24 hours/day had higher fetal death rates and lower pregnancies/rat ratios than did the controls. Also, two groups exposed at 1,000 ppm for 8 hours/day had a fetal death rate significantly higher than that of the controls. The 8-hour daily exposures did not significantly alter the pregnancies/rat ratio.

Bruce [123] exposed male and female mice of three different strains to air or to air containing 16 ppm halothane for 7 hours/day, 5 days/week for 6 weeks. Male and female animals were then paired and exposed daily to the same conditions. No significant difference was found between the exposed animals and the controls on examination for splenic weights and any histologic changes of liver, spleen and testes in the males, and the number of pregnancies, implantations/pregnancy, and resporption/pregnancy in females.

In 1976, Kripke et al [124] reported the effects of chronic exposure to nitrous oxide at subanesthetic concentrations on spermatogenesis in male rats. The study was conducted to determine what, if any, toxic effect nitrous oxide might have on dividing cells, possibly giving some indication of the teratogenic potential of the agent. The investigators exposed 135 male LEW/f Mai rats to an atmosphere of 20% (200,000 ppm) nitrous oxide, 20% oxygen, and 60% nitrogen for either 8 hours or 24 hours/day for up to 35 days. After 14 days, evidence of damage to the seminiferous tubules was found in all animals. The toxic effect appeared to be confined to the

spermatogenic cells with a reduction in the number of mature spermatozoa and the appearance of multinucleated forms. Recovery of normal spermatogenesis occurred after a return to room air for more than 3 days. Other cells within the testes were not damaged.

Kennedy et al [125] investigated the effect of halothane at anesthetic concentrations on reproduction in rats exposed before mating, on fetal development in rats and rabbits exposed during various stages of gestation, and on fetal survival in rats whose dams were exposed during late stages of pregnancy. The fertility and general reproduction studies used three control and six experimental groups of Charles River albino rats, CD strain, with at least 10 male and 20 female rats in each group. The experimental groups were exposed to halothane for 1 hour/day prior to pairing for 1-5, 6-10, or 11-15 days at mean concentrations of 1.48% (14,800 ppm), 1.34% (13,400 ppm), and 1.40% (14,000 ppm), respectively. The authors reported neither an indication of any effect on mating and fertility nor any differences in population and survival data for offspring between control and exposed groups. No gross abnormalities were observed on examination of offspring at birth and at weaning.

Teratologic studies were conducted by the same investigators [125] in rats and rabbits. Pregnant Charles River rats, CD strain, and impregnated New Zealand albino rabbits were used. In the study, 24 rats were exposed for 1 hour/day on gestation days 1-5, 12 rats on days 6-10, or 12 rats on days 11-15 to mean halothane concentrations of 1.35% (13,500 ppm), 1.43% (14,300 ppm), and 1.43% (14,300 ppm), respectively. Separate groups of 15 rabbits were exposed on gestation days 6-9, 10-14, or 15-18 to mean halothane concentrations of 2.16% (21,600 ppm), 2.16% (21,600 ppm), and

2.30% (23,000 ppm), respectively. No significant differences were reported between rat control and test groups when killed at an interim or terminal period with respect to the number of corpora lutea, implantation and resorption sites, and viable fetuses. Fetuses from rats exposed on days 11-15 showed increases in the percentages of incompletely ossified or nonossified sternum sections. In rabbits, the reactions were essentially the same as in rats. No apparent exposure-related effect was reported regarding reproductive data. Skeletal findings included incompletely ossified or nonossified sternum sections, supernumerary ribs, and thickened ribs. The authors considered these skeletal effects to be incidental and not specific drug-related structural defects.

Pregnant Sprague-Dawley rats were exposed by Lansdown et al [126] to subanesthetic concentrations of halothane to determine any effect on fetal development. Groups of eight pregnant rats were exposed to halothane at 50, 100, 200, 800, 1,600, and 3,200 ppm for 8 hours/day on days 8-12 of gestation. In a second series of experiments, groups of 8 pregnant rats were exposed to halothane at 1,600 or 3,200 ppm for 8 hours/day on days 1-21 of pregnancy. Control groups were used for each exposure concentration. All rats were killed and examined on day 22. Animals exposed at 3,200 ppm from day 1 became drowsy and failed to feed normally resulting in data from this group being excluded from the reported results. Exposure to halothane at 50-3,200 ppm on days 8-12 of gestation caused no statistically significant reduction in the mean litter size or in the fetal and placental weights. However, rats exposed at 1,600 ppm throughout pregnancy had statistically significant reductions in fetal weight and crown-rump length ($P<.001$), even though there was no reduction in placental weight. The

authors observed that maternal food intake was reduced and may have been a factor in these results. No appreciable differences between control and experimental groups were reported in the number of centers of ossification in the skull or postcranial skeleton. Several skeletal anomalies were identified in both groups but their frequency was independent of exposure.

Halothane was tested for possible mutagenicity by Baden et al [127] in an in-vitro microbial assay system using two histidine-dependent mutants of Salmonella typhimurium, TA98 and TA100. Halothane, in concentrations ranging from 0.1% to 30% (1,000 ppm to 300,000 ppm), was incubated with the bacteria in the presence or absence of a metabolic activation system prepared from either rat liver treated with Aroclor 1254 or from human liver. Trifluoroacetic acid, a major metabolite of halothane, and urine from patients anesthetized with halothane were also tested. Halothane, trifluoroacetic acid, and patients' urine were reported to have no mutagenic effect on the bacterial systems.

Several studies utilized chick embryos to determine the effects of exposure to anesthetic gases on fetal development [105-107,114,115]. Anesthetic agents used in the chick embryo exposure studies included methoxyflurane, halothane, fluroxene, diethyl ether, nitrous oxide, and cyclopropane. The exposure concentrations were very high compared to those in other animal studies and, in some cases, exceeded the levels normally used in clinical anesthesia. The value of these studies may be limited to an indication of gross effects or of increasing trends of abnormal fetal development resulting from such excessive exposures. Major effects seen in the chick embryos included a death rate among exposed chicks higher than in controls, a significant increase in fetal anomalies, a reduction of the

neural tube mitotic index, and a decreased growth rate.

(e) Carcinogenicity

The carcinogenicity of specific inhalation anesthetic agents, ie, chloroform and trichloroethylene, has been identified. Eschenbrenner [128] in 1945 reported the effects of repeated oral doses of chloroform on induction of hepatomas in mice. Hepatomas were produced in 7 of 10 female mice fed 30 doses of 600 or 1,200 mg/kg at 4-day intervals over a 4-month period. Male mice receiving similar doses died within the first week of the experiment.

The National Cancer Institute (NCI) released results of the chloroform carcinogenicity bioassay program in March 1976 (Report on Carcinogenesis Bioassay of Chloroform, National Cancer Institute, March 1, 1976). Osborne-Mendel rats were fed chloroform in corn oil (at 90 and 180 mg/kg body weight for males and at 100 and 200 mg/kg for females) for 111 weeks. A significant increase in epithelial tumors of the kidneys in treated male rats was observed. Of the 13 tumors of renal tubular cell epithelium seen in 12 of the 50 high-dose male rats, 10 were carcinomas and 3 adenomas; 2 of the carcinomas were found to have metastasized. Two carcinomas and two adenomas of renal tubular epithelium were observed in the 50 low-dose male rats. The tubular cell adenocarcinomas were widely metastasized. An increase in thyroid tumors in chloroform-treated female rats was also seen which NCI did not consider significant.

In the same bioassay, mice (B6C3F1) were fed chloroform for 92-93 weeks at doses of 138 and 277 mg/kg for males and at 238 and 477 mg/kg for females. A highly significant increase in hepatocellular carcinomas was observed in both sexes of treated mice when compared with control animals.

The incidence of hepatocellular carcinoma was 98% for males and 95% for females at the high dose, and 36% for males and 80% for females at the low dose compared with 6% in both matched and colony control males, none in matched control females, and 1% in colony control females. Nodular hyperplasia of the liver was observed in many of the male mice fed low doses that had not developed hepatocellular carcinoma. A bulletin on chloroform was released by NIOSH in March 1976 to alert the occupational health community of these findings (J Finklea, written communication, March 15, 1976).

In 1975, Lloyd et al described information released by the NCI [129] about the carcinogenicity of trichloroethylene in rats. Male and female rats (Osborne-Mendel) and mice (B6C3F1) exposed by gastric intubation were used in the study. Both sexes of rats were given doses at either 1,000 mg/kg or 500 mg/kg, 5 times/week for 18 months, with an observation period of 6 months following exposure. Male mice were given 2,400 or 1,200 mg/kg and female mice 1,800 mg/kg or 900 mg/kg doses 5 times/week for 18 months, followed by an observation period of 3 months. Hepatocellular carcinomas were not seen in the rats; 30 of the 98 (30.6%) mice given the low dose, and 41 of the 95 (43.2%) mice given the higher dose had hepatocellular carcinomas. Only 1 (2.5%) of the 40 control mice developed a carcinoma. Various tumors also were found in other organs in the exposed mice. Although the investigators did indicate that their study results were preliminary, they expressed a definite concern about occupational and environmental exposures to trichloroethylene. Therefore, NIOSH alerted the occupational health community about the carcinogenic potential of trichloroethylene [129]. The suspected carcinogenicity of chloroform and

trichloroethylene and their limited availability for clinical use should encourage the curtailment and elimination of their use as anesthetic agents. The interpretation of these results remains to be defined because the agents were administered by a route different from normal anesthetic delivery and in concentrations approximately 25 times the anesthetizing dose.

Corbett [130] reported a study in which "timed-pregnant" Swiss/ICR mice were exposed to either 0.5% isoflurane on days 12, 14, 16, and 18 of pregnancy (Group I) or to 0.1% isoflurane on days 12, 14, and 16 of pregnancy (Group II). The offspring of these two groups were then exposed to 0.1% isoflurane every other day beginning at 5 days of age for 25 exposures. Each exposure period lasted 2 hours. A control group and their offspring were exposed to room air only. The investigators found more pulmonary adenomas in the exposed groups than in the control groups, but the difference was not statistically significant. At the end of 15 months, 27% (10/37) of the males in Group I had hepatic neoplasms with three of the animals having multiple tumors. Seventeen percent (5/30) of the Group II males had hepatic neoplasms, while none were observed in the 23 male control animals. These differences were statistically significant. Hepatic neoplasms were not observed in the females of any group. The author concluded that, even though there were certain experimental design deficiencies and the results did not prove isoflurane to be a carcinogen, the data are ample to consider isoflurane as highly suspicious of being carcinogenic. On pathologic examination of the liver tissue of affected animals, Farber (written communication, September 1975) indicated that the animals showed a neoplastic process in the liver which may have been either

benign or malignant and recommended that the use and marketing of isoflurane be stopped until further tests could be conducted.

Correlation of Exposure and Effect

Early reports in the medical literature attributed adverse health effects seen among anesthetists and operating room personnel to exposure to vapors of anesthetic agents [16,18-20], and anesthetists and surgeons began implementing venting and control procedures as early as 1922 [19]. Health effects reported in 1929 among surgeons, anesthetists, and operating room nurses included headache, fatigue, and heart complaints [19]. The common anesthetics in use at that time were nitrous oxide, diethyl ether, and chloroform. A 1949 report [20] attempted to ascribe signs and symptoms of three members of a German surgical team, including fatigue, headache, lymphocytosis, eosinophilia, and electrocardiographic evidence of myocardial damage to their exposure to anesthetic vapors over periods of 4-14 years. The most relevant information on current occupational exposure is in the epidemiologic [12,13,69,72-80] and mortality studies [70,71] reported between 1967 and 1975. The epidemiologic studies, conducted among operating room and dental personnel, suffer from a lack of quantitative data on exposure levels and identification of anesthetic agents used.

In most cases, workers were exposed to a mixture of agents and possibly several different agents throughout the day. Environmental measurements [131-150] placed usual operating-room exposures at 1-10 ppm for halothane and other volatile agents, and 400-3,000 ppm for nitrous oxide. Usual dental exposures were approximately 3,000 ppm nitrous oxide, 15 ppm halothane, and 25 ppm trichloroethylene. Epidemiologic findings,

supplemented by known acute exposure effects on human and supportive animal studies, permit some correlation between anesthetic gas exposure and observed health effects. The five most commonly used anesthetic agents in the 1975 survey conducted by NIOSH were nitrous oxide, halothane, enflurane, methoxyflurane, and diethyl ether (Table XIII-10). Nitrous oxide, diethyl ether, and chloroform were the primary anesthetics used until the introduction of the fluorinated hydrocarbon anesthetics in the mid 1950's.

An increased incidence of spontaneous abortion in exposed female workers and of congenital abnormalities among their children, and the same increases among the wives and children of exposed men are the major adverse health effects identified in the epidemiologic studies. Effects on the liver, kidneys, and CNS have also been described following exposure to anesthetic gases. Increased risk of cancer is also suggested in three epidemiologic studies and one mortality study.

(a) Spontaneous Abortion

Vaisman [69] reported that 18 of 31 pregnancies in a group of Russian female anesthetists, exposed 25 hours/week or more, ended in spontaneous abortion. Nitrous oxide, diethyl ether, and halothane were the main anesthetics in use at the time of the survey. This particular survey involved a small population and had no control group, but it was the first study to identify a high incidence of spontaneous abortion among exposed women. A survey of Danish nurses and female anesthetists [72] revealed a 10% spontaneous abortion rate in pregnancies started before exposure in anesthesia practice, and a 20% spontaneous abortion rate in pregnancies started after exposure. These data allowed the first attempt at

correlating an increased incidence of spontaneous abortion with exposure to anesthetic gases. A new factor surfaced in the Danish study when it was learned that the wives of exposed anesthetists also had a higher than normal incidence of spontaneous abortion. This finding was also seen in later studies [12,13]. Table III-5 summarizes the spontaneous abortion rate data gathered from various groups occupationally exposed to anesthetic gases. Groups chosen for study were anesthetists, operating room nurses, oral surgeons, and dentists, representing the personnel most heavily exposed in a chronic manner to waste anesthetic gases.

TABLE III-5

SUMMARY OF SPONTANEOUS ABORTION RATES
FROM EPIDEMIOLOGIC STUDIES* IN PERSONNEL
EXPOSED TO ANESTHETIC GASES

Exposed Group (E)	Spontaneous Abortion Rate,% E	C	Control Group (C)	Ref.
Operating room nurses	29.7	8.8	General duty nurses	73
Female anesthetists	37.8	10.3	Female physicians	73
"	18.2	14.7	"	74
"	17.1	8.9	"	12
Nurse-anesthetists	17.0	15.1	General duty nurses	12
Operating room nurses	19.5	15.1	"	12
Wives of exposed dentists	16.0	9.0	Wives of unexposed dentists	13
Female anesthetists	17.9	10.6	Before exposure	77
Maternal exposure	15.5	10.9	Neither parent exposed	80

*Studies listed are limited to those utilizing a control group.

Several investigators have described increased rates of fetal resorptions in animals following exposure to anesthetic gases [108,109,116,117,122]. The majority of these studies used anesthetic

quantities of nitrous oxide , alone or with halothane. Significant increases in resorptions were reported [108,109,116,117] but the exposure levels greatly exceeded those normally associated with occupational exposure. In a study by Corbett et al [122], pregnant rats were exposed to nitrous oxide at levels of 15,000, 1,000, and 100 ppm, within the range of occupational exposure. The most significant increases in fetal death rate followed 8-hour exposures at 1,000 or 100 ppm on days 10-13 or 14-19 of pregnancy. Fetal death rates of 14.5-18.4% were seen among the exposed animals compared to 11.1% among controls.

(b) Congenital Abnormality

The survey by Knill-Jones et al [74] was the first to attempt a correlation between occupational exposure to anesthetic gases and congenital abnormalities in the children of exposed personnel. The incidence of such abnormalities, 6.5%, was significantly greater among children of female anesthetists who had worked during the first six months of pregnancy than in the children of those who had not worked during this period, 2.5% ($P<0.02$). Care must be taken in interpreting these results as the control group had a congenital abnormality incidence of 4.9%. The greatest value in the study [74] is that a health effect was seen in the most heavily exposed persons, the anesthetists, while exposed, but was reduced in the same group when not exposed. The same trend was seen by Corbett et al [76] when they surveyed a group of nurse-anesthetists. Out of the total number of births in the survey, the mothers worked at some time during 62.4% of the pregnancies, and did not work during 37.6% of the pregnancies. The rate of congenital abnormalities among children of mothers who worked during pregnancy was 16.4%, while the rate of such

abnormalities among children of mothers who did not work during pregnancy was 5.7%. The difference was statistically significant ($P<0.005$). The incidence of birth defects in the general population, 8.4%, was used as a control value. Epidemiologic studies that followed [12,13,80] also identified a higher than normal incidence of congenital abnormalities among the children of personnel exposed to anesthetic gases. A summary of these data is presented in Table III-6. The studies indicate a trend toward higher rates of congenital abnormality among the children of exposed personnel but, once again, no direct causal relationship between the effect seen and extent of exposure to anesthetic agents can be drawn.

TABLE III-6

SUMMARY OF CONGENITAL ABNORMALITY RATES FROM EPIDEMIOLOGIC STUDIES* IN PERSONNEL EXPOSED TO ANESTHETIC GASES

Exposed Group (E)	Congenital Abnormality Rate,% E	C	Control Group (C)	Ref.
Female anesthesiologists	5.9	3.0	Female pediatricians	12
Nurse-anesthetists	9.6	7.6	General duty nurses	12
"	9.6	5.9	Unexposed nurse-anesthetists	12
Wives of exposed dentists	4.7	4.1	Wives of unexposed dentists	13
Maternal Exposure	5.5	3.6	Neither parent exposed	80
Paternal Exposure	4.5	3.6	"	80

*Studies listed are limited to those utilizing a control group.

The teratogenic potential of specific anesthetic agents has been demonstrated in several animal exposure studies [105-116,118,120,121]. Exposing pregnant rats and mice to halothane and nitrous oxide at levels

well above those encountered in an occupational environment resulted in significant developmental anomalies in the offspring [108-110,118,120,121]. While these studies establish the teratogenic potential of halothane and nitrous oxide at high levels, they do not permit a correlation between occupational exposure and congenital malformation. Reports by Chang et al [111-113], attempted to duplicate occupational exposure conditions by exposing pregnant female Sprague-Dawley rats to halothane at 10 ppm for 8 hours/day , 5 days week throughout pregnancy. Liver, kidney, and brain tissues from the offspring were examined for anomalous development. Focal cytoplasmic degradation and areas of necrosis were reported in 50% of the liver tissues [111]. Damage to the proximal convoluted tubules was observed when kidney tissues were examined [112]. Ultrastructural examination of the neonatal brain tissues [113] revealed several anomalies, including occasional neuronal necrosis and failure of the postsynaptic membrane density to form. The anomalies seen after exposure to halothane at 10 ppm were not as immediately visible as the rib and vertebral malformations occurring after exposures at higher levels, yet they do establish a teratogenic effect following exposure at levels encountered in an occupational situation.

(c) Liver and Kidney Effects

Hepatic dysfunction and massive hepatic necrosis have been documented in patients following clinical anesthesia [21-32]. These cases involved exposure conditions completely different from those of occupational exposure. Occupational exposure has been suggested as the cause of two cases of hepatic dysfunction [151,152] in anesthetists, but substantial proof was not provided.

Effects on the liver and kidneys have been reported in three epidemiologic studies following occupational exposure to anesthetic gases [12, 13, 78]. Exposure in two of the studies [12,13] was defined as at least 1 year's duration but not necessarily the year immediately before onset of the signs and symptoms. Hepatic disease, excluding serum hepatitis, was reported more frequently in exposed female operating room personnel than in unexposed controls. Table III-7 summarizes the hepatic disease rate in various exposed groups in the US [12,13]. A survey of anesthetists and operating room nurses [78] in Czechoslovakia revealed a trend toward an increased rate of hepatic disease with increased length of service and, presumably, increased exposure. The only control utilized by the Czechoslovakian survey was the group of exposed personnel with less than 1 year of service.

TABLE III-7

SUMMARY OF HEPATIC DISEASE RATES FROM EPIDEMIOLOGIC STUDIES* IN PERSONNEL EXPOSED TO ANESTHETIC GASES

Exposed Group (E)	Hepatic Disease Rate,% E	C	Control Group (C)	Ref.
Female anesthesiologists	4.9	2.9	Female pediatricians	12
Female nurse-anesthetists	3.8	1.7	General duty nurses	12
Operating room nurses	2.1	1.7	"	12
Male anesthesiologists	4.1	2.6	Male pediatricians	12
Male nurse-anesthetists	4.7	5.1	Male nurses	12
Male operating room nurses	4.2	5.1	"	13
Exposed male dentists	5.9	2.3	Unexposed male dentists	13

*Studies listed are limited to those utilizing a control group.

Possible toxic damage to the kidneys following methoxyflurane anesthesia has been described [34-39] but, once again, the exposure conditions were different from occupational exposure. The same three surveys discused above [12,13,78] examined the incidence of kidney disease among exposed personnel. Among the US personnel surveyed [12,13], only the exposed female groups reported rates of renal disease higher than those among controls. The nurse -anesthetists showed the most statistically significant rate increase. Kidney damage reported in the Czechoslovakian study [78] showed only a small increase consistent with increased length of service.

Adverse effects on the liver and kidneys have occurred in animals following exposure to anesthetic agents at levels near or equal to those needed to produce anesthesia [83-90,93,94]. The value of these studies is that they demonstrate effects on the liver and kidneys after high-level exposures and raise the question of the same effects following exposure to levels associated with an occupational environment. Animal studies attempting to investigate the effects of chronic low-level exposure to various volatile halogenated anesthetics have shown minimal to moderate toxicity to the liver and kidneys [61,96,98,99]. Exposure studies, using the range of occupational exposure for volatile agents of 1-10 ppm, reported only slight damage in liver and kidneys on ultrastructual exami nation. More readily apparent were the organ lesions seen at higher levels, 500 ppm and above; such levels were not found in an occupational environment. Situations such as the anesthesia induction period may produce levels of halogenated anesthetics high enough to be a factor in the reported increased incidence of liver and kidney diseases in exposed personnel.

(d) Central Nervous System Effects

Anesthetic agents act on the CNS by producing narcosis. Two epidemiologic studies have suggested CNS effects in exposed personnel [69,78] who reported an increased incidence of headache, fatigue, irritability, and disturbance of sleep. Damage to cerebral cortical neurons was seen following the exposure of young adult rats to halothane at 10 ppm for 8 hours/day, 5 days/week for 8 weeks [97]. Permanent learning deficits and neuronal damage was seen in rats exposed to halothane at 8-12 ppm, 8 hours/day, 5 days/week from conception to 60 days of age [119]. Exposure of pregnant rats to halothane at 10 ppm, 40 hours/week throughout pregnancy produced ultrastructural anomalies in cerebral cortical neurons in the offspring [113]. Neuronal damage and learning deficits diminished when exposure to halothane was begun at a progressively increasing age of the exposed animals [119], suggesting that the developing fetus may be most susceptible to permanent CNS damage as a result of exposure to anesthetic gases.

(e) Carcinogenicity

A retrospective mortality study among members of the American Society of Anesthesiologists [70] and an epidemiologic survey of a group of nurse-anesthetists [75] have raised the question of a possible increased incidence of cancer among persons occupationally exposed to anesthetic gases. A prospective mortality study did not substantiate the increased malignancy rate among the ASA members [71]. The range of age at diagnosis (27-62), the year in which anesthesia practice began (1926-1968), and the year of diagnosis (1935-1971) have raised a question as to the significance of the nurse-anesthetist study [75]. However, a more recent epidemiologic

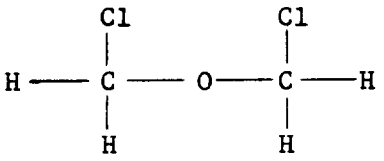
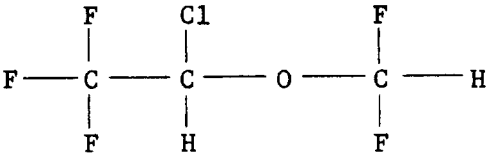
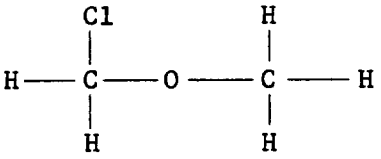
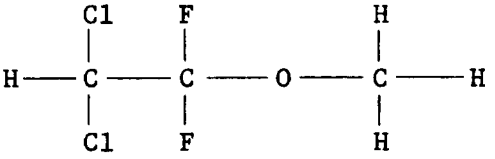
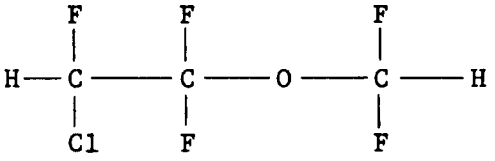
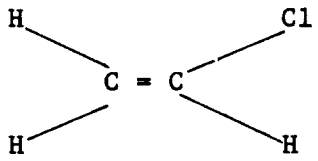
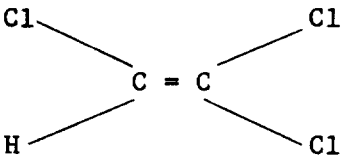
study involving a large population of exposed (7,136) and unexposed (6,560) female hospital personnel [12] has indicated that there is a significant increased occurrence of cancer in exposed females compared with unexposed controls. The increases ranged from approximately 1.3- to 1.9-fold, with probabilities of 0.05, < 0.01, and 0.07 for the female anesthesiologists, nurse-anesthetists, and operating room nurses, respectively. The increased occurrence of cancer was not observed in exposed males.

The carcinogenic potential of chloroform, trichloroethylene, and isoflurane has been demonstrated at levels well above those considered to be occupational exposures [128-130, Carcinogenesis Bioassy of Chloroform, NCI, March 1976]. This does not mean that a correlation could not be drawn between exposure at occupational levels to these agents, or any other anesthetic agents, and an increased incidence of cancer in exposed persons.

Structural similarities exist between some known human carcinogens and several inhalation anesthetic agents. Chloromethyl methyl ether and bis(chloromethyl) ether were predicted by Van Duuren [153] to be carcinogenic and later identified as being responsible for an outbreak of lung cancer among industrial workers exposed to bis(chloromethyl) ether [154,155]. Some halo-ether anesthetics have structures similar to these chlorinated ethers. Trichloroethylene is structurally similar to vinyl chloride, a known carcinogen [156,157]. Table III-8 demonstrates the structural similarities between the known human carcinogens and several inhalation anesthetics. No conclusions can be drawn, nor are they intended to be, from the presentation of these elementary structural similarities.

TABLE III-8

STRUCTURAL COMPARISON OF SEVERAL KNOWN HUMAN CARCINOGENS
WITH CERTAIN INHALATION ANESTHETIC AGENTS

Human Carcinogens	Inhalation Anesthetics
	
Bis(chloromethyl)ether	Isoflurane
	
Chloromethyl methyl ether	Methoxyflurane
	
	Enflurane
	
Vinyl chloride	Trichloroethylene

Adapted from reference 130

Summary Tables of Exposure and Effect

The effects of exposures on humans to inhalation anesthetics that e presented in Chapter III are summarized in Table XIII-14. The effects of short- and long-term exposures on animals to inhalation anesthetics are summarized in Table XIII-15.