III. BIOLOGIC EFFECTS OF EXPOSURE

Extent of Exposure

Chloroform ($CHCl_3$) is a nonflammable, clear, colorless, volatile liquid at ordinary temperature and pressure. It has a pleasant ether-like, nonirritating odor. [1] The more important physical properties are presented in Table X-1. [1-4]

Chloroform was originally made from acetone and bleaching powder and can also be made by reduction of carbon tetrachloride. The principal method of manufacture is now chlorination of methane. [1]

Prior to World War II, chloroform was used primarily as an anesthetic and a pharmaceutical. The annual production of chloroform in the United States at that time was between 2,000,000 and 3,000,000 lbs. It is now seldom used as an anesthetic, [1] but it is used by many manufacturers for pharmaceutical purposes. [5,6] The production of chloroform for the manufacture of chlorodifluoromethane has grown over the years from 40,396,000 lbs in 1955 to 230,766,000 lbs in 1971, the latest year for which data are available. [7-23]

In 1971, chloroform was manufactured by 6 chemical companies in the United States. Two of these companies and 3 others used chloroform in the manufacture of chlorodifluoromethane. [23]

NIOSH estimates that 80,000 people are potentially exposed to chloroform in their working environment.

Historical Reports

Simpson, [24] a surgeon and obstetrician in Edinburgh, reported in an 1847 issue of Lancet on the merits of chloroform as an anesthetic agent. He advocated its use because it was pleasant to inhale, its action was more rapid and complete than that of ether, only a small amount was needed to produce narcosis, and it did not require the use of a special inhaler or instrument for its administration. He especially recommended it for use in obstetric practice because it alleviated maternal pain and recovery from anesthesia was rapid.

In 1874, Witte [25] observed that it required less chloroform to anesthetize frogs by absorption through the skin of the abdomen or thigh than by inhalation. He reported that rabbits also could be anesthetized by application of chloroform to the shaved abdomen, and he recommended that humans be anesthetized by absorption through the skin rather than by inhalation.

One of the first detailed descriptions of death from liver damage following chloroform anesthesia published in the English literature appeared in the January 26, 1894 issue of Lancet. [26] A 4-year old boy seemed to be well after an operation which lasted about 1 hour, but early the next morning he began to vomit, his pulse became weak, breathing was shallow and irregular, and he lapsed into unconsciousness. Vomiting continued (the vomitus being dark brown in color), little urine was passed, consciousness was never regained, and death occurred 30 hours after the operation. At autopsy, the liver was found to be small (14 1/2 oz), pale buff color, studded with minute purple dots, and greasy to the touch.

Microscopy revealed intense fatty infiltration with no apparent fatty degeneration.

In 1898, Desgrez and Nicloux [27] reported that carbon monoxide was formed in dogs during chloroform anesthesia. They considered that the amount of carbon monoxide in the blood after 3-5 hours of anesthesia was equivalent to that present after exposure for 30 minutes to 100 ppm of carbon monoxide in air. Carbon monoxide was measured by a "grisoumeter" and chloroform did not interfere with the analytical method.

In 1904 Schwenkenbecher [28] reported that immersion of white mice up to their necks in aqueous solutions of chloroform was lethal. The solutions ranged from 0.3%-0.7% and the immersion time from 45-165 minutes. A special collar was used to preclude inhalation, and the genital and anal openings were closed.

Moore and Roaf [29] in 1906 reported that chloroform added to hemoglobin solutions at body temperature caused a change in color and a precipitation of the hemoglobin.

In 1909, Whipple and Sperry [30] published studies of liver necrosis in dogs following anesthesia with chloroform. They compared the liver damage observed in dogs to that seen in a 19-year old woman who died 3 days after anesthesia with chloroform for 35 minutes. They concluded that in dogs chloroform anesthesia for 1-2 hours would invariably cause central liver necrosis, and that the anatomic changes in the human autopsy were identical to those observed in the experimental dogs.

Lehmann [31] was the first to mention the industrial hygiene aspects of chloroform. He stated that in Germany more than 100 tons of chloroform

was produced annually and that its manufacture in well designed factories did not create any danger to workers.

Lewin [32] in 1920 reported that the use of chloroform as a solvent for fats and resins was associated with a feeling of being "stoned", headache, dizziness, breaking of the voice, sometimes an increase in saliva flow, bronchial catarrh, pounding of the heart, and with continued exposures, disturbances of metabolism, leading to a "real change in substance in the kidney and even to albumin in the urine."

Effects on Humans

(a) Central Nervous System Effects

The most outstanding effect of chloroform on the central nervous system is narcosis. Because of this property, chloroform was extensively used as an anesthetic. Featherstone [33] concluded from a review of the literature that 20,000 ppm of chloroform was usually used to produce anesthesia and that 40,000 ppm, if continued for several minutes, could be an overdose. He suggested, for induction of anesthesia, gradually increasing the concentration of chloroform during the first 2 or 3 minutes to attain and maintain a concentration of 25,000 or 30,000 ppm until full anesthesia developed. The concentration used to produce anesthesia is usually not maintained for the duration of the operation but is replaced by a lower maintenance concentration such that the integrated exposure was actually much lower than 20,000 ppm.

In the experiments reported by Lehmann and Hasegawa [34] in 1910, dizziness and light intoxication were experienced during 20-minute exposures to chloroform concentrations of 4,300-5,100 ppm (20.8-25 mg/liter)

(Table X-2). During exposure for 15 minutes to 7,200 ppm (35.3 mg/liter) (Table X-3), these effects became so pronounced that experimental exposure of humans to more concentrated chloroform atmospheres was deemed unsafe.

Lehmann and Schmidt-Kehl [35] reported human exposures to 10 concentrations of chloroform, all lower than those used by Lehmann and Hasegawa. [34] On 6 separate days they set up fans in a 10 x 10 x 10 meter "waterproof" room to evenly distribute chloroform spray from a dispersing apparatus with an oxygen bulb attached. The chloroform concentrations were determined by alkaline hydrolysis. [35] Other than odor there were no responses until a concentration of 920 ppm was achieved; exposure at this level for 7 minutes and at all higher levels up to 3,000 ppm caused symptoms of central nervous depression. Other symptoms including "headache and heart pounding" were observed at the highest levels.

Heilbrunn et al [36] tabulated 31 cases of "chronic chloroform poisoning" from the literature and recounted one case of a 33-year old male who habitually had inhaled chloroform for 12 years. The psychiatric and neurologic symptoms reported in this latter case were depression, loss of appetite, hallucinations, ataxia, and dysarthria.

Other symptoms from habitual use of chloroform such as moodiness, mental and physical sluggishness, nausea, rheumatic pain, and delirium are presented in Table X-4. These effects were reported from nonoccupational exposures. [36-40]

Three days after a 19-year old patient ingested an unknown amount of chloroform, Storms [41] noted cerebellar damage characterized by an instability of gait and a slight tremor on finger-to-nose testing. These signs disappeared in 2 weeks.

Fokina [42] reported effects of chronic exposure in the work environment to mixtures of chlorinated methanes, including chloroform. Although the concentrations were not given, it was stated that the maximum permissible concentrations of individual components were exceeded at times. (There was no MAC for chloroform in Russia in 1965. [43]) The majority of workers showed signs of autonomic dysfunction, including diminution or disappearance of the corneal reflexes, dissociation between the deep (exaggerated) and superficial (sluggish) reflexes, marked persistent dermographism, general hyperhydrosis, acrohyperhydrosis, blotchiness of the skin of the hand and forearms, tenderness when pressure was applied to specific cervical points, and arterial hypotension. Autonomic dysfunction was mainly found in workers employed for less than 3 years. Workers employed for more than 5 years showed diencephalic disturbances and autonomic polyneuritis. [42]

(b) Hepatotoxic Effects

Toxic effects of chloroform on the liver have been studied most often in conjunction with its use as an anesthetic, or in a few cases where a person either accidentally or intentionally ingested chloroform. Liver damage has been evaluated by liver function tests, and by macroscopic and microscopic observations.

Cullen et al [44] studied prothrombin in 6 patients before and after light to moderate chloroform anesthesia of 60-90 minutes' duration. Concentrations of chloroform in the blood were found to be 15-18 mg%. Prothrombin, determined by a 2-stage titration technique, was normal in all cases prior to operation and decreased in all cases following operation, by

an average of 18% with a range of 11-40%. Icteric indices were markedly elevated in 2 of the patients.

Elevated serum transaminase activity and prothrombin time were observed by Storms [41] in a 19-year old patient who had accidentally ingested an unknown amount of chloroform. The patient was comatose and cyanotic, breathing was labored, diastolic pressure reduced, pulse was 108, and there were decreased deep tendon reflexes. Liver function tests performed over a period of several days following ingestion are summarized in Table X-5. The values of all tests were normal 8 weeks after ingestion.

Delayed chloroform poisoning often occurred in obstetrical cases, [45-48] and many authors have described instances of this occurring after delivery. This delayed chloroform poisoning is usually characterized by a latent period of a few hours to a day before symptoms develop; then drowsiness, restlessness, jaundice, and vomiting may occur, followed by fever, elevated pulse rate, liver enlargement, abdominal tenderness, delirium, coma, and abnormal findings in liver and kidney function tests. Death often ensues, usually from 3-10 days post partum. Autopsy reports generally describe the liver as having a bright yellowish color, and on microscopy, fatty infiltration with necrosis.

Examples of delayed chloroform poisoning following obstetrical anesthesia in which vomiting, jaundice, delirium, coma, and sometimes death occurring are presented in Table X-6. Results of urine and blood analyses of 2 cases are presented in Table X-7. Gibberd [45] studied 3 women given chloroform prior to delivery, who died from acute diffuse necrosis of the liver. Each had received prenatal care by a physician and had apparently been in normal health. Lunt [49] reported on 3 obstetrical exposures to

chloroform from which all patients recovered. Obstetrical exposures, such as reported by Gibberd [45] and Lunt, [49] were generally multiple in nature, with the first chloroform concentrations administered to reduce pain but not to produce deep narcosis. Inhalations of chloroform from capsules or bottles were given for relief of pain at various intervals during labor, often separated by several hours. Furthermore, chloroform was often used as the anesthetic agent during delivery. Obstetrical patients were exposed to chloroform intermittently over long periods of time.

Surgical exposures differ from obstetrical exposures in that in the former there is usually an initial, high concentration great enough to produce unconsciousness, [48,50-53] followed by maintenance levels for the duration of the operation, usually lasting for 1/2 hour-2 hours.

Whipple and Sperry [30] recorded a fatal case of chloroform poisoning following a minor surgical procedure lasting only 35 minutes. Vomiting began the night after the operation and cramps developed on the following day. Muscle tremors, jaundice, and restlessness also developed. Three days after the operation the patient became comatose, his pulse rate and temperature increased and he died. Autopsy showed advanced central necrosis of the liver and fatty degeneration of the kidneys and heart.

Liver damage has also been reported following ingestion of chloroform. [52,54] Schroeder [52] described an acute case in which a man ingested 4 oz of chloroform. When first observed, his pupils were dilated, he was cyanotic and perspired profusely. An electrocardiographic tracing obtained a few hours after admission showed only occasional extrasystoles and minimal S-T depression. On the following day, there was no abnormality

to be found in the tracing. He developed hepatomegaly with jaundice and vomiting. There were initial rises in serum bilirubin, alkaline phosphatase, and SGOT. Recovery began on the fifth day after ingestion.

Bomski et al [55] reported enlarged livers in 17 of 68 workers exposed to chloroform in concentrations ranging up to 205 ppm. In 3 of the 17 workers with enlargement of the liver, toxic hepatitis was diagnosed on the basis of elevated serum enzyme activities and elevated serum gamma globulin. In the remaining 14 cases of liver enlargement fatty liver was diagnosed, but not confirmed by liver biopsy.

(c) Renal Effects

Although the cause of death in most cases of chloroform poisoning has been attributed to necrosis of the liver, there has also been evidence at autopsy of renal damage, including albumin and red blood cells in urine, elevated blood urea, necrosis, and fatty degeneration. [30,45,49]

In the case of delayed chloroform poisoning reported by Whipple and Sperry, [30] urine specimens contained a trace of albumin, a few hyaline casts, and considerable quantities of leucine and tyrosine. At autopsy, the convoluted tubules of the kidneys showed a definite fatty degeneration with some of the epithelial cells having undergone necrosis. The capillaries were also found to be congested.

In the studies by Gibberd [45] and Lunt [49] laboratory findings indicated renal dysfunction; there were albumin, red blood cells, and pus in the urine, congestion of cortical vessels, fatty deposits, and necrosis.

(d) Cardiovascular Effects

Cardiac irregularities were found frequently by Kurtz et al [56] in 1936 during electrocardiographic (ECG) surveillance of 113 surgical

procedures. Arrhythmias were reported in all 6 cases in which chloroform was used for anesthesia and in 83 of 107 operations in which other anesthetics were used, including ethyl ether, cyclopropane, nitrous oxide, tribromoethanol, procaine, vinyl ether, and ethylene. The authors [56] did not report the durations of anesthesia.

In 1951, Orth et al [57] reported results of ECG surveillance during surgery on 52 patients given chloroform for anesthesia. Among the 52 patients, many developed more than one type of cardiac irregularity during anesthesia and 7 patients were symptom free. There were 4 instances of sinoauricular block, 11 of sinoauricular extrasystoles, 4 of auricular fibrillation, 22 of auriculoventricular block, 14 of auriculoventricular extrasystoles, 32 of auriculoventricular rhythm, 36 of ventricular extrasystoles, 6 of slow ventricular rhythm, 2 of bundle branch block, 20 of ventricular tachycardia, and 4 cases of cardiac arrest. The authors [57] stated that "this is a higher incidence of irregularities than is observed clinically with any other anesthetic agent except trichloroethylene". The irregularities were attributed to both reflex effects on cardiac automaticity and a direct depressant effect on the myocardium. In view of the high incidence of ECG abnormalities with all studied anesthetics, [56] the possibility of all other factors in the etiology of the abnormalities needs to be considered in drawing conclusions from this study. For example Orth et al [57] did not report the durations of anesthesia while Whitaker and Jones [58] considered this to be important.

Whitaker and Jones [58] in 1965 found less frequent cardiovascular effects among 1,502 cases where chloroform was administered by a precision vaporizer in concentrations not exceeding 22,500 ppm. There were 9 cases

of arrhythmia in the 1,164 operations in which anesthesia lasted 30 minutes or less and there were 10 cases of arrhythmia in 338 operations in which the duration of anesthesia was greater than 30 minutes.

(e) Hemolysis

Belfiore and Zimmerman [59] found that chloroform could affect the fragility of the red blood cell membrane without first being metabolized in the liver. Erythrocytes from 12 healthy adults were suspended in saline solution with chloroform ranging from 0.0125-0.10 mole/liter. The control was erythrocytes in a saline solution. At concentrations of 0.0125, 0.02, and 0.025 mole/liter there was no demonstrable effect. In concentrations of 0.05 mole/liter, erythrocyte leakage of hemoglobin, lactic dehydrogenase (LDH), and malic dehydrogenase began at 7 minutes and reached a maximum at 20 minutes for hemoglobin and at 10 minutes for the enzymes.

Belfiore and Zimmerman [59] found that incubation of cells with reduced glutathione (GSH) and oxidized glutathione (GSSG) inhibited hemolysis as measured by a loss of hemoglobin. They found that inhibition increased as the concentration of GSSG increased, but did not increase as the concentration of GSH increased. It has been suggested more recently, however, that it is only the GSSG that enters the intact red blood cell; the effect of GSH is due to its oxidation to GSSG. [60]

(f) Effects on the Skin

Malten et al [61] measured injury and regeneration of forearm skin exposed to liquid industrial solvents, including chloroform. The solvents were contained in glass cylinders 2 cm in diameter which were fixed to the skin by agar-agar for 15 minutes a day for 6 consecutive days. An unspecified number of sites served as controls. The rate of water evaporation

from these sites was determined daily to evaluate the degree of skin damage and regeneration. Exposure to chloroform was reported to be similar in effect to ethanol and to cause an increase in the rate of water evaporation with repeated exposures. Recovery of normal water retention, indicative of the formation of a new horny layer, occurred slowly during the 30 days after the last exposure.

Hoffman [62] suggested that chloroform could be used on skin to combat mosquitoes and other biting insects. It seems doubtful that present-day dermatologists would recommend this use of such an irritating substance. Hoffman cautioned against getting chloroform into eyes and mucous membranes because of irritating effects.

Oettel [63] carried out systematic experiments with chloroform administered to the skin by way of a small glass vessel, 1 cm in diameter, one end of which was open, with glass hooks fused into it. Vessels were filled with chloroform and tied onto the arms of 5 subjects. Pure chloroform was used for various exposure times. Three minutes after application of chloroform, there was a sensation of burning and stinging. When exposure time was increased to 6 minutes, the pain became more intense and then subsided quickly. When the chloroform was removed, the pain increased again, only to be replaced by a loss of feeling. Erythema was noted and the hyperemia which also occurred after 3 minutes of exposure was somewhat stronger, more cherry red with a light yellow undertone. In a 30-minute period after removal of the chloroform, there was a fading away of the erythema and hyperemia, and 5 hours later, little blisters formed at the edge of the area of application. Erythema and pigmentation disappeared 7 days after the exposure.

From these studies, [61-63] it can be concluded that exposure of the skin to liquid chloroform will cause irritation, erythema, hyperemia, and destruction of the epithelium. Much of this information on skin irritation from chloroform is based on prolonged contact. However, it should be noted that repeated, brief contact can cause skin defatting.

(g) Respiratory Absorption

Absorption of inhaled chloroform was studied by Lehmann and Hasegawa [34] in 1910. In a series of 3 experiments they computed the amount of chloroform absorbed as indicated by the difference between the concentrations in the inhaled and exhaled air. Each subject inhaled chloroform vapor through the mouth and exhaled air was collected in alcohol and subsequently analyzed for chloroform by hydrolysis with alkali. In the first experiment one subject inhaled 3 different concentrations of chloroform from a vessel containing a weighed amount of chloroform and the other inhaled 4 different concentrations. Each trial was performed at intervals of at least 3 hours. At exposure concentrations ranging from 2,700-6,500 ppm and exposure times of 3-10 minutes, the percent absorption ranged from 54-73.

In the second experiment 2 subjects each inhaled chloroform for 20 minutes on 3 separate days. The exposure concentrations were between 4,300 and 5,000 ppm (20.8 and 25 mg/liter). The percentages of chloroform absorbed which ranged between 49.4 and 77 are presented in Table X-2.

In the third experiment one subject inhaled chloroform on 2 different occasions. The percentages of chloroform absorbed which ranged from 73.8-80.7 are presented in Table X-3.

(h) Breath and Tissue Concentrations

Several investigators have measured the concentration of chloroform in exhaled air, blood, and other tissues, after inhalation or ingestion of chloroform. [34,41,44,50,64-66]

The concentrations of chloroform found in exhaled air by Lehmann and Hasegawa [34] after exposure were a function of the amount inhaled and the elapsed time after exposure (Table X-3).

In 1951, Morris [50] studied chloroform in exhaled air and peripheral venous blood of patients undergoing surgery. Exhaled breath samples from 11 patients maintained for various times in the third stage (surgical level) of chloroform anesthesia were collected in alcohol and evaluated by a modified Fujiwara reaction. The data are presented in Table X-8. The concentrations found were greater in plane 3 (deep surgical anesthesia) than in plane 1 or 2 (light or moderate surgical anesthesia) and they were greater at the beginning of anesthesia than at later times during anesthesia. Since chloroform is administered intermittently during surgery, the exhaled breath concentrations probably reflected time-weighted average exposure.

Peripheral venous blood was also collected from 58 patients when blood concentrations of chloroform necessary to maintain anesthesia were thought to have been achieved. Analysis was by ether extraction and a modified Fujiwara method. Chloroform concentrations of 2.0-23.2 mg % were found. Thirty to 50% of chloroform in the blood at the end of exposure was removed in the first 15 minutes following cessation of exposure. Thereafter, the rate of elimination decreased, and "small amounts" were reported to have been detected in the blood 8 hours after exposure.

Concentrations of chloroform in the blood were also measured by Cullen et al [44] in 1940 in 3 persons undergoing surgery. Two patients were anesthetized by the absorption technique, with the inhaled gas containing at least 50% oxygen. In one subject, 60 minutes after induction of anesthesia, chloroform was 16 mg %; in the other subject it was 18 mg % 70 minutes after induction of anesthesia. In the third patient, anesthesia was induced by the open drop technique and then maintained by pharyngeal insufflation. In this patient, at 75 minutes of anesthesia, 15 mg % chloroform was found in the blood.

Storms [41] reported a case in which a 19-year old boy accidentally ingested an unknown amount of chloroform and was found to have 20 mg % of chloroform in the blood 10 hours after the ingestion. This case is reported in more detail in the section on hepatotoxicity.

Breath concentrations of chloroform in a worker after an industrial exposure to a mixture of solvents including chloroform, carbon tetrachloride, trichloroethylene, and perchlorethylene were measured by Stewart et al [64] in 1965. The exposure was defined as a "few minutes" without a gas mask followed by an unspecified time with a general purpose chemical respirator, to unknown concentrations with "strong" odors. The exposed worker experienced dizziness, weakness, nausea, and finally unconsciousness. The duration of unconsciousness is not known, but "10 minutes later" he was coherent, though uncoordinated and nauseated. Thirty minutes after his collapse, infrared analysis of his expired breath contained: perchlorethylene, 11 ppm; carbon tetrachloride, 9.5 ppm; chloroform, 7 ppm; and trichloroethylene, 11 ppm. Three days later, 0.1

ppm chloroform was found in the exhaled breath; 12 days after exposure, there was no chloroform found in the exhaled breath.

Several authors have reported on the concentration of chloroform in tissues following suicide, homicide, or death during or after an operation. Gettler [65] measured brain concentrations of chloroform from 390-480 mg/kg in 4 cases of suicide by inhalation, 372-384 mg/kg in 3 cases of homicide by inhalation, and 70-182 mg/kg in 10 cases of death during or after surgery.

Gettler and Blume [66] used a modified Fujiwara method to estimate chloroform content of tissues following death during or after an operation, or by suicide or homicide. In 7 cases where death was reported to be due solely to excessive amounts of administered chloroform, concentrations in mg/kg tissue were: brain, 372-480; lungs, 355-485; and liver, 190-275. In 9 cases of death from shock, concentrations in mg/kg were: brain, 60-182 (most were between 120 and 182); lungs, 95-145; and liver, 65-88.

Epidemiologic Studies

There are very few reports of industrial workers exposed to chloroform. The studies of Challen et al [67] and Bomski et al [55] are the only studies that contain exposure concentration measurements, descriptions of symptoms and diagnoses, and comparisons with control groups.

In 1958, Challen et al [67] reported a study of a confectionery firm in England that manufactured medicinal lozenges. In 1950, the operators began to complain of the chloroform vapor given off during the production of the lozenges. A system of part-time work was initiated to alleviate

complaints of lassitude, flatulence, water brash (British term indicative of symptoms of dyspepsia), dry mouth, thirst, depression, irritability, and frequent and "scalding" micturition, but this was not successful, and finally the operators refused to work on that particular process. In 1954, a new team of operators was engaged and in 1955 a system of exhaust ventilation was installed, after which the work proceeded without interruption.

In order to confirm the effectiveness of the ventilation system, Challen and his associates [67] were asked to ascertain the concentrations of chloroform. Additionally, clinical investigations were performed and an attempt was made to simulate the original conditions in order to compare the chloroform concentrations before and after remedial measures were introduced. The original conditions were simulated by closing the doors and windows and shutting off the ventilation system.

A single air sample was taken continously in the breathing zone of the operator of the ingredient mixing process during a period of 20 minutes coinciding with the duration of the process. Sampling at the rate of 2 liters/min was done by drawing air through 2 U tubes containing dried silica gel. Additionally, at a point in the operation where a peak concentration was expected, a 6-liter "grab" sample was taken. Air samples of 30-minute durations each were also taken in the breathing zones of cutting room operators. The samples were taken during 2 periods of production for 3 different operations on the same day (under current conditions) and during 3 periods on another day (during the simulated conditions). The samples were analyzed by alkaline hydrolysis. Results in ppm of chloroform were as follows [67]:

Operation	Period 1	Period 2	Period 3
Mixing room during normal operation:			
Continuous sampling during mixing period	128	---	---
Grab sampling during emptying period	1,163	---	---
Cutting room during normal operating conditions:			
Feeding operation	71	57	---
Dusting operation	35	31	---
Removing trays	23	29	---
Cutting room during simulation of original conditions:			
Feeding operation	219	237	161
Dusting operation	110	158	155
Removing trays	77	92	---
General atmosphere at center of room	82	98	---

Clinical investigations of 3 different groups of workers were performed by Challen et al. [67] One group of 8 employees was termed the "long service operators". These were people who refused to continue in the lozenge department after they experienced the previously described symptoms. This group of workers, when exposed to chloroform vapor in probable concentrations ranging from 77 to 237 ppm, had been observed staggering about the work area. After terminating work in the lozenge department the "long service operators" reported experiencing nausea and stomach upset after even short exposures to the smell of chloroform.

A second group of 9 employees in this study, [67] termed the "short service operators", were the replacements of the "long service operators". Two of these 9 employees did not report unpleasant experiences from chloroform exposure. Among the other 7, 5 reported dryness of the mouth

and throat at work; 2 were subject to lassitude in the evening; 1 complained of lassitude and flatulence at work, and the experiences of 2 others were similar to those of the "long service operators". The "short service operators" worked in locations where the chloroform concentrations ranged from 23-71 ppm.

A third group of 5 employees in this study [67] who worked in other departments of the firm served as controls and exhibited no symptoms. Neither tests of liver function (thymol turbidity, thymol flocculation, direct van den Bergh, and serum bilirubin), clinical examinations, nor urinary urobilinogen showed significant differences among the 3 groups of workers.

In 1967, Bomski et al [55] reported on liver injury from chloroform among workers in a pharmaceutical factory in Poland. The study included the entire group of 294 workers who used chloroform in the course of production; of these, 68 were exposed to chloroform for 1-4 years and still had contact with chloroform, 39 had chloroform contact at one time, 23 had viral hepatitis with icterus 2-3 years earlier and were designated as posticterus controls and were working in a germ-free area, and 165 worked in a germ-free area with no history of viral hepatitis. Blood pressure, blood morphology, urinalysis, blood albumin, serum protein, thymol turbidity, zinc sulfate turbidity, the "Takata-Ara" sulfate (colorimetric) test, urobilinogen, SGOT, and SGPT were measured in all. A complete medical history was taken. Sixty of the people were hospitalized for determination of BSP clearance and urinary urobilinogen.

The air in the production room was sampled and chloroform concentrations were determined using the Grabowicz [68] method. The concentration

of chloroform ranged from 2-205 ppm. No other concentration measurements were reported nor was there any mention of the frequency of sampling.

The authors [55] compared the frequency of viral hepatitis and jaundice among a group of inhabitants of the city, 18 years and older, with that of the same 68 pharmaceutical workers who used chloroform. The results showed that in 1960, 0.35% of city inhabitants had viral hepatitis, while 16.67% of the chloroform exposed workers had viral hepatitis. In 1961, the frequency for city inhabitants was 0.22% and the frequency among the chloroform workers was 7.50%. In 1962 the frequency of viral hepatitis was 0.38% for city inhabitants and 4.4% for workers using chloroform. The authors suspected that the toxic liver changes occurring as a result of exposure to chloroform promoted a viral infection in such cases, but they did not give information on the incidence of viral hepatitis among other plant workers, which might have helped resolve questions about sanitary practices and facilities in the plant.

The majority of the workers who were in contact with chloroform during the investigation period covered in this study complained of headache, nausea, belching, and loss of appetite.

Among the 68 workers using chloroform, 10 cases of splenomegaly were found compared to none in the controls. They did not explain the splenomegaly but point out it was not present in controls.

The frequency of enlarged livers (17 out of 68) among workers exposed to chloroform exceeded the frequency of enlarged livers in the other groups (5 out of 39, and 2 out of 23). Livers were judged to be enlarged if they extended at least 1 cm beyond the rib arch in the midclavicular line. The upper margin was apparently not measured. In 3 of

the 17 chloroform workers with enlargement of the liver, toxic hepatitis was diagnosed on the basis of elevated serum enzyme activities and elevated serum gamma globulin. The measured amounts of these serum constituents in these 3 workers were not reported. In the remaining 14 cases of liver enlargement, fatty liver was diagnosed. It was claimed that the latter diagnoses were substantiated by a 79% reduction in the incidence of hepatomegaly in the people studied during a 12-month period when hygienic work conditions were improved as a consequence of the studies. [55]

In a continuing study [LD Pagnotto, written communication, December 1973] by the Department of Labor and Industries, Commonwealth of Massachusetts, worker exposure to chloroform, methylene chloride, and toluene in a plant manufacturing plastic film was investigated. The workers were exposed to levels of chloroform from 7-170 ppm, with a mean of 47 ppm.

Physical examinations and the following laboratory tests were performed regularly on all employees connected with the process: SGOT, LDH, alkaline phosphatase, blood bilirubin, BUN, creatinine, cholesterol, serum protein, urobilin, urobilinogen, urine bilirubin.

The blood tests were performed yearly and urine tests quarterly. The liver function tests have been done for more than 2 years, the kidney function tests for only 1 year. Some of the employees at times experienced what was termed "dry heaves". Laboratory findings have been normal so far, but there appeared to be a significant number of findings in the upper normal range, particularly for the blood bilirubin and BUN. There was no evidence, though, that there was a progressive increase in the values found. However, the evaluations of the laboratory findings were based on

the normal range for the general population rather than a specific control group for this particular investigation.

It is not apparent from this study what the time-weighted average exposures were.

Animal Toxicity

The majority of animal studies of chloroform toxicity have been conducted to provide supplementary information relevant to the clinical use of chloroform as an anesthetic. Consequently these animal studies include concentrations of chloroform many times greater than would be experienced in daily occupational situations except in the case of accidents. The following subsection contains a summation of some of these studies.

(a) Central Nervous System Effects

Chloroform has been reported to cause narcosis in a variety of experimental animals. [69-72] Fuhner [71] exposed 30 mice to vapor concentrations of chloroform ranging from 2,500-7,400 ppm (12-36 mg/liter). Each mouse was individually exposed in 10- to 11-liter bottles with the chloroform vaporized to the concentration desired. The method of ascertaining the concentration was not stated. The following effects were observed:

2,500-2,700 ppm (12-13 mg/liter) - some loss of reflexes, rarely narcosis, recovery in 2 hours

4,000 ppm (20 mg/liter) - narcosis in 1 hour and recovery

4,700-6,000 ppm (23-27 mg/liter) - narcosis in 3/4 hour, recovered, some died

6,800 ppm (33 mg/liter) - narcosis in 1/2 hours, death in most

7,400 ppm (36 mg/liter) - narcosis in 10 minutes

In 1936 Lehmann and Schmidt-Kehl [35] studied the acute effects of chloroform on cats. Fully grown cats weighing 2.5-3.5 kg were fasted before the experiment then exposed to concentrations ranging from 7,200-22,000 ppm (35-105 mg/liter). The concentrations of chloroform were determined by hydrolysis with alkali in alcohol. Upon exposure to chloroform, toppling, loss of righting ability, and loss of leg reflexes were observed. At 7,200 ppm (35 mg/liter) light narcosis was observed at 78 minutes and deep narcosis after 93 minutes. Exposure to 22,000 ppm (105 mg/liter) brought about light narcosis after 10 minutes and deep narcosis after 13 minutes. Depression of nervous and muscular activity was accompanied by irritation of the mucous membranes of the eyes, nose, and mouth.

Lehmann and Hasegawa [34] studied absorption of inhaled chloroform in rats exposed to concentrations of 4,100, 4,300, 11,000, and 16,400 ppm (20, 21, 54 and 80 mg/liter) for 4, 12, 10, and 10 hours, respectively. Concentrations of chloroform were determined by hydrolysis with alkali in alcohol. At a concentration of 4,000 ppm (20 mg/liter), 30.6% of the chloroform inhaled during the first 15 minutes was absorbed, 25.4% after

the first hour, and so forth, decreasing to a minimum of 4.8% after 4 hours. Concentrations of 11,000 and 16,400 ppm (54 and 80 mg/liter) similarly showed absorption decreasing with time of exposure. The initial absorption was never greater than 40%.

(b) Hepatotoxicity

Numerous studies have shown that chloroform causes fatty infiltration and necrosis of the liver. [30,73-77] None of these studies involved long-term inhalation exposures to low concentrations. However, these studies do show that the hepatotoxic effects of chloroform can occur as the result of ingestion, inhalation, or intravenous administration.

Whipple and Sperry [30] pointed out the similarity of microscopic changes in the livers of humans and experimental animals including fatty degeneration and centrilobular necrosis. They administered 1-2 oz of chloroform for 1-2 hours (implying inhalation) to dogs, often on successive days. In general, the animals recovered from the anesthesia and initially appeared to be quite well. However, vomiting began to occur (sometimes with blood) 1-4 days after exposure to chloroform and some of the animals developed diarrhea. Death then ensued. Autopsies revealed lesions of the liver, including central hyaline necrosis, acute yellow atrophy, and subcapsular hemorrhage. Less frequently the kidneys showed fatty degeneration of the convoluted tubules. Where death due to chloroform did not occur, liver repair was noted within 7-12 days.

Jones et al [74] in 1958 studied the relative hepatotoxicity of inhalation anesthetic drugs using chloroform as a standard of reference. Chloroform was given orally to 350 white mice, each weighing approximately 20 g. The mice were killed 72 hours after exposure and livers were fixed

in formalin. The authors [74] were able to estimate the following effects of acute injury from esophageal instillation of chloroform:

0.35 mg/g minimum narcotic dose (MND 50)

1.1 mg/g minimum lethal dose (MLD 50)

0.035 mg/g threshold hepatotoxic effect- minimal midzonal fatty changes

0.07 mg/g minimal central fatty change - fatty infiltration

0.14 mg/g moderate liver changes - massive fatty infiltration

0.35 mg/g severe liver changes - fatty infiltration, centrilobular necrosis

Kylin et al [78] in 1963 studied the hepatotoxic effects of inhalation exposure of 20 mice to chloroform for 4 hours. The experiments were performed on female albino mice, of a single strain, with a mean weight of 23 g. The mice were exposed to chloroform concentrations of 100, 200, 400, or 800 ppm for 4 hours in a chamber approximately 15 liters in volume. The mice were killed 1 or 3 days after exposure and tissues studied microscopically. Evaluation was limited to assessing the extent of necrosis and the degree of fat infiltration of the liver. Mice exposed to 100 ppm for 4 hours and killed 1 or 3 days later showed moderate infiltration of fat in the liver. Specifically, the liver underwent fatty degeneration involving a thin cell layer, usually in the periphery of the liver lobules, and up to 3 to 5 cell-widths in size. These alterations were seen more frequently in mice killed 1 day after exposure than in those sacrificed after 3 days. Exposure of mice to 200, 400, or 800 ppm caused increased liver alteration including some necrosis at 200 ppm, increasing in extent at 400 and 800

ppm. Associated with the microscopically demonstrable liver necrosis was the increase in activity of serum ornithine-carbamoyl transferase.

(c) Nephrotoxicity

Kidney involvement as a result of exposure to chloroform was reported as early as 1929 by Whipple and Sperry. [30] Dogs inhaling 1-2 oz chloroform during 1-2 hours often showed on successive days fatty degeneration of the convoluted tubules of the kidneys.

Plaa and Larson [79] in 1964 studied the nephrotoxic properties of chlorinated hydrocarbons in mice. In assessing kidney function, phenol-sulfonphthalein (PSP) excretion and urinary protein and urinary glucose were measured. The chlorinated hydrocarbons were dissolved in corn oil and the doses administered intraperitoneally in 10 ml corn oil/kg body weight. In mice treated with chloroform the effect on the kidney, as measured by decreases in PSP excretion 24 hours later, was dose-dependent. The presence of any glucose or 100 mg % of protein 24 hours after administration, or less than 40% PSP excretion in 2 hours, was considered significant and indicative of impaired renal function. The extent of kidney impairment, expressed in terms of the 3 function tests, increased with the dose of chloroform. The incidence of reduced PSP excretion in 2 hours decreased as the dose of chloroform decreased from 1 ml/kg-0.016 ml/kg. With a 0.5 ml/kg dose of chloroform, PSP excretion although initially reduced, returned to normal after 4 days. Tables X-9 and X-10 show the relationship of the 3 variables studied to the chloroform dosage.

Microscopic examination of kidney sections showed correlation between the percentage of mice in each group showing abnormal kidney function tests and the percentage showing necrosis of the proximal tubules.

The median effective dose of chloroform (ED50) for significant PSP excretion was 0.12 ml/kg and for increasing urinary protein and glucose, it was 0.07 ml/kg. [79]

Of all the chlorinated hydrocarbons tested, chloroform and 1,1,2-trichloroethane possessed the most marked nephrotoxic properties, causing proteinuria and glucosuria with nonlethal doses and a decrease of PSP excretion, and necrosis of the convoluted tubules in 100% of the animals at higher doses. [79]

(d) Chronic Ingestion Study

The study by Miklashevskii et al [80] exposed 18 male albino rats with an initial weight of 150-180 g, and 18 male guinea pigs with an initial weight of 220-250 g; they were divided into 3 groups of 6 animals of each species. Animals of the first group received a peroral chloroform dose of 0.4 mg/kg. In the second group, the guinea pigs received a dose of 35 mg/kg (1/50 LD50), while the albino rats received a dose of 125 mg/kg (1/50 LD50). The third group served as a control. No mention was made of the dosing schedule although the experiment ran for 5 months and daily administration was implied.

Rats and guinea pigs given the 0.4 mg/kg dose of chloroform showed no changes in conditioned reflexes or in autonomic or cardiac activity, blood protein ratios, catalase concentrations, or phagocytic capacity. There was an increase in ascorbic acid in the adrenals of the guinea pigs. [80]

Some guinea pigs given doses of 35 mg/kg died during the course of the experiment. Five of the guinea pigs lived longer than 2 months, but only 2 of these lived longer than 3 months. The ratio of blood protein

fractions in the guinea pigs given 35 mg/kg was altered by the end of the first month. These changes consisted of an increase in the globulin content (from 32.9±1.09 to 40.9±2.22%) involving the alpha and gamma fractions and a decrease in the albumin content, so that the albumin-globulin ratio decreased from 2.1-0.4. The change in this ratio was even more pronounced at the end of the second month. The guinea pigs in the 35 mg/kg groups also showed a decrease in blood catalase activity from 2.0±0.13-1.2±0.11 (no units given) in the second month of the experiment. [80]

The guinea pigs which had died from a dose of 35 mg/kg of chloroform had structural lesions of the liver, heart muscle, and stomach wall upon microscopic examination. Microscopic changes included fatty infiltration, necrosis and cirrhosis of the liver parenchyma, lipoid degeneration, proliferation of interstitial cells in the myocardium and acute edema of the submucosal and muscular layers of the stomach. [80]

The rats of the group that received doses of 125 mg/kg showed no significant changes in the conditioned reflexes after one month, but during the fourth and fifth month, ability to develop new conditioned reflexes was impaired.

Studies of the autonomic regulation of cardiac activity indicated a decrease in cholinergic activity. [80]

(e) Carcinogenicity

Eschenbrenner [81] in 1945 studied the effect of repeated oral doses of chloroform on induction of hepatomas in mice. This study followed the format of an additional study by the same author that showed induction of hepatomas by repeated feeding of carbon tetrachloride in olive oil solution, in amounts sufficient to produce liver necrosis. [82]

Accordingly, a graded series of necrotizing and nonnecrotizing doses of chloroform were administered. Three-month-old strain A mice which had an incidence of spontaneous hepatomas of less than 1% at 16 months were given intragastric doses of oil solutions of 5 μl/kg body weight. The chloroform content of the solutions varied so that the chloroform doses were respectively 1.6, 0.8, 0.4, 0.2, or 0.1 μl/kg.

The presence or absence of liver necrosis was determined by microscopic examination of liver sections taken 24 hours after a single dose of chloroform. The livers of animals receiving doses of 0.2 and 0.1 μl/kg of chloroform showed no necrosis. However, with these doses, necrotic areas were observed in the kidneys of males, but not of females. This sex difference of renal necrotic lesions was observed at all concentrations. No sex difference was observed for liver necrosis. Twenty-four hours after a single dose of 0.4 μl/kg or more of chloroform there was extensive necrosis of liver cells around the central veins. Thirty doses were given at 4-day intervals to test for any carcinogenic effect. (This was the schedule under which a hepatoma incidence of 100% was obtained when carbon tetrachloride was used.) Hepatomas were found only in animals that received necrotizing doses of chloroform and which were killed 1 month after the last dose. [81] These were seen only in female mice, which could reflect the greater sensitivity of males, ie the males might have died earlier, before onset of malignant changes. The authors inferred that necrosis was a prerequisite to tumor induction. The significance of this study on occupational exposures is not clear; more studies to clarify questions of carcinogenicity of chloroform need to be conducted.

(f) Teratogenicity

Schwetz et al [83] in 1973 evaluated the effects of repeated exposures to chloroform on rat embryo and fetal development. Pregnant Sprague-Dawley rats were exposed to 30, 100, or 300 ppm of chloroform for 7 hours a day on days 6 through 15 of gestation. Day 0 of pregnancy was considered to be the day on which sperm were seen in vaginal smears. Concentrations of chloroform in the exposure chambers were continuously monitored by combustion analysis. An infrared spectrophotometer with a multipath gas cell was used 3 times daily to analyze the chamber air and substantiate the concentration calculations.

The effect of chloroform on rats exposed to 300 ppm was confused by changes in dietary intake. It was not possible to determine whether decreased food consumption was the result of loss of appetite or the inability to eat due to narcosis. [83]

Exposure of pregnant rats to 100 ppm on days 6 through 15 of gestation revealed a significant incidence of fetal abnormalities as compared to controls. There were significant increased incidences of acaudia, imperforate anus, subcutaneous edema, missing ribs, and delayed skull ossification.

Rats exposed to 30 ppm showed significant incidences of delayed skull ossification and wavy ribs, but no other effects. [83]

The teratogenicity of oral doses of chloroform was studied in rats and rabbits. [84] There was no evidence of teratogenicity in either species at any dosage level tested. However, in both species reduced birth weights (7.5% in rats and 1.1% in rabbits) were observed with the highest dosages, 126 and 50 mg/kg, respectively.

(g) Metabolism and Mechanism of Action

In 1961, Butler [85] demonstrated that chloroform is found in the expired air from dogs receiving carbon tetrachloride and that this conversion can be reproduced by the incubation of carbon tetrachloride with tissue homogenates or with reduced glutathione or with cysteine. When chloroform was incubated for a day with homogenates of mouse liver, chromatograms revealed the presence of methylene chloride in concentrations of 30-90 μg/ml. However, no methylene chloride was found in the expired air of dogs receiving chloroform by inhalation.

Paul and Rubinstein, [86] in 1963, studied the metabolism by rats of carbon 14 labeled carbon tetrachloride and chloroform. With intraduodenal doses of 1 ml/kg, some carbon tetrachloride was converted to chloroform. There was no evidence that other chloromethanes were formed from either administered carbon tetrachloride or administered chloroform. Eighteen hours after dosing, 74% of the radioactivity from chloroform had appeared in the exhaled air as chloroform and 3.6% as carbon dioxide. More carbon dioxide was formed from chloroform than from carbon tetrachloride, both in vivo and in vitro, but never more than 5% of the administered chlorinated hydrocarbon was metabolized to carbon dioxide. Homogenation of the liver markedly diminished carbon dioxide production from both carbon tetrachloride and chloroform.

The mechanisms of the biochemical effects of chloroform on the liver were studied by Scholler [87] and Reynolds and Yee. [88] Scholler [87] tried to determine whether structural damage affecting protein synthesis occurs when chloroform acts on the liver of a rat. Twenty Sprague-Dawley rats were fasted for 10 hours and 4 were exposed to 1 vol % chloroform

(10,000 ppm) for a period of up to 5 hours in an anesthesia chamber. Preliminary examinations of all animals anesthetized showed a slight increase in respiratory acidosis but no signs of arterial hypoxemia or metabolic acidosis. After 4 1/2 hours of chloroform anesthesia, the livers of most exposed rats showed gross enlargement of the centrilobular hepatic cells. The cells also showed a striking paleness, and upon staining of frozen sections, fatty degeneration was also observed. On electron microscopy it was found that chloroform produced an early dilation of the granular endoplasmic reticulum with detachment of the ribosomes producing a marked reduction of centrilobular protein synthesis. Additionally, after anesthesia with chloroform, extensive necrosis of portions of the renal tubular epithelium was found, while the lung revealed severe leucocytic infiltrations in the alveolar septa. In light of this latter finding, it should be noted that in 1966 Wattenberg [89] stated that kidney and lung tissue also contain hydroxylating enzyme systems. This enzymatic activity is less intense than in the liver, but can be increased by some lipid-soluble compounds. Scholler [87] concluded that the toxic effect of chloroform on the liver, kidney, and lung as observed in animal experiments and in humans can be explained by the formation of toxic metabolites by hydroxylating enzyme systems in the cells of damaged organs.

Reynolds and Yee in 1967 [88] compared the patterns of incorporation of isotopic carbon from chloroform, carbon tetrachloride, methylene chloride, and methyl chloride into chemical constituents of liver organelles 2 hours after oral introduction of the chloromethanes. This investigation was based on the hypothesis that the hepatotoxicity of the compounds was related to the binding of reduction products of the chemical

components to the endoplasmic reticulum and to the formation of chloromethylated lipids and proteins. They used 2 indicators of early damage to the endoplasmic reticulum: 1) The ability to suppress glucose-6-phosphatase activity in the centrilobular portion of the liver in 1 hour; and 2) the ability to cause increases in cellular ribonucleic acid (RNA) content 2 hours after exposure. These indicators were compared with labeling of cellular constituents and with the ability of these agents to cause centrilobular necrosis 24 hours after exposure.

In the experiment, young male rats weighing between 100 and 300 g each were fed doses of either 830 or 2,600 μmoles of chloromethanes per 100 g of rat weight in an equal volume of mineral oil, by polyethylene stomach tube. Control animals received an equal volume of mineral oil. Animals were killed 1, 2, and 24 hours after dosing. Radioactive carbon incorporation from labeled chloroform, carbon tetrachloride, methylene chloride, or methyl chloride dissolved in 0.24 ml of mineral oil and fed to animals after a fast of 16 hours was studied. Control animals received either labeled sodium bicarbonate in 0.5 ml of 0.01 N NaOH or labeled formaldehyde in 0.25 ml of water. Bicarbonate and formaldehyde are chloromethane oxidation products and these served as comparisons to the incorporation patterns of the 4 agents used. [88]

Two hours after oral introduction both carbon tetrachloride and, to a lesser extent, chloroform caused an increase in RNA content of the liver and centrilobular necrosis at 24 hours. The concentrations of chloroform found in the liver within the first few hours after dosing were slightly greater than those of carbon tetrachloride. However, the extent of necrosis from chloroform was less. The carbon labeled chloroform was re-

covered in an amino acid locus corresponding to methionine. The amount of labeled radioactive carbon found in lipids and microsomes was related to the chlorine content of the chloromethanes.

(h) Potentiation

Alcohols, barbiturates, and some other chemicals such as DDT when administered before chloroform increase the toxic effects of chloroform, apparently by lowering the threshold for its necrotic action. [90-93]

Kutob and Plaa [90] found that ethanol pretreatment of mice increased the toxic effect of chloroform on the liver. Male Swiss albino mice weighing 20-30 g were initially treated with an oral dose of ethanol (5 g/kg) in a 25% aqueous solution. The ethanol pretreatment was followed by single, subcutaneous, minimally hepatotoxic doses of chloroform (0.08 ml/kg) given in a 1.6% olive oil solution. Time interval between ethanol pretreatment and dosing with chloroform was systematically shortened from 15 days to 12 hours to determine the shortest period before administration of chloroform in which administration of ethanol would increase the susceptibility to chloroform. The time lag between ethanol and chloroform was purposely chosen so that the ethanol would be metabolized by the time the chloroform was administered. Each experiment consisted of 4 groups of animals: 1) untreated; 2) treated with ethanol; 3) treated with chloroform; and 4) treated with ethanol followed by chloroform. Liver function was studied 24 hours after the administration of chloroform by determining 1) pentobarbital (45 mg/kg) sleeping time, 2) BSP retention, and 3) liver succinic dehydrogenase activity. Tissue slides were also studied to corroborate the liver function tests.

All experiments showed reduced liver function as indicated by prolonged pentobarbital sleeping times and elevated BSP retention. The liver succinic dehydrogenase activity was depressed when chloroform was administered 12 or 24 hours after ethanol, but not when it was administered 48 hours after ethanol. Ethanol alone or chloroform alone did not significantly depress succinic dehydrogenase activity.

In mice pretreated with ethanol 15 hours, or 1, 2, or 4 days before the administration of chloroform, microscopic examination revealed livers with cytoplasmic vacuolization in pericentral cells. The pericentral cells were also enlarged and almost completely devoid of eosinophilic material. [90]

In a similar study, Sipes et al [91] pretreated rats with isopropanol and reported enhanced ability to covalently bind radioactive carbon labeled chloroform to microsomal protein.

Dingell and Heimberg [92] studied the hepatic metabolism of aminopyrine and hexobarbital in rat liver microsomes after the administration of chloroform or carbon tetrachloride or methylene chloride. The chlorinated hydrocarbons were administered in equimolar doses by gastric intubations and killed 24 hours later. Liver microsomes were prepared from rat livers weighing from 250-375 mg. Either 5 μmoles of aminopyrine or 1.9 μmoles of hexobarbital was added to a mixture of enzyme substrates.

The rate of metabolism of hexobarbital was measured by estimation of the disappearance of substrate. The rate of demethylation of aminopyrine was measured by estimation of the amount of formaldehyde formed. For both the aminopyrine and hexobarbital pretreatment before carbon tetrachloride decreased the rate of metabolism significantly to 14 and 29%, respectively,

of control values. Chloroform, however, only decreased metabolism of aminopyrine to 61% and hexobarbital to 95% of control values.

McLean [93] fed male mice either stock diet or protein free diets for 1 week before intragastric administration of chloroform. Some of the mice of each group were also given sodium phenobarbital in the drinking water (1 mg/ml) for 1 week before chloroform administration; others were given a single subcutaneous injection of DDT (100 mg/kg) 1 week before chloroform. The purpose of DDT and the phenobarbital administration was to stimulate liver hydroxylating enzyme activity. The purpose of the protein deficient diet was to reduce the liver hydroxylating enzyme activity, but in this experiment this was not realized. Phenobarbital and DDT increased the liver hydroxylating enzyme activity and the toxicity of chloroform was more than doubled by the phenobarbital and DDT pretreatment as measured by the LD50.

Correlation of Exposure and Effect

The use of chloroform as an anesthetic agent has provided information about the effects to be expected from acute exposure. Exposures during anesthesia have usually been to concentrations of around 20,000 ppm, [58] and exposure times have been from 30-240 minutes. [30,45,94] One or 2 days after chloroform anesthesia, nausea, jaundice, and vomiting may develop, often followed by elevated temperature and pulse, epigastric pain, muscle twitching, delirium, and coma. In some cases, death has occurred 3-10 days after anesthesia. [30,45,46,51] Autopsies have demonstrated fatty infiltration of the liver with diffuse central necrosis, and enlarged, soft, congested kidneys with cloudy swelling of the

epithelial lining of the convoluted tubules, and yellow striations marking the pyramids. [24,45,51,54,94]

Habitual inhalation of 1 oz daily of chloroform for 7 years followed by 2 oz/day for 5 more years was associated with delusions, restlessness, depression, convulsions, ataxia, dysarthria, tremor of the tongue and fingers, and insomnia; at autopsy, the brain showed slightly thickened meninges in the frontal lobe, many fibroblasts and dilated blood vessels. [36] In other cases of habitual chloroform inhalation for periods of time ranging up to 30 years, hallucinations, delirium, and tremors were common manifestations. [36] The exposures (Table X-4) are difficult to evaluate, but they were chronic and the effects on the central nervous system were definite.

Lehmann and Hasegawa [34] and Lehmann and Schmidt-Kehl [35] performed the only controlled exposure experiments with humans. The exposures were for a maximum of 30 minutes, and response measurements were limited to subjective responses of the subjects. These experiments provide information about responses of people exposed to 160 ppm (0.8 mg/liter or 800 mg/cu m) through a range of concentrations up to 7,200 ppm (35.3 mg/liter), as follows:

160 ppm (0.8 mg/liter) for unspecified time - no odor
205 ppm (1.0 mg/liter) for unspecified time - light transient odor
390 ppm (1.9 mg/liter) for 30 minutes - light transient odor
920 ppm (4.5 mg/liter) for 7 minutes - stronger, lasting odor; dizziness, vertigo after 3 minutes

680 ppm (3.3 mg/liter) to 1,000 ppm (5.0 mg/liter) for 30 minutes - moderately strong odor; taste

1,100 ppm (5.4 mg/liter) for 5 minutes - still stronger, permanent odor; dizziness, vertigo after 2 minutes

1,400 ppm (6.6 mg/liter) to 1,800 ppm (8.57 mg/liter) for 30 minutes - stronger odor, tiredness, salivation, giddiness, vertigo, headache, taste

3,000 ppm (14.46 mg/liter) for 30 minutes - all above plus pounding heart, gagging

4,300 ppm (20.8 mg/liter) to 5,000 ppm (25 mg/liter) for 20 minutes - dizziness and light intoxication

5,100 ppm (25 mg/liter) for 20 minutes - dizziness and light intoxication

7,200 ppm (35.3 mg/liter) for 15 minutes - dizziness and light intoxication as above but more pronounced

The data presented by Challen et al [67] provide some quantitative information about exposure and effect, even though some of the information about exposure was obtained after the fact. Employees in a confectionery manufacturing medicinal lozenges had complained of nausea, flatulence, loss of appetite, frequent and burning micturition, lack of mental concentration, depression, and irritability. Measurements were made of an atmosphere created under conditions which were considered to simulate those in existence at the time the employees were affected. The average chloroform concentrations based on 30-minute samples ranged from 77-237 ppm. It was noted that reducing work to part time in this environment relieved the employees' complaints. No studies were made on the exposed

workers during the time of the exposure which covered the years 1950-1954. Measurements of liver and kidney function made in 1958, 3-4 years after the exposure, were normal.

Another group of 10 workers were later engaged in the same jobs after ventilation was improved. These workers had been working 4 hours a day for 10-24 months under conditions where 30-minute average chloroform concentrations ranged from 23-71 ppm, except for 1 operation which ran 4 times a day for a total of about 2 hours in which the exposure during operation averaged 128 ppm, with 4 exposures each lasting 1 1/2-2 minutes at 1163 ppm.

In 9 of the latter workmen studied by Challen et al, [67] only 2 did not report unpleasant experiences. Among the other 7, 5 reported dryness of the mouth and throat at work; 2 were subject to lassitude in the evening; 1 complained of lassitude and flatulence at work; and the complaints of 2 others were similar to those experienced by the original workmen. Liver function tests were normal in these workers.

In the study of Bomski et al, [55] workroom concentrations of chloroform in a pharmaceutical industry fluctuated between 2 and 205 ppm, but no further information about environmental exposure concentrations was given. The incidence of viral hepatitis (4.4-16.7%) was much higher among the people working in the chloroform environment than among the general population (0.22-0.35%); and 10 cases of splenomegaly and 17 cases of enlarged liver were found among the 68 chloroform workers.

Sixty-eight workers who had been exposed to this chloroform environment for 1-4 years were studied in detail, along with 3 control groups: 1) 30 previously chloroform-exposed workers; 2) 23 nonchloroform exposed-

workers who had previously had viral hepatitis; and 3) 165 nonchloroform exposed workers with no history of viral hepatitis. Seventeen of the 68 chloroform-exposed workers had enlarged livers. Three of these 17 workers had toxic hepatitis on the basis of elevated serum enzyme activities and elevated serum gamma globulin. The other 14 workers with enlarged livers were judged to have fatty liver, though admittedly without confirmation by biopsy.

The incidence of enlarged livers among these 68 workers was significantly higher than in the controls. It was noted that there was a reduction in hepatomegaly in 79% of the people studied during the 6-12 month period when hygienic conditions at work were improved. [55]

Schwetz et al [83] studied the effects of 30, 100, and 300 ppm of chloroform on pregnant rats for 7 hours/day on days 6 through 15 of gestation. There were significant incidence of delayed skull ossification and "wavy ribs" in litters from dams exposed to 30 ppm of chloroform.

The authors [83] found that in litters from dams exposed to 100 ppm there were significant incidences of acaudia, imperforate anus, subcutaneous edema, missing ribs, and delayed sternebrae ossification. Rats exposed to chloroform at 300 ppm ate so little food (1 g/day) that it would be impossible to consider any of the other effects as characteristic of chloroform. [83]

Kylin et al [78] found that mice exposed to 100 ppm chloroform for 4 hours showed moderate fatty infiltration and degeneration at the periphery of the liver lobules, one day after exposure. These effects were found less frequently 3 days after exposure, indicating a certain amount of regeneration. A direct dose-response relationship, evidenced by increasing

liver alterations and necrosis, was observed at concentrations ranging from 100 to 800 ppm. Further indication of a dose-response relationship was the increase of the serum ornithine carbamoyl transferase with increasing concentrations of chloroform.

Animal studies [95] have shown that chloroform is capable of causing liver and kidney injury after 130 repeated 7-hour exposures to concentrations as low as 25 ppm. Injury at that exposure level was minor and reversible; at higher concentrations (50 and 85 ppm) repeated exposure produce more severe injury but no changes in clinical chemical tests for such injury. Repeated 4-hour exposures to 25 ppm caused no effect. From this study, it was suggested that a ceiling of 25 ppm would be more appropriate, with the TWA concentration not to exceed 10 ppm.

A summary of concentration-responses data is presented in Table X-11.