III. BIOLOGIC EFFECTS OF EXPOSURE

Extent of Exposure

Dioxane ($C_4H_8O_2$), also known as p-dioxane, is a colorless combustible liquid with a faint pleasant ethereal odor similar to that of absolute ethyl alcohol. Other names commonly used for dioxane are 1,4-dioxane, diethylene dioxide, diethylene ether, and glycol ethylene ether. The more significant physical and chemical properties are listed in Table XII-1 [1-3].

Dioxane was first prepared by Lourenco [4] in 1863 by heating ethylene glycol with ethylene dibromide in sealed tubes for several days at 160 C. At about the same time, Wurtz [5] prepared dioxane by treating dioxane dibromide with hydrogen sulfide or mercury at room temperature. The dibromide of dioxane resulted from the reaction of one mole of bromine with two moles of ethylene oxide at low temperatures [6].

Dioxane is produced commercially by dehydrogenation of ethylene glycol [7]. It can also be produced by catalytic dimerization of ethylene oxide in the vapor phase or by reaction of bis(2-chloroethyl) ether or 2-chloroethyl-2'-hydroxyethyl ether with strong aqueous sodium hydroxide [8].

Dioxane became available in commercial quantities around 1929 and has been used industrially as a solvent for fats, waxes, greases, paint and varnish strippers, resins, polyvinyl polymers, dyes, lacquers, varnishes, paints, mineral oil, celluloid, and similar products where nitrocellulose, cellulose acetate, or other cellulose esters or ethers are used. It has had a minor use as a wetting agent and dispersing agent in dye baths, textile processing, and stain and printing compositions [1,7,9,10]. It has

been used in cleaning and detergent preparations, adhesives, cosmetics, emulsions, polishing compositions, and as a stabilizer for chlorinated solvents. Dioxane has also been used as a preservative, fumigant, deodorant, and as the nonaqueous solvent with lead-ion selective electrodes [11]. It was formerly used in the preparation of tissue sections for histology [12,13].

Dioxane has also been used as a solvent in the purification of organic compounds and in molecular weight determinations [14]. In 1974, Ziegler and Wilke [15] described a method for radioimmunoassay of glucagon after extraction of blood with dioxane.

There are two large and two small dioxane-manufacturing plants in the United States [16]. The two larger plants produce annually about 10 and 5 million pounds of dioxane, respectively, and one of the smaller plants produces about 1 million pounds. Most of the dioxane produced is used as a solvent as previously discussed [1,7,9,10] and as a stabilizer in chlorinated solvents [written communication, R Daniels, March 1977]. NIOSH estimates that 100,000 US workers are exposed to 1,1,1-trichloroethane [17], and many of these are also exposed to dioxane. NIOSH further estimates that an additional 2,500 people in the United States are exposed to dioxane in their work environment.

A summary of some operations where dioxane exposure may occur is presented in Table XII-2 [18].

Historical Reports

Dioxane was prepared as early as 1863 [4,5] but it was not used commercially until 1929 [9].

The earliest studies of the biologic effects of dioxane on guinea pigs and humans were reported in 1930 by Yant et al [19]. The first report of adverse effects from industrial use of dioxane appeared in 1934 in which five fatalities occurred when a change was instituted in a process where dioxane was used as a solvent [20].

A 1934 report by Fairley et al [21] on human exposures to dioxane at 1,000 and 2,000 ppm for 3-5 minutes concluded that, beyond an unpleasant sensation of warmth in the throat and chest, there were no subjective responses to warn workers of dioxane exposure. In a series of 15-minute exposures to dioxane at 200 and 300 ppm, Silverman and coworkers [22] reported in 1946 that, although the odor was not objectionable, the majority of subjects complained of irritation of the eyes, nose, and throat at dioxane concentrations above 200 ppm. The dioxane occupational literature was reviewed by Estler in 1935 [23], Gross in 1943 [24], Smyth in 1952 [25], Rowe in 1963 [2], and Browning in 1965 [1].

Effects on Humans

(a) Experimental Studies

Experimental studies to determine the effects of dioxane on humans have been limited to identifying sensory responses during exposures lasting 15 minutes or less [19,21,22,26-28]. Yant et al [19], in 1930, described the effects on five volunteers after they inhaled air containing 5,500 ppm dioxane for 1 minute. Signs and symptoms of exposure included eye

irritation, a burning sensation in the nose and throat, and, in three subjects, a slight vertigo that disappeared "quickly" after they left the exposure chamber. When the same five subjects were exposed to air containing 1,600 ppm dioxane for 10 minutes, they experienced an immediate slight burning of the eyes with lacrimation and a slight irritation of the nose and throat. The alcohol-like odor of dioxane, easily noticeable at first, decreased in intensity with continued exposure. Lacrimation and nasal irritation persisted throughout the test. No vertigo was noticed, but one subject complained of an upset stomach after the exposure had been completed. The specifications of the exposure chamber, the purity of dioxane, and the methods of generating and measuring the dioxane atmospheres were not reported.

A 99.8% dioxane-0.2% water preparation, completely free of aldehydes and other impurities, was used in studies reported in 1936 by Wirth and Klimmer [26]. Several subjects were simultaneously exposed in a 10-cu m glass and stoneware exposure chamber at dioxane concentrations of 0.7, 1.4, 2.8, 5.6, 8.4, 280, 1,400, and 2,800 ppm. The lower concentrations (8.4 ppm and less) were generated by evaporating the calculated amount of dioxane from a filter paper with the aid of a fan. The higher concentrations were attained by dispersing dioxane using a compressed-air sprayer. The duration of the exposures was not reported. The detection threshold, the concentration at which dioxane was perceived as an unidentified odor, was 2.8 ppm. At a concentration of 5.6 ppm, the odor, which was described as fruity and not unpleasant, was identified as that of dioxane. As the concentration was increased, the odor was eventually described as that of methylated spirits. Slight mucous membrane irritation

occurred at 280 ppm, while at 1,400 ppm the irritation was quite distinct with slight stinging in the nose and scratchiness and dryness in the throat. At 2,800 ppm, irritation was initially very strong and complaints of slight pressure in the chest were expressed. The subjects became accustomed to the irritation and odor after a few minutes, but continued to experience an unpleasant, metallic, bitter taste. The application of 0.05 ml of either pure or technical grade dioxane three times daily to the underside of the forearm dried the skin but caused no other signs of irritation. When liquid dioxane was dropped on the mucous membrane of the mouth, a temporary, slight burning sensation was experienced [26].

In experiments reported in 1946, Silverman and coworkers [22] found, in 15-minute exposures to dioxane, that a concentration greater than 200 ppm was required to cause eye, nose, or throat irritation in the majority of subjects. The subjects were exposed at either 200 or 300 ppm technical grade dioxane in a 1,200-cu ft chamber. Air-vapor concentrations were produced in a dynamic exposure chamber by continuously adding a known quantity of air saturated with dioxane to the measured flow of air being continually forced into the chamber. Motion pictures were shown during the exposure to occupy the subjects' attention. The exact number of subjects was not given, but an average of 12 men and women were used for each of a number of exposures to different concentrations of dioxane. The majority experienced eye, nose, and throat irritation at a dioxane concentration of 300 ppm, although they did not find the odor objectionable. No further details or experimental results were reported.

A dioxane odor threshold of 170 ppm was reported by May [27] in 1966, with a pronounced odor being noticed at 270 ppm. In this experiment, a

panel of eight men and eight women between the ages of 31 and 63 sniffed graded dilutions of dioxane from wide-mouth flasks. This 170-ppm odor threshold was considerably higher than either the 2.8-ppm threshold for detection or the 5.6-ppm threshold for recognition reported by Wirth and Klimmer [26], or the 100% odor recognition threshold of 5.7 ppm reported by Hellman and Small [28]. An odor fountain, used by the latter investigators [28], was placed about 14 inches below the vent pipe which carried the odorous stream out of the exposure chamber. With the exception of valves and couplings made of stainless steel, the system was constructed of glass and Teflon to minimize absorption of the odorant. The concentration of the odorant at the odor fountain could be changed within 1 minute. The detection threshold was 1.8 ppm and the recognition threshold was 5.7 ppm [28].

Studies of dioxane exposure of four healthy male volunteers were conducted at a concentration of 50 ppm (48-52 ppm) for 6 hours and reported by Young et al [29] in 1976. An extensive physical examination including a chest X-ray, an electrocardiogram, respiratory function tests, conventional blood chemistry determinations, and urinalysis were conducted on the volunteers prior to the study. Following the exposure, all the tests except the chest X-rays were repeated at 24 hours and at 2 weeks. Samples of blood and urine collected during and after the exposure were analyzed for dioxane and its metabolite, beta-hydroxyethoxyacetic acid (HEAA), by gas chromatography and mass spectrometry.

A dynamic chamber, 2.49 x 2.64 x 4.06 m (26.7 cu m), was used for the exposure studies, and the airflow was maintained at 3.68-4.16 cu m/minute throughout the exposure. A circulating fan was used inside the chamber to

provide uniform distribution. The concentration of dioxane in the chamber was determined and monitored throughout the exposure analytically by an infrared analyzer.

The half-life for elimination of dioxane was found to be 59 $\pm$ 7 minutes; 99.3% of the dioxane was eliminated in the urine as HEAA and 0.7% as dioxane. Dioxane and HEAA were detectable in the urine until 6 and 18 hours, respectively, after the end of exposure. The total absorbed dose of dioxane during the 6-hour period was 5.4 $\pm$ 1.1 mg/kg.

The investigators [29] constructed a one-compartment open system model to characterize the kinetics of dioxane and HEAA in humans; they used this model for simulating daily repeated 8-hour exposures at 50 ppm [30]. Using this pharmacologic profile in humans, they concluded that, at concentrations of 50 ppm or lower, dioxane is rapidly metabolized to HEAA and will not accumulate in the body even after continuous or repeated exposures. Mild eye irritation which persisted throughout the exposure was noted in all the volunteers, according to Chenoweth (written communication, December 1976); however, no itching of the eyes or lacrimation, or any other sensory responses were noted.

(b) Occupational Case Histories

In 1959, Johnstone [31] described an occupationally related fatality in which a man had been exposed to dioxane for just 1 week. The 21-year-old man, who had a history of heavy beer consumption, had used dioxane to clean a workbench and to keep his hands free of resin-type glue. He kept an uncovered bucket of dioxane between his knees for these purposes. Neither a respirator nor an exhaust ventilation system was employed. After 1 week of dioxane exposure, he was hospitalized with severe epigastric pain

and rising blood pressure which reached 220/120 about 3 hours after admission. Within a few hours, he developed severe convulsions followed by unconsciousness and anuria. The anuria lasted 5 days during which time his blood nonprotein nitrogen rose to 181 mg/100 ml compared to a normal value of 15-40 mg/100 ml. His condition continued to deteriorate even after he regained kidney function, and he died on the 6th day of hospitalization. Autopsy findings included centrilobular liver necrosis and necrosis of the renal cortex with extensive interstitial hemorrhage and occasional hyalinization of glomeruli. There were microscopic findings in the brain that were considered to be secondary to anoxia and cerebral edema [31].

The exact dioxane concentrations to which the deceased worker was exposed were not known, but they ranged from 208 to 650 ppm and averaged 470 ppm in the 75 X 50 X 25 ft workroom. When and where the samples were taken, the number of samples, and the method of collection and analysis were not mentioned. The worker may have been subjected to absorption of dioxane through the skin in addition to possible inhalation exposure at higher vapor concentrations because of the close proximity of his breathing zone to the open bucket of dioxane. The plant foreman was reported to have been exposed to dioxane for many months without evidence of ill effects [31].

A 47-year-old woman who had worked in the dioxane distillation department of a chemical concern for several weeks developed inflammatory skin abnormalities. The case was reported by Sonneck [32] in 1964. Concentrations of dioxane to which the woman was exposed were not reported nor was it mentioned whether liquid dioxane came into direct contact with her skin. Her right arm was most affected but her face and left hand were

also involved. Low-grade, vaguely defined, reddened areas with isolated pinhead-size, reddish papules were found on her face. The dorsal surface of her left hand was almost all reddened with pinhead-size papules on the sides of the fingers. Six to eight months previously, the woman's right arm had suffered first and second degree burns from isoprene when an explosion occurred in a distillation process. Sonneck [32] considered the healed area to be especially sensitive and the reason why most of the right arm was affected. Skin sensitivity tests demonstrated the patient's pronounced hypersensitivity to dioxane as compared to skin sensitivity tests in two controls which were negative. The author's [32] diagnosis from the skin tests and microscopic studies was contact eczema due to dioxane.

Epidemiologic Studies

The deaths of five men who were exposed to dioxane in an artificial silk plant in England were the subject of a report by Barber [20] in 1934. The deceased workers were 29-38 years of age and were stated to have been apparently healthy. The work conditions in the factory were partly described by Barber [20] and further described by Henry [33]. The exposures occurred in an experimental plant where two similar machines were used to treat cellulose acetate yarn with dioxane during the manufacture of artificial silk. The process had been in operation since July 1932. Early in October 1933, the process in one of two machines was altered. In the Annual Report, the Chief Inspector of Factories [33] stated that, on one machine, the vessel containing dioxane solution was enclosed between the 14th and 18th of September 1933, without exhaust ventilation. Dioxane

vapor was thereby concentrated in the air above the liquid dioxane and, when the enclosure was opened for manipulation of the yarn, the men were exposed at this higher vapor concentration [33]. The time of exposure to the vapor was also increased at the end of October 1933, with some of the men working approximately 12 hours on Saturdays and Sundays [20]. These work conditions continued during the period in which the fatal exposures occurred (November 5-19, 1933). Dioxane concentrations before, during, or after the change in process were not reported.

Prior to the change in process, evidence of liver or kidney damage was not noted although a few men had stayed away from work occasionally because of anorexia, nausea, and vomiting. For example, one of the men, who had an exceptionally high exposure in April 1933, stayed home with "stomach trouble" for several days. He was the first of the five men to die after the October 1933 process change.

According to Barber [20], 16 men were definitely exposed to dioxane, and 8 or 9 of these had worked on the machine where the process was altered. Seven of these sixteen men became ill between the 5th and 19th of November, and five men died between the 11th and 25th of November. The first two men died at home after being attended by different physicians, and the other three were admitted to a hospital. A general examination of the latter cases revealed that they had enlarged livers and suffered from a hemorrhagic condition of the kidneys. In the histories of their illnesses, signs and symptoms of poisoning such as nausea and vomiting were followed by oliguria and anuria. Clinical blood findings included 21,000-38,000 leukocytes/cu cm (sic) with 80-94% neutrophils compared to normal values of 5,000-10,000 leukocytes/cu cm (sic) and 60-70% neutrophils. Necropsy

findings included enlarged pale livers, swollen hemorrhagic kidneys, and edematous lungs and brains. Microscopic examinations revealed centrilobular liver necrosis, almost symmetrical necrosis of the outer part of the renal cortex, and hemorrhages around the glomeruli.

Eighty men who worked in the factory were studied by Barber [20] 7-14 days after work with dioxane was stopped. These 80 men were all those who had any potential for exposure to dioxane during the time it was used in the factory. The kinds of jobs these men had and the extent of their exposures were not well described. It was reported that other processes were going on in the room that housed the dioxane machines [33]. The number of men working on the other processes and the nature of the processes were not stated.

Of the 80 men, 11 were classified as "much exposed," with 3 or 4 of them having had exposures similar to those of the men who died. It could be interpreted that two of these three or four men worked on the same machine as those who died. One interpretation could be that one or two men had worked on the same machine as those who died and that the other seven or eight worked on the machine that was not altered. Another interpretation could be that some of the 11 men worked on other processes in the vicinity of the dioxane machine. Of the remaining 69 workers, 13 were classified as "old exposures" since they had not had any potential for exposure to dioxane for at least 2 months. It was not indicated in the report whether any of these 13 men had worked on the dioxane machines. Of the other men, all may have worked in the room where the dioxane machines were housed, or they may have included managerial, maintenance, and other personnel with potential for exposures to dioxane. These 80 men may be

considered as representing four exposure groups: those 3 or 4 with near lethal exposure, those 7 or 8 with less exposure, those 56 with occasional or incidental exposure, and those 13 with past exposure. The 80 workers were queried for symptoms of poisoning and were examined for enlarged livers. Blood studies included urea nitrogen, the indirect van den Bergh test which was apparently used to check for liver changes, and blood cell counts; the urine was tested for albumin and RBC. The most significant findings in this study were in 1 of the 11 men classifed as "much exposed." It was not stated whether or not this man worked on the same machine as those who died. He complained of being unwell, had a positive indirect van den Bergh reaction, a trace of albumin and a few RBC in the urine, and a leukocytosis of 13,600/cu cm(sic). His was the only palpable liver among the 80 workers. Two other men, who had worked on the machine with those who died and who had been away from work because of illness for a day or two during the critical exposure period, did not have palpable livers at the time of the examination. These two men did have a trace of albumin in their urine and leukocyte counts of more than 10,000/cu cm(sic). Among the 11 most exposed workers, one had RBC in the urine, four had a trace of albumin in the urine, and all but one had blood leukocyte counts in excess of 10,000/cu cm(sic). By comparison, RBC were not found in the urine of any of the other 69 exposed workers, and a trace of albumin was found in nine of them. Barber [20] considered the leukocytosis in the 11 most exposed workers to be due to their exposure. In the 13 men with past exposure, a tendency toward eosinophilia was noted.

Thiess et al [34] conducted an epidemiologic study of 74 workers who had been exposed to dioxane in a dioxane-manufacturing plant in Germany.

As a group, these workers had a cumulative potential exposure of 1,840 man-years and an average potential exposure of 24.9 years. A series of measurements of dioxane concentrations was taken in 1974 at various locations in the production plant. Sampling and analytical methods were not described. The results of these measurements are presented in Table III-1. During a cleaning operation in one of the stills, an attempt was made to simulate earlier working conditions by evaporating dioxane until its odor was considered similar to that of past years. At the simulated level, the odor was described as being highly offensive and the concentration of dioxane was found to be 3.5 ppm (odor threshold, 2.8 ppm and recognition threshold, 5.6 ppm [26]).

The 74 employees included 24 workers who were then working at the dioxane plant, 23 former employees who were then working elsewhere, 15 who had retired but still alive, and 12 who had died.

The current workers had been exposed to dioxane for 5-41 years and their ages varied between 32 and 62 years. Six of them had a history of exposure to ethylene chlorhydrin and dichloroethane by inhalation or from burns of the skin and mucous membranes due to contact with butanol or acetic acid. Two of the workers had suffered slight facial burns as a result of contact with dioxane-containing sodium hydroxide. All 24 employees were given clinical examinations during 1974 and 1975 that included a chest X-ray. Clinical data from these 24 current workers in the dioxane plant and from the 23 who had previously worked with dioxane are presented in Table III-2.

TABLE III-1

CONCENTRATIONS OF DIOXANE IN THE WORKPLACE
OF A PRODUCTION PLANT IN GERMANY

Dioxane Work Area	Dioxane Concentration	
	mg/cu m	ppm
Synthesis cauldron R301 normal operation	0.8	0.22
" " " " "	1.0	0.28
Synthesis cauldron installation shut off	0.2	0.06
Stirring cauldron R302 normal operation	1.8	0.50
" " " " "	1.5	0.42
Sample taken from cauldron R302 during normal operation	2.0	0.60
Old building	1.5	0.42
"	12.6	3.50
"	0.1	0.03
"	3.7	1.03
New building separated by firewall near dioxane installation	0.02	0.01
With dioxane apparatus shut off	0.07	0.02
Dioxane distillation point	1.4	0.39
	2.3	0.64
	0.1	0.03
	0.2	0.06
During cleaning operation (simulation of working conditions of previous years)	12.6	3.50
Measurement station	5.4	1.50
" "	2.3	0.64
Measurement at 30 cm distance from end of production line	47.8	13.28
Small leak in pump	13.7	3.81
Small leak in pump (normal operation)	0.06	0.02
" " " " " "	0.2	0.06
" " " " " "	1.0	0.28
Small leak (installation shut off)	0.02	0.01
Drumfilling room using exhaust	24.0	6.67

Adapted from reference 34

TABLE III-2

CLINICAL MEASUREMENTS IN 24 CURRENT DIOXANE WORKERS AND IN 23 PREVIOUS DIOXANE WORKERS

Clinical Measurement	Author's Normal Range	Currently Exposed Subjects		Previously Exposed Subjects	
		Range	Number Abnormally High	Range	Number Abnormally High
SGOT mU*	5-17	7.0-25.0	2	7.0-53	4
SGPT mU*	5-23	6.0-54.0	6	8.0-63	4
Alkaline Phosphatase**	40-190	71-178.5	0	69-163	0
Gamma glutamyl transferase*** mU*	6-28	6-128	6	7.0-54	7
Creatinine**	0.8-1.2	0.8-1.6	5	00.9-1.4	1
Urea**	20-50	19-60	2	19-45	0
Thymol turbidity test	0-3.0	0.3-5.0	1	0.2-3.75	2

*Milliunits
**Units not described
***The authors classified this as a test of liver function but did not otherwise describe the test.

Adapted from reference 34

Two employees had reduced hemoglobin (Hb) and RBC counts, another had a slightly elevated leukocyte count, and still another had reduced Hb and RBC counts as well as a slightly elevated leukocyte count. The thrombocyte counts were slightly reduced in four employees. No hemorrhagic tendency was noticed in any of the 24 employees nor was enlargement of the liver or

jaundice noted. Serum glutamic-pyruvic transaminase (SGPT) levels were elevated in six men and in two of these six men serum glutamic-oxalacetic transaminase (SGOT) levels were appreciably elevated. An abnormal thymol turbidity level was noted in one employee. All six who had elevated transaminase levels were known to have consumed daily for several years about 80 g of alcohol, either in the form of beer or wine. No liver complaints were noted among the six employees. When five of these men reduced their alcohol consumption, their transaminase levels returned to normal. Serum creatinine was slightly higher than normal in four employees and two others showed a slight increase in serum urea content. A follow-up examination of these workers, 6 months later, showed normal values for these two serum tests. Proteinuria, with no abnormal components, was found in one employee who had just recovered from an influenza infection.

Chromosome analyses were performed on six employees then working at the dioxane production plant as well as on six control persons. The sex of the subjects was not given. Lymphocyte cultures were prepared for both groups, incubated at 37 C for 70-72 hours with phytohemagglutinin, and subsequently processed. Thirty to fifty metaphases were investigated for each person. A total of 230 metaphases evaluated in the exposed group had a gap rate (cells with achromatic lesions) of 5.65% and other aberrations (fragments, deletions, dicentric chromosomes, and fractures) amounting to 1.74%. In comparison, 306 metaphases evaluated in the control group had a gap rate of 5.24% and other aberrations amounting to 2.62%. No statistical evaluation was made.

The 23 workers working elsewhere and thus no longer exposed to dioxane had been exposed to dioxane for 3-38 years. Both SGOT and SGPT

levels were elevated in five employees and serum creatinine was higher than normal by 0.1-0.2 mg in two other employees. The daily consumption of alcohol by each of these seven employees was in excess of 80 g.

The 15 retirees, still alive in 1975, exhibited no evidence of liver or kidney disease. Retirement occurred when employees reached the normal retirement age or because of medical reasons such as emphysematous bronchitis, articular arthrosis, degenerative spinal diseases, or cardiac and circulatory problems. No liver or kidney diseases or any cases of carcinoma were detected in the 15 retirees who were still alive. No details regarding alcohol consumption or results of laboratory investigations of these retirees were reported.

Twelve deaths occurred during 1964-74 among the seventy-four exposed workers. The causes of death and other pertinent information are presented in Table III-3.

TABLE III-3

CAUSES OF DEATH OF EMPLOYEES EXPOSED TO DIOXANE
IN A DIOXANE PRODUCTION PLANT IN GERMANY*

Age at Death	Duration of Exposure at dioxane plant (mo)	Reported Cause of Death and Post-Mortem Analysis
51	188	Acute heart attack
54	204	Heart and circulatory failure
58	420	Cirrhosis of liver, bleeding of esophageal varices
60	264	Right heart failure due to pyloric stenosis, duodenal ulcer (histology, negative)
66	496	Liver and kidney insufficiency
67	264	Acute heart infarct
67	502	Heart infarct with lung edema
67	511	Pneumonia and sepsis, right heart failure, chronic interstitial nephritis
69	408	Pericardial flux, uremia, bronchopneumonia
69	414	Heart infarct
73	111	Suspicion of cerebral injury
73	372	Heart and circulatory failure

*As shown in official death certificates and other sources

Adapted from reference 34

For the 74 workers, 14.5 deaths were expected using German Federal Republic mortality statistics for 1970-73 as compared to the 12 deaths observed. Cancers found in 2 of the 12 employees, who died between 51 and 73 years of age, were a lamellar epithelial carcinoma of the left lumbar region, and a myelofibrotic leukemia. According to the authors [34], these deaths were not statistically different from the number of expected cancer deaths.

A study of 165 employees who worked in a dioxane-manufacturing plant in Texas was undertaken by Buffler et al [35] in 1975. This included 100 employees of the dioxane-production unit and 65 of the dioxane-processing subunit (closed system) within the vinyl chloride division. These men were all those who had worked in dioxane manufacture for more than 1 month between April 1, 1954 and June 15, 1959.

During the dioxane-manufacturing process, control operators, loading operators, and maintenance personnel were potentially exposed. The control operators monitored the operations of the plant from an enclosed control room. This included periodic monitoring of the control dials, and conducting sampling and analysis of the product twice each shift. Exposure to dioxane vapor could have occurred during sampling as well as during chemical analysis. The loading operators were responsible for loading three tank cars with dioxane weekly, the time required being approximately 30 minutes/car. Escape of dioxane vapor occurred when loading lines were being connected or disconnected to the dioxane tank cars.

To repair or replace worn or damaged equipment, the appropriate dioxane line had to be drained by maintenance personnel and exposure to dioxane was likely to occur during these operations. Specialized

laboratory personnel, engineers, foremen, and supervisors were also potentially exposed to dioxane [35].

In the dioxane-processing area, loading operators and laboratory personnel were potentially exposed to dioxane. The samples were drawn by the loading operators at various stages and analyzed by the laboratory personnel. The employees not included in the cohort were the maintenance personnel and those who barreled the finished product, chlorothene, which contained about 3.5% dioxane.

In the dioxane-production plant, area monitoring was conducted in 1968, and both personal and area monitoring were conducted in 1973 and 1974. The concentrations found in these studies were considered by the authors [35] to be representative of dioxane exposures since 1959 because no changes had been made in the dioxane-manufacturing plant after production began.

The area monitoring in 1968 was accomplished by sampling in impermeable plastic bags outside the control room and around storage tanks located approximately 50 feet from the dioxane control room. In 1973, area monitoring was conducted for 48 hours at 6 feet above ground level in the dioxane-production area, the loading area, and the storage area. In 1973 and early 1974, the loading operators and various control operators were monitored with personal samplers as were the control operators and unloaders in 1974 and 1975. The data from these samplings are presented in Table III-4.

TABLE III-4

DIOXANE CONCENTRATIONS (ppm) IN VARIOUS WORK LOCATIONS (1968-1975) IN A DIOXANE-MANUFACTURING PLANT

Location	Year and Type of Sample					
	1968 Grab Range	1973 Area Avg	1973 Area Max	1973-74 Personal Range	1974 Personal Average	1975 Personal Range
Outside control room	<0.6-2.0					
Around storage tanks	100 and 800	0.2	11			
Production area		0.3	16			
Loading area		0.5	22			
Loading operator and control operators				0.1-1.5		0.8-32
Control operator				8.3		
Unloading tank cars*						
Worker 1					2.9	
Worker 2					16.8	
Worker 3					2.3	
Worker 4					1.4	
Worker 5					<0.1	
Three measurements, one worker**						1.5-5.0

*Sampling time 5-26 minutes
**Sampling time 3-6 minutes

Adapted from data supplied by Buffler et al [35]

Of the 100 employees in the production plant, 68 were still employed in the plant, 21 were employed elsewhere, 2 had retired by choice, 1 had disability retirement, 1 was known to be alive but his employment status was not known, and 7 had died.

The case summaries of the seven employees from the production plant who died are presented in Table III-5. The exposure histories showed that they had been exposed to other chemicals of possible significance for longer periods than their exposure to dioxane. They had been employed at the plant for longer than 15 years with an average duration of exposure to all chemicals of 18.5 years, including an average of 4.2 years exposure to dioxane compared to an average of 4.4 years of dioxane exposure for the total group of 100 workers. Diseases of the stomach, liver, heart, and lungs were listed as causes of death. Two of the deaths were due to cancer, a carcinoma of the stomach, and an alveolar cell carcinoma. These cancers were different from those reported by Thiess et al [34]. The employees who died of cancer had been exposed to dioxane for less than 4 years; one of them had also been exposed to hydrogen chloride, carbon tetrachloride, perchloroethylene, and trichloroethylene, and the other to vinyl chloride and methylene chloride. No statistically significant differences ($P<0.05$) were seen between the two observed deaths from cancer and an expected number of 0.9 as calculated for the study group from Texas vital statistics for the years 1960-69, based on a Poisson distribution ($P=0.22$).

TABLE III-5

CASE SUMMARIES OF DECEASED EMPLOYEES EXPOSED TO DIOXANE IN A DIOXANE-PRODUCTION PLANT IN TEXAS*

Age at Death	Exposure to		Death Certificate Statement of Cause of Death
	Dioxane (mo)	Other Chemicals (mo)	
39	59	Perchloroethylene (60) Trichloroethylene (49) Methylene chloride (19)	Stomach hemorrhage (no indication of malignancy)
45	77	Perchloroethylene (70) Trichloroethylene (79)	Cardiovascular (natural cause-heart attack)
49	28	Hydrogen chloride (3) Carbon tetrachloride (4) Perchloroethylene (131) Trichloroethylene (71)	Carcinoma of stomach
50	40	Iron oxide fumes (212) Sodium hydroxide (7)	Chronic hepatitis (pneumonitis due to chronic hepatitis, cirrhosis, and liver failure)
51	23	Hexachlorobenzene (3) Carbon tetrachloride (17) Mineral seal oils (83) Sodium hydroxide (42) Perchloroethylene (41)	Cardiovascular (myocardial infarction)
52	38	Vinyl chloride (131) Methylene chloride (18)	Lung cancer (alveolar cell carcinoma)
64	89	Hydrogen chloride (20) Perchloroethylene (157)	Cardiovascular (natural cause-heart failure)

*As of June 30, 1975

Adapted from reference 35

In the dioxane-processing subunit, 5 of 65 employees died. Table III-6 presents the case summaries of the deceased employees. All these employees were exposed to vinyl chloride simultaneously with their exposure to dioxane. Three of the deaths were attributed to accidents, the fourth to myocardial infarction, and the fifth to cancer (malignant mediastinal tumor). No statistically significant difference ($P<0.05$) was seen between the one cancer death and the 0.8 expected.

TABLE III-6

CASE SUMMARIES OF DECEASED EMPLOYEES IN THE DIOXANE-PROCESSING SUBUNIT*

Age at Death	Exposure (mo)	Cause of Death	Autopsy Done
21	12	Malignant mediastinal tumor, unclassified with generalized metastasis	Yes
22	6	Tubular necrosis in both kidneys due to extensive third degree burns (automobile accident)	Yes
39	66	Accidental drowning	No
43	13	Cardiac arrest due to electric shock	No
52	28	Myocardial infarction	No

*As of June 30, 1975

Adapted from reference 35

In another study of the same plant [36], the urine of five workers was collected daily for 10 days and analyzed for dioxane and its potential

metabolite, HEAA. Both dioxane and HEAA were found in the urine of the five workers. The average concentrations of dioxane and HEAA in the urine, as well as the exposure concentrations of dioxane, are presented in Table III-7. The average concentrations of dioxane and HEAA in samples of urine collected at the end of each workday were 3.5 and 414 μmol/liter, respectively. This indicated that the biotransformation of dioxane in humans was apparently to the same product (HEAA) as found previously by the same investigators [37] in rats. The authors [36] stated that the high ratio of HEAA to dioxane, 118 to 1 in human urine, suggested that at low exposure concentrations dioxane was rapidly metabolized to HEAA. The previous study conducted on rats demonstrated that the toxicity of dioxane was observed only when the metabolism of dioxane to HEAA was saturated [30]; hence, the high ratio of HEAA to dioxane in human urine indicated that the metabolic pathway was not saturated. This and animal findings [30,37] led the investigators [36] to believe that exposure to dioxane vapor at low concentrations posed a negligible health hazard.

TABLE III-7

DIOXANE AND HEAA CONCENTRATIONS IN URINE OF FIVE EMPLOYEES AND IN WORKROOM AIR IN A DIOXANE-MANUFACTURING PLANT IN THE USA*

Employee Body Weight (kg)	Urine		Air
	Dioxane (μmol/liter)	HEAA* (μmol/liter)	Dioxane (ppm)
74.8	4.3	60	1.0
110.7	3.3 ± 2.0	230 ± 8.5	1.6 ± 0.5
74.4	3.6 ± 0.4	622 ± 154	2.0 ± 1.0
79.4	3.7 ± 1.4	470 ± 229	1.8 ± 0.4
78.5	3.0 ± 0.7	445 ± 144	1.1 ± 0.6
Overall mean	3.5 ± 1.2	414 ± 216	1.6 ± 0.7

*Values are the means ±SD

Adapted from reference 36

Two representatives of another manufacturing concern separately provided information on exposure to dioxane among employees in their dioxane manufacturing plant (written communications, CU Dernehl, April 1976, RE Peele, January 1977).

Area monitoring was conducted in 1974 and 1975 in both production and drumfilling facilities. Samples were taken by carbon tube as well as by grab sampling. Grab sampling was accomplished using 50-ml glass syringes and the sample was directly injected into the gas chromatograph. The areas that were sampled were divided into three categories: The breathing zone, the general workroom, and point sources (leaks, vents, sewers, etc). Grab sampling in the breathing zone showed an average dioxane concentration of 11.36 ppm (range, 0.05-51 ppm) for 30 samples and an average of 4.28 ppm (range, 0.05-36.7 ppm) for 44 samples in the general

drumming-type operations, an average of 9.0 ppm was obtained for 46 samples, the range of dioxane concentration being 0.05-51 ppm. Only three samplings each were done using carbon tube in the breathing zone and general workroom atmosphere. Carbon tubes were primarily used for detection of dioxane concentrations in point sources and these did not necessarily result in occupational exposure. Five samples, ranging from 12.1-108.9 ppm, gave an average of 45.52 ppm at point sources.

During the 42 years of dioxane production in the plant, about 80 workers were thought to have been potentially exposed to dioxane. In 1976, 42 persons, who were identified as having worked in the dioxane unit at some time or other, were given complete physical examinations, chest X-rays, electrocardiograms, and a series of liver profile tests. It was reported that abnormalities were not found in any of the 42 employees. The company had begun cancer surveillance of all of its employees about 20 years ago. A total of 67 reported deaths from malignancy was reported among the company employees. Four of these deceased employees were known to have been potentially exposed to dioxane; one died with cancer of the colon, one with lymphosarcoma, one with lung carcinoma, and one with glioblastoma.

Animal Toxicity

(a) Acute and Subacute Studies

(1) Parenteral Injection

A dose-response relationship was noted by two investigators [21,38] when dioxane was injected iv into rabbits. Target organs were the kidneys and liver, in which the damage, characterized by cellular

degeneration, became progressively more severe with increasing doses. Similar findings were observed when mice, rabbits, and guinea pigs were injected sc with dioxane [24,39,40]. Mice and rats were also injected ip to study the narcotic effects, incidence of tumors and LD50 of dioxane [41-44].

Effects of dioxane administered iv to rabbits were reported by Fairley et al [21] in 1934. Four rabbits received a single dose of 1, 2, 3, or 5 ml of 80% dioxane diluted with saline to a total volume of 10 ml. Three other rabbits each were given two 5-ml injections of dioxane mixed with 5 ml of saline with an interval of 48 hours between injections. One rabbit, used as a control, received 10 ml of saline. The immediate effect of dioxane injection in all the rabbits was violent struggling, which began as soon as the first few drops were injected. With doses of 4 or 5 ml of dioxane, the struggling was followed by convulsions and collapse; then the rabbits rapidly returned to normal. The four rabbits given the single doses of 80% dioxane were killed 1 month later. Degeneration of the renal cortices with hemorrhages was observed by microscopic examination. In the rabbit administered the 3-ml dioxane dose, the degenerative changes extended into the medulla, and the liver showed extensive and gross cellular degeneration starting at the periphery of the lobules. No abnormality was found in other organs. The livers of the rabbits given the 1- and 5-ml doses showed no microscopic abnormalities, and areas of cloudy swelling were seen in the liver of the rabbit given 2 ml of dioxane.

One of three rabbits given two 5-ml doses of 80% dioxane was killed for necropsy when it seemed acutely ill, 5 days after the second injection. The two remaining rabbits appeared to be ill on the 7th day when one died

and the other was killed. Macroscopically, the kidneys of the three rabbits were enlarged and the three livers appeared either normal, mottled, or pale, respectively. In all three animals, microscopic examination revealed almost total destruction of the renal cortex with only a few glomeruli remaining in a meshwork of connective tissue. Many of the medullary tubules were blocked with blood casts and hyaline material, but degenerative changes were not observed. Hemorrhages were seen in both the cortex and medulla. There were extensive degenerative changes in the hepatic cells of the livers of all three rabbits, apparently starting from the edges of the lobules [21].

Two more rabbits were given a single 4-ml dose of pure dioxane mixed with an equal volume of saline. The pure dioxane was prepared by the investigators [21] from redistilled dehydrated ether. The red cell counts and hemoglobin measurements were slightly lower, viz, 2,490,000 and 3,170,000, and 65 and 70%, respectively, and the white cell counts were high, viz, 34,100 in one rabbit 24 hours after the iv injections. Forty-eight hours after the injections, the RBC and WBC counts had returned toward normal but the hemoglobin count remained low. Both rabbits developed hind-limb paralysis that continued until the 7th day after dioxane administration at which time they were killed. The average blood urea concentrations in the two rabbits, when measured at 24 hours (38 and 40 mg/100 ml) and 6 days (37 and 36 mg/100 ml), were not significantly different from the pre-injection values (35 mg/100 ml). Microscopically, both rabbits showed advanced cellular degeneration with hemorrhages in the renal cortex. The medullary tubules were blocked with casts. Well-marked cellular degeneration in the liver was found in one rabbit but no definite changes were found in the other.

Dioxane was administered iv to rabbits by De Navasquez [38] in doses of 0.2 ml/kg in 10 ml of water or in doses of 1.0 or 1.5 ml/kg in 5 ml of water. A single dose of 0.2 ml/kg induced no visible effects in an unspecified number of rabbits. A few minutes after an unstipulated number of rabbits had been injected with 1.0 ml/kg of dioxane, they became flaccid in stature and appeared dazed. They moved slowly, exhibited poor coordination, and had weak reflexes. When they leaned against a structure such as a wall for support, they slowly collapsed to the bottom of the cage. A gradual improvement usually occurred within 4 hours. The author [38] stated that cats were similarly affected although the details of the cat experiments were not given.

Five rabbits were administered a single dioxane dose of 1.5 ml/kg [38]. Signs, similar to those described for the rabbits given the 1.0 ml/kg dose, disappeared within a few hours. However, in contrast to the 1.0 ml dose, a polyuric condition developed within 24-48 hours, with a corresponding decrease in the specific gravity of the urine. The rabbits then developed anuria, usually within 96 hours after the beginning of the experiment, with concomitant increase in blood urea that reached 300 mg/100 ml or higher within 4-5 days of the dioxane injection. The animals lost weight, became lethargic, and eventually died after 3-4 days of complete anuria. Microscopic findings were acute hydropic degeneration of the convoluted tubules and vacuolization of liver cells, apparently because of glycogen accumulation.

A tolerance to acutely toxic doses of dioxane was observed in rabbits first given repeated sublethal doses of dioxane in an experiment conducted by De Navasquez [38]. For example, in one animal initially given eight

doses of 0.5 ml/kg of dioxane at weekly intervals, the only visible effect was temporary polyuria when the rabbit was subsequently given five doses of 1.5 ml/kg. Both the kidneys and liver were reported to be normal at autopsy; the method of examination was not reported. This phenomenon of developing tolerance was observed in other rabbits, although the author [38] did not specify the mode of administering the dioxane.

A single 1.5 ml/kg dose of dioxane in a 25% solution of unspecified solvent was administered to each of two rabbits in an experiment reported by Kesten et al [45] in 1939. One rabbit died 2 days after the injection. The other was killed for examination on the 4th day. The kidneys of both rabbits had hydropic degeneration of the convoluted tubular epithelium. The liver of the rabbit that died appeared normal; that of the other rabbit had hydropic cellular degeneration around the efferent hepatic veins and diffused glycogen deposits in some of the liver cells. There was no fat present in the vacuoles or vacuolated liver cells although small amounts of finely divided fat were occasionally found in both the epithelial and Kupffer cells. The nonprotein nitrogen of this rabbit had risen to 242 mg/100 ml before it was killed.

Von Oettingen and Jirouch [39] injected 5 ml/kg dioxane subcutaneously (sc) into two white mice, and 7.5 and 10 ml/kg, respectively, into two additional mice in an experiment reported in 1931. One of the two mice given the 5 ml/kg dose died whereas the other three were alive after 24 hours. No additional information was provided.

In 1943, Gross [24] reported that rabbits tolerated 1 or 2 ml/kg sc injections of dioxane with temporary albuminuria being the only deleterious sign. In a cat, a 2-ml/kg dose, dissolved in 50% oil (unspecified),

produced signs of illness including a loss of appetite and weakness. Microscopic examination revealed hydropic degeneration of the tubular epithelium of the kidneys, hydropic distension of the liver cells with glycogen deposits. Five rabbits and six guinea pigs were administered dioxane at 2, 4, or 6 ml/kg. All those animals given dioxane doses of 6 ml/kg died as did most of those given 4 ml/kg; one guinea pig given 2 ml/kg died after several days. Microscopic findings were similar to those reported for the cat. No further details were given.

An experiment was reported in 1942 by Cortese [40] in which two groups of eight guinea pigs were administered sc injections of dioxane diluted with equal parts of distilled water. Animals in one group received 1 ml of the dioxane-water mixture/guinea pig for 10 successive days. Each animal in the other group received the 1:1 mixture in amounts ranging from 0.20 to 0.75 ml each day for 35 days. Although the body weights of the guinea pigs were not given in this report [40], in two other reports [46,47], it was stated that the body weights of guinea pigs that were used ranged from 152 to 192 g. Evaluation of these three reports [40,46,47] indicates that the same guinea pigs may have been the subject of more than one report.

Three of eight guinea pigs given the 1-ml dose died between the 7th and 9th days of the experiment and the other five were killed for examination on the 10th day. One guinea pig from the 2nd group died on the 30th day of the experiment, and the remainder of the animals were killed at the end of the experiment on the 35th day.

Microscopic findings in the guinea pigs from the 10-day study were pronounced. The glomeruli appeared congested, with extravasations in

Bowman's capsules. The most marked changes were seen in the tubules where, in some cases, the nuclei were discolored and, in others, cells were found in the tubular lumen. Besides the cellular alterations, the tubular lumen also revealed a reticular formation, probably due to the presence of albumin. The lobular structure of the liver was barely recognizable; the cytoplasm showed many vacuoles, predominantly in the periphery of the lobules. Hemorrhages were present in the spleen, the pulp of which was congested with hematic pigments; the splenocytes were quite numerous, and some were filled with hemosiderin. The pancreatic tissue showed a regressive phenomenon, characterized by the disappearance of the protoplasm and barely visible nuclei; there were no hemorrhages and no microscopic changes evident in the islets of Langerhans. The lungs revealed a congested diffuse phenomenon with marked hemosiderin in the parenchyma. The adrenal cortices showed extensive cellular vacuolization and, in some cases, the nuclei also seemed to be in a phase of degeneration or had disappeared. Numerous hemorrhages were found in the adrenal medullas but cellular regression, though present, was less marked than in the cortices.

The myocardial fibers of the heart were separated because of edema and had lost their transverse structure. The nuclei were well preserved in some places while in others they were pyknotic or swollen or somewhat discolored. Edema was more pronounced around the coronary vessels, but no significant microscopic findings were seen in the vessels themselves.

The microscopic findings were similar in both the 10-day and 35-day studies of the guinea pigs; the major difference was that the adrenals were more severely affected in the 35-day study. There was an increase in WBC count in the 10-day study group and a decrease in the 35-day study group.

An increase in RBC showing excessive variation in size was observed in both groups of guinea pigs but no hematologic values were given [40].

The narcotic effects of dioxane administered intraperitoneally (ip) to rats were studied and reported by Knoefel [41] in 1935. On administering dioxane at 1.76 ml/kg to three rats, he noted that sleep was not induced. With a dose of 2.64 ml/kg, one rat slept for 8 minutes, another for 10 minutes, and the third was wide awake. When a dioxane dose of 4.40 ml/kg was administered to three other rats, all of them died. Microscopic findings were not reported.

The LD50 of dioxane, determined from studies on 60 female albino mice weighing between 18 and 27 g, was reported by Karel et al [42] in 1947. Pure dioxane injected ip was used in order to avoid any possible toxic interaction of dioxane with a diluting medium. The LD50 for dioxane was found to be 0.76 ml/kg based on deaths occurring within 7 days after dioxane administration. Moderate renal tubular degeneration was observed in animals that died from the 1st day through the 4th day of the experiment.

In a determination of the LD50 of dioxane in Sprague-Dawley rats, Argus and associates [43] administered dioxane in saline, ip, at concentrations ranging from 5.30 ml/kg to 5.80 ml/kg. In six other groups, each rat received ip 10 mg of methylcholanthrene (MC), a microsomal enzyme-inducing agent, 23 hours before the injection of dioxane at 4.90 ml/kg to 5.30 ml/kg. The LD50 values were calculated based on deaths that occurred 48 hours after dioxane administration. Though a statistically significant increase ($P<0.001$) in dioxane toxicity was observed when rats were pretreated with MC, as indicated by a reduction in the LD50 value from 5.60

± 0.06 ml/kg to 5.18 ± 0.06 ml/kg, this increase in toxicity does not seem to be great. No results by the use of MC alone were reported.

For microscopic examination of dioxane-induced tissue and organ damage, 15 additional Sprague-Dawley rats were used. Five animals were each injected ip with doses of 10 mg MC, 5.18 ml/kg dioxane, or 5.18 ml/kg dioxane, 23 hours after having received the MC treatment. No changes were detected in the kidneys of the rats given MC alone but vacuolization of the tubular epithelium of the kidney was seen in two rats that were administered dioxane. All five rats given both dioxane and MC showed such vacuolization of the extensive tubular epithelium that the investigators [43] attributed the deaths to the combined action of dioxane and MC.

Weil et al [44], in a 1968 report, studied the production of tumors in dioxane-treated A/J mice, a strain with a normally high incidence of spontaneous pulmonary tumors. A preliminary study was undertaken to determine the maximum dose that could be used without causing excessive mortality. Dioxane doses (ip) of 1.25, 2.5, or 5.0 ml/kg, after a maximum of 6, 5, and 3 doses, respectively, killed all three mice in each of the three groups. Tricaprylin, a triglyceride, was used as the vehicle. Two of three animals died when administered a maximum of nine injections of dioxane at 0.625 ml/kg in tricaprylin solution, whereas none of three mice died when administered a maximum of five injections of 0.3125 ml/kg in tricaprylin solution. On the basis of these preliminary studies, doses of 0.3125 and 0.156 ml/kg of dioxane in a 1:7 dilution with tricaprylin were selected for the tumor experiments. Groups of 40 male A/J mice, at a median age of 69 days at the first injection, were administered three injections of dioxane/week for 4 weeks. The mice received 0.06 or 0.03 ml

of the dioxane-tricaprylin solution/injection. One mouse in the higher dose group died whereas all mice in the lower dose group survived. Surviving mice were killed 38 weeks after the 1st injection. A control group of 40 mice received 12 injections of tricaprylin (0.1 ml/mouse/injection) but controls without tricaprylin were not used.

Twenty-four of thirty-nine and 21 of 40 mice that received 0.156 or 0.3125 ml/kg/injection of dioxane, respectively, had neoplasms compared to 18 of 36 tricaprylin-injected controls. When expressed as percentages of mice with gross neoplasms, these were 61.5, 52.5, and 50.0%, the higher dose level of dioxane and control groups being almost equal. Similarly, the number of growths/tumor-bearing mouse was 1.5, 1.3, and 1.4, respectively, for the same three groups. The authors [44] concluded that the incidence of pulmonary tumors, predominantly adenomas, was not increased by repeated ip injections of dioxane, and no gross tumors were observed in other organs.

(2) Oral

Narcotic effects, toxic effects on the liver and kidneys, and LD50's were studied by a variety of investigators by the oral route, ie, intragastric intubation (ig), or in drinking water [38,41,45,48-54]. Mice, rats, guinea pigs, rabbits, and dogs were used for these oral administrations.

Effects of dioxane, administered intragastrically at doses of 0.88, 1.76, 4.51, 6.62 or 8.82 ml/kg to groups of one to three rabbits in an experiment to determine the narcotic potency, were described by Knoefel [41] in 1935. Two rabbits that received either a dose of 0.88 or 1.76 ml/kg and two of three rabbits given 4.51 ml/kg appeared to be normal and

remained erect in posture. The third rabbit administered 4.51 ml/kg remained semierect and in a staggering position. Two of three rabbits administered dioxane at 6.62 ml/kg died but the third one remained in a staggering position. The rabbit administered 8.81 ml/kg of dioxane died. Microscopic findings were not reported.

Effects of nonlethal and lethal doses of dioxane administered ig to an unspecified number of rabbits were reported by De Navasquez [38] in 1935. Dioxane doses of 0.2 ml/kg in 10 ml water, 1.0 ml/kg in 5 ml water, or 2 ml/kg in 20 ml water were administered as single doses to an unspecified number of rats. Doses of 0.2 ml/kg, repeated at weekly intervals for an unspecified number of weeks, did not appear to affect the rabbits at all; one rabbit received as many as 15 weekly doses of 0.2 ml/kg without effect. A state described as drunkenness was seen with 1-ml doses, but single doses of 2 ml/kg administered to five nonfasting rabbits killed them. The minimal lethal ig dose of dioxane was found to be 2.0 ml/kg in 20 ml water. Microscopic changes observed in the kidneys and liver were similar to those seen in rabbits given iv injections of dioxane; they were hydropic degeneration of the convoluted tubules of the kidneys and vacuolization of liver cells.

The effects of repeated doses of dioxane on renal function of one rabbit were described in greater detail by the investigator [38]. This rabbit received 1 ml/kg on days 1, 3, 5, 7, 10, 12, 16, and 18, and 2, 2.5, 3, 4, and 5 ml/kg on days 20, 27, 38, 42, and 47, respectively. Blood urea and the volume of urine excreted were checked each day. The rabbit was found to be anuric on day 8, polyuric on days 20, 40, and 42, and oliguric on days 7, 10, 11, 27, 38, 39, and 48. The blood urea rose to 81 mg/100 ml

after the administration of 4 ml dioxane on day 42 and increased to 175 mg/100 ml after a 5-ml dose was administered on day 47, which produced a terminal uremia.

The ig administration to a dog of 25 ml of commercial dioxane in 75 ml water followed 50 minutes later by 100 ml of a 50% solution was reported in 1936 by Schrenk and Yant [48]. The investigators attributed the immediate effect as excitation of the "sexual centers" but presented no evidence to that effect. Twenty minutes after the second administration, the dog appeared unsteady and vomited; its hindquarters were stiff after 2.5 hours. A half-hour later, it lay down and was unable to rise; its respiration was characterized by prolonged expiration. After 10 hours, the dog began to improve and, by 15 hours, the only apparent sign was rigidity during walking. The dog ate well and appeared in good condition for 3 days, then its condition began to deteriorate. The dog died 6 days after having received the 2nd dose of dioxane. No microscopic examination was conducted.

Laug and coworkers [49], in 1939, reported the ig LD50 of dioxane in male and female mice, rats, and guinea pigs. The animals died between 24 hours and 5 days after the administration, with the highest average mortality occurring at about 36 hours. Limpness and distinct signs of intoxication with muscular incoordination were observed immediately upon administration of the lower dioxane doses followed by an apparent recovery; some animals died several days later. Animals given the larger doses showed similar signs of intoxication followed by coma and death within 24 hours. The ig LD50 of dioxane was found to be 5.7 ml/kg for mice, 5.2 ml/kg for rats, and 3.9 ml/kg for guinea pigs. Gross post-mortem

observations included hemorrhagic areas near the pyloric end of the stomach, distension of the bladder, and somewhat enlarged kidneys. Microscopic changes in the livers and kidneys of some of the animals were slight, while extensive damage was noted in others. The other organs, not specified, appeared to be normal.

Nelson [50], in 1951, reported experiments in which he administered ig doses of dioxane in a total volume of 10 ml to rats and rabbits. Deaths were recorded 2 weeks after administration. Eight rats and two rabbits were used as controls. Doses of 0.2, 1.0, 3.16, 5.63, or 10 ml/kg were administered to 2-8 rats and 0.1, 1.0, 3.16, or 10 ml/kg to 2-3 rabbits. No details regarding the strain, sex, age, or weight of the animals were given. There was an increased anesthetic effect ranging from unsteadiness to narcosis with very weak or absent corneal reflexes in both rabbits and rats with increasing doses; similarly, with increasing doses, the survival rate decreased, death occurring within 24 hours in animals administered 10 ml/kg of dioxane. The approximate LD50 for rats was stated to be 6 ml/kg and 2 ml/kg for rabbits.

Preliminary studies on the toxicity of dioxane were reported by Smyth et al [51] in 1941. Dioxane was administered in aqueous solution by stomach tube to albino male Wistar strain rats weighing 90-120 g and guinea pigs of both sexes, weighing 250-300 g. Different concentrations of dioxane were used, the highest concentration being 50%. Details of the actual amounts given to the rats were not stated. In most cases, 10 animals were used to determine the toxicity of each dose, and the range of doses included those that produced 0 and 100% mortality. All deaths that occurred within 14 days after administration of dioxane were used for

calculations of LD50 unless the signs of poisoning were inconsistent with those of similarly treated animals, or if infection was found on autopsy. Microscopic studies of the tissues were not made. All the doses caused some degree of irritation of the digestive tract. The primary action was upon the kidneys but did not proceed beyond bloody urine. The liver was less affected. Narcosis was observed only at the LD50 or at higher doses. The LD50 of dioxane for rats was found to be 7.1 ml/kg and 3.15 ml/kg for guinea pigs.

Smyth et al [52,53] attempted to study the joint toxic action of several industrial chemicals by intubation in rats in all possible pairs using equitoxic and equivolume mixtures. An equitoxic mixture was defined as a mixture of two chemicals in volumes directly proportional to their respective rat oral LD50 values, so that each component contributed the same degree of toxicity to the mixture.

For this purpose, pairs of industrial chemicals were administered to an unspecified number of albino female rats in two separate experiments. In the first experiment, 350 pairs of 27 chemicals, mixed in equal volumes, were administered. In the second experiment, 53 pairs of 26 chemicals mixed in volumes proportional to their LD50 values were administered. The chemical pairs were given to groups of five rats in doses differing by a geometric factor of 2. Chemical pairs that were not soluble were administered separately with a minimum time delay between the two doses. The adjusted ratios of predicted to observed LD50 values of the four chemicals that did not act additively with dioxane when mixed in both equal volumes and equal toxicity are presented in Table III-8.

TABLE III-8

RATIOS OF PREDICTED TO OBSERVED LD50 VALUES OF CHEMICALS MIXED WITH DIOXANE (LD50 = 7.07 ml/kg) IN EQUAL VOLUMES, AND IN VOLUMES PROPORTIONAL TO EQUAL TOXICITY

Component	LD50 of Component	Unadjusted Ratio of Predicted to Observed LD50	Adjusted Ratio of Predicted to Observed LD50	
			Equal Volume	Equal Toxicity
Acetonitrile	8.27	3.15	2.15	1.49
Propylene glycol	42.90	0.51	-0.96	-0.42
Tetrachloroethylene	8.00	2.83	1.83	0.41
Ucon fluid 50-HB-260	5.00	0.40	-1.52	-0.77

Adapted from references 52,53

A positive adjusted ratio showed a greater than additive effect as compared to the predicted combined toxicities of the two chemicals, whereas a negative adjusted ratio showed a less than additive effect. From Table III-8, it can be seen that acetonitrile and tetrachloroethylene, in combination with dioxane, had a greater than additive effect in both equal volume and equal toxicity experiments, whereas propylene glycol and Ucon fluid with dioxane had a less than additive effect. The investigators [53] concluded that the greater the difference between adjusted ratios for equivolume and equitoxic mixtures the less likely it was that the pairs would act additively.

Fairley et al [21] reported in 1934 studies in which they gave six rats and six mice each a 5% solution of dioxane in water in place of their regular drinking water. The animals either died or were killed by the 67th day of the experiment.

All six rats and three of six mice showed severe lesions in the renal cortex, of the patchy type of degeneration. Cellular degeneration was very advanced and showed large necrotic areas in many cases. Degeneration was slight or absent in the renal medulla, but many tubules were blocked with casts. Hemorrhages and vascular congestion were seen throughout the kidneys. In the remaining three mice, vascular congestion was quite marked with hemorrhages in both cortex and medulla, but cortical degeneration was not evident.

The liver also showed cellular degeneration in five rats and three mice; it was extreme in two rats, well-marked in one, and in an early stage in the other two rats and three mice. Vascular congestion was noted in all rats and mice. However, the animals that were killed on days 60 and 67 showed cellular degeneration in the kidneys and vascular congestion in the livers. This may indicate that tolerance was achieved in these animals, more so in mice than in rats, or that liver cell regeneration may have commenced in these animals. No other organs showed any injurious effect with the exception that the spleens of two mice were slightly affected though no gross changes were observed. The rats seemed to be more severely affected than the mice and two rats died with an acute gastroenteritis. No details regarding control animals were given.

Drinking water containing 5% commercial dioxane was administered to two dogs, and water containing 5% specially purified dioxane was given to a third dog in an experiment reported by Schrenk and Yant [48] in 1936. A measured quantity of the 5% dioxane solution was placed in the water pan which was securely fastened to the cage. The solutions were placed in the cages twice daily only long enough for the dogs to satisfy their thirst.

The remaining portion was removed and measured. Though there were variations, each of these dogs had a greatly reduced water intake. No details regarding the use of controls were reported.

The dogs did not show any specific effects for about 4 days, then signs of poisoning were noticed in the following order: loss of aggressiveness, quietness, sluggishness, loss of appetite, vomiting, weakness, inability to stand, labored breathing, and unconsciousness. Two of the dogs died on the 9th and 10th days after administration. The third dog, which was returned to regular drinking water on day 6, was killed for autopsy on day 45. The dog had recovered from the signs of intoxication, based on its appearance and blood nonprotein nitrogen determinations, the values of which were not reported. The blood counts of the two dogs that died, which included Hb, RBC, WBC, and differential and platelet counts, revealed a tendency towards leukocytosis. The blood nonprotein nitrogen was markedly increased shortly before death. The kidney cortices of the dogs that died had an abnormal appearance, while those of the dog that was killed on day 45 showed fibrosis. The authors [48] stated that fibrosis of a varying degree was frequently found in dogs; hence, fibrosis observed in the dog that was killed may not have been due to dioxane poisoning. The livers were enlarged in the dogs that died. Gastrointestinal tracts were congested with many hemorrhagic areas, and the stomachs contained bloody fluid. The brains appeared edematous with areas suggestive of hemorrhages. The lungs were congested.

The effect of an estimated dioxane intake of 1 ml/kg/day on 10 rats given water containing 5% dioxane by volume was reported in 1939 by Kesten et al [45]. Eight of ten rats died during days 5 to 12; the other two were

killed on days 6 and 8. The kidneys of all rats showed extensive hydropic degeneration of the convoluted tubular epithelium. High levels of blood nonprotein nitrogen were also noted. The livers of six rats showed swelling and cellular hydropic degeneration. Two other rats that were maintained on 1% dioxane in drinking water for 110 days followed by 3% dioxane for 41 and 48 days, showed patchy areas of degeneration in the kidneys, and the liver of one rat showed damage.

Electron microscopic studies of the kidneys of white rats after ingestion of dioxane were reported by David [54] in 1964. Forty white rats of an unspecified inbred strain, weighing between 150-180 g, were given drinking water containing 5% dioxane for 1-10 days. Surviving animals were killed for microscopic studies on days 1, 3, 5, 7, 8, and 10 and on days 2, 4, 7, and 9 after discontinuing dioxane treatment. A total of 15 rats were examined. The 35 rats that died were not studied. Studies on control rats were not mentioned.

No macroscopic changes were seen in rats killed during the first 7 days, but, in rats killed later, there were frequent enlargements of the kidneys and small to relatively large retracted areas of a grayish-yellow coloration giving the renal surface a pock-marked appearance. Microscopic examination of the kidneys from the first 3 days showed swelling of the tubular epithelium in the proximal section of the nephron, causing the lumen to appear closed. Vesicular degeneration of tubular epithelium was first observed at 5 days and became more severe from the 7th day on. An accumulation of intracellular hyaline droplets was observed by electron microscopy, followed by enlargement of the basal labyrinth. Subsequent changes were noted in the tubular epithelium followed by degeneration and

ultimately resulting in necrosis. The mitochondria showed accumulation of fluid, osmiophilic deposits, and needle-shaped crystal formations that developed from the cristae. The size of the crystals was not sufficient for chemical analysis. By comparison with electron micrographs from other studies, David [54] considered that the crystals were formed by the addition of calcium salts to the protein component of the cristae and were not calcium oxalate.

(3) Inhalation

Inhalation of dioxane caused a variety of signs in guinea pigs, rabbits, cats, rats, and mice, including eye and nose irritation and narcosis. Tumors, behavioral modification, and microscopic changes in kidneys, livers, lungs, and brains were also observed [19,21,24,26,55].

Yant et al [19] exposed an unspecified number of guinea pigs to dioxane at concentrations of 1,000, 2,000, 3,000, 10,000, and 30,000 ppm, and observed the duration of exposure in minutes up to a maximum of 8 hours required to produce signs such as nasal irritation, eye irritation, retching movements, changes in respiration, and narcosis. The composition of the dioxane vapor-air mixture was calculated from the quantity of liquid vaporized and the quantity of air contained in, or flowing through, the animal exposure chamber. The calculated composition of the dioxane-air mixture was always checked by sorption of the vapor from a measured volume of the mixture by air-equilibrated activated charcoal and determination of the gain in weight. Guinea pigs that were exposed at 30,000 ppm for 3 hours developed a state of marked narcosis during exposure, and the animals died within 2 days. Congestion and edema of the lungs, hyperemia of the surface of the brain, and paleness of the liver were seen in guinea pigs

that were killed immediately after the exposure at 30,000 ppm for 30 minutes; patches of congestion in the lungs and hyperemia of the surface of the brain occurred in those that were killed 4 or 5 days after the test; and there were no pathologic changes in the animals killed 9 or 10 days after exposure. Congestion of the lungs and a few hemorrhagic areas in the mucous membranes of the stomach were seen in the guinea pigs that died within 1 day of exposure at 30,000 ppm dioxane and bronchopneumonia and severe congestion of the surface vessels of the brain in the animal that died 2 days after exposure. Fifteen control animals showed no gross pathologic changes resembling those seen in the exposed animals.

The authors [19] concluded that, since exposure at 30,000 ppm for 2-3 hours caused death in the guinea pigs and an 8-hour exposure at 10,000 ppm did not cause death, workers would not voluntarily tolerate atmospheres which would cause serious acute poisoning and that no acute trouble would be experienced if the symptoms of eye and nasal irritation were regarded as a warning to avoid further exposure. However, from Barber's report [20], it was noted that workers continued to work throughout the shift despite the symptoms during the exposure, although they stayed away from work for a couple of days thereafter because of illness.

The effects of inhaling dioxane at 1,000, 2,000, 5,000, and 10,000 ppm on guinea pigs, rats, mice, and rabbits were described by Fairley and his associates [21] in 1934. Animals were placed in a 1-cu m static chamber and the calculated amount of the dioxane-water mixture was then introduced. The dioxane concentration was obtained by vaporizing the calculated quantity of the dioxane-water mixture. The 1,000-ppm vapor was obtained by heating the mixture; for the other concentrations, the mixture

was sprayed into the chamber. The mean temperature of the chamber was maintained at 27 C. Each week, the animals were exposed for 1.5 hours twice daily for 5 consecutive days, once on the 6th day, and were rested on the 7th day.

In general, the guinea pigs were the most affected and the mice the least affected at all the concentrations studied. Most of the animals exposed at 10,000 ppm died after 2-7 exposures, while, at the lower concentrations, the animals were killed after various exposure times. The kidneys showed cortical lesions ranging from patchy swelling to complete necrosis as the dioxane concentration increased. Hemorrhages and vascular congestion were also observed. The livers showed changes ranging from vascular congestion to cellular degeneration as the concentrations increased. At 10,000 ppm, the lungs showed pulmonary lesions that varied from an acute vascular congestion to an advanced infiltration of RBC and these pulmonary lesions were the cause of death in these animals. At the lower concentrations, the lungs showed only vascular congestion or were normal. All other organs appeared to be normal.

Mice were exposed to two grades of dioxane in inhalation experiments reported by Wirth and Klimmer [26] in 1936. One grade was a very pure product that contained 99.8% dioxane with 0.2% water and was completely free of aldehydes and other impurities. The other, a technical grade of dioxane of 96.4% purity, contained 1.5% aldehyde and acetal, 2.1% water, and trace amounts of alcohol and acids. Experiments were carried out with both grades of dioxane at concentrations ranging from 1,400 ppm for about 8.3 hours to 39,000 ppm for approximately 1 hour on white mice of an unspecified strain. A static gas mixture in two 32-liter anesthesia flasks was employed.

None of the mice died during the exposure, but deaths occurred as early as 12 minutes and as long as 67 hours after removal from exposure. Signs of labored breathing followed by disturbance in the sense of equilibrium and irritation of the eyes occurred during exposure at all concentrations. With one exception, all mice died at dioxane concentrations of 8,300 ppm and above. The pathologic changes noted at autopsy were signs of inflammation in the respiratory tract and lung nodes in a few of the mice. When other mice (numbers not reported) were exposed at 1,400 ppm, 8 hours/day, for 17 days, no deaths occurred.

In their experiments with cats, Wirth and Klimmer [26] admitted a continuous flowing gas mixture into the exposure chamber at a constant flow. Again, the same two grades of dioxane were used. Two cats in each experiment were exposed at concentrations of 12,000 ppm for 7 hours, 18,000 ppm for 4.3 hours, 24,000 ppm for 4 hours, and 31,000 ppm for 3 hours. With both grades of dioxane, a loss of equilibrium, increased salivation, and lacrimation were manifested during all exposures. Narcotic effects appeared earlier in the exposure period as the dioxane concentration increased. After removal from exposure, the cats became progressively less active and died in 3-10 days. Necropsy findings were fatty livers and inflamed respiratory organs.

Three male cats were also exposed at an average of 1,400 ppm for about 6.5 hours/day for 14 days. From the 4th day to the end of the experiment, the cats seemed sleepy during exposure. Retching and vomiting were observed occasionally, with slight polydypsia. None of the animals died as a result of exposure. Urinalysis was negative for protein, sugar, and blood; bilirubin and urobilinogen were normal. Increased Hb

concentrations, RBC, and lymphocyte counts without substantial changes in total leukocyte counts occurred and had not returned to normal three weeks after removal from exposure. In the experiments with mice and cats, only slight differences were observed between the two grades of dioxane, the paralyzing and narcotic effects occurring a little earlier in the case of pure dioxane. No significant changes were observed at autopsy between the two grades of dioxane used.

Experiments reported by Gross [24] showed that 21 of 28 animals (mice, rats, guinea pigs, and rabbits) died from single 8-hour exposures at concentrations of about 4,000-11,000 ppm of dioxane. The animals died 7.5 hours at the earliest and 5 days at the latest after the completion of the experiment. Ten animals (mice, rats, guinea pigs, and rabbits) lived through exposure at 37,500 ppm for 3 hours; four of them died within 6 days. The signs of intoxication included signs of irritation of the mucous membranes, labored breathing, and narcosis. There was occasional albuminuria. Hyperemia and edema of the lungs and liver and kidney injuries, stated by Gross [24] to be similar to those observed by De Navasquez [38], were found by microscopic examination. Similar toxic and pathologic signs were observed at 2,700 ppm and, though less severe, at 1,350 ppm.

In unpublished work conducted between 1937 and 1955, Smyth [56] noted that rabbits were particularly susceptible to eye and nose irritation, and that repeated inhalation at 800 ppm killed some guinea pigs from kidney injury within 30 days. No details regarding the experimental design, the use of controls, or microscopic observations were presented.

Effects in rats exposed in groups of five to dioxane vapor at concentrations ranging from about 6,000 ppm to 19,000 ppm for about 4-6 hours were reported in 1951 by Nelson [50]. The strain, age, and sex of the rats used were not mentioned. The survival time decreased with increase in dosage as reflected by the product of concentration and exposure time, while the anesthetic-narcotic effect increased with dosage.

The LC50 of dioxane for 4-hour exposures of female Carworth Farms-Nelson rats, a specific pathogen-free (SPF) strain, was found to be 14,250 ppm (51.3 mg/liter) by Pozzani et al [57] in 1959. The LD50 by the oral route was found to be 6.16 ml/kg.

The effect of inhalation of dioxane vapor on avoidance-escape behavior and on the growth rate of pretrained 30- to 40-day-old female rats was studied by Goldberg et al [55] and published in 1964. The concentrations of dioxane were 1,500, 3,000, or 6,000 ppm. Eight to ten rats were used in both control and experimental groups in a series of experiments with different compounds. The rats were exposed 4 hours/day, 5 days/week, for 2 weeks. Responses examined were determined each day before, during, and 2 hours immediately after removal from exposure. Behavioral studies included the effect of dioxane on conditioned pole-climbing avoidance response to a buzzer and an unconditioned escape response to the buzzer and an electrical shock. The normal response time to both stimuli was 2 seconds. It was noted that only one animal was affected at 2,500 ppm and its responses were not consistent from day to day. In the group exposed at 3,000 ppm, the avoidance reaction was delayed in two to three rats/exposure. About 75% of the rats showed delay of the avoidance response after one exposure at 6,000 ppm, but the escape response

was unaffected. After two exposures, the avoidance reaction was blocked in all animals and the escape response was blocked in three of eight rats. Three or more exposures completely blocked the avoidance response in 37-62% of the rats; the escape response was not affected.

It was noted from these experiments that the delay in avoidance response increased with increase in concentration and, in multiple exposures, the escape responses were also blocked in many cases.

(4) Skin Application

Experiments in which liquid dioxane was applied to the skin of rabbits, guinea pigs, and mice demonstrated that the chemical was rapidly absorbed and produced signs of incoordination, narcosis, and erythema [21,50].

To study the effects of dioxane through the skin, Fairley et al [21] applied an 80% dioxane-water mixture to the shaved skin of four rabbits and four guinea pigs. Each rabbit received 10 drops and each guinea pig 5 drops of the solution for 7-14 weeks. Two applications were given daily for 5 days, one application on the 6th day, and none on the 7th day of each week. No skin irritation was observed from these applications. The animals remained in apparently normal health. One rabbit and one guinea pig were killed on days 49, 66, 77, and 101.

Macroscopically, nothing abnormal was noticed. On microscopic examination, the renal and hepatic lesions were progressively worse in the first three pairs of animals killed, ranging from no cellular degeneration to gross degeneration. The animals killed on day 101 (160 applications) indicated only an intermediate degree of injury with both kidneys and livers showing patchy cloudy swelling, but no definite recovery or repair.

In other words, injury was greatest after 121 applications (day 77) but, when checked after 160 applications, it seemed to be moderate. This may indicate that either tolerance or regeneration occurred.

The effects of dioxane resulting from its absorption through the skin of rabbits were reported by Nelson [50] in 1951. Undiluted dioxane was applied to a shaved area of the belly which represented approximately 15% of a rabbit's body surface. Dioxane was in contact with the skin of two to four rabbits, used in groups, for 1.7, 3.0, 4.1, and 4.8 hours. A control rabbit was treated with water for 6 hours and another for 7.7 hours. Three rabbits showed evidence of intracutaneous and subcutaneous hemorrhages at the end of exposure. When dioxane was administered to two rabbits for 1.7 hours, unsteadiness and incoordination were noticed in both animals; one died within 24 hours and the other was alive 2 weeks after exposure. When four rabbits were exposed to undiluted dioxane for 3 hours, all showed unsteadiness and incoordination; two died within 3 days, one on the 5th day, and the other was alive 2 weeks after exposure. One rabbit exposed for 4.1 hours and another for 4.8 hours appeared to be in a state of narcosis with very weak or absent corneal reflexes and both died at the end of exposure. Dioxane caused skin erythema in the two surviving rabbits which cleared in a matter of days with a superficial scaling and without any evidence of permanent damage.

(5) Other Studies

In 1931, von Oettingen and Jirouch [39] noted hyperemia and pus formation and edema of the conjunctiva, and edema of the nictitating membrane when a drop of pure dioxane was placed into one eye of a rabbit.

The effects of dioxane at various concentrations as well as effects of some other solvents on the eyes of rabbits were reported by Carpenter and Smyth [58] in 1946. Dioxane was applied to the eyes that had earlier shown no grossly visible stains from a fluorescein solution. During and for 1 minute after the application of dioxane to the center of the cornea, the lids were held open. On examination 18-24 hours later, it was found that dioxane caused severe corneal necrosis and that dioxane was more injurious than ethanol but less so than either acetone or isobutanol.

The toxicity of dioxane to Eagle's KB strain of human carcinoma cells was observed by Smith et al [59] in 1959. In this study, the concentrations of dioxane added to the culture were 0.8, 4, 20, 100, and 500 μg/ml. It was not stated what solvent was used in this experiment. The ID50 (dose for 50% inhibition) of dioxane was estimated to be in the range of 200-800 μg/ml. By comparison, acetone, methanol, and ethanol had ID50 values greater than 5,000 μg/ml.

The effect of dioxane on growth and development of 150 chicken embryo tibial buds in organotropic cultures was reported by Franceschini [60] in 1964. Dioxane was added to the culture medium in amounts of 24, 47, 94, 180, 375, 750, and 1,025 μliters. In the same study, thalidomide was added to the culture medium of 150 other tibias in amounts of 1,500, 2,000, 2,500, 3,000, 3,500, 4,000, 5,000, and 7,500 μliters. Hypertrophic and vacuolized chondroblasts in diaphysis and reappearance of mitosis in the metaphysis were the first signs of poisoning in tibias examined after 2 days' growth on media containing either dioxane or thalidomide.

A study relative to the teratogenicity of dioxane was reported by Schwetz and his colleagues [61] in 1975. Sprague-Dawley rats and Swiss-

Webster mice were exposed to a commercial preparation of 1,1,1-trichloroethane that contained dioxane and other additives and impurities for 7 hours/day on days 6-15 of gestation. The exposure concentration of 1,1,1-trichloroethane averaged 875 ppm and that of dioxane about 32 ppm. Although a few terata were found in the fetuses of both rats and mice, they were not of a consistent type in the two species and did not occur more often than in untreated litters of the colonies.

(b) Chronic Studies

Chronic studies were conducted with rats and guinea pigs administered dioxane orally and with mice exposed to the chemical by dermal application and by inhalation [43,62-70]. Results have demonstrated the induction of tumors in the nasal cavity and liver of rats [43,62,64,65], in the gall bladder, lungs, and kidneys of guinea pigs [63], and in the skin and lungs of mice [66,70]. Other reported findings in rats were bronchitis, bronchopneumonia, and kidney and liver damage [66,67,70].

A group of investigators at Tulane University [62] noted that some chemical carcinogens, such as dimethyldinitrosophthalamide, heptylamine, diethylacetamide, and diethylformamide, were also potent protein-denaturing agents. Though no tumors from dioxane administration had been found in earlier studies by Kesten et al [45], the Tulane group decided to study the carcinogenic potential of dioxane because of postulated carcinogenicity, based on its protein-denaturing characteristics.

In their first study, in 1965, Argus et al [62] used adult male Wistar rats, weighing between 150 and 200 g, and housed two to a cage. The animals were pretreated with 75 mg/liter terramycin for 1 week in their drinking water, probably to prevent infection. The animals were then fed

1% dioxane in their drinking water for 63 weeks at an average estimated total dose of 132 g/rat. Nine rats were used as controls. The weights of the dioxane-treated rats increased to 500-580 g by the end of the experiment; no details regarding weight gain in control rats were given. Complete necropsy was performed on all rats.

The changes found in the kidneys resembled glomerulonephritis. The tubular epithelium, especially in the convoluted tubules, contained a granular precipitate. Many arteries were obliterated and a thickening of arterial walls was observed. Epithelial proliferation was quite prominent in the glomeruli of some kidneys. One rat showed an early transitional cell carcinoma in the renal pelvis 65 weeks after the start of the experiment; the surface epithelium showed marked proliferation. This same rat also had a 2-cm diameter tumor in the liver but no further details were given.

Hepatomas were observed, apparently by microscopic examination, in 6 of 26 rats, but no details were given except that these hepatomas developed 64 and 65 weeks after the start of the experiment. In the lungs of many rats (number unspecified), both control and treated, there were cases of severe bronchitis with epithelial hyperplasia, marked peribronchial infiltration, and multiple abcesses. A lymphosarcoma, found in one of the control animals, was of a large cell type and localized in the retroperitoneal lymph nodes. Leukemia was found in one rat treated with dioxane.

In view of the observed hepatomas in 6 of 26 rats, the investigators concluded that dioxane is a hepatic carcinogen. However, the following points need to be considered regarding the experimental design of Argus et

al [62]: (1) Animals were pretreated with terramycin for 1 week before the start of the experiment. Whether a possible action of terramycin on liver microsomes affected the subsequent action of dioxane is not clear. (2) Four other candidate carcinogens, diethylacetamide, diethylformamide, dimethyldinitrosophthalamide, and heptylamine were tested at the same time as dioxane. It is not known whether the dioxane-treated rats were housed in the same room as the others, and whether cross-contamination could have had a significant effect. (3) The dose of dioxane which produced tumors was 737 times that producing tumors in diethylacetamide-treated rats, which also caused kidney damage. (4) Only nine control rats were used.

In 1970 Hoch-Ligeti and Argus [63] studied various candidate carcinogens including dioxane and observed the induction of tumors and hyperplasias described as tumor-like in 9 of 22 male guinea pigs given dioxane orally. The concentration of dioxane in drinking water ranged from 0.5 to 2% and was regulated to maintain the normal growth of the guinea pigs. The exact amount of dioxane absorbed from the drinking water was not known; however, the total amount received by a guinea pig over a 23-month period was 588-635 g. The animals were killed within 28 months. Ten guinea pigs were used as controls.

Peri- or intrabronchial epithelial hyperplasia and nodular mononuclear infiltration were present in the lungs of nine guinea pigs. In addition, two guinea pigs had carcinoma of the gall bladder, three animals had early hepatomas which were not described, and one had an adenoma of the kidney.

The lungs of 4 of 10 control guinea pigs showed peripheral mononuclear cell accumulation, one showed hyperplasia of the bronchial

epithelium, and one showed the formation of a cartilaginous substance in the bronchus. Both dioxane-treated and control animals were very small in number.

The induction of carcinomas in the nasal cavities of 6 of 120 rats fed dioxane in drinking water was reported by Hoch-Ligeti et al [64] in 1970. Five groups of 30 male Charles River CD-strain rats, 2-3 months of age and 110-230 g in weight, were used. These were SPF animals derived from a Sprague-Dawley line. Four groups of rats were given drinking water containing 0.75, 1.0, 1.4, or 1.8% dioxane for 13 months; the fifth group served as a control. The average daily fluid consumption, determined for each group over a 3-day period on two occasions, was approximately 36 ml. The rats were killed with ether at 16 months or earlier if the nasal cavity tumors were clearly observable. Autopsies were performed on all rats. Table III-9 presents the total dosage, latent periods, and incidence of liver and nasal cavity tumors in six rats.

Table III-9 shows that four of the rats, two that received 1.4% dioxane and two that received 1.8% dioxane, had liver tumors as well as tumors of the nasal cavity. One rat at each of the other doses developed tumors only in the nasal cavity. The nasal cavity tumors, found to be heavily necrotic on dissection, were squamous cell carcinomas with marked keratinization and formation of keratin pearls. They appeared to arise in the anterior part of the nasal cavity as evidenced by dysplasia and in situ carcinomatous changes of the squamous epithelium. Neurologic signs were not observed. A small, firm, well circumscribed tumor was found on the back of the nose of one control rat, which on microscopic examination proved to be a subcutaneous fibroma.

TABLE III-9

TOTAL DOSES AND LATENT PERIODS OF NASAL AND HEPATIC TUMORS FOUND IN SIX DIOXANE-TREATED CHARLES RIVER STRAIN RATS

Concentration of Dioxane Administered (%)	Total Dose* (g)	Dioxane mg/kg/day	Latent Period (Days)	Nasal Cavity Tumor Present	Hepatic Tumor Present
0.75	104	1350	385	Yes	No
1.00	142	1750	407	Yes	No
1.40	191	2500	382	Yes	Yes
1.40	198	2170	456	Yes	Yes
1.80	213	3255	329	Yes	Yes
1.80	256	2630	256	Yes	Yes

*Calculated on the basis of an average daily fluid intake of 36 ml

Adapted from reference 64

No tumors were found in the livers or nasal cavities of the control animals. The investigators [64] concluded that dioxane was a hepatic carcinogen in the rat and that it possessed the versatility of inducing tumors at various locations and hence was of possible carcinogenic hazard to humans. In this study, two rats, each given 1.4 and 1.8% dioxane in drinking water, showed hepatic tumors which were not described either in location or microscopically. The rats given 1% dioxane for 68 weeks did not show any hepatic tumors; yet, in the earlier study [62], six undescribed hepatomas at the 1% level were observed at 64 and 65 weeks. The investigators [64] suggested that a free radical or a carbonium ion may be formed during dioxane metabolism which may represent a proximate carcinogen.

In another report, apparently of the same experiment, Argus et al [43] reported that the hepatocarcinogenicity of dioxane in male rats was a

function of the total oral dose administered. Five groups of 28-32 Charles River CD strain male rats, 2-3 months old, and weighing 110-230 g at the beginning of the experiment, were used in each group. The rats were housed two to a cage. The experimental animals were given drinking water containing 0.75, 1.0, 1.4 or 1.8% dioxane for 13 months with one group used as a control. Fresh dioxane solutions were prepared daily. The weights of the rats were recorded weekly, and all rats that survived for 16 months were killed with ether. Autopsies were performed on all rats. The exact number of animals used in each group was not stated and hence the number of animals having tumors or the number of tumors/animal could not be determined. Four "incipient" tumors were found in the livers in the group that received 0.75% dioxane and nine in the group that received 1% dioxane. These so-called "incipient" tumors were described as nodules with the histologic characteristics of hepatomas. Thirteen "incipient" liver tumors and three hepatomas were found in the 1.4% group and 11 "incipient" liver tumors and 12 hepatomas were found in the group administered 1.8% dioxane. The total dioxane doses administered were about 90, 118, 162, and 205 g in the four groups, and the tumor incidence, apparently the percentage of animals with either "incipient" or frank hepatomas, was 14, 28, 53, and 82, respectively. The hepatomas were manifested grossly as white lesions, 0.5-1.0 cm in diameter. Microscopically, these lesions consisted of long strands of large cells, 2-4 cells thick. A conglomeration of small nodules was seen in some tumors. Metastases were not observed. At all doses of dioxane, the kidneys showed marked alterations; in many, the changes were those of glomerulo- and pyelonephritis with characteristic epithelial proliferation of Bowman's capsule, periglomerular fibrosis, and distension of tubules [43].

In their earlier studies, the authors [64] had observed nasal tumors at all the four levels, 0.75, 1.0, 1.4 and 1.8% dioxane, one tumor at each of the two lower levels and two each at the two higher levels of dioxane. In the present study, no nasal tumors were reported and, although lung tumors were not found in any of the rats, the lungs of one rat given 1.4% dioxane in drinking water showed early peripheral adenomatous change of the alveolar epithelium; papillary hyperplasia of the bronchial epithelium was found in another rat receiving the same level of dioxane.

For electron microscopic studies, the investigators [43] administered 1% dioxane in drinking water to 10 male Sprague-Dawley rats. Five of them were killed after 8 months and the other five after 13 months of treatment. Although microscopic examination revealed no evidence of liver tumors in any of the rats killed after 8 months, "incipient" liver tumors, which they termed precancerous changes, were seen in two rats consuming dioxane for 13 months.

The investigators [43] concluded that hepatocarcinogenicity did not appear to be strain-specific since both Wistar [62] and Sprague-Dawley [43,64] rats seemed to be susceptible. In these studies, 1 of 26 Wistar rats showed a transitional cell carcinoma of the kidney pelvis [62], but no mention was made of alterations or kidney tumors in their second report [64]. The third report [43] showed no kidney tumors, though alterations occurred at all four doses. They also stated that, from their third study [43], dioxane was considered to be a weak to moderate hepatocarcinogen. They also suggested that the combination of repeated exposure to dioxane vapor and to an enzyme inducer such as MC could increase the toxicity and possibly the carcinogenicity of dioxane. However, as seen earlier in their

studies with MC followed by dioxane, there was no great increase in toxicity.

In earlier work with dioxane [43,62,64], the effects of low doses were not studied. Since the doses which the rats received were massive, some greater than 1,000 mg/kg/day to compensate for the small test animal population, it was difficult to estimate the possible hazard in a work environment. The need for dose-response data following exposure at lower levels of dioxane prompted Kociba et al [65] to conduct a long-term toxicity study in male and female Sherman strain rats given dioxane in their drinking water, the results of which were published in 1974. Three groups of rats, each consisting of 60 males and 60 females, were given drinking water containing 1.0, 0.1, or 0.01% dioxane for up to 716 days (2 years). A fourth group consisting of 60 rats of each sex was used as a control. Periodically, samples were collected from individual water dispensers and analyzed for dioxane content by gas-liquid chromatography. Autopsy was conducted on all rats that died or appeared moribund.

Rats that were fed 1% dioxane in drinking water were found to consume dioxane at a daily rate of approximately 1,000 mg/kg (males) and 1,600 mg/kg (females) over the 2-year period. There were more deaths during the first 4 months of the study among experimental animals of both sexes than in the control group. After the 5th month, the mortality rate of test animals was not statistically significantly different from that of the controls. Only one male rat out of 60 fed 1% dioxane was alive during the last 60 days of treatment. Rats that received 0.1% dioxane in drinking water consumed dioxane daily at rates of about 94 mg/kg (males) or 148 mg/kg (females) [65].

Water consumption by rats of both sexes in the 1% group and in females of the 0.1% group was significantly lower ($P<0.05$) than that of the controls. The body weights of both male and female rats of the 1% group were significantly lower ($P<0.05$) than those of controls within 2 days after the start of the study, and remained depressed throughout the 2-year period. The body weights of rats that received 0.1 or 0.01% dioxane in drinking water were not significantly different from those of controls.

Gross and microscopic examination of tissues from rats that received 1.0 or 0.1% dioxane revealed variable degrees of renal tubular, hepatocellular, and epithelial degeneration and necrosis; these effects were similar to those seen by Fairley et al [21], De Navasquez [38], Schrenk and Yant [48], and Kesten et al [45], who administered dioxane for shorter periods. In addition, rats that received 1.0 or 0.1% dioxane showed evidence of hepatic regeneration as indicated by hepatocellular hyperplastic nodule formation and evidence of renal tubular regeneration as indicated by increased tubular epithelial regenerative activity [65]. Nasal carcinomas were observed in 3 of 66 rats of the 1% group ($P=0.055$). No evidence of tumor formation or other toxic effects were observed in rats maintained on water containing 0.01% dioxane from which they consumed dioxane doses of approximately 9.6 mg/kg/day (males) and 19.0 mg/kg/day (females).

In the control group of 106 rats, 1 showed a hepatocellular carcinoma, another a cholangiocarcinoma, and 2 had hepatic tumors. Since earlier work [43,62,64] involved the use of massive doses to demonstrate tumor formation and carcinomas, the investigators [65] in this study used both high and low doses of dioxane to obtain a dose-response relationship.

There was a significant increase in hepatocellular carcinomas in rats given 1% dioxane in drinking water (10/66 vs 1/106) and a suggestive increase in the same group in squamous cell carcinoma of the nasal turbinates (3/66 vs 0/106). There were no significant increases in tumors of other types compared to those seen in controls, or in rats given lower doses of dioxane.

A report on the effects of orally ingested dioxane on Osborne-Mendel rats and B6C3F1 mice was made by Holmes in 1976 [67]. Groups of 35 male and 35 female rats and 50 mice of each sex received 1.0 or 0.5% of dioxane in drinking water; an additional 35 rats and 50 mice of each sex served as controls. Surviving rats were killed after 110 weeks and mice after 90 weeks. An air-conditioning failure approximately 1 year after the study had begun made it necessary to restart the experiment for male rat controls and for male rats given 1% dioxane dose. The daily dose of dioxane ingested by the experimental animals was measured during the second year of the experiment. For rats given 0.5% of dioxane, the average dose was calculated as 0.25 and 0.35 g/kg/day for males and females, and 0.60 and 0.64 g/kg/day for males and females given the 1.0% dose. For mice, the calculated average doses were 0.72 and 0.38 g/kg/day for males and females given 0.5% dioxane. For male and female mice which drank water containing 1.0% of dioxane, the average doses were 0.83 and 0.86 g/kg/day.

A high fatality rate was reported for both male and female rats [67], with about 80% deaths in the 1.0 and 0.5% dioxane groups and 50% in controls. The rats, especially females, were reported to have been highly susceptible to murine and other bronchial pneumonias. Many of the deaths in the treated groups were attributed to renal and hepatic toxicity. Renal

toxicity was described as tubular nephrosis with vacuolar degeneration and necrosis of the tubular epithelial cells. Liver toxicity was characterized as fatty change or frank hepatocellular necrosis beginning as a centrilobular lesion and often involving the entire lobule. In females only, these hepatic lesions led to formation of hyperplastic nodules, and occasionally to hepatocellular carcinomas.

An increase in the number of malignant tumors was seen with increasing dioxane doses [67] with 11 tumors in controls, 28 in the 0.5% dioxane group, and 41 in the 1.0% group. These tumors were not compared statistically. Most of the malignant tumors occurred in the nasal epithelium. Tumors were confirmed by microscopic examination but the findings were not described. Squamous cell carcinomas of the nasal cavity were seen in 0/32 controls, 23/57 of the 0.5% dioxane group, and 23/51 of the 1% group. Two animals of the 0.5% group had olfactory neuroblastomas and one had a rhabdomyosarcoma. Seven animals of the 1.0% group had olfactory neuroblastomas and two had seromucous adenocarcinomas. The two seromucous adenocarcinomas metastasized, one to the brain and cervical lymph node and the other to the lung. Four of the nine neuroblastomas and 4 of 46 squamous cell carcinomas also metastasized, mainly to the brain.

Most of the mice, except for 11 of 50 females given 1% of dioxane, survived to the end of the experiment. According to the author [67], these 11 deaths may have been due to chronic rhinitis and murine pneumonia which had an increased occurrence among female mice.

The target organ in the mouse was the liver [67]. One of 99 examined controls had chronic hepatitis compared to 26 of 94 in the 0.5% dioxane group and 14 of 87 in the 1.0% group. An increase in malignant liver tumor

incidence was reported with increasing dose. Three of 99 controls, 11/94 of the 0.5% dioxane group and 10/87 of the 1% group had hepatic nodular hyperplasia. Hepatocellular carcinomas were seen in 4/99, 24/94, and 44/87 of controls, 0.5%, and 1.0% groups, respectively. The tumors were confirmed microscopically. The hyperplasias were described as small, circumscribed, single or multiple areas demarcated by surrounding compressed liver tissue. Hepatocellular carcinomas were characterized by general disruption of normal liver structure with abnormally formed trabeculi, sinusoid-like blood spaces, anisocytosis, abnormal staining properties, and intranuclear and intracytoplasmic inclusion bodies.

To determine the effects of long-term repetitive inhalation of dioxane, Torkelson et al [68] conducted a "lifetime" 2-year study with Wistar strain rats. At the time the study was started and until 1971, the ACGIH threshold limit value for dioxane was 100 ppm (360 mg/cu m); hence this concentration was selected for the 2-year study. In addition, earlier studies of animals exposed at 50-100 ppm for shorter periods had shown no adverse effects [68].

In the inhalation experiment conducted by Torkelson et al [68], 288 male and 288 female rats were exposed at 0.4 mg/liter of air (111 $\pm$ 5 ppm of dioxane) for 7 hours/day, 5 days/week, over the 2-year period, with 192 males and 192 females used as controls. The dioxane vapor concentration was monitored by an on-line infrared spectrometer analyzer.

Animals were monitored for evidence of toxicity including changes in activity, eye and nasal irritation, skin changes, respiratory distress, weight changes, and tumor formation. Hematologic values, including packed cell volume, RBC, Hb, WBC and differential WBC, were within normal

physiologic limits. Blood urea nitrogen (BUN), alkaline phosphatase, total protein, and SGPT values were normal. The slightly lower BUN and alkaline phosphatase values found in exposed males as compared to control males were not considered to be of toxicologic significance. There was no evidence of changes in activity, body weights, or in gross or microscopic appearance of organs and tissues of the animals because of dioxane exposure [68]. No hepatic or nasal tumors occurred in either control or exposed rats. Neither any one type of tumor nor the total number of tumors occurred more frequently ($P<0.05$) in the exposed rats than in the controls.

Torkelson et al [68] stated that the absence of a detectable increase in the tumors of rats in the inhalation experiment supported their earlier hypothesis [65] that liver injury precedes tumor development in the liver.

A comparative calculation of possible daily doses of dioxane was made by the investigators between their inhalation study [68] and their earlier ingestion study [65]. In the oral study [65], male and female rats that received 1% dioxane in their drinking water ingested an average of 1,015 and 1,599 mg/kg/day, respectively; those that received 0.1% ingested 94 and 148 mg/kg/day and rats that received 0.01% ingested 9.6 and 19 mg/kg/day of dioxane. In the inhalation study, about 105 mg/kg/day of dioxane would have been inhaled during a 7-hour period, if dioxane was totally absorbed from the respiratory tract. This inhalation dose was based on a respiratory rate of at least 250 ml/min for male rats weighing 400 g, or about 105 liters of air/7 hours of exposure. This calculated inhalation dose of dioxane was very similar to that of male rats ingesting water containing 0.1% dioxane, or 94 mg/kg/day. However, only the latter exposure caused pathologic changes in the liver.

As discussed by the investigators [68], a few differences were noted between the inhalation and ingestion studies: (1) the administration of dioxane in the ingestion study was 7 days/week as compared to 5 days/week in the inhalation study, (2) although dioxane is very soluble in water, less than quantitative absorption may have occurred in the respiratory tract, and (3) the gastrointestinal absorption may have exposed the liver to a higher concentration of dioxane than absorption from the lungs.

Skin painting experiments were conducted by King et al [66,70] for 78-110 weeks on mice and rats for a carcinogenesis bioassay study of chlorinated dibenzodioxins and dioxane. Two sets of experiments were undertaken, one to determine whether dioxane was a carcinogen, and the other to determine whether it promoted the carcinogenicity of dimethylbenzanthracene (DMBA). Acetone was used as a vehicle and as a control for the chlorinated dibenzodioxins, where 0.2 ml of the sample was dissolved in acetone. It is not apparent whether dioxane was also dissolved in acetone. Dioxane was applied topically three times weekly to the backs of 30 male and 30 female mice. For the study of promotional activity, the same number of mice were initially treated with 50 μg DMBA 1 week prior to the application of dioxane.

Twenty-two of 30 male mice and 25 of 30 female mice survived 60 weeks of dioxane treatment. One suspected carcinoma, not confirmed microscopically, was seen in the surviving male group, and one subcutaneous tumor was seen in the surviving female group. No description of the type of tumor was given. One reticulum cell malignant lymphoma was seen among eight mice that died, but there was no evidence of skin lesions.

In the group administered dioxane after pretreatment with DMBA, 4 of 30 male mice and 5 of 30 female mice survived 59 weeks of test; two papillomas and three suspected carcinomas each were found among both male and female mice and two subcutaneous tumors were observed among the males. Lesions of the liver included megalocytosis, distended bile canaliculi, centrilobular necrosis, and cuffed triad vessels. General mononuclear periportal infiltration and mild peripherolobular fibrosis were identified in a number of the dioxane-treated mice. The skin at the dioxane-treated area showed conditions ranging from hyperplasia to dermal fibrosarcoma. Squamous cell carcinoma of the nasal septum was observed in one mouse that had skin papilloma. Seven of nine mice had malignant lung lesions, three of which were considered metastatic from other unspecified sites. No studies were performed by the use of DMBA alone; hence, it is difficult to ascertain whether lesions and tumors observed were due to dioxane, DMBA, or both.

Acetone was used as a vehicle for the study of the dioxins and perhaps for dioxane too. No skin tumors were seen in the surviving mice in the carcinogenic study, but promotional studies showed the presence of two undescribed subcutaneous tumors, and a reticulum cell malignant lymphoma was found in one of the female mice.

In a later report of the same experiment, King et al [70] stated that mortality was high in the dioxane-promotion group (55/60). The average number of papillomas/mouse was about 30% lower than that seen for croton oil-treated animals, yet the comparative rate of promotional activity for croton oil and dioxane on male mice seemed to be similar.

Skin painting with dioxane following DMBA initiation led to the development of a number of alveolar adenocarcinomas which were not metastatic from the skin tumors. On the other hand, in the studies of dioxane without DMBA, the incidence of tumors was greatly reduced but certain of the systemic responses comparable to those seen in the dioxane promotion experiments were found, especially alveolar adenocarcinomas.

The investigators [70] stated that the high incidence of lung adenocarcinomas in the dioxane-treated groups suggested the possibility that inhalation of the compound may have affected the results. Because of the caging system, appreciable air concentrations of dioxane were produced, a fact which was verified by gas chromatographic analysis. A peak concentration of 5,278 ppm was found within 3 minutes of the skin application, which decreased to 694 ppm at 15 minutes and to 146 ppm at 1 hour. By 3 hours, the concentration of dioxane had decreased to 55 ppm.

To determine any possible effects of inhalation and to recheck some of the earlier promotional studies, further work was conducted by King et al [70] using both acetone and dioxane. In the dioxane promotion experiment without the use of filters in the cages, 12 of 30 male mice died and one tumor on one of the survivors was noted; 2 of 30 females died and no papillomas were observed in the survivors after 54 weeks of treatment. With the use of filtered rack, no papillomas were noted and four females died during the experiment.

In the acetone promotion experiment, no papillomas were observed in mice housed in unfiltered racks. When filtered racks were used, four mice showed papillomas and one other developed a papilloma prior to death. The repetition of the experiment did not show the high degree of promotional

activity that was found in the initial study. Microscopic details were not completed at the time the report was made.

The total number of tumors, unspecified as to type and site, was slightly greater in the 0.5% dioxane group than in controls. The male rats given 1% dioxane were not kept for the full term because of laboratory difficulties and this would account for the lack of an increased incidence of tumors in males fed 1% dioxane. It was stated that liver tumors were seen but the distribution between the various treatment groups was not described. The lack of sufficient detail makes this study difficult to interpret.

Because of the divergent results obtained by various investigators and the unknown purity of dioxane used via ingestion, inhalation, and skin applications, Perone and coworkers [69] conducted a study to determine the toxicity of dioxane and its cutaneous and percutaneous carcinogenic potential. For this purpose, mice were given topical applications of various grades of dioxane, with and without impurities, and with ethyl alcohol as a control. The samples were analyzed by gas chromatography. Mice of the C3H/HeJ Agouti strain weighing about 20 g each were used.

A preliminary 4-week study was conducted to determine the minimum lethal dose defined as the concentration that killed 10% of the animals. Different concentrations were used, and 0.05 ml of each concentration was applied topically to the shaved interscapular area of each mouse three times weekly. The animals were observed daily for toxic effects and for weight gain. All mice in each group survived. For the testing of the carcinogenic potential of dioxane, 30 male mice were used for each of the four grades of dioxane and for ethyl alcohol used as solvent control. Each

mouse was housed individually to prevent male mice from fighting. The study was continued for 78 weeks.

There was no gross evidence of tumor formation in any of the five groups tested, nor was there any evidence of skin irritation. Weight gains were similar for all groups. A large number of mice from all groups died because of a malfunction in the heating system; no tumors were grossly visible, and necropsy showed respiratory failure as the cause of death. Another group of mice died from a respiratory infection caused by contamination of some other mice not used in the dioxane study and no tumors were observed at necropsy [69].

Of the 40 mice that survived the treatment, five hepatic tumors and one pulmonary tumor were observed. In the 17 ethyl alcohol-treated mice that were used as solvent controls, two showed hepatic tumors and one developed a pulmonary tumor. The investigators [69] concluded that the remarkably normal microscopic appearance of the skin of mice of all test groups suggested that the treatments of the four dioxane groups and ethyl alcohol group were not deleterious. Untreated controls were not used. Since the solvent-treated control mice also showed hepatic and pulmonary tumors, no conclusion can be drawn about the carcinogenic potential of dioxane from this experiment. The high death rate from intercurrent disease may have helped obscure the presence or absence of real differences between control and test animals.

(c) Mechanism of Action

Oxalic acid and diglycolic acid, which are in vitro oxidation products of dioxane, were considered by several investigators [21,24,71,72] to be likely mammalian metabolites from dioxane exposure. Fairley et al

[71] provided support for this argument by a comparative study of the toxicities of salts of oxalic and diglycolic acids with that of dioxane. The sodium salts of oxalic acid and diglycolic acid were administered iv to rabbits, and ethyl oxalate was applied to the skin of rabbits and guinea pigs. Renal and hepatic changes were similar to those seen with dioxane. Wiley et al [72] were unable to demonstrate an increase in the urinary content of oxalic acid in dogs and rabbits when 2 ml of 40% dioxane was injected daily for 7 days. This suggested that oxalic acid was not a metabolite of dioxane.

Some evidence that dioxane metabolism may be involved in its toxicity was provided by experiments performed by De Navasquez [38] and Argus et al [43]. A tolerance to dioxane at doses that had been determined to be lethal was observed by De Navasquez [38] in rabbits first given repeated sublethal doses of dioxane. The kidneys and livers were normal in the animals given eight dioxane doses of 0.5 ml/kg at weekly intervals followed by five "lethal" doses of 1.5 ml/kg. The only observed effect was temporary polyuria. In contrast, animals given a single 1.5 ml/kg dose, without any pretreatment, died. As reviewed earlier in this section, the toxicity of dioxane was reported [43] to have been increased in rats pretreated with MC, a microsomal enzyme-inducing agent, but the enhancement of toxicity was not marked.

In 1976, Braun and Young [37] demonstrated that beta-hydroxyethoxyacetic acid (HEAA), rather than either oxalic acid or diglycolic acid, was the major urinary metabolite of dioxane in rats. Uniformly labeled C-14 dioxane was administered to two rats by oral intubation. Composite 24-hour urine samples were collected and analyzed

for metabolites using thin layer ion exchange column chromatography and gas chromatography, gas chromatography-mass spectrometry, and by nuclear magnetic resonance spectroscopy. One major and two minor C-14-containing compounds were confirmed by thin layer chromatography. The two minor compounds were identical to C-14 dioxane and C-14 diethylene glycol. The major compound was identified as HEAA using the other four analytical methods. HEAA accounted for approximately 85% of the radioactivity in the urine, with dioxane and diethylene glycol accounting for the remaining 15%.

In a study reported by Young and Gehring [30] in 1975, the pharmacokinetics of dioxane in rats differed depending upon the dose and regimen of administration. A single dose of 10 mg/kg of C-14 dioxane was rapidly metabolized and excreted in the urine as about 95% HEAA and 4% dioxane. At a dose of 1,000 mg/kg, dioxane was eliminated slowly, with over 25% excreted unchanged into the urine. The renal and pulmonary clearance rates of C-14 radioactivity were the same with doses of 10 or 1,000 mg/kg C-14 dioxane, but the plasma clearance rate of dioxane decreased from 2.88 to 0.25 ml/minute. Multiple daily doses of dioxane of 1,000 mg/kg were excreted more rapidly than an equivalent single dose. This was not the case when multiple daily doses of 10 mg/kg dioxane were administered.

Studies [29,30,37] in 1976 in both rats and humans have shown that HEAA is the major metabolite whether dioxane is given in large or small doses. Other investigators [43,64] predicted the formation of a carbonium ion but this requires a great amount of energy and is a less likely intermediate. A mechanism for the metabolism of dioxane, consistent with available data, may be postulated as follows:

1) An oxonium ion may be first formed, 2) a nucleophilic attack by water may take place, thus opening the ring, with the formation of the alcohol, 3) reduction of the alcohol to the corresponding aldehyde, which is a fast reaction, and 4) oxidation of the aldehyde to the acid, HEAA, which is also a fast reaction. The metabolism of dioxane is presented in Figure III-1.

The formation of HEAA without the appearance of radioactivity in either the aldehyde or the alcohol [37] gives evidence that HEAA is the main product formed. Secondly, the excretion of dioxane per se in the larger doses used [30] indicates that neither an alternate pathway nor a blockage of the formation of aldehyde or acid occurs. Thus, at the present time, no alternate pathway has been noted since the entire radioactivity was found in dioxane and HEAA combined when both large and small doses of C-14 dioxane were used. The investigators [30] also stated that extrapolation of effects from high doses of dioxane to the effects anticipated to be associated with exposure to small amounts in the work environment could not be correctly done because the pharmacokinetics of both doses were different.

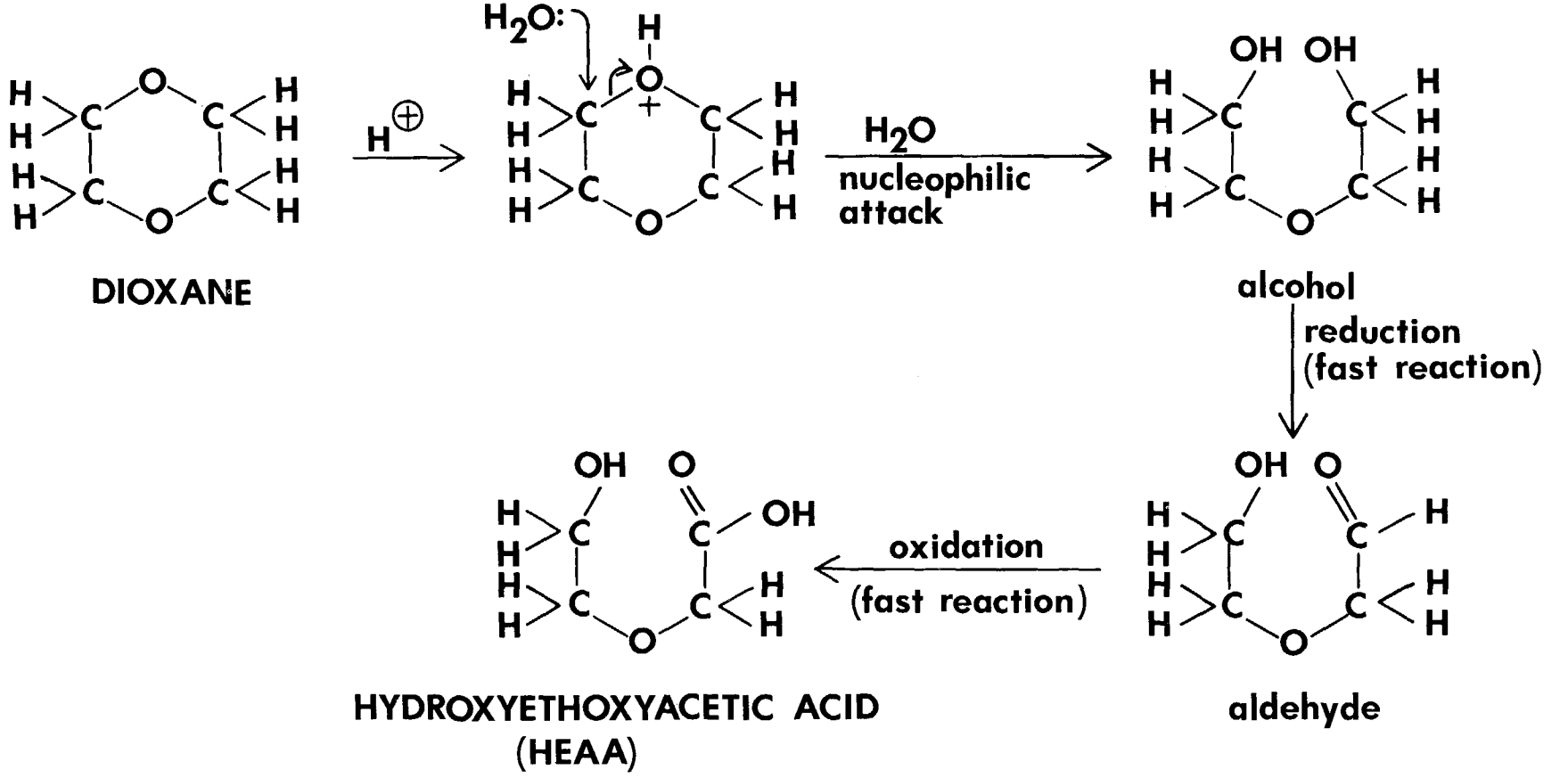

FIGURE III-1. METABOLISM OF DIOXANE

Correlation of Exposure and Effects

(a) Acute and Subacute Effects Related to Occupational Exposure

Dioxane has been used industrially for about 40 years without generating a large number of reports of adverse effects to workers. The first such report appeared in England in 1934 as a result of an episode of illnesses and deaths in a factory where dioxane was used [20]. In this episode, no estimates were made of dioxane concentrations. Historical records of the workers who had been exposed during the first 15 months that the process was operative indicated some cases of nausea and vomiting with lost worktime. Some changes were then made in one of two similar machines where dioxane was used. The changes probably increased the exposure concentrations and, at the same time, the workers increased the hours/day and days/week that they worked. After 5 weeks of this increased exposure, the men working on the altered machine became ill with symptoms of nausea, anorexia, vomiting, and "stomach trouble;" five of them died. Signs and symptoms of poisoning, such as nausea and vomiting, were followed by oliguria for 3-4 days, anuria, coma, and death. Enlarged pale livers, swollen hemorrhagic kidneys, and edema of the lungs and brain were found at autopsy. Microscopic findings included centrilobular liver necrosis, necrosis of the renal cortex, and hemorrhages around the glomeruli. Jaundice was not observed.

The 80 men who worked in the factory and who had any potential for exposure to dioxane during the period it was used were examined 7-14 days after work was stopped in the dioxane unit. Three or four of these men had exposures similar to those who died. When they were examined, there were histories of nausea and vomiting, traces of albumin and a few RBC in the

urine, and a significant leukocytosis. In seven or eight other men, who had probably worked directly with dioxane and received less exposure, there were also findings of leukocytosis and albumin and RBC in the urine. Among these 11 "most exposed" workers, there were also findings of positive indirect van den Bergh reactions probably attributable to liver changes, and one man had a palpable liver; however, there was no clinically apparent jaundice in any of the 80 workers. Thirteen men who had not had any exposure to dioxane for at least 2 months showed a tendency toward eosinophilia. In the other 56 men, who were considered to be valid controls, a faint trace of albumin was found in the urine of 25, and a positive indirect van den Bergh reaction in 7.

Another report of an occupational fatality appeared in 1959 [31]. The worker became ill after 1 week of exposure to dioxane and the course of his illness was similar to that of the English workers [20]. In this case, estimates of exposure concentrations were made after the worker's death. Concentrations of 208-650 ppm, with an average of 470 ppm, were found in the 75 x 50 x 25 foot workroom. No respirator or exhaust ventilation was provided. Details of the number of samples, when or where they were taken, or the method of collection and analysis were not reported. The worker had used the dioxane, which he placed in an uncovered bucket between his knees, to clean a workbench and to keep his hands free of resin-type glue. By this practice, he may have absorbed dioxane through the skin.

A report of dermatitis associated with occupational exposure to dioxane was published in 1964 [32]. A 47-year-old woman developed inflammatory skin abnormalities after several weeks of exposure to dioxane. She may have been sensitive to dioxane because, 6-8 months earlier, she had

suffered first and second degree burns from isoprene. Skin sensitivity tests in the patient demonstrated a pronounced hypersensitivity to dioxane whereas in two control persons the tests were negative.

The application of 0.05 ml of either pure or technical grade dioxane [26] three times daily to the underside of the forearm of a volunteer dried the skin but caused no other signs of irritation. A drop of dioxane placed on the mucous membrane of the mouth produced a temporary, slight burning sensation [26]. When a drop of pure dioxane was placed in an eye of a rabbit, it produced hyperemia and pus formation of the conjunctiva, and edema of the conjunctiva and nictitating membrane [39]. In another experiment, undiluted dioxane was found to be more irritating to a rabbit's eye than ethanol and less irritating than acetone or isobutanol [58].

That dioxane can be absorbed through the skin has been demonstrated in experimental animals [21,50,66,69,70]. An 80% dioxane-water mixture was applied twice daily to the skin (rabbits, 10 drops, and guinea pigs, 5 drops) for up to 101 days. Extensive degenerative changes in the kidneys and livers were found that were similar to changes caused when dioxane was given by other routes of administration. In another report [50], application of undiluted dioxane to rabbits for 3 hours caused unsteadiness and incoordination and they died within 5 days. Evidence of liver and kidney damage was inconsistent. It is evident from animal studies that skin absorption of dioxane does occur and it is concluded from this and evidence from human studies [31,32] that skin contact is a potentially hazardous route of absorption in man. Skin absorption may be prevented by the use of proper protective equipment like gloves, aprons, etc, which are described in Chapter V.

One of the reasons why workers may have been exposed to fatal concentrations of dioxane is that it has poor warning properties. Although it has a low odor threshold (2.8-5.7 ppm) [26,28], its odor is not unpleasant and individuals get accustomed to it after the first few minutes [19-22,26]. Dioxane vapor is also relatively nonirritating to the mucous membranes [19,22]. Irritation of the eyes, nose, and throat was experienced by volunteers exposed to dioxane at concentrations of 5,500 ppm for 1 minute or at 1,600 ppm for 10 minutes [19]. Silverman et al [22] observed that a concentration greater than 200 ppm for 15 minutes was required to cause eye, nose, or throat irritation in the majority of experimentally exposed subjects. The symptoms of irritation, although initially distinct, diminished rapidly both in experimental subjects and in occupationally exposed people [26,33].

Concentrations of dioxane in the air that are immediately dangerous to the worker's life have not been determined from occupational histories. The highest concentration that has been reported in human exposure studies was 5,500 ppm. Subjects exposed at this concentration for 1 minute [19] experienced a slight vertigo during exposure. In animal experiments, guinea pigs that were exposed at 30,000 ppm for 3 hours showed a state of narcosis after 87 minutes and died within 2 days [21]. The LC50 for rats exposed to dioxane for 4 hours was reported to be about 14,000 ppm [57]. Guinea pigs, rats, and mice exposed at 10,000 ppm for 1.5 hours twice daily seemed drowsy after the first exposure and died after 2-7 exposures [21]. Such exposures killed all mice and rats after 6-34 exposures and one guinea pig after 29 exposures; the remaining guinea pigs survived 63 exposures, and all the rabbits survived 33 exposures. All rats, mice, guinea pigs,

and rabbits survived at least 45 exposures at 2,000 ppm [19]. All mice and cats exposed at 1,400 ppm, 8 hours/day, 5 days/week, for 2 weeks survived [26].

Young et al [29], in 1976, stated that no adverse effects were seen in four subjects exposed at 50 ppm dioxane for 6 hours. Laboratory tests conducted before and 14 days after the exposure did not show changes. The only subjective effect noted was mild eye irritation which persisted throughout the exposure as reported by Chenoweth (written communication, December 1976). The authors constructed a pharmacokinetic model describing the fate of dioxane and HEAA in humans on repeated exposure at 50 ppm. A comparison of the assessment of the hazard to humans exposed repeatedly to dioxane was made by extrapolation of the toxicologic information from their rat data [30]. They concluded that exposure at the current TLV of 50 ppm dioxane [73] will not cause adverse effects on repeated exposure. This conclusion is probably based on considerations of the effects of dioxane alone and may not be valid if the workers in the dioxane manufacturing unit are also exposed to various other solvents in their work environment; in addition, the effects of alcohol consumed after working hours should be taken into consideration. It is not known yet whether the toxicity of dioxane in humans is increased with exposure to other solvents and with alcohol consumption.

The acute studies indicate that at high levels of dioxane (above 2,000 ppm) symptoms of "stomach trouble" occur, which, on continued exposure, are followed by oliguria, anuria, coma, and death. Those exposed at lower concentrations show symptoms like nausea and vomiting. The single human inhalation study reported in 1976 [29] showed no adverse effects

other than mild eye irritation on the volunteers at 50 ppm.

(b) Chronic Effects Related to Occupational Exposure

Epidemiologic studies of workers exposed to dioxane in its manufacture have been reported [34,35]. In these studies, it has been found that death rates in dioxane-exposed workers have not exceeded those of the general population, that there has been no common cause of death among them, and that the number of deaths from cancer are not significantly different from the number expected from statistics on the normal population. Examination of the health status of the workers then employed in dioxane manufacture have not resulted in indications of the existence of chronic diseases associated with dioxane exposure. Among the workers examined were some who were exposed for as long as 17-50 years. Estimates of concentrations of dioxane of the exposed workers were 0.1-23.6 ppm in one plant, 0.01-13.28 ppm in another, and 0.2-0.5 ppm in the third.

Chronic inhalation experiments with animals have been limited. One experiment was conducted [68] to determine the effects of long-term repetitive inhalation of dioxane vapor around 100 ppm, the TLV at the time of the experiment. In addition, preliminary studies at exposure concentrations of 50 and 100 ppm did not show any adverse effects when compared with controls. Hence, rats were exposed to dioxane at a concentration intended to be 100 ppm but found to be 111 ppm, 8 hours/day, 5 days/week, for 2 years. There were no indications of a developing chronic disease in the rats exposed to 111 ppm dioxane.

To study the long-term absorption of dioxane through the skin, skin painting experiments were conducted by King et al [66,70] and Perone et al [69]. Both investigators used solvents which themselves might have been

responsible for changes in the liver, kidney, or lungs. It is not certain from the first report of King et al [66] whether acetone was used as the solvent vehicle in all their studies. They stated that acetone was originally included as a solvent control in the carcinogenesis study but, when their promotional studies were started 3 months later, they noted that skin tumors and a reticulum cell malignant lymphoma occurred in control mice. A repetition of their second study did not confirm their initial observations of skin tumors.

Perone et al [69] used ethyl alcohol as the solvent control in a study which also showed hepatic tumors and pulmonary adenomas in both test and in solvent control animals.

Though these long-term studies of dermal absorption were inconclusive, it seems reasonable to interpret that absorption through the skin can take place without erythema or other external signs, and that excessive absorption of dioxane through the skin may cause liver and kidney damage similar to that seen in the acute human case described by Johnstone [31] and those observed in animal experiments of Fairley et al [21] and Nelson [50].

(c) Effects on the Kidneys

The six men who died after exposure to dioxane had all developed anuria [20,31]. The deaths occurred about 5-8 days after the initial symptoms of illness. In one case, there was a record of kidney dialysis and other treatment during hospitalization. In the other cases, there were no reports of treatment for the disease. In all six cases, microscopic findings were necrosis in the renal cortex, RBC in the lumens of tubules, and interstitial hemorrhages. The cells of the renal medulla were intact.

The concentrations of dioxane at which the five English workers were exposed were not known [20]; in the Johnstone report [31], the exposure concentrations were estimated at 205-650 ppm, and absorption through the skin had apparently occurred [31].

The only indications of kidney injury found in other workers exposed to dioxane in the English factory were traces of albumin in the urine of seven or eight men and a few RBC in the urine of two or three [20]. The concentrations of dioxane and other exposure characteristics of these men were not known.

Animal experiments demonstrated probable species differences in susceptibility of the kidney to dioxane [21,24], particularly at lower exposures. Cortical damage was apparent with vascular congestion and hemorrhages also being present. No cell degeneration was noted in the renal medulla and hemorrhages, though present, were less marked than in the cortex. Similar microscopic findings were found in the kidneys of the four species exposed at 10,000 and 5,000 ppm. At 2,000 ppm, microscopic findings in the rabbits and guinea pigs were still severe, whereas the changes in rats did not include cellular degeneration and, in mice, only vascular congestion was found. The findings at 1,000 ppm were not described in detail; it was only stated that well-marked cortical lesions were seen in all animals. In another report, rabbits repeatedly exposed to dioxane at 800 ppm died with kidney injury within 30 days [56]. Neither sufficient details of the experimental design nor the nature of the kidney injury were given in this report. In another inhalation experiment [24], no significant microscopic changes in the kidneys were found at 1,000 ppm. Torkelson et al [68] observed no changes in kidneys when rats were exposed

at 111 ppm, 7 hours/day, 5 days/week, for 2 years.

When given drinking water containing 5% dioxane, two of three dogs died after 8 days, most rats died within 30 days, and mice survived up to 67 days [21,45,48]. Grossly, the kidneys were swollen and hemorrhagic but microscopic examination of the dogs were not conducted in all studies. All rats and some mice showed severe cortical degeneration, and some of the mice showed no cell changes except vascular congestion in the kidneys even after 67 days. Hemorrhages were present throughout the kidneys. A tolerance was noted when two rats were fed 1% dioxane in drinking water for 110 days followed by 3% dioxane for 41 and 48 days; only patchy areas of degeneration were observed in the kidneys.

One of 22 guinea pigs which ingested 0.5-2% dioxane in drinking water for 23 months [63] developed an adenoma in the kidney. No other microscopic studies of the kidneys were reported in this study.

Many rats that ingested 1% dioxane in drinking water for 63 weeks [62] showed extensive changes in the blood vessels of the kidneys and the tubular epithelium, particularly in the proximal convoluted tubes, and had a granular precipitate. An early transitional cell carcinoma was found in the renal pelvis of one rat.

Rats that ingested 0.75-1.8% dioxane in drinking water for 13 months [43] showed marked alterations; in many, the changes were those of glomerulo- and pyelonephritis with characteristic epithelial proliferation of Bowman's capsules.

Rats that ingested 0.1 and 1% dioxane in drinking water [65] showed varying degrees of renal tubular epithelial degeneration and necrosis and evidence of regeneration. No microscopic changes were seen at the 0.01%

dioxane level. A renal carcinoma was found in one of two rats but this carcinoma appears not to have been related to dioxane ingestion.

Kidney changes due to repeated exposures were also found in rabbits and guinea pigs when dioxane was applied to the skin [21]. After 49 days of application, the changes consisted of cloudy swelling, congestion, and hemorrhages. After 66 days, there was extensive cortical cell degeneration and necrosis with hemorrhages. After 77 days, the cortical necrosis was more extensive and the hemorrhages extended to the medulla. After 101 days, the changes seemed less severe, but vascular congestion was extreme.

Among the workers exposed to dioxane who have been the subject of recent reports, there is little evidence of kidney involvement (only one case of kidney insufficiency) in two studies [34,35] involving exposure concentrations of dioxane less than 25 ppm. However, in view of evidence at higher concentrations, perhaps including skin penetration [20,31], and from evidence from animal experiments [21,24,43,62,64,65], it is concluded that both tubular and glomerular changes may occur in workers sufficiently exposed to dioxane.

(d) Effects on the Liver

The only evidence of liver injury among the 80 English workers examined by Barber [20] was a palpable liver in 1 of the 11 "highly exposed" workers. Livers were not palpable in two employees who had worked long shifts on the altered machine and who were away from work due to "stomach trouble." Six of the 24 German workers then employed in dioxane production had elevated SGPT levels, and the SGOT levels of 2 of these 6 workers were slightly elevated. All six workers were known to have consumed about 80 g of alcohol daily in the form of beer or wine. When

five of these workers reduced their beer consumption, their transaminase levels returned to normal. Serum creatinine was slightly elevated in five workers.

Animal experiments have shown probable species differences in the susceptibility of the liver to dioxane [21,26,43,45,48,56,62,64-68].

Injury to the liver was noted to vary from a normal condition at 1,000 ppm to vascular congestion and cell degeneration as the concentrations were increased to 10,000 ppm with repeated inhalation exposure of 1.5 hours twice daily for 5 days, once on the 6th day and no exposure on the 7th in a repeated pattern [21]. The narcotic-anesthetic effects also seemed to increase with the total dosage as represented by the product of concentration and exposure time [50]. The signs of narcosis seemed to appear earlier in experiments where pure dioxane was used but no differences between the pure and commercial grades were noted on microscopic observation of the liver [26]. No evidence of damage to liver was found after chronic inhalation exposures of rats at 111 ppm for 7 hours/day, 5 days/week, for 2 years.

These studies indicate that chronic inhalation at concentrations of 2,000 ppm and above cause liver damage and increase the narcotic-anesthetic effect in animals.

Acute and subacute administration of dioxane by ingestion [21,45,48,54] caused liver injury that varied with the species as well as the amount of dioxane used. Mice seemed to be the least affected and showed only vascular congestion compared to rats, rabbits, or guinea pigs which showed cellular degeneration as well as extreme vascular congestion. Cellular degeneration was more marked when 5% dioxane was given to the animals in drinking water.

In chronic experiments, concentrations of 1.4 and 1.8% dioxane in drinking water for 13 months [64] produced hepatic "incipient" tumors as well as hepatomas, while ingestion of 0.75 and 1.0% dioxane did not cause any changes. In another report [43], some rats given 1% dioxane for 13 months also showed "incipient" tumors which the investigators considered precancerous changes, but rats given the same amount for 8 months did not show any changes. In an earlier report [62], 6 of 26 rats were stated to have produced hepatic tumors but no further description of the tumors was provided.

In a more recent report, a dose-response relationship was seen when rats were fed 0.01, 0.1, and 1.0% dioxane in drinking water [65]. Hepatic tumors and carcinomas were seen at the 1.0% dose; in addition, there was evidence of hepatic regeneration as indicated by hepatocellular hyperplastic nodule formation at both 0.1 and 1.0% levels. No changes were observed among rats which ingested 0.01% dioxane. However, 1 of 120 control rats showed a hepatocellular carcinoma, another showed cholangiocarcinoma, and two others showed hepatic tumors.

Varying degrees of liver damage were also seen when dioxane was applied to the skin of rabbits and guinea pigs, the total number of applications varying from 75-160 [21]. Two applications were given each day for 5 days, one on the 6th, and none on the 7th, in a repeating pattern. After 75 applications, the liver was still normal on microscopic examination but vascular congestion, later followed by cellular degeneration, was noted after 105 and 121 applications. However, in both rabbits and guinea pigs, when tested after 160 applications, only vascular congestion was noted. This may again mean that either a tolerance had been

developed or that cellular regeneration may have taken place.

In the long-term skin painting experiments, King et al [66] stated that liver lesions of a mild nature were seen which included megalocytosis, occasional distended bile canaliculi, occasional centrilobular necrosis, cuffed triad vessels, and mild peripherolobular fibrosis. No details were given as to how many mice showed these changes.

In the skin-painting experiments of Perone et al [69], hepatic tumors and pulmonary adenomas were noted in alcohol-treated control mice and in dioxane-treated mice.

Despite the fact that these skin application studies did not show definite hepatic changes compared to controls, dioxane can penetrate through the skin and is a hazard if proper protection against skin absorption is not taken.

In conclusion, liver changes such as vascular congestion and cellular degeneration have been noted at concentrations higher than 2,000 ppm by inhalation and above 1.0% in drinking water. The skin absorption experiments were inconclusive, but it is evident that dioxane can penetrate the skin and cause damage if absorbed in large quantities, similar to the damage observed as a result of ingestion or inhalation.

(e) Effects on Other Organs and Tissues

Edema of the brain was seen at autopsy in the three workers exposed to dioxane who had been hospitalized prior to their deaths [20,31]. Two of the three also had edema of the lungs [20]. These brain and lung changes were probably terminal, possibly agonal, rather than specific toxic effects of dioxane. No changes in tissues and organs other than to the kidneys and liver were found among workers exposed to nonlethal concentrations of

dioxane [20,31] nor were any noted among workers in the two epidemiologic studies [34,35]. However, in animals exposed to dioxane, changes in tissues and organs, other than kidneys and liver, have been reported [19,21,24,26,63,66,70].

In inhalation experiments where animals were exposed at 30,000 ppm for 3 hours, hyperemia of the brain and congestion of the lungs were seen when animals were killed immediately after exposure [19]. Animals that died within 1 day of exposure at a concentration of 30,000 ppm for 30 minutes showed congestion of the lungs and hemorrhagic areas in the mucous membranes of the stomach. An animal that died 2 days after the exposure at 30,000 ppm showed a beginning bronchopneumonia and severe congestion of the surface vessels of the brain. When animals were killed 9-10 days after the experiment had been completed, no brain or lung changes were seen. In the studies by Fairley et al [21], animals survived only 2-7 exposures at 10,000 ppm, pulmonary edema being the main cause of death. At 5,000 ppm and up to 94 exposures, signs of bronchopneumonia were seen only in one rabbit, while the remainder of the animals showed liver and kidney damage. No changes were seen in the lungs or organs other than the liver and kidneys of rabbits, guinea pigs, rats, and mice exposed to dioxane at 2,000 or 1,000 ppm for up to 102 and 202 exposures [21]. Hyperemia and edema of the lungs were seen when mice, rats, rabbits, and guinea pigs were exposed at 37,500 ppm for 3 hours or 4,000-14,000 ppm for 8 hours [24].

In view of the evidence of irritation of the respiratory tract at only very high levels, injury to lungs is judged not likely to be an effect at concentrations in an occupational environment.

When 5% dioxane was given in drinking water [21], gastroenteritis was seen in two rats that died after 31 and 34 days of exposure. Four other rats similarly exposed for 17-67 days showed no such damage. Gross examination of six mice given 5% dioxane in drinking water for up to 67 days showed enlarged spleens [21]. When three dogs were given 5% dioxane in drinking water, two died after 8 days and the other was killed on day 45. Gastrointestinal tracts of the two dogs that died after 8 days were congested with many hemorrhagic areas and their stomachs contained bloody fluid. Brains were edematous with areas suggestive of hemorrhages and the lungs were congested. Six of twenty-two guinea pigs given 0.5-2.0% dioxane in drinking water for 23 months showed peri- or intrabronchial epithelial hyperplasia, nine showed nodular mononuclear infiltration in the lungs, and gall bladder carcinomas were seen in two of the animals [63]. However, 6 of 10 control animals showed changes including mononuclear cell accumulation (4/10), hyperplasia of bronchial epithelium (1/10), and cartilaginous formation in the lung (1/10). When rats were given 0.75, 1.0, 1.4, or 1.8% dioxane in drinking water for 13 months, one, one, two, and two, respectively, developed tumors of the nasal cavity [43]. Chronic bronchopneumonia, including murine pneumonia, was seen in rats that died after exposure to 0.5-1.0% dioxane in drinking water for up to 42 weeks [66].

Pulmonary edema and edema of the brain were seen at concentrations of 10,000 ppm by inhalation and 5% dioxane when ingested. At lower levels, no changes in any organs other than the liver or kidney were seen.

(f) Synergistic Effects of Dioxane

Studies have been reported which suggest that the toxicity of dioxane

may be enhanced when workers are exposed to other chemicals [35,43,52,53,66,70]. Smyth et al [52,53] reported that acute toxicity was greater than additive when dioxane was given intragastrically to rats in pair-combinations with acetonitrile or tetrachloroethylene, whereas the toxicity was less than additive when dioxane was given with propylene glycol. These differences occurred when the two chemicals were given in equal volumes as well as in volumes proportional to their respective LD50's.

The acute toxicity of dioxane was also reported to have been increased when the chemical was given in conjunction with microsomal enzyme-inducing agents [43], where the LD50 of dioxane was reduced from 5.6 to 5.2 ml/kg and kidney changes were more severe when rats were treated with MC 23 hours previously. This change in LD50 from 5.6 to 5.2 ml/kg, though statistically significant, is not large where toxicity is concerned. Animals pretreated with DMBA showed a higher incidence of skin, lung, and lymphatic tissue lesions when mice were treated topically with dioxane [66,70], but no details with DMBA alone were provided. It is not known whether the incidence of lesions seen in the skin, lung, and lymphatic tissue were due to DMBA, dioxane, or both.

It is not known whether alcohol promoted the toxicity of dioxane in the workers who died, became ill, or showed subclinical evidence of dioxane toxicity [31,34]. In the case of the worker who died after 1 week's exposure to dioxane and who was known to be a heavy beer drinker, it is evident that dioxane penetrated the skin, apparently causing liver and kidney damage, since he dipped his hands in a bucket containing dioxane during his work. Secondly, six workers who consumed more than 80 g of

alcohol/day showed elevated transaminase levels, but these levels became normal in five of them when they reduced their alcohol consumption. If, indeed, a synergistic effect had occurred, the transaminase levels would not likely have returned to normal, and toxic effects on tissues or organs would have been expected.

Studies indicate that dioxane may markedly increase the effects of known carcinogens or microsomal enzyme stimulators like DMBA, MC, etc. Evidence of significant enhancement of toxicity by other materials is not strong but is at least suggestive. However, this evidence is from acute toxicity, and potentiation of chronic toxicity is not interpreted from available information. As a result, a standard based in part on potentiation of dioxane toxicity by other substances is not proposed. However, it does seem appropriate that dioxane workers exposed to other chemical substances, especially other solvents, and workers who drink significant amounts of alcohol should be studied for evidence of potentiating toxicity. Experimental research to elaborate interaction is also important.

Carcinogenicity, Mutagenicity, Teratogenicity, and Effects on Reproduction

(a) Carcinogenicity

The carcinogenic potential of dioxane was first reported by Argus et al [62] in 1965 when they gave the chemical to rats in drinking water. Since then several other studies [43,63-67,70] have shown the induction of tumors in guinea pigs, rats, and mice when dioxane was administered in drinking water or applied to the skin. However, a description of the types of tumors observed was not given in some of the reports [43,62,64,66,70].

A summary of cancers and other tumors observed by various investigators is presented in Table III-10. The second report of King et al [70] has not been included in the table since the authors stated that later studies in both skin painting and oral administration did not show the same high degree of promotional activity of dioxane as was seen in the initial study. Also, it is not known whether acetone was used as the solvent vehicle. The authors [70] stated that tumors were induced in mice when acetone alone was administered. These studies have been reviewed in more detail in the previous section and are summarized here.

Neoplastic lesions most often described in rats given dioxane in drinking water were tumors of the liver and of the nasal cavity [43,62,64,65,67]. In the liver, they were described as "incipient" liver tumors, hepatomas, and hepatocellular carcinomas. In the nasal cavity, epithelial carcinomas, olfactory neuroblastomas, seromucous adenocarcinomas, and rhabdomyosarcomas were observed. Another important finding was the dose-response relationship reported by two groups of researchers in rats given dioxane in drinking water [43,65]. In the study of Kociba and coworkers [65], no tumors were observed at the 0.01% dioxane concentration. At 0.1%, one hepatocellular carcinoma (statistically not significant) was seen. At 1.0% dioxane doses, a significant increase ($P<0.05$) was reported in hepatic tumors of all types, in hepatocellular carcinomas, and in nasal carcinomas as compared to those seen in control animals (12/66 vs 2/106, 10/66 vs 1/106, and 3/66 vs 0/106, respectively). In the second study [43], the incidence of liver tumors was 14, 28, 53, and 82% when rats were given 0.75, 1.0, 1.4, and 1.8% of dioxane, respectively.

TABLE III-10

SUMMARY OF CANCER AND OTHER TUMORS REPORTED IN ANIMALS ADMINISTERED DIOXANE*

Ref No.	Species	Route	Dose	Duration (wk)	Neoplastic or Preneoplastic Changes
62	Rat	Drinking water	1% after 75 mg terramycin	63	6 hepatomas, 1 renal adenoma, 1 leukemia, lung hyperplasia
44	Mouse	ip	0.16-0.31 ml/kg	3 inj/wk for 4 wk	24 gross pulmonary tumors
63	G pig	Drinking water	0.5-2.0%	100	3 hepatomas (undescribed), 1 renal adenoma, 9 lung hyperplasia, 2 gall bladder carcinomas
64	Rat	"	0.75-1.8%	56	1 epithelial carcinoma, 1 squamous cell carcinoma, 2 adenocarcinomas of nasal cavity, 2 hepatic carcinomas
43	"	"	"	56	Increase in "incipient" hepatic tumors with increasing dose, marked alterations in kidney
67	"	"	0.5-1.0%	110	Nasal cavity carcinomas, rhabdomyosarcomas and seromucous adenocarcinomas

TABLE III-10 (CONTINUED)

SUMMARY OF CANCER AND OTHER TUMORS REPORTED IN ANIMALS ADMINISTERED DIOXANE*

Ref No.	Species	Route	Dose	Duration (wk)	Neoplastic or Preneoplastic Changes
67	Mouse	Drinking water	0.5-1.0%	90	Chronic hepatitis, hepatocellular carcinomas, hyperplasia
65	Rat	"	0.01-1.0%	102	Carcinomas of the nasal cavity, hepatic, renal, and bronchial carcinoma, and lymphosarcomas
69	Mouse	sk	Various grades with and without impurities	78	Hepatic cell adenomas
68	Rat	inh	111 ppm, 7 hr/d, 5 d/wk	104	Fibromas of kidney and lung, 1 lung carcinoma, subcutaneous fibrosarcomas and lymphosarcomas

ip = intraperitoneal, sk - skin application, inh = inhalation

In another report of what appears to be the same experiment, these authors [64] reported 1, 1, 2, and 2 squamous cell carcinomas in rats given water containing 0.75, 1.0, 1.4, and 1.8% of dioxane, respectively.

In the Holmes report [67], a dose-response relationship was also apparent in rats. A total of eleven malignant tumors were seen in the control group, 28 in the 0.5% dioxane group, and 41 in the 1.0% group. One of 32 examined controls, 23/57 of the rats given 0.5% dioxane in drinking water, and 23/51 of the 1% dioxane group had squamous cell carcinomas of the nasal cavity. Two of the 0.5% dioxane group had olfactory neuroblastomas, and one had a rhabdomyosarcoma. Seven of the 1% group had olfactory neuroblastomas, and two had seromucous adenocarcinomas. The two seromucous adenocarcinomas, 4/9 neuroblastomas, and 4/46 squamous cell carcinomas had metastasized to other sites.

In mice given dioxane in drinking water [67], a dose-response relationship was seen with 4/99 of the examined controls, 24/94 of the 0.5% dioxane group, and 44/87 of the 1.0% group having developed hepatocellular carcinomas.

In 22 guinea pigs given 0.5-2.0% dioxane in drinking water, two had gall bladder carcinomas, three had early hepatomas, nine had lung hyperplasia, and one had an adenoma of the kidney [63]. Four of ten controls showed mononuclear cell accumulation and one epithelial hyperplasia in the lung.

Evidence of cancer induction and promotion were reported when dioxane was applied to the skin of mice [66]. In mice that survived 60 weeks of dioxane treatment, one suspected carcinoma, not confirmed microscopically, was reported in 1 of 22 males and one unspecified subcutaneous tumor was

seen in 1 of 25 females. One reticulum cell malignant lymphoma was seen in the eight mice that died. In animals pretreated with DMBA 1 week prior to dioxane application, 2 papillomas and three suspected skin carcinomas were reported among 9 of 60 surviving mice. A squamous cell carcinoma of the nasal septum was observed in one mouse that had a skin papilloma. Seven of the nine surviving mice had malignant lung lesions, three of which were considered metastatic from other unspecified sites. These findings of cancer induction and promotion are judged to be inconclusive, as results of this study [66] were not duplicated when the study was repeated [70]. It is unclear whether acetone was used as a solvent vehicle in these two studies. In the second study [70], acetone alone appeared to induce tumors. In the skin painting study by Perone et al [69], results were also inconclusive; hepatic tumors and pulmonary adenomas were observed in both control and dioxane-treated animals.

There was no statistically significant evidence ($P<0.05$) of increased tumor incidence in 423 rats exposed at 111 ppm dioxane for 2 years [68]. However, an experimental animal population of several hundred will usually be too small to detect a difference of tumor incidence of 10/100,000, which is a realistic cancer incidence to be concerned with in man [74]. If rats are much more sensitive to dioxane-induced cancer than man, an experimental population of several hundred rats might suffice, but information on relative sensitivities of the two species is lacking. In fact, a speculation that rats are much less sensitive than man cannot be disproved by available data. Therefore, the results from the exposure of rats at 111 ppm [68] do not provide enough assurance that similar exposure of humans will not result in an increased incidence of tumors.

In the epidemiologic studies of Dernehl (written communication, April 1976), of Thiess and coworkers [34], and of Buffler et al [35], no significant differences were seen by the authors between the observed and expected number of cancer deaths in the worker population. In the epidemiologic study conducted on 74 workers, Thiess et al [34] noted no statistically significant difference between the 12 deaths observed in the plant and the 14.5 deaths expected. Two men, aged 66 and 71 years, respectively, apparently died from cancer, the former from a lamellar epithelial carcinoma of the left lumbar region and the latter from a myelofibrotic leukemia. In the study conducted by Buffler and associates [35], 2 of 7 deaths among the 100 workers in the production area were due to cancer, a carcinoma of the stomach and an alveolar cell carcinoma. These two men had been exposed to dioxane for less than 4 years; one had also been exposed to hydrogen chloride, carbon tetrachloride, perchloroethylene, and trichloroethylene, and the other to vinyl chloride for about 11 years and to methylene chloride for 1.5 years. The two cancer deaths were not significantly different at a P value of 0.05 or less from expected deaths due to cancer. One of five deaths among 65 workers in the processing area was also due to cancer, a malignant mediastinal tumor. This death was not significantly different from expected cancer deaths from processing area workers. In the unpublished data provided by the other manufacturing concern (written communication, CU Dernehl, April 1976), four cancer deaths in the dioxane unit occurred; one man died with cancer of the colon, one with lymphosarcoma, one with lung carcinoma, and one with glioblastoma.

Although no statistically significant increase was seen between the total number of observed and expected cancer deaths in the reports from Dernehl (written communication, April 1976), Thiess et al [34], and Buffler and coworkers [35], these results are not conclusive evidence that the reported concentrations of dioxane in air represented safe worker exposure levels. The populations at risk were very small, 74, 165, and 80 workers. Statistical tests may be too insensitive to detect very small increases in cancer death rates in such populations. Furthermore, these studies gave total cancer mortality rates, rather than rates from tumors of specific kinds and sites. While understandable in these cases in consideration of the small populations studied, appropriate mortality studies should classify tumors, and compare by classification with rates in the normal population.

From the animal studies, development of tumors from large doses of dioxane seems apparent in rats and guinea pigs. Findings in rats have been confirmed by independent investigators [43,62,64,65], although shortcomings exist in some of the studies. Results at lower doses are inconclusive in that the test populations of animals may have been of insufficient size to detect those lower incidences of cancer induction that are typical of humans exposed to environmental chemical carcinogens. One inference from the human and animal metabolism studies [29,30,36] is that dioxane is carcinogenic only at high doses that overwhelm the ability of the organism to detoxify and excrete dioxane adequately. This inference, drawn by the authors [29] of the studies as a conclusion, is impressive, but not completely persuasive. Their data can also be interpreted to suggest that, even at lower concentrations, metabolism of dioxane was not complete. If

so, metabolic defenses against the possible cancer-inducing properties of dioxane at low doses may not be complete, assuming that metabolic defenses against induction of cancer by dioxane exist. Thus, although the evidence from animal studies suggests that dioxane is a carcinogen in man, no quantitative data have been found which delineate safe and unsafe concentrations of dioxane for humans.

(b) Mutagenicity and Teratogenicity

Franceschini [60] studied the effect of dioxane on the growth of chick embryo tibial buds. The tibia were removed from 7- to 8-day-old embryos and studied in pairs as described in an earlier section. In the same study, thalidomide was also used. Hypertropic and vacuolized chondroblasts in diaphysis and reappearance of mitosis in the metaphysis of the tibia were seen in media containing dioxane or thalidomide. The relevance of this study to any conclusion on human teratogenesis is not now clear. Direct injection of chemicals into the avian embryo precludes maternal modification, such as metabolism, detoxication or activation, excretion, or placental transfer. Thus, confirmation in mammals is needed before extrapolating these data to a standard for occupational exposure to dioxane.

The other study related to teratogenesis conducted by Schwetz et al [61] showed a few terata in fetuses of both rats and mice, both control and treated, but the terata were not of a consistent type. Dioxane (about 3.5%) was added to 1,1,1-trichloroethane as an additive and the exposure concentration of dioxane was 32 ppm. These results are inconclusive, at least so far as they pertain to dioxane exposure.

No studies of mutagenicity in animals or humans exposed to dioxane have been reported.

Summary Tables of Exposure and Effects

Case summaries of humans occupationally exposed to dioxane as well as inhalation experiments on humans are presented in Tables III-11 and III-12. The summaries of animal experiments with regard to parenteral injection, ingestion, percutaneous administration, and inhalation are presented in Tables III-13 and III-14.

TABLE III-11

CASE SUMMARIES OF OCCUPATIONAL EXPOSURE TO DIOXANE IN HUMANS

Ref. No.	Exposure Concentration (ppm)	Time	Effects
31	208-650	1 wk	Liver and kidney damage, death
20	Unknown	5 d	Liver and kidney damage, lung edema, death
"	"	4-6 wk	Anuria, death
"	"	"	Liver and kidney damage, death
"	"	"	Liver and kidney damage, coma, death
"	"	7-8 wk	Liver and kidney damage, lung congestion
32	-	-	Skin erythema, no death

TABLE III-12

EXPERIMENTAL INHALATION EXPOSURES IN HUMANS

Ref. No.	Exposure Concentration (ppm)	Exposure Time (min)	Effects
19	5,500	1	Irritation of eye, nose, throat vertigo
21	2,000	3	Strong odor, tolerance
19	1,600	10	Mucous membranes, burning
21	1,000	5	Odor
22	300	15	Irritation of eye, nose, throat
"	200	480	Safe concentration for sensory tests
27	170, 270	-	170 ppm - odor threshold 270 ppm - pronounced odor
26	2.8, 5.6	-	2.8 ppm - detection threshold 5.6 ppm - recognition threshold

TABLE III-13

INJECTION, PERORAL AND PERCUTANEOUS EXPERIMENTS IN ANIMALS*

Ref. No.	Species	Route	Dose; Duration	Effects
38	Rabbit	iv	0.2 ml/kg; single dose	No visible effect
"	"	"	0.2 ml/kg; weekly intervals	"
"	"	"	1.0 ml/kg; single dose	Temporary narcosis
"	"	"	1.5 ml/kg; single dose	Minimum lethal dose
"	"	"	0.5 ml/kg, 8 doses + 1.5 ml/kg, 5 doses	Tolerance, transient polyuria
45	Rabbit	"	1.5 ml/kg; single dose	Liver and kidney damage
39	Mouse	sc	10 ml/kg; single dose	Minimal fatal dose
24	Rabbit	"	1-2 ml/kg; single dose	Albuminuria
"	G pig	"	2-6 ml/kg; single dose	Liver and kidney damage
"	Cat	"	2 ml/kg; single dose	"
40	G pig	"	(a) 1 ml/g pig; 10 d (b) 0.2-0.75 ml/g pig; 35 d	Internal organ damage, blood changes "
42	Mouse	ip	1.65 ml/kg; single dose	100% mortality
"	"	"	0.76 ml/kg; single dose	LD50

TABLE III-13 (CONTINUED)

INJECTION, PERORAL AND PERCUTANEOUS EXPERIMENTS IN ANIMALS*

Ref. No.	Species	Route	Dose; Duration	Effects
43	Rat	ip	5.6 ml/kg; single dose	LD50
"	"	"	10 mg MC + 4.9-5.3 ml/kg; single doses	After MC, LD50 5.18 ml/kg
44	Mouse	"	1.25-5 ml/kg; 3 inj/wk/4 wk	Fatal to mice
"	"	"	0.16-0.63 ml/kg; 3 inj/wk/4 wk	0-66% deaths
41	Rat	"	1.8-4.4 ml/kg; single doses	0-100% mortality
21	Rabbit	pc	10 drops/rabbit; 5 drops/g pig; 2/d/5d;1/6th, 0/7th, repeated	Renal and hepatic lesions in some
50	"	"	Pure, 1.75-4.8 hr/2 wk	Temporary erythema; high doses, death
50	"	ig	0.8-8.8 ml/kg; Single doses	0-100% mortality
38	"	"	1 ml/kg; single dose	Temporary narcosis
38	"	"	2 ml/kg;single dose	Minimal lethal dose
49	Mouse	"	4.5-7.5 ml/kg; single doses	Mortality increased with dose
49	G pig	ig	3.90 ml/kg; single dose	LD50
50	Rat	"	6.0 ml/kg; single dose	"

TABLE III-13 (CONTINUED)

INJECTION, PERORAL AND PERCUTANEOUS EXPERIMENTS IN ANIMALS*

Ref. No.	Species	Route	Dose; Duration	Effects
50	Rabbit	ig	2.0 ml/kg; single dose	LD50
52	Rat	"	6.47 ml/kg; single dose	"
21	Mouse	po	5% daily; 11-67 d	Liver and kidney damage
45	"	"	1 ml/kg, 1-12 d	"
45	"	"	1%/110 d, + 3%/41, 48 d	Liver and kidney damage, slight, tolerance
48	Dog	"	5%/10d, twice daily	Lung, liver, kidney damage, slight
54	Rat	"	(a) 1-10 d (b) 9 d + ad lib water/2, 4, 7, 9 d	Kidney damage

iv = intravenous, sc = subcutaneous, ip = intraperitoneal, pc = percutaneous, ig = intragastric, po = drinking water

TABLE III-14

ANIMAL INHALATION EXPERIMENTS

Ref. No.	Species	Exposure Concentration, ppm; Duration	Effects
24	G pig, rabbit, rat, mouse	37,500; 3 hr	50% death; lung, liver, and kidney damage
26	Mouse	1,400-39,000; single doses	Death above 8,300 ppm
"	Cat	12,000-31,000; until irritation noted in min	Narcosis
24	G pig, rat, rabbit, mouse	4,000-11,000; 8 hr	Liver and kidney damage
19	G pig	1,000-30,000; maximum 480 min	Mucous membrane irritation; congestion, edema of lungs
55	Rat	1,500-6,000; Avoidance to buzzer, shock, in min	Behavioral changes above 3,000 ppm
21	G pig, rat, mouse	1,000-10,000; twice/5 d, once/ 6th and 0/7th d	Liver and kidney damage increased with doses; death at 10,000 ppm after 2 exposures
24	Cat, mouse, rabbit, g pig	1,350-2,700; 8 hr/34-45d	Liver and kidney damage
26	Cat	1,400; 6.5 hr/ 14 d	Increased blood counts
68	Rat	111; 7 hr/d, 5 d/wk, for 2 yr	Various tumors in both control and dioxane-treated