III. BIOLOGIC EFFECTS OF EXPOSURE

Ethylene dibromide, also known as 1,2-dibromoethane and ethylene bromide, is a clear, colorless, heavy liquid at room temperature with a distinctive odor described as "characteristic, mildly sweet" [1]. The minimum concentrations of ethylene dibromide in air reported to be detectable by odor range from 10 ppm (77 mg/cu m) [2] to 25 ppm (192.5 mg/cu m) [3]. Selected chemical and physical properties of ethylene dibromide are listed in Table XII-1 [1,3,4].

Ethylene dibromide is a reactive molecule which may form covalent bonds under biologic conditions [5,6]. Because of the two replaceable bromine atoms, ethylene dibromide is considered to be a bifunctional alkylating agent that is capable of introducing cross-links into biologic materials [6]. It tends to react with nucleophilic organic compounds [7] more readily with those that are relatively easily polarized, such as those containing sulfhydryl or amino groups, than with those that are less readily polarized, such as acids and ketones.

The half-life of ethylene dibromide in water at 20 C and at a pH of 7 is about 14 years [7]. Although the biologic half-life of ethylene dibromide in humans is unknown, the presence of large numbers of nucleophiles in biologic tissue suggests that the half-life would be considerably shorter than that in water. The approximate biologic half-life of ethylene dibromide after intravenous (iv) injection in rats was less than 2 hours, and in chicks, it was less than 12 hours [8]. An approximate biologic half-life of 14C-labeled ethylene dibromide in mice and in guinea pigs can be estimated from the data presented by Edwards et

al [9] and Plotnick and Conner [10] as less than 48 hours. This indicates that either spontaneous reactions with nucleophiles, enzymatically catalyzed degradative reactions, or efficient excretory mechanisms predominate in biologic systems.

Alkyl bromides, such as ethylene dibromide, readily react with thiols, amines, alcohols, and other nucleophilic biochemical constituents [11-13]. The initial monoalkylation product between ethylene dibromide and substrate heteroatoms, such as nitrogen, oxygen, or sulfur, is a "half-mustard" (a thiane) reagent which may spontaneously cyclize under biologic conditions to form a strained three-membered ring. This highly reactive intermediate product may then undergo a second alkylation reaction with cellular constituents. When both alkylation reactions occur with cellular DNA, the covalent cross-links between the DNA strands may prevent the normal separation of the strands during DNA synthesis and subsequent cell division [11,13]. Thus, such bifunctional alkylating agents as ethylene dibromide tend to possess a considerably greater biologic activity than monofunctional agents of the same primary reactivities [11].

The covalent reaction of ethylene dibromide with biologic materials may alter the chemical behavior and physical characteristics of the cellular constituents so as to prevent the altered molecules from functioning normally in physiologic processes. The formation of stable reaction products may account, in part, for the subsequent deleterious effects observed in biologic systems exposed to ethylene dibromide. The alkylation by foreign chemicals of the biologic materials controlling cellular metabolism is the most plausible basis for the induction of genetic and neoplastic alterations after the exposure of biologic

populations to alkylating agents. When the risk of induction of adverse biologic changes, such as mutation and neoplasia, increase as a cumulative function of the total dosage, then the observation of measurable effects may not be possible until after long periods of exposure to low concentrations of the inducing agent [7,14].

Extent of Exposure

Ethylene dibromide-manufacturing processes are based on the bromination of ethylene [1]. Natural bromide-containing brines are treated with chlorine to release elemental bromine through anionic replacement. The reaction between gaseous ethylene and liquid bromine (which may contain traces of chlorine) yields a mixture of ethylene dibromide and reaction side-products, including very small amounts of vinyl bromide, ethyl bromide, and ethyl chlorobromide [15]. If the reaction temperatures and pressures are carefully controlled, the purity of the ethylene dibromide can approach 99.95% [15]. Ethylene dibromide can also be produced by the hydrobromination of acetylene [2], although this process is of little commercial value today.

In 1921, the antiknock properties of tetraalkyl lead compounds in the internal combustion engine were discovered [16]. To prevent the deposition of lead on the cylinder walls, a substance capable of reacting with the lead to aid its removal from the engine was needed. Ethylene dibromide was found to be such a substance and has been used since then in leaded gasoline as a lead scavenger.

The US production of ethylene dibromide increased from an estimated 64 million pounds in 1940 to over 331 million pounds in 1973 [17]. This

fivefold increase can be related to the increased consumption of gasoline containing ethylene dibromide as an additive, which has always been its largest use [1]. About 85% of the ethylene dibromide produced during the last few years has been used as a constituent of antiknock mixtures containing tetraethyl lead [17,18]. Other applications (about 15%) include use as a fumigant-insecticide or nematocide, as a synthetic intermediate, and as a specialty solvent for resins, gums, and waxes [18]. Ethylene dibromide has previously been used as a gauge fluid, in fire extinguishers, and as an industrial solvent [2,19]; however, these applications are now limited or nonexistent. Although over 100 pesticides registered with the Environmental Protection Agency contain ethylene dibromide [19,20], the compound is not available as an over-the-counter product [18].

The production of ethylene dibromide is the largest single use of bromine, and, as such, the locations of manufacturing plants have usually been near the major sources of bromine [1]. Until 1961, the major source of bromine for ethylene dibromide manufacture was seawater; since 1961, the major source of bromine has been underground brine deposits, and the manufacturing of ethylene dibromide has been redistributed to be near these natural bromide brine wells in Michigan and Arkansas [1]. A number of other occupations in which a potential for ethylene dibromide exposure exists are listed in Table XII-2 [21].

NIOSH estimates that approximately 9,000 employees (manufacturing, formulating, fumigating) are potentially exposed to ethylene dibromide in the workplace. If one includes gasoline station attendants, this figure becomes much larger (about 660,000).

Historical Reports

One of the first instances of ethylene dibromide intoxication was described by Marmetschke [22] in 1910. This case history described the accidental use of ethylene dibromide instead of ethyl bromide as an anesthetic or narcotic agent. Although this report is historical rather than clinical, it is one of the few relating to human exposure which give the amount of ethylene dibromide administered to each patient and the clinical symptoms resulting from the exposures. Thus, this report will be discussed in detail in Effects on Humans.

Another early case of occupational exposure to ethylene dibromide, which will also be discussed in Effects on Humans, was described in 1928 by Kochmann [23].

Effects on Humans

In 1910, Marmetschke [22] wrote about a case of ethylene dibromide poisoning that occurred as the result of the mistaken administration of ethylene dibromide to a patient instead of ethyl bromide. A woman was administered the contents of a 70-g bottle of ethylene dibromide in aliquots of about 10 ml (22.0 g) through a gauze mask without producing the expected anesthesia. She coughed constantly during the administration and was dizzy at the completion of the application. She began to vomit and continued vomiting throughout the night. The patient complained of a burning sensation in her chest and of inability to sleep. During the night, she had diarrhea, and the next day she became dizzy again after exertion. She became very restless and nervous and complained of having trouble in breathing. She continued to complain of thirst, abdominal pain,

and a burning sensation in her chest. The next day, she had uterine hemorrhaging, and died approximately 44 hours after she received the ethylene dibromide.

The results of the autopsy showed that the skin was a very pale blue and that the vessels of the skin were filled with blood [22]. The body cavities contained a clear reddish liquid. Examination of the internal organs showed advanced stages of parenchymatous degeneration of the heart, liver, and kidneys. Signs of upper respiratory tract irritation and extensive surface hemorrhage were found, in addition to swelling of the pulmonary lymphatic glands. Several hemorrhages were also noted in blood vessels of the mediastinum. Microscopic examination showed fatty degeneration of the cardiac musculature and of the liver cells. Marmetschke [22] concluded that the woman became ill from inhalation of ethylene dibromide, which produced general weakness, nausea, vomiting, diarrhea, chest pains, coughing, shortness of breath, cardiac insufficiency, and hemorrhagic diathesis with bleeding from the uterus.

In 1928, Kochmann [23] described a case history of an employee exposed repeatedly to ethylene dibromide vapor in the course of his work in the production of the chemical. The employee was exposed at an unknown concentration of what was described as a foul-smelling, pungent vapor for only short periods. He was suffering from an irritation of the conjunctiva with external swelling of the lower lids and swelling of the glands under the chin and in the angle of the jaw. He was disturbed and seemed pale and fatigued.

The employee returned to his work shortly thereafter, but soon became ill again [23]. This time, he suffered from conjunctivitis, pharyngeal and

bronchial irritation, severe loss of appetite, headaches, and depression. His condition improved rapidly on cessation of work and resumption of treatment. The concentration of airborne ethylene dibromide at which he was exposed was not stated.

In 1938, Pflesser [24] reported that a seaman aboard a destroyer accidentally spilled some gauge fluid in his boots. This fluid contained 55 parts of ethylene dibromide, 30 parts of tetrachloroethane, 15 parts of paraffin oil, and a trace of Sudan Red B. He continued working without emptying or removing his boots. During the following night, burning pain developed in both feet, and, the next morning, his feet were reddened, extremely painful, and blistered between the toes. The attending physician observed that the feet were inflamed and very sensitive to pressure, but that no vascular disturbances or neurogenic lesions were evident. With extensive treatment, complete healing occurred after 14 days.

Pflesser [24] subjected eight human volunteers and himself to the gauge fluid and to each of the components separately to determine their effects on the skin. One milliliter of the gauge fluid was placed on the hands of the volunteers and rubbed between the hands for about 1 minute. Two hours later, the hands were washed with soap and water. None of the subjects developed any symptoms of adverse effects on the day of the test or later. Pflesser observed that, after rubbing the fluid between the hands for 1 minute, the liquid could no longer be seen on the skin, and he concluded that it had been absorbed or had evaporated.

In an additional experiment [24], the same volunteers rubbed 1 ml of the gauge fluid into the skin of the right forearm for 1 minute. Again, washing with soap and water was done after 2 hours. No symptoms of

irritation were seen immediately after the exposure or later.

In a third experiment [24], the same volunteers were subjected to 1 ml of the gauge fluid on a cotton swab that was placed on the left forearm and covered; exposure time was 2 hours. After a few minutes, all eight subjects noted a sensation of heat at the application site, sometimes complaining of a pronounced burning of the skin. At the end of the 2-hour exposure, a strong reddening of the skin had appeared at the application site in all cases, and, in one case, pinhead-sized blisters had developed. During the next 12 hours, a severe burning pain developed in addition to small blisters which merged into large ones. The blisters initially contained a clear, amber-yellow, thick liquid, but the liquid had a pronounced tendency to coagulate because of its high protein content. The area immediately surrounding the application site was edematous and swollen, and, in some cases, the swelling extended from the fingertips to the upper arm. In one case, the axillary lymph nodes were moderately swollen and painful. Even with topical cod liver oil treatment, the skin did not grow back until 13-17 days after exposure, and surface scars persisted for at least 4 months. Pflesser [24] stated that all subjects felt severely ill during the first days following exposure. In two subjects, dermatitis, consisting of severe swelling and reddening of the skin accompanied by intensive itching, persisted in the area of the application for several weeks.

To determine which component of the gauge fluid was responsible for the adverse effects on the skin, Pflesser [24] subjected the same eight subjects and himself to each of the individual components. The results of the tests with the paraffin oil, Sudan Red B, and tetrachlorethane were

negative. During the 1-hour exposure to 0.5 ml of tetrachlorethane, several individuals noted a slight sensation of heat at the application site, but no symptoms similar to those for the gauge fluid were subsequently noted. All volunteers rubbed 0.5 ml (1.1 g) of ethylene dibromide into the skin of the forearm for 1 minute and washed with soap and water 30 minutes later. All subjects developed swelling and reddening of the exposed area, as well as itching, during the 24 hours immediately after exposure. The symptoms subsided without supportive treatment within 2-3 days.

In an additional experiment [24], the subjects received 0.5 ml (1.1 g) of ethylene dibromide applied to the skin on a swab, the swab and the application site being covered for 10 minutes. The swab was then removed and the area was washed with soap and water. A sensation of heat or slight burning was noticed during the exposure; and, during the next 24 hours a painful reddening and swelling of the skin developed. However, no blistering was noticed in any of the subjects. The injuries disappeared in 3-5 days without leaving visible traces.

In a subsequent experiment [24], this procedure was repeated except that the application site was covered for 30 minutes. After this exposure period, the swab was removed and the area was washed with soap and water. During the 30-minute exposure, the subjects reported the customary heat or burning sensation at the application site. After exposure, a very painful inflammation of the skin occurred, including reddening, swelling, and blistering, within 15-20 hours. The damaged skin grew back after 7-13 days of supportive treatment. The author experienced such a strong burning pain in his skin immediately after application that increased so rapidly that

the swab containing the ethylene dibromide had to be removed after 3 minutes. The pain and reddening of the skin subsided, but then increased again after a few hours until a blister the size of a crab apple had formed at the application site after 18 hours. Very strong pain was associated with the blister. When the blister was opened, Pflesser collected 21 ml of a clear exudate, which coagulated soon afterward, and over 245 ml of fluid within the first 3 days after application. In addition, the author stated that when the blister appeared on the right arm, all places that had previously been exposed to ethylene dibromide or to the gauge fluid on both the left and right arms began swelling, reddening, and itching. A moderately strong, painful glandular swelling occurred in the left armpit (opposite the arm of the application site). Pflesser also reported that only a slight edema had occurred surrounding the application site after the 1st day, but that, on the 2nd day, an extensive edema of the entire right forearm and right hand suddenly developed. During these first few days after exposure, there was a pronounced feeling of illness and a slight temperature increase in addition to the physical manifestations at the exposure site. Healing of the damaged skin was complete after 17 days of zinc-sulfur ointment supportive treatment, leaving only a surface scar.

From this series of experiments, Pflesser [24] concluded that ethylene dibromide was the component of the gauge fluid that was responsible for the swelling, reddening, and blistering of the skin of the seaman on the destroyer. He also concluded that the effects and their intensities depended on the duration of exposure to the skin, and that covering the application site to prevent evaporation greatly increased the extent of damage from exposure. Pflesser concluded that ethylene dibromide

was absorbed through the skin, causing tissue death, general inflammation, and plasma exudation. He further postulated that ethylene dibromide possessed a sensitization potential, citing the symptoms that appeared at previous application sites after his own exposure for 3 minutes to 0.5 ml of ethylene dibromide as proof of the assumption.

In 1960, Olmstead [25] reported the death of a 43-year-old woman who was hospitalized 48 hours after ingesting 4.5 ml, or approximately 140 mg/kg, of ethylene dibromide in capsular form. She vomited almost immediately after swallowing the capsules; vomiting recurred periodically during the next 48 hours. Twenty-four hours after ingestion, watery diarrhea was first noted and recurred frequently. About 12 hours later, the patient noted a decrease in the volume and a darkening of the urine. She was completely anuric by the time of hospitalization. She had tachypnea and marked agitation after ingesting the capsules. At the time of hospitalization, the patient complained of abdominal pain, nausea, vomiting, and diarrhea. Physical examination showed normal temperature and blood pressure. Her appearance was that of an acutely agitated, disheveled, very ill person. Her pulse was thready, respiration was increased, and systolic murmurs and sinus tachycardia were present. The lungs were clear and the results of abdominal, rectal, and neurologic examinations were normal.

The patient did not improve even after supportive treatment [25]. Her pulse became weaker and more sporadic, and she died 54 hours after the ingestion of the ethylene dibromide. An autopsy, 3 hours after death, disclosed no excess fluid in any of the body cavities, that all organ weights were normal, and that there were no gross abnormalities in the

heart. The surface texture of the liver was more friable than normal and it was studded with small, bright yellow areas; the usual structure was destroyed. The kidneys were intensely congested, particularly in the medullary area. The lungs were moderately congested and had some edema. A diffuse reddening of the gastric mucosa and of the small and large intestines was noted, but without associated evidence of necrosis or ulceration. Microscopic examination showed centrilobular necrosis of the liver, with masses of red blood cells in the sinusoids and scattered yellow pigment, but remarkably little inflammation. Also noted was damage in the proximal tubular epithelium of the kidney with focal vascular congestion, but this damage was local and not widespread. From the results of the autopsy, Olmstead [25] concluded that ingestion of ethylene dibromide resulted primarily in changes in the liver and kidneys. The hepatic lesions were characterized by massive necrosis with minimal cell inflammation, whereas the kidney lesions displayed patchy and local necrosis. He further concluded that these changes paralleled those found in various animals after oral administration of ethylene dibromide.

Epidemiologic Studies

In 1977, Ott et al [26] submitted to NIOSH the results of a preliminary epidemiologic study on the mortality experience of 161 chemical company employees at two ethylene dibromide manufacturing sites. Plant A was opened in 1926 and was closed in 1976; Plant B began operation in 1942 and closed in 1969. Plant A continuously produced ethylene dibromide during its 50-year operation. Employees were potentially exposed over this period, either during the manufacture of ethylene dibromide or indirectly

through the production of other chemicals, to approximately 25 substances including bromine, benzene, substituted phenols, vinyl bromide, carbon tetrachloride, and numerous other halogenated hydrocarbons. Industrial hygiene estimates of ethylene dibromide exposure for the plant A reactor and still operators included in the study group were based on area and personal sampling in 1950, 1952, 1971-1972, and 1975. Plant B operations consisted of the batch production and distillation of ethylene dibromide. Both functions in Plant B were performed in the same building until the early 1960's when the batch reactors were replaced by continuous ones. No industrial hygiene surveys were conducted with respect to plant operations at Plant B. Exposures in Plant B were estimated to be primarily to ethylene dibromide, bromine, ethylene, sulfur dioxide, and chlorine. In addition to the ethylene dibromide operators, lead burners in Plant B may have been exposed to ethylene dibromide, since they could have spent up to 90% of their time in the ethylene dibromide production area repairing glass and lead lines.

In Plant A, 18 breathing zone samples taken in 1950 ranged from 1.0 to 7.4 ppm for reactor operators and from 2.2 to 10.6 ppm for still operators [26]. In 1952, area samples between the stills ranged from 19 to 24 ppm and up to 31 ppm on warm days. Breathing zone determinations taken while filling drums ranged up to 13.4 ppm and, after a spill, up to 71 ppm. Extensive monitoring during 1971-1972 using continuous infrared soectrophotometry indicated TWA concentrations of 2.0 ppm (range 0.4-38 ppm) for the reactor operators and 3.5 ppm (range 0-23 ppm) for the still operators before a shed was fixed. The still operator exposures were consistently higher than those of the reactor operators. The 1975 sampling

indicated that reactor operators were exposed to concentrations ranging from 1.8 to as high as 96 ppm, with an 8-hour TWA concentration of 5 ppm for 22 samples. The authors stated that occasional excursions to concentrations beyond 75 ppm were suspected, based on symptomatology. Past records indicated that employees sensed a strong odor and reported respiratory irritation at about 75 ppm of ethylene dibromide. Gastrointestinal discomfort and vomiting were probably induced by short exposures to ethylene dibromide at 100-200 ppm for up to 1 hour, or by lower exposures over longer periods of time. Between 1954 and 1970, three episodes of acute exposure with respiratory or gastrointestinal involvement were reported by individuals in the study.

Employees were identified for this study by reviewing annual census lists and selecting all reactor and still operators from both plants, and foremen and lead burners from Plant B [26]. In Plant A, all individuals who had left the company prior to 1950 were not included in the study because of the difficulty of conducting followups on individuals from their social security numbers. Expected deaths were calculated from observed mortality rates for US white males, by 5-year groups, for the years 1942-1971. Five employees working with arsenicals in addition to ethylene dibromide were analyzed separately because of prior indications that arsenical employees were at an increased risk of developing respiratory malignancies. All of the arsenical-exposed employees were located in Plant A. Interestingly, three deaths have occurred among these five employees as compared with 0.6 expected deaths, and two of the three deaths were due to respiratory cancer.

By January 1976, deaths had occurred in 15 of 57 Plant A workers and in 20 of 99 Plant B workers [26]. In Plant B workers, no increase in malignancy was noted (total malignant neoplasms: 1 observed, 3.8 expected) and deaths from all causes were not elevated over expected deaths and were not clustered in any cause. The one death was attributed to pancreatic cancer (foreman, 18.7 years of exposure); another death was listed as arteriosclerotic heart disease with metastatic lymph node cancer (operator, 26 months of exposure). A total of eight deaths occurred due to malignant neoplasms, which included a father and son who both died of stomach cancer. This highly unusual occurrence indicates that a heritable family trait may have been a contributing factor in these two cases.

In Plant A workers, increased malignancies and respiratory deaths were observed. Total malignant neoplasms: 5 observed, 2.2 expected ($P<0.072$); malignant neoplasms, digestive system: 2 observed, 0.7 expected ($P<0.157$): all other malignant neoplasms: 3 observed, 0.9 expected ($P<0.063$); influenza and pneumonia: 2 observed, 0.3 expected ($P<0.037$). The levels of significance were calculated using a Poisson distribution.

An examination of mortality in relation to duration of exposure and interval since the first exposure for Plants A and B combined indicated no statistically significant increase in deaths; however, an increase in total deaths was observed in the subgroup categorized as 15-24 years since the first exposure (18 observed, 12.2 expected) as well as the total deaths from malignancies in this same category (4 observed, 2.2 expected). This increase, although not statistically significant, seems to have been most apparent in employees having 6 or more years' exposure in ethylene dibromide operations (total deaths: 6 observed, 3.5 expected; total

malignancies: 3 observed, 0.7 expected).

Ott et al [26] recognized the difficulty in the interpretation of these data due to limitations in the size of the study group (161 employees) and the variety of toxic agents to which individuals may have been exposed. Nevertheless, they noted that an indication of increased mortality may exist in the population (Plant A) with presumably higher ethylene dibromide exposure. Investigation of the deaths from nonmalignant respiratory disease indicated no apparent influence from exposure to ethylene dibromide.

The findings reported from this study group were equivocal because of the small population size, the exclusion of employees who were not routinely exposed to ethylene dibromide but probably had occasional exposures, and the lack of comparative data on employees in Plants A and B who did not work with ethylene dibromide yet had exposures to the numerous other chemical agents used.

Animal Toxicity

(a) General

In 1927, Thomas and Yant [27] investigated the toxic effects of ethylene dibromide vapor on guinea pigs. Three groups of three guinea pigs each were subjected to ethylene dibromide vapor at concentrations of 0.8, 0.4, or 0.2% (61,600, 30,800, or 15,400 mg/cu m) for 30, 60, or 150 minutes, respectively. The animals were observed for external appearance while being exposed; both macroscopic and microscopic examinations were performed after death. All the guinea pigs died within 6-18 hours after the exposure at each concentration. Evidence of nasal irritation and a

generalized weakened condition were noted during exposure; external appearance was normal for all nine animals when examined at autopsy. Macroscopic and microscopic examinations of the internal organs showed a pronounced granular degeneration of the parenchymal tissue of the kidneys and smaller amounts of damage in the pancreas, spleen, heart, liver, and adrenals. In addition, the authors observed that ethylene dibromide exposure produced swelling and a generalized interstitial edematous degeneration of the endothelial lining of the abdominal vascular system that was not described further.

Thomas and Yant [27] noted that commercial and purified ethylene dibromide applied to the shaved abdomens of three groups of two rats each over a 2-cm square area killed all of the animals within 6-18 hours at doses of 0.25, 0.50, or 1.00 ml (0.55, 1.1, or 2.2 g)/animal. No attempt was made to determine the minimum lethal dose. The application site showed marked hyperemia of the small cutaneous blood vessels, and the abdominal muscles became contracted and remained tense. By 20 minutes, the reflexes became weak and the animals were scarcely able to stand. A slight, temporary increase in activity was noted during the next 10 minutes, but the general appearance remained that of great weakness. Macroscopic and microscopic examinations of tissues were similar to those in guinea pigs after vapor inhalation. The only difference observed between the two routes of exposure concerned the spleen: gross examination of the guinea pigs exposed to the vapor of airborne ethylene dibromide revealed that spleens were pale and edematous, whereas spleens of the dermally exposed rats were highly congested and edematous. No microscopic characterization of these differences was given.

In 1928, Lucas [28] published the results of an experiment in which two adult rabbits inhaled ethylene dibromide vapor in quantities sufficient to produce light or deep anesthesia (concentrations not reported). Very light anesthesia was maintained in one rabbit by inhalation of ethylene dibromide for about 10 minutes. The rabbit vocalized during exposure, presumably responding to the irritating effects of ethylene dibromide. Respiration became extremely rapid during exposure and there was considerable phonation. The mucous membranes of the mouth were a peculiar "old rose" color after recovery from the anesthesia. This may have been due to a combination of vascular congestion and cyanosis. Death occurred within 18 hours, and, at autopsy, the liver was enlarged and mottled. Microscopic examination of the liver showed slight-to-moderate diffuse fatty changes, which tended to be more marked in the portal regions.

Another rabbit deeply anesthetized by inhalation of ethylene dibromide for about 12 minutes exhibited signs of marked irritation from the gas, considerable phonation, rapid breathing progressing as the anesthesia continued, and snuffling in its breathing after recovery from the anesthesia [28]. Death occurred within 15 hours. Autopsy showed the lungs to be enlarged and filled with a frothy exudate. About 10 ml of fluid was found in the pleural cavity. The liver was swollen and markedly congested. Lucas postulated that these results may have been caused by the decomposition of ethylene dibromide to hydrogen bromide and that local high concentrations of this material would be sufficient to bring about the observed changes.

In 1928, Kochmann [23] subjected rabbits and cats to ethylene dibromide vapor to determine the toxic effects of repeated exposures. An

unspecified number of cats and rabbits was exposed to ethylene dibromide at an aerosol concentration of approximately 0.01% (770 mg/cu m) for 30 minutes each day. The author stated that the actual concentration was somewhat lower, because the fine fog was partially condensed and the substance did not pass into the vapor state completely. The survival period for rabbits varied from 4 to 22 days and for cats, it lasted approximately 10 days. Kochmann reported that the same effects were observed in all experiments. In cats, there was a reddening of the nasal mucosa and frequent sneezing after the first exposure. Two exposures later, the animals developed agitation, locomotor impairment, and excessive salivation. The next day, the animals began to tremble while in the cage, tears began forming, and the nasal mucosa was strongly reddened. After 11 exposures, the survivors were very weak and maintained a reclining posture, and strong trembling was observed, especially in the extremities. These conditions persisted until death occurred. There was a general weight loss during the exposures. Autopsy showed that the body cavities contained a clear, yellowish liquid. The lungs contained dark red discolorations and were judged by the author to be partially nonfunctional. The spleen was slightly enlarged and the kidneys were swollen and yellow colored. Kochmann diagnosed the condition of the cats after exposure to ethylene dibromide as follows: rhinitis, conjunctivitis, ascites, pleural effusion, pneumonia in the left superior lobe of the lung, and incipient fatty degeneration of the liver and possibly degeneration of the tubules of the kidneys. Similar adverse signs of intoxication, including rhinitis, conjunctivitis, anorexia, and loss of weight, appeared in rabbits. Autopsies of the rabbits showed that the small intestine contained an

excessive amount of liquid, that the colon contained blood, and that the liver and kidneys were hyperemic.

In an additional experiment, the animals inhaled ethylene dibromide at a concentration of 0.01% (770 mg/cu m) once for 30 minutes, and the author [23] noted that there was a reduction in appetite, a corresponding loss of weight, a distinctly reduced hemoglobin content in the blood, and a slow recovery to the pretreatment conditions of the test animals.

Kochmann [23] exposed an unspecified number of cats and rabbits to ethylene dibromide at concentrations of 0.005 or 0.007% (385 or 538 mg/cu m, respectively) for 4 hours every 2nd day until death occurred or the experiment was terminated. The cats exposed to 538 mg/cu m (70 ppm) lost weight, developed general trembling, agitation and spasms in the rear legs, and died after 14 days. Autopsies showed a yellowish discoloration of the liver parenchyma but no other remarkable abnormalities. The rabbit exposed to 538 mg/cu m (70 ppm) lost weight during the experiment but was alive after 40 days. No other signs of toxic effects were reported by the author. Another rabbit exposed to 385 mg/cu m (50 ppm) died after 7 days. The results of the autopsy showed slight amounts of serous liquid in the body cavities and a swelling of the intestines, but other organs appeared to be normal.

Kochmann [23] concluded that the results of the animal experiments agreed substantially with the effects described by and observed in humans, as presented in the Effects on Humans. He concluded that, even when ethylene dibromide was inhaled in concentrations as low as 385 mg/cu m (50 ppm), it would cause local irritation, absorptive metabolic interference, and possible deterioration of the parenchymatous tissues of organs through

long-term exposures. Kochmann indicated that death was probably from injury to the circulatory system, especially to the heart and vessels, and to the secondary paralysis caused by damage to the nervous system. Because of the imprecise measurements of the concentrations in this study, it is difficult to draw quantitative conclusions as to a dose-effect relationship. Data show that ethylene dibromide at concentrations as low as 385 mg/cu m (50 ppm) may produce death and pronounced systemic injuries in mammals when inhaled repeatedly for up to 4 hours daily.

In 1929, Glaser and Frisch [29] published the results of an experiment with a group of guinea pigs to determine the effects of repeated exposure to ethylene dibromide vapor. The guinea pigs, weighing approximately 650 g, were exposed to ethylene dibromide at measured concentrations of 19.6, 24.6, 8.0, 29.0, 25.5, and 16.5 mg/liter (19,600, 24,600, 8,000, 29,000, 25,500, and 16,500 mg/cu m, respectively) for 15-minute periods on 6 consecutive days. The authors noted that nose rubbing and muscular spasms of the diaphragm occurred during the exposures, but disappeared immediately after the guinea pigs were removed from the exposure chamber. Paralysis of the rear extremities occurred 24 hours after the sixth exposure, but it had partially disappeared after 5 additional days and was completely gone after another 8 days. The authors concluded that the adverse effects, such as paralysis of the extremities and spasms of the diaphragm, appeared at a lower concentration and in a much shorter time than did the adverse effects in a concurrent experiment with methyl bromide. They also concluded that the signs of toxicity remained longer than those observed from methyl bromide.

In 1929, Kistler and Luckhardt [30] reported the effects on respiration, muscle reflexes, and blood pressure in dogs exposed to ethylene dibromide by inhalation, ingestion, and injection. Dogs, anesthetized with sodium barbital, were sequentially injected iv with 0.2-1.0 ml (0.44-2.2 g) of ethylene dibromide for a total of 5.5 ml (12 g). Marked decreases in respiratory rate, blood pressure, and muscle reflexes resulted. Ethylene dibromide at 0.3 ml (0.66 g) injected iv caused cessation of respiration which was "momentary" or lasted as long as 40 seconds in the dogs tested. The cessation was followed by panting and a gradual return to almost normal breathing rate. The same dose produced a 75% decrease in blood pressure, the fall being immediate and sharp. The blood pressure gradually returned toward normal in a period of 2-12 minutes; but, in some cases, it remained 30% below normal. Ethylene dibromide at doses of 0.1-0.3 ml (0.22-0.66 g) injected iv caused a profound and rapid depression of the knee jerk reflex, which never returned to normal after the exposure.

The effects on dogs exposed to 1.0-10.0 ml (2.2-22.0 g) of ethylene dibromide vaporized from an ether bottle were essentially the same as those caused by the iv injections, differing only slightly in the extent of the decreases [30]. The recovery times were described as being prolonged because of the longer exposure times, but complete experimental details were not given.

The investigators [30] found that each unanesthetized dog receiving oral doses of 0.0625, 0.125, 0.250, or 0.55 ml/kg (approximately 135, 270, 540, or 1,200 mg/kg, respectively) of ethylene dibromide vomited immediately and several times more during the next 4 hours. Definite

depression and increased salivation began 20 minutes after the administration of ethylene dibromide, but the animals did not become excited or uncoordinated at any time during the study. Blood was found in the feces when defecation occurred. The dogs died within 22 hours. Autopsies were performed, but the results were not given specifically for ethylene dibromide. However, all dogs that died had slight lung edema and engorged blood vessels in the lungs and liver. The authors concluded that the effects of exposure to ethylene dibromide on blood pressure, respiratory rate, and muscular reflexes were similar after injection or inhalation. The extent of the influence of the preexposure injection of sodium barbital on the results of the iv experiments is not known because concurrent control experiments were not described.

Merzbach [31], in 1929, subjected dogs for 1 hour to the vapor from 1, 2, or 5 ml (2.2, 4.4, or 11.0 g, respectively) of ethylene dibromide in a 100-liter glass bell jar to determine the effects of inhalation on each dog. Ethylene dibromide was placed within the glass bell jar as a liquid and allowed to vaporize. The dog exposed to 5 ml (11.0 g) of vaporized ethylene dibromide became very restless and began to salivate strongly after 20 minutes. Five minutes later, the respiratory rate increased markedly to 120 breaths/minute. At that time, the dog lay on its side, and clonic twitches of the legs were noted. After 45 minutes of exposure, it lay quietly but responded to acoustic stimuli, the twitches ceased, and the respiratory rate dropped to 66 breaths/minute. When the 1-hour exposure ended, the dog immediately vomited several times, trembled severely, and fell immediately while trying to stand. Rales and rattling were audible in the chest. It lost consciousness 35 minutes after the exposure ended and

died during the night. Autopsy indicated abundant blood in the right lung and in the lower lobe of the left lung. The heart was found in systole, and hemorrhages had occurred in the subendocardium and in the mucous linings of the intestines and rectum. The liver was congested. Fresh hemorrhages were noted on the surface of the dura mater, and both corneas were clouded.

The dog exposed to 2 ml (4.4g) of vaporized ethylene dibromide for 1 hour developed toxic effects similar to those of the dog exposed at 5 ml (11.0 g), but the extent of effects was less pronounced [31]. This dog also died between 12 and 18 hours after exposure. Autopsy results were similar to those for the other dog exposed to 5 ml (11.0 g), although the extent of cellular damage was slightly less and no blood was seen in the lungs.

The dog exposed to 1 ml (2.2g) of vaporized ethylene dibromide for 1 hour showed signs of restlessness, ocular irritation, labored respiration, and increased respiratory rate during the exposure [31]. Five hours after the exposure ended, a milky-blue opacity developed in the corneas and became progressively more pronounced, developing into purulent conjunctivitis in both eyes with an ulcer in one cornea. The dog lost considerable weight, completely stopped eating, and died after 3 weeks. The autopsy showed bronchopneumonic foci and severe hyperemia in both lungs. A spherical thrombus was found in the heart, and the liver showed pronounced fatty degeneration.

The effects noted above in the hearts of the dogs exposed to ethylene dibromide were also found in isolated, perfused hearts from male frogs (Rana temporaria) subjected to 400, 800, or 1,600 ppm of ethylene dibromide

in Ringer's solution [31]. Immediate diastolic arrest occurred at 800 and 1,600 ppm with no recovery of function. At 400 ppm, there was diastolic arrest after 1 minute, but washing produced a gradual recovery to normal function. No cardiac arrest or change in the heart rate was found at a concentration of 200 ppm of ethylene dibromide.

In 1946, Aman et al [32] studied the effects of repeated oral administration of ethylene dibromide on rats and guinea pigs. Nineteen animals were administered ethylene dibromide at average daily doses of 0, 3.4, 4.4, 7.1, 15.0, or 20.0 mg in oil or alcohol solution by gavage for 77-95 days in approximately 4 months. Total accumulated dosages of ethylene dibromide ranged from 0 to 1,420 mg. One rat, given 20 mg of ethylene dibromide daily, died after 3 months. All other rats and guinea pigs appeared to have gained weight normally during the experimental periods, and no untoward signs of toxicity were noted. Autopsy and microscopic examinations were not mentioned. The authors concluded that daily administration of ethylene dibromide for periods up to 4 months did not adversely affect the growth and outward physical appearance of rats and guinea pigs. The lack of experimental detail and data, the ambiguity of the data given, the inadequate number of control and experimental animals, and the absence of autopsies make this study difficult to evaluate and its importance questionable.

In 1952, Rowe et al [33] investigated the effects of ethylene dibromide administered to rats, guinea pigs, rabbits, mice, chickens, and monkeys by single oral intubations, eye contact, dermal contact, dermal absorption, single exposure inhalation, or repeated exposure inhalations. The ethylene dibromide used in these studies was the 99% pure, commercial

quality product, except that the inhalation studies were conducted with a repurified commercial product of essentially 100% purity. Ethylene dibromide was administered in undiluted form for eye and dermal contact, dermal absorption, and inhalation studies; in olive oil and acacia emulsion or olive oil solution for oral intubation studies; in propylene glycol solution for eye contact studies; or in butyl carbitol acetate solution for dermal contact studies.

Fifty-five female rabbits, 28 chicks, 40 male and female guinea pigs, 40 female rats, 60 male rats, and 20 female mice were administered ethylene dibromide at sufficient doses to enable calculation of single oral-dose LD50's of 55 mg/kg, 79 mg/kg, 110 mg/kg, 117 mg/kg, 146 mg/kg, and 420 mg/kg, respectively [33]. The LD50's for male and female rats were significantly different ($P<0.05$). Of the five species tested, rabbits were the most sensitive to ethylene dibromide and mice were the least sensitive.

Rowe et al [33] found that undiluted ethylene dibromide promptly caused obvious pain and conjunctival irritation when introduced into the eyes of rabbits. The undiluted material was in contact with the eyes for 30 seconds before one eye was thoroughly flushed for 3 minutes with running water; the other eye was not washed. Both eyes of each rabbit were then observed for injury. The conjunctival irritation cleared within 48 hours, and a very slight amount of superficial necrosis of the cornea healed promptly and completely. When a 10% solution of ethylene dibromide in propylene glycol was tested in rabbits by the same procedure used with the undiluted material, it produced a more severe response than did the undiluted material. Moderate conjunctival irritation developed within 2 hours and persisted for 48 hours before remission began to occur.

Moderate-to-severe corneal injury also persisted for about 48 hours before tissue repair became evident. Healing was complete 12 days after exposure without corneal scarring being evident. No injury to the iris or the lens of the eye was noted. A 1% solution in propylene glycol elicited a response in the rabbit eye very similar to that of the undiluted material. The authors noted that prompt washing of the treated eyes had a beneficial effect in all cases, slightly reducing the intensity of the response and shortening the healing time.

Application of undiluted ethylene dibromide or 10% solutions in butyl carbitol acetate to the shaved skins of rabbits killed the animals within 24 hours [33]. It was apparent that a few hours of confined contact of the material with the skin caused marked erythema and edema. When evaporation was not inhibited by a covering, only slight erythema was noted. A 1.0% solution of ethylene dibromide in butyl carbitol acetate applied to rabbit ears 10 times in 14 days caused only slight erythema and exfoliation. When the solution was applied repeatedly for 10 times in 14 days onto the shaved abdomen of the rabbit and then bandaged, marked erythema and edema, which progressed to necrosis and exfoliation, were observed. Healing was complete without scarring within 7 days after termination of both exposures.

Rowe et al [33] found that absorption of undiluted ethylene dibromide at doses of 210, 300, 650, or 1,100 mg/kg through the intact skin killed 1 of 15 rabbits at 210 mg/kg and all 5 rabbits at 1,100 mg/kg when the exposures lasted for 24 hours. In all of the exposed animals, the material produced a moderate-to-severe erythema, edema, and necrosis of the skin and caused scar formation in the survivors. Marked central nervous system

(CNS) depression was seen in rabbits at all of the doses. At the 650 and 1,100 mg/kg doses, the animals felt cold to the touch. Deaths occurred within 4 days or not at all. The authors did not calculate an LD50 for dermal absorption in the rabbits; however, an LD50 of approximately 400 mg/kg for ethylene dibromide can be estimated from the dose-response data.

These investigators [33] also studied the effects of single exposures of ethylene dibromide vapor to groups of 4-30 rats of both sexes at concentrations of 100, 200, 400, 800, 1,600, 3,000, 5,000, or 10,000 ppm (770, 1,540, 3,080, 6,160, 12,300, 23,100, 38,500, or 77,000 mg/cu m, respectively) and to guinea pigs of both sexes at 200 or 400 ppm (1,540 or 3,080 mg/cu m). At each concentration, the rats were exposed to the vapor for durations ranging from 0.02 to 16.0 hours to allow for the determination of concentration versus period of exposure values that represent death in essentially all of the rats, death in 50% of the rats, and death in essentially none of the rats. The findings of this study are presented in Table III-1. These data suggest that at concentrations above about 5,500 mg/cu m (715 ppm), the Haber product [the product of the exposure concentration (LC50) and the duration of exposure] becomes actually almost a constant but that, at lower concentrations of ethylene dibromide, the Haber product increases rapidly as the concentration is lowered further. This suggests, in turn, that exposure of the rat to a concentration of about 5,500 mg/cu m (715 ppm, 29.2 μmoles/liter) of ethylene dibromide saturates some detoxification mechanism. Whether this is metabolic or excretory in nature cannot be judged from these data.

TABLE III-1

VAPOR TOXICITY OF ETHYLENE DIBROMIDE IN RATS

Vapor Concentration		Calculated Lethal Time (Hours)		
mg/cu m	ppm	LC99.99	LC50	LC0.01
77,000	10,000	0.15	0.04	0.01
38,500	5,000	0.35	0.09	0.03
23,100	3,000	0.60	0.18	0.06
12,300	1,600	1.10	0.30	0.10
6,160	800	2.20	0.75	0.28
3,080	400	7.50	2.00	0.62
1,540	200	42.00	12.00	2.00
770	100	-	-	>10.00

Adapted from Rowe et al [33]

Ethylene dibromide produced only slight anesthetic effects at the concentrations used [33]. Depression of the CNS was observed in rats exposed at the higher concentrations (unspecified). Deaths usually occurred within 24 hours at the higher concentrations and were caused by respiratory or cardiac failure. Deaths occurring from exposures at lower concentrations (unspecified) were generally delayed, sometimes for as long as 12 days after exposure. The majority of these deaths were caused by pneumonia. These animals usually lost weight, appeared rough and unkempt, became quite irritable, discharged what appeared to be a blood-tinged fluid

from the nose, and finally died. Animals surviving the exposure at the lower concentrations exhibited a similar progression of toxic signs for several days before recovery was apparent. Rats exposed at concentrations producing mortality and killed for autopsy 16-24 hours after exposure showed an increase in the weight of the lungs, liver, and kidneys. The lungs were congested, edematous, hemorrhagic, and inflamed; the liver had cloudy swelling, centrilobular fatty degeneration, and necrosis; and the kidneys exhibited slight interstitial congestion and edema, with slight cloudy swelling of the tubular epithelium in some cases. Guinea pigs appeared to be slightly less susceptible than rats to the effects of ethylene dibromide vapor when exposed to the same concentrations. All guinea pigs exposed at 400 ppm (3,080 mg/cu m) for 7 hours died, whereas all those exposed at 400 ppm (3,080 mg/cu m) for 7 hours and at 200 ppm (1,540 mg/cu m) for 7 hours lived. The authors made no attempt to determine concentrations and corresponding exposure durations that would produce an LD99.99, LD50, or LD0.01 in guinea pigs, but they did postulate that 30 ppm (231 mg/cu m) for 5 hours was the most severe repeated exposure without detectable adverse effects.

Rowe et al [33] subjected rabbits, monkeys, guinea pigs, and rats to 7-hour exposures, 5 days/week, for approximately 6 months to ethylene dibromide vapor at a concentration of 25 ppm (192.5 mg/cu m). To facilitate comparison of the data, a calculation of the amount of inhaled ethylene dibromide has been made by assuming a minute volume of 0.61 liters/min/kg [34]. A rat maintained in a 25-ppm (192.5 mg/cu m) atmosphere would inhale approximately 0.12 mg/minute/kg or 49 mg/kg during a 7-hour exposure. Groups of 20 male and 20 female rats were given 151

exposures in 213 days without evidence of adverse effects as judged by general appearance and behavior, growth rate, final body and organ weights, blood urea nitrogen values, periodic hematologic examinations, and macroscopic and microscopic examination of tissues. The amount of inhaled ethylene dibromide during 151 7-hour exposures would total about 7.4 g/kg. Two groups of controls, well-matched with the experimental animals with respect to number, age, sex, and body weight, were used in the experiment. One group was exposed to the same experimental regimen as the group exposed to ethylene dibromide, but in a chamber ventilated with clean air. The second control group was simply maintained in the animal quarters.

During the test period, 10 of 20 ethylene dibromide-exposed male rats died, primarily from pneumonia and upper respiratory tract infections, and 3 of 20 ethylene dibromide-exposed female rats died from unspecified causes [33]. Twenty-three additional female rats subjected to 13 7-hour exposures at 25 ppm (192.5 mg/cu m) in 17 days showed no ill effects and were found to have total liver lipid contents similar to those of control rats. Groups of 8 male and 8 female guinea pigs tolerated 145 7-hour exposures at 25 ppm (192.5 mg/cu m) in 205 days without evidence of adverse effects as judged by the same criteria used to evaluate the rat data, except that liver lipid determinations were not conducted. Mortality during the experiment, caused by pulmonary infections, was 50% in male and 25% in female guinea pigs exposed to ethylene dibromide. Eighteen of the 20 animals in both the male and female control groups exposed to clean air survived the experimental regimen, but only 7 and 8 of the 20 females and males, respectively, in the unexposed control groups survived. An additional group of 8 female guinea pigs received 13 7-hour exposures at 25

ppm (192.5 mg/cu m) in 17 days without exhibiting adverse effects. Rabbits, 3 male and 1 female, and monkeys, 1 male and 1 female, were subjected to 152 7-hour exposures in 214 days and 156 7-hour exposures in 220 days, respectively, at 25 ppm (192.5 mg/cu m). There were no signs of adverse effects in the rabbits and monkeys when judged by the above criteria for the guinea pig data. Since an unusually high mortality rate was observed in the nonexposed control rats but not in the air-exposed control animals, no decisive conclusions can be drawn from the published data with respect to the hazard resulting from exposure to ethylene dibromide at 25 ppm (192.5 mg/cu m).

Rowe and his colleagues [33] found that rats, guinea pigs, monkeys, and rabbits did not tolerate ethylene dibromide well at a concentration of 50 ppm (385 mg/cu m) administered 7 hours/day, 5 days/week, for 70-90 days. The group of 20 male and 20 female rats receiving 63 exposures in 91 days exhibited increased liver and kidney weights in both sexes, increased lung weights in males, decreased spleen weights in females, and decreased testis weights in males at autopsy. The group of 8 male and 8 female guinea pigs receiving 57 exposures in 80 days showed decreased rates of growth and final body weights, increased organ weights, slight central fatty degeneration of the livers, and slight interstitial congestion and edema with degeneration of the tubular epithelium in the kidneys. The 1 male and 3 female rabbits receiving 59 exposures in 84 days showed only small increases in liver and kidney weights; no other adverse effects were noted. The male and female monkeys receiving 49 exposures in 70 days appeared ill, nervous, and unkempt throughout the experimental period. Liver weights were increased and very slight central fatty degeneration of the liver was

noted; no other adverse effects were noted from microscopic examination of the body organs.

At concentrations of 100 ppm (770 mg/cu m) of ethylene dibromide vapor, 10 female rats steadily lost weight and 3 died after 1, 5, and 7 exposures, respectively, for 7 hours/day [33]. The remaining rats appeared thin and unkempt at the time of autopsy after seven exposures in 9 days. The stomachs were full of food which appeared blood tinged, and lung, liver, and kidney weights were increased markedly. Microscopic examination showed some thickening of the alveolar walls with slight leukocytic infiltration of the lungs, widespread cloudy swelling of the liver, and slight congestion and hemosiderin deposition in the spleen. Rabbits subjected to the same concentration suffered severe intoxication to the extent that two of four died after the second 7-hour exposure and the third died while receiving the third exposure. The fourth animal was killed after receiving the fourth exposure in 4 days. Microscopic examination of tissues from the last two rabbits showed widespread central fatty degeneration of the liver with some necrosis.

The authors [33] concluded from the above series of experiments that ethylene dibromide was a fairly toxic, markedly irritating material. They postulated that ingestion of 1.5-2.0 ml (3.3-4.4 g) of undiluted ethylene dibromide could jeopardize the life of the average human and that this amount of material could easily be swallowed by accident. Rowe et al pointed out that, although ethylene dibromide was not likely to cause permanent injury to the eye, it probably would cause appreciable pain and suffering; thus, eye protection was strongly recommended for those handling ethylene dibromide. Although the hazard of contact with uncovered skin was

not thought to be particularly serious unless repeated or prolonged, marked irritation and rapid absorption of ethylene dibromide occurred when it was confined to the skin. Ethylene dibromide vapor at 50 ppm (385 mg/cu m) repeated daily was not well tolerated by the species tested, whereas adverse effects were not believed to have resulted from exposure at 25 ppm (192.5 mg/cu m). It is difficult to accept this conclusion because both the colony controls and the groups exposed at 25 ppm (192.5 mg/cu m) had similar pulmonary involvement, whereas the control animals exposed in the chamber to clean air had none. The numbers of rabbits and monkeys used in the repeated inhalation experiments are too small to allow an adequate assessment of the value of the data concerning these two species.

Adams et al [35], in 1952, reported the results of an investigation to determine the single-exposure vapor toxicity of an ethylene dibromide fumigant mixture in albino rats. Dowfume EB-15, composed of 20.4% ethylene dibromide, 19.6% ethylene dichloride, and 60.0% carbon tetrachloride, was mixed with the air entering an exposure chamber at a constant rate. Vapor concentrations were calculated on the basis of an "average" molecular weight of 143 for the mixed vapor and expressed as ppm. Groups of 10-20 adult albino rats were each exposed at concentrations of 200, 500, 1,100, 2,400, or 4,800 ppm (1,540, 3,850, 8,470, 18,480, or 36,960 mg/cu m, respectively). The exposure duration depended on the concentration of Dowfume EB-15 being administered and ranged from 0.15 hour for 4,800 ppm (36,960 mg/cu m) to 7.0 hours for 200 ppm (1,540 mg/cu m). All of the concentrations of Dowfume EB-15, except the 200-ppm (1,540 mg/cu m) concentration, produced visible signs of drowsiness, unsteadiness, weakness, and incomplete anesthesia. Mortality usually occurred within 24

hours at the higher concentrations and within 1-6 days at the lower concentrations. An additional group of male rats exposed to the 500- and 1,100-ppm (3,850 and 8,470 mg/cu m) concentrations for durations that produced 50% mortality showed consistent moderate microscopic injuries in the liver and kidneys at autopsy, 1 and 3 days after exposure. Liver damage consisted of centrilobular fatty degeneration with general congestion and occasional hemorrhagic necrosis in rats at both concentrations, but it was more marked in the rats exposed at 500 ppm (3,850 mg/cu m). Kidney damage included a moderate-to-severe reaction consisting of general congestion, cloudy swelling, and necrosis, as well as degeneration of the tubular epithelium. The injury to the kidneys was more severe in the rats killed and autopsied 3 days after exposure than in those killed and examined 1 day after exposure. Minor abnormalities, consisting of congestion and slight cloudy swelling, were observed in the adrenal glands and the epididymis. There were also increases in the absolute and relative weights of the liver and kidneys. No attempt was made by the authors to explain the significance of the marked microscopic changes present in the liver at 500 ppm (3,850 mg/cu m) but absent at 1,100 ppm (8,470 mg/cu m).

In another experiment, Adams et al [35] examined the effects of concentrations of the ethylene dibromide-containing vapor mixture (Dowfume EB-15) below the lethal range for male rats to determine the maximum concentration at which the rats could be exposed without developing detectable adverse effects. Rats were exposed to Dowfume EB-15 vapor at concentrations of 50 ppm (380 mg/cu m) for 7.0 hours, 100 ppm (770 mg/cu m) for 7.0 hours, 200 ppm (1,540 mg/cu m) for 1.5 or 2.5 hours, 500 ppm (3,850

mg/cu m) for 0.4 or 0.6 hour, and 1,100 ppm (8,470 mg/cu m) for 0.2 hour. Three to seven male rats from each exposure concentration were autopsied 16-24 hours after single exposures to discern detectable injury. The criteria used to evaluate injury were organ weight and microscopic changes in the liver and kidneys. Adverse effects occurred with Dowfume EB-15 at 100 ppm (770 mg/cu m) for 7.0 hours, 200 ppm (1,540 mg/cu m) for 2.5 hours, 500 ppm (3,850 mg/cu m) for 0.6 hour, and 1,100 ppm (8,470 mg/cu m) for 0.2 hour. No adverse effects were noted with Dowfume EB-15 at 50 ppm (385 mg/cu m) for 7.0 hours, 200 ppm (1,540 mg/cu m) for 1.5 hours, or 500 ppm (3,850 mg/cu m) for 0.4 hour.

The investigators [35] concluded that the toxic effects caused by Dowfume EB-15 were essentially the same as those expected from a simple summation of the separate actions of its constituents, as reported in the literature; hence, there was no potentiation. In addition, they concluded that an industrial hygiene standard for single exposures of employees to Dowfume EB-15 vapor, which contains 20.4% ethylene dibromide, could be established, and that exposure should not exceed 7 hours at 75 ppm (577.5 mg/cu m), 1 hour at 300 ppm (2,310 mg/cu m), and 0.1 hour at 1,500 ppm (11,550 mg/cu m). These conclusions, drawn in 1952, are of questionable relevance today since the current NIOSH recommendation for an occupational exposure limit for carbon tetrachloride (2 ppm) [36], which is 60% of the Dowfume EB-15 mixture, is well below these authors' recommendations.

In 1956, McCollister et al [37] described an investigation conducted to compare the effects of fumigant mixtures containing ethylene dibromide with those of ethylene dibromide alone. A total of 100 mature young adult male and female albino rats were given one of six amounts of ethylene

dibromide administered as single oral doses in corn oil and acacia by intubation. A second group of 135 rats were given one of eight doses of a suspension containing 7.2% ethylene dibromide, 29.2% ethylene dichloride, and 63.6% carbon tetrachloride (Dowfume EB-5). A third group of 90 rats were given five doses of a suspension containing 20.4% ethylene dibromide, 19.6% ethylene dichloride, and 60.0% carbon tetrachloride (Dowfume EB-15), as described above. The LD50's for the three dosage regimens were 140 mg/kg for 100% ethylene dibromide, 290 mg/kg for Dowfume EB-15, and 660 mg/kg for Dowfume EB-5. As the percentage of ethylene dibromide in the mixtures increased, the mixtures became more and more toxic to the rats, and the apparent toxicity of ethylene dibromide increased, based on the untrue assumption that it was the only toxic compound in the mixtures and on its percentages in the mixtures. The toxicity increased from an oral LD50 of 140 mg/kg for the pure chemical to one of 59.2 mg/kg in Dowfume EB-15 and to one of 47.5 mg/kg in Dowfume EB-5.

In a second experiment, McCollister and coworkers [37] compared the vapor toxicities of fumigant mixtures containing ethylene dibromide with the toxicity of ethylene dibromide alone in young adult albino rats. The concentration-duration of exposure lines to produce 50% mortality in rats were only slightly displaced from those calculated from the individual toxicities of the components, Dowfume EB-5 being slightly more than half as toxic as Dowfume EB-15 with 1-hour exposures. The lines for the two mixtures were not parallel, so it is inaccurate to try to compare their vapor toxicities in any general statement. At relatively high vapor concentrations, the mixtures produced CNS depression. Injury to the liver and kidneys was similar to that produced by the individual components of

the mixtures when administered separately. It appears that potentiation occurs after ingestion of ethylene dibromide-containing mixtures with carbon tetrachloride and ethylene dichloride but not after inhalation of the same mixtures.

The authors [37] estimated safe concentrations and exposure times for human subjects exposed to undiluted ethylene dibromide at a single exposure to be 50 ppm (385 mg/cu m) for 7 hours, 200 ppm (1,540 mg/cu m) for 1 hour, and 800 ppm (6,160 mg/cu m) for 0.1 hour. The corresponding values for Dowfume EB-5, which contains 7.2% ethylene dibromide, and Dowfume EB-15, which contains 20.4% ethylene dibromide, were both 75, 300, and 1,500 ppm (577.5, 2,310, and 11,550 mg/cu m), respectively. Maximum safe daily exposure concentrations for 7 hours/day were estimated to be 25 ppm (192.5 mg/cu m) for ethylene dibromide and for mixtures containing ethylene dibromide. It is difficult to accurately assess the additive effects of carbon tetrachloride and ethylene dichloride in relation to the lethal effects that may have been caused by ethylene dibromide alone. In a similar study by these investigators [38] on rats with Dowfume EB-5, they pointed out the similar capacities of ethylene dibromide and carbon tetrachloride to cause tissue injury after the rats inhaled the vapors.

Since ethylene dibromide is used as a soil nematocide, Schlinke [39], in 1969, administered ethylene dibromide to yearling sheep and to 5- to 7-day-old calves. Single oral doses of 10, 25, or 50 mg/kg each were given in capsules to one calf and one sheep, or of 25 mg/kg to two sheep, after initial weights and preexposure blood samples had been taken. Both the calf and sheep receiving the 50 mg/kg dose developed stiffness, prostration, and anorexia and died within 3 days after administration. The

calf receiving the 10 or 25 mg/kg dose showed no ill effects. One of the two sheep given 25 mg/kg developed signs similar to those occurring at the 50 mg/kg dose and died within 2 days. The second sheep showed no signs of ill effects and appeared well. The one sheep receiving the 10 mg/kg dose also appeared normal. Animals dying as a result of ethylene dibromide poisoning required about 24 hours for signs to appear and become prominent, death occurring after 2-3 days. Ethylene dibromide caused a slight decrease in whole blood cholinesterase activities in some of the animals, but the author indicated that this was probably not significant. Schlinke concluded that similar effects appeared at a given dosage in both sheep and calves. Autopsies were not performed on enough animals to develop conclusions regarding gross abnormalities.

In 1970, Schlinke [40] reported the effects of ethylene dibromide administered orally to chickens reared from a specific pathogen-free flock. Ethylene dibromide at doses of 0, 50, 100, or 200 mg/kg/day was administered in capsules or as an oral drench to groups of five to seven chickens between 6 and 7 weeks of age for 10 consecutive days. The chickens were weighed before and after administration to determine weight gain, and visual observations were made daily. All chickens receiving the 200 mg/kg dose had anorexia and general depression prior to death, which occurred before the fourth dose could be given. The results of autopsies showed inflammation of the crop, excess pericardial fluid, and congestion of the liver. All chickens given the 50 or 100 mg/kg doses appeared normal and gained weight normally during the experiment. The short period of exposure in this study does not permit an estimation of possible effects from longer exposures.

In 1977, Ter Haar (written communication, February 1977) submitted the results of a study to determine the effects of repeated inhalation of ethylene dibromide at concentrations of 3, 15, or 75 ppm (23.1, 115.5, or 577.5 mg/cu m) on B6C3F1 mice. Groups of 10 male and 10 female mice were exposed for 6 hours/day, 5 days/week, for 13 weeks in 100-liter plexiglass chambers operated at an airflow of 10 liters/minute inside a 6,000-liter chamber operated at an airflow of 1,500 liters/minute. A group of 10 male and 10 female mice serving as controls were similarly exposed but without the toxicant. Animals were observed daily for adverse effects and were weighed weekly. Autopsies were planned to be performed on all animals that died during the experiment or that were killed at the termination of the experiment.

According to Ter Haar, four of 10 males exposed at 3 ppm (23.1 mg/cu m) of ethylene dibromide died during the experiment on weeks 8, 10, 11, and 13. One of 10 females exposed at 75 ppm (577.5 mg/cu m) was killed on week 5 because it was moribund. The others survived the experimental period. No tissues were examined from the animals receiving 3 ppm (23.1 mg/cu m) of ethylene dibromide, although four mice died during the experiment. Tissues were considered to be normal in those from the 15-ppm (115.5 mg/cu m) group. The lungs of the animals exposed at 75 ppm (577.5 mg/cu m) showed megalocytosis of the bronchiolar epithelium in both males and females, and the lungs of females had regenerative epithelial hyperplasia and cellular debris in the lumens. No other findings were reported and complete experimental data were not given.

Ter Haar (written communication, February 1977) stated that, of the rats exposed at concentrations of 3 and 15 ppm (23.1 and 115.5 mg/cu m) of

ethylene dibromide for 90 days, only the males developed swelling of the hepatocytes. Animals exposed at 75 ppm (577.5 mg/cu m) had decreased thyroid follicular size and swelling or increased vacuolation of the zona reticularis cells of the adrenals. No further information was presented. Experimental design, data, and results of the autopsy were not included.

(b) Reproduction

In 1965, Amir and Volcani [41] described the effects of oral administration of ethylene dibromide on spermiogenesis in four Israeli-Friesian bulls fed an average of 2 mg/kg/day from the age of 4 days to approximately 24 months. For the first 3 months, 2 mg/kg of ethylene dibromide in an ethyl alcohol solution was added to the milk given daily to the bull calves. From the age of 3 months to 12 months, 2 mg/kg of ethylene dibromide was dissolved in soybean oil and administered daily in the feed concentrates, and after 12 months, the ethylene dibromide was administered in an oil mixture at the rate of 4 mg/kg every other day by capsule. Animal weight and height were measured throughout the study. Semen collections began when the bulls reached 14-16 months of age and continued weekly thereafter. Semen was collected from control bulls of the same age.

Ethylene dibromide did not affect growth or health of the bulls when compared with the controls [41]. Spermatozoic density of the semen collected from experimental bulls was low and spermatozoic motility was poor throughout the 8-10 months of semen collection. However, the reaction time until ejaculation was similar to that of the control animals. Semen smears revealed abnormal spermatozoa in experimental animals, with various degrees of degeneration and malformations, such as coiled tails, pyriform

heads, and tailless spermatozoa.

Ethylene dibromide administration was discontinued in two of the four bulls to ascertain the reversibility of the exposure, and was later readministered to one of these two to determine the time needed for ethylene dibromide's action to be effective [41]. In the first animal, almost normal spermatozoa (the acrosomes were not entirely restored) were obtained after exposure had been discontinued for 1 month; semen of normal density, spermatozoic motility, and sperm cell forms was obtained 3.5 months after discontinuation. In the second bull, normal semen was obtained 10 days after administration was stopped. After 2 further weeks of renewed exposure, semen from the second bull began exhibiting the same abnormalities as described above for bulls receiving ethylene dibromide on the continuous schedule. A previously unexposed 16-month-old bull of the same breed was fed ethylene dibromide at 2 mg/kg/day for 3 weeks. After 2 weeks, his semen exhibited the abnormalities described above, which persisted for a month after exposure ended. The semen quality improved, but normal semen was not obtained until 2-3 months after administration stopped.

From these experiments, the authors [41] concluded that quantities of ethylene dibromide, equivalent to two or three times that expected in insufficiently aired grains, caused definite reproductive impairment when fed daily or every other day to bulls. Semen density and spermatozoic motility decreased sharply and the spermatozoa were of abnormal shape after only 2 weeks of exposure. Recovery occurred in 10 days-3 months in the animals tested. Resumption of exposure caused the abnormalities to return. Studies to determine whether the reproductive impairment was prolonged

after repeated removal and reinstatement of ethylene dibromide diets were not conducted. However, in the one bull that was reexposed after a period of discontinuation, the time necessary for recovery of normal spermatozoic production increased from 10 days after the first discontinuation to 2-3 months after the second.

In 1973, Amir [42] reported the effects of ethylene dibromide on spermiogenesis and sperm maturation in six 15- to 20-month-old Israeli-Friesian bulls by oral and intraperitoneal (ip) administration. Two bulls were given ethylene dibromide orally by capsule at a dose of 4 mg/kg on alternate days. One bull was killed after 12 days (7 doses) and the other castrated after 21 days (10 doses). Smears of spermatozoa from the testis, different parts of the epididymis, and the ductus deferens were observed microscopically for abnormalities. Samples of spermatozoa examined from the bull killed on the 12th day showed that more than 50% of the spermatozoa in the testis and about 10% of the spermatozoa in the first two segments of the head of the epididymis had deformed heads. Abnormal spermatozoa, almost all of which had tail and acrosomal defects, were found in differing percentages in the various segments of the epididymis. Samples of spermatozoa examined on the 21st day from the bull receiving 10 doses indicated that spermatozoa with deformed heads constituted almost the entire population of the testis and head of the epididymis. The body and tail of the epididymis contained a mixture of abnormal and normal spermatozoa, the abnormal spermatozoa exhibiting head, tail, and acrosomal defects. Abnormal spermatozoa constituted 67% of the population in the ductus deferens and 77% of the ejaculate on day 21, with almost all of the abnormal spermatozoa showing tail and acrosomal defects.

Tritium-labeled (3-H) ethylene dibromide was given orally by capsule to one bull on alternate days for 10 doses of 2 g each, and a second bull was anesthetized and injected once beneath the tunica vaginalis of each testis with 120 mg of 3H-ethylene dibromide [42]. Two other bulls were similarly injected with carbon-labeled (14 C) ethylene dibromide at doses of 220 or 350 mg. Ejaculates were collected two or three times a week before and during the oral administration, and for 2-3 months after the administration. Spermatozoa were observed microscopically for abnormalities. The seminal plasma was separated by centrifugation and the spermatozoa were resuspended for determination of the radioactivity by liquid scintillation spectroscopy. Two days after the last 3H-ethylene dibromide oral dose, or 4 days after the 3H- and 14C-ethylene dibromide injections, both of the bulls given 3-H and one of the bulls given 14-C were unilaterally castrated. Samples of the testes and parts of the head of the epididymis were sectioned, autoradiographed for 60 days, and then examined microscopically. Maximum concentrations of 3H- and 14C-radioactivity appeared in the seminal fluid samples collected 7-9 days after administration and in the spermatozoa about 1 week later. The percentage of abnormal spermatozoa resulting from 3H- and 14C-ethylene dibromide administration increased to a maximum after the radioactivity of the spermatozoa was minimal or not detectable. After the last 3H-oral dose or 3H- and 14C-injections, autoradiographic results showed labeling of the basal cells of the head of the epididymis and the smooth muscles enveloping the epididymal duct for oral 3H- and both 3H- and 14C-ethylene dibromide injections at 2 and 4 days, respectively. No 3H- or 14C-radiolabeling of the testes was noted through autoradiography. From the distributional

pattern found in the genital tract of the two bulls given unlabeled ethylene dibromide, Amir concluded that the spermatozoa with deformed heads apparently originated in the testes under the influence of ethylene dibromide and gradually replaced spermatozoa with tail and acrosomal defects as they advanced through the epididymis. Furthermore, he noted that ethylene dibromide appeared to affect spermiogenesis while having no measurable direct toxic action on the spermatozoa. He postulated that the toxic action of ethylene dibromide most probably occurred through interference with the absorptive and secretory functions of the epididymis or by interfering with the process of nuclear chromatin condensation, but no experimental basis was given for this postulation.

In 1975, Amir [43, and written communication, August 1976] investigated the spermicidal effects of ethylene dibromide in Israeli-Friesian bulls administered 4 mg/kg every 2nd day in capsules for 10 doses. Eleven bulls (D Amir, written communication, August 1976) given ethylene dibromide in olive oil were 15-24 months of age and weighed 400-500 kg. The two remaining bulls given ethylene dibromide in olive oil were 4.5 and 5 years of age and weighed 950 and 1,050 kg, respectively. Six young bulls, 15-24 months of age, were used as controls; three were given olive oil and three remained as negative controls. Six of the 11 young bulls given ethylene dibromide and the 6 control bulls were castrated or killed (number killed or castrated not specified) 1 day after receiving the last oral dose. Semen was collected from the seven remaining bulls two or three times a week for 2 months after the first dose and once or twice a week for the next 2 months. Spermatozoic concentration, motility, and malformation were determined microscopically from at least 400 randomly chosen

spermatozoa from each semen sample. In the control bulls, 2-4% of the spermatozoa taken from various places along the genital tract had misshapen heads, and distribution of the malformed spermatozoa was uniform throughout the tract. In the animals that were killed or castrated, the number of spermatozoa exhibiting abnormal, mainly pear-shaped heads was markedly increased in the ethylene dibromide bulls over that of the controls and varied in distribution in the genital tracts of the different bulls. Amir [43] attributed the distributional pattern of abnormal spermatozoa in the genital tract to the variation in the time of release of the spermatozoa through the ductus deferens caused by individual variations in the dose-response threshold of the bulls.

In the semen samples collected from the five young bulls, maximum numbers of spermatozoa with misshapen heads appeared in the ejaculates 2-10 days after the cessation of ethylene dibromide administration [43, and written communication, August 1976]. Again, these differences were thought to be caused by differences in the sperm transit time through the epididymis as well as to the variation in release time of the affected spermatozoa from the testes. The effect of ethylene dibromide was more acute in the adult than in the young bulls. The concentrations of spermatozoa in the younger bulls were only slightly affected by the doses, but were greatly reduced in the older bulls. Normal spermatozoic concentrations were regained after 1 and 4 months in the two older bulls. During the period of low spermatozoic concentration, the ejaculates of the older bulls contained much spermatozoic debris and many spermatids and spermatocytes; the debris presumably came from further disintegration of the abnormal spermatozoa. The number of abnormal spermatozoa in the young

bulls decreased to about normal within 3 weeks after the last dose, but remained elevated in the two adult bulls for about 15 weeks. The author concluded that ethylene dibromide affected spermiogenesis in the young bulls since the abnormalities in the spermatozoa observed in the ejaculates persisted for a period that coincided with the duration of spermiogenesis in the bull, which is about 3 weeks. Amir also concluded that either ethylene dibromide affected earlier stages of the spermatogenic process in adult bulls or its elimination from the circulation of young bulls was more rapid.

In 1955, Bondi et al [44] fed fumigated grain to laying hens to determine its effects on egg laying and on the number and weight of eggs. The grain contained 10-320 ppm of ethylene dibromide mixed equally with standard laying rations that resulted in total ration concentrations of 5-160 ppm of ethylene dibromide. Twenty-five hens, 1.5 years of age, were divided into five groups of five hens each. Average egg weights and numbers were determined at the beginning of the experiment and weekly thereafter for 9 weeks. Sorghum grain for feeding was prepared by fumigation with different amounts of ethylene dibromide to give grain concentrations of 0, 50-60, 80-100, 180-220, or 270-320 ppm of unchanged ethylene dibromide. The fumigated grain was then given with equal portions of a standard laying ration to each group. The weight and number of eggs decreased progressively in all ethylene-dibromide groups during the 5th and 9th weeks (only data given) in relation to the concentration of ethylene dibromide in the feed. Cessation of egg laying occurred after 46 or 56 days of feeding in the five hens consuming ethylene dibromide in grain at concentrations of 270-320 or 180-220 ppm, respectively. The authors also

stated that the eggs of five hens receiving ethylene dibromide concentrations of 50-60 ppm weighed less than eggs from control hens after 3 weeks. They also observed that hens that had ceased egg production could not be induced to lay again by feeding them a bromide-free diet, even after feeding continued for several months.

To determine the effects of small amounts of ethylene dibromide in grain on egg laying, the authors [44] subjected 4 groups of 16 6-month-old hens each at concentrations of ethylene dibromide in grain ranging from 0 to 30 ppm (total ration concentrations of 0-15 ppm). In addition to the ethylene dibromide, small amounts of "residual bromide," defined as the bromine-containing material remaining in sorghum grain that had been fumigated with ethylene dibromide and then aerated to remove free ethylene dibromide, were added to each feeding mixture, thus giving concentrations of 0 ppm (control), 0 ppm of ethylene dibromide plus 120 ppm of residual bromide, 10-15 ppm of ethylene dibromide plus 20 ppm of residual bromide, and 20-30 ppm of ethylene dibromide plus 50 ppm of residual bromide. The prepared grains were then fed with equal portions of a standard laying ration to each group of hens. Mean egg weights for each group were averaged over 4-week periods for 16 weeks. The 16 hens receiving the ethylene dibromide-free grain which contained 120 ppm of residual bromide did not differ from the control group, both showing the expected increase in egg weights as the hens grew older. Both groups of hens receiving ethylene dibromide were affected, but the group receiving the 20- to 30-ppm grain was influenced the most. Significant ($P<0.01$) differences were seen in egg weights after 8 weeks of exposure. In the 12th week, the group receiving ethylene dibromide grain at 10-15 ppm also differed significantly

($P<0.05$) from the control group in the weight of eggs laid. Although no experimental data were given, the authors stated that the number of eggs laid by the different groups was "practically the same" during the experimental period. When hens in the groups receiving ethylene dibromide were returned to control feed, the weight of the eggs laid increased to the same weight as those of the controls in 3 weeks for the group that had received grain concentrations of 10-15 ppm and in 6 weeks for the group that had received grain concentrations of 20-30 ppm.

The authors [44] concluded from the results of these two experiments that ethylene dibromide alone caused a significant reduction in the weight and number of eggs laid by hens fed grain containing ethylene dibromide. Even grain concentrations as small as 10-15 ppm (total concentrations of 5-7.5 ppm) caused significant egg weight reductions within 10-12 weeks, but these effects were reversible and produced no long-term sequelae. Large amounts of ethylene dibromide in the grain, greater than 180 ppm (total concentrations of 90 ppm), caused an irreversible cessation of egg laying within 46-56 days. Bondi et al [44] also concluded that these observed effects on egg production were the result of free ethylene dibromide in the diet, and were not caused by "residual bromides."

In 1968, Alumot et al [45] reported on the effects of prolonged feeding with ethylene dibromide-fumigated mash on the growth rate and sexual development of chicks and on the fertility of mature chickens. Sixty 1-day-old female chicks were divided equally into two groups, the first was fed twice daily a commercial mash containing 40 ppm of ethylene dibromide and the second served as controls in a paired-feeding design. Weights and feed consumption were recorded weekly up to 10 weeks of age.

Feed intake, weight gain, onset of egg production at 4.5-5 months, and incidence of double-yolked eggs were the same for the experimental and control groups. The weight of eggs from the group receiving ethylene dibromide was significantly lower ($P<0.01$) than from the controls and the number of eggs laid by the hens receiving ethylene dibromide approached statistical significance, lowering below that of the control hens.

In a second experiment [45], two groups of 12 1-year-old hens in full egg production were fed for 4 weeks a commercial mash with or without 100 ppm of ethylene dibromide. At the end of 4 weeks, egg weight in the hens fed ethylene dibromide had significantly decreased from an average of 63 to 43 g. Both groups were then artificially inseminated twice at 7-day intervals with 0.1 ml of semen/hen. Eggs collected between the 2nd day after the first insemination and the 7th day after the second insemination were examined for embryos. A striking reduction in the fertilization rate, with no live embryos at all, was noted in the ethylene dibromide-fed group; only 2 of 16 eggs were fertilized as compared with 48 of 56 eggs from the control group, and both fertilized eggs from the ethylene dibromide group contained dead embryos.

Similarly, a third experiment [45] was conducted to determine the effects of ethylene dibromide on male chicken growth rate and sexual development. Three groups of 20 3-day-old cockerels were fed mash containing 0, 80, or 180 ppm of ethylene dibromide in which the amount of feed intake by the 180-ppm group determined the amounts of food given to the control and 80-ppm groups. Three additional groups of 25 cockerels were each fed mash containing 0, 150, or 300 ppm of ethylene dibromide without restrictions on the intake. At 6 weeks, the cockerels fed 150 ppm

of ethylene dibromide showed a reduced weight gain when compared with the controls. Only cockerels fed the 300-ppm ethylene dibromide diet without restrictions on food intake showed significant growth retardation and an apparent feed intake depression at 12 weeks of age; however, the weight gain to feed intake ratio was not significantly different. The cockerels receiving the regulated diets were killed at 3 months of age, and the bromide content of the testes, spermatozoic count and activity, and testes weight were determined. Significant amounts of bromide were found in the testes of the ethylene dibromide-fed groups, but differences were not observed in spermiogenic activity, spermatozoic count, or testes weight between the control and ethylene dibromide-fed groups. The remaining unrestricted-intake cockerel groups were examined at the age of 9 months by collecting semen samples three times at weekly intervals and were killed at 12 months of age for testicular examination and measurement of body, testes, and comb weights. Semen collected and examined from ethylene dibromide-fed cockerels did not differ significantly from that of the controls. Comb weights at death declined sharply with increasing concentrations of ethylene dibromide in the diet; however, body and testes weights were not affected by the ethylene dibromide.

Another experiment [45] was conducted to determine if ethylene dibromide administration to adult cockerels would affect fertility rates and hatchability of eggs. Eleven mature cockerels each were fed for 105 days a control mash or mash containing 300 ppm of ethylene dibromide. Semen was collected about 2, 4, 8, and 10 weeks after the beginning of the experiment. No significant differences were seen between the semen of the controls and that of the cockerels fed ethylene dibromide with respect to

ejaculated volume and spermatozoic motility and concentration. Subsequent fertilization tests in hens conducted with the pooled semen collected from the ethylene dibromide-fed and control groups gave no significant differences in fertilization rate or hatchability of eggs at 60 or 105 days.

The authors [45] concluded that prolonged feeding of mash containing ethylene dibromide significantly depressed growth of male chickens when fed without restrictions, but that the depression seemed to result from reduced food intake and not from the direct action of ethylene dibromide. They also concluded that ethylene dibromide had no effect on the onset of egg production in hens fed from birth, on sexual development in males and females, and on sperm characteristics or fertility in mature males. However, statistically significant reductions in egg size and egg fertility were noted in hens fed ethylene dibromide.

In 1969, Alumot and Mandel [46] published the results of an investigation designed to measure the effects of ethylene dibromide administration on the formation and release of follicle-stimulating hormone (FSH) by the anterior pituitary gland in laying hens. FSH activity was determined by bioassay in groups of 20 1-week-old male White Rock cockerels by injecting pooled pituitary gland extracts. The changes in testicular weight were directly compared with those of control cockerels, and the increase in testes weight was used as an indication of FSH activity.

Ninety 3-month-old pullets were divided equally into three groups [46]. The first group was killed and FSH activity determined at the start of the experiment. The pullets in the second group were each fed approximately 10 mg/day of ethylene dibromide in fumigated feed for 4

weeks. The third group served as controls for FSH activity at 4 months of age. No changes in the FSH concentration or activity were detected in the pullets fed mash containing ethylene dibromide.

In a second experiment [46], a group of 30 mature hens were each fed approximately 10 mg/day of ethylene dibromide in treated mash for about 2 months until egg laying had almost ceased. FSH concentrations were examined as above and compared with those from 30 control hens. There were no appreciable differences between FSH concentrations in the hens fed ethylene dibromide and in the control hens. The authors [46] concluded that ethylene dibromide did not affect FSH formation or concentrations in the pituitary gland.

A further experiment [46] was conducted to determine whether hormone injections could counteract the decrease in egg sizes caused by ethylene dibromide. Four hens, laying very small eggs as a result of ethylene dibromide administration, were injected with a freeze-dried pituitary extract obtained from 7- to 11-week-old freshly killed broilers. Each hen received daily 4 mg of the preparation for 6 days. Egg weights recorded before, during, and 2 weeks after the last injection showed no significant differences. A purified FSH preparation, Ovine FSH-S2 from the National Institutes of Health, was injected into another group of four laying hens previously given ethylene dibromide as described above. The weights of whole eggs and yolks were compared for each hen prior to, during, and for 10 days after the injections. Injection of the purified hormone did not change the total egg weight or the weight of the yolks, which both remained lower than comparable control values. The authors concluded from the above four experiments that ethylene dibromide did not affect pituitary FSH

concentrations in treated hens. The injection of FSH preparations did not reverse the adverse effects of ethylene dibromide; therefore, the effects of ethylene dibromide on egg laying did not seem to be connected with impaired formation or release of FSH.

In an attempt to clarify the causes of smaller eggs in laying hens fed ethylene dibromide-fumigated mash [44], Alumot and Harduf [47] separated chick serum proteins into two crude fractions, globulin and albumin. The proteins in each fraction were labeled with 125-I and injected iv into 14 hens that had been previously fed fumigated mash containing about 100 ppm of ethylene dibromide and into 14 control hens. The labeled proteins were injected into the hens when egg weights had decreased in the ethylene dibromide-fed hens to about 40 g as compared with 60 g in the controls. The amount of radioactivity was measured in egg yolks collected daily for 2 weeks after injection and in ovarian follicles of hens killed 40 hours after injection. Eggs from control and ethylene dibromide-fed hens differed in the amount of radioactivity in the yolk protein fractions. The amount of radioactivity in yolks of eggs from hens fed ethylene dibromide was about half that of the controls when the albumin fraction was injected iv and about a third that of the controls when the globulin fraction was injected iv. Similar results were observed in the ovarian follicles, particularly in the radioactive uptake per unit of membrane area, but no differences were found between results obtained from albumin and globulin injections. The authors postulated that the impaired passage of proteins to the follicle may be related to the decreased egg weight caused by ethylene dibromide in hens. Since the globulin fraction was composed of high molecular weight proteins and the albumin fraction of

low molecular weight proteins, the different ratio of radioactive incorporation into the yolk was thought by the authors to be an indication of an impairment of the permeability of the vitelline or follicular membranes to proteins induced by ethylene dibromide. These results indicate a significant difference in the metabolism of 125-I-labeled protein between the hens maintained on mash containing about 100 ppm of ethylene dibromide and the control population.

In 1970, Edwards et al [9] investigated the antifertility effects of ethylene dibromide in an unspecified number of adult male Wistar rats. Doses of 10 mg/kg were injected ip into 250-g rats for 5 consecutive days. The average live litter size from six female rats serially mated on a weekly schedule with the males receiving ethylene dibromide decreased substantially during the 3rd week and drastically (to zero) during the 4th week after administration. From the authors' data, the average litter size appears normal during the 5th week and for every week thereafter. The authors concluded that the antifertility effects resulted from ethylene dibromide selectively damaging the spermatid cells, since the short course of exposure produced only transient sterility in rats and corresponded in time to changes that would result from spermatid damage. In an additional study [9], the major metabolite of ethylene dibromide in urine, S-(2-hydroxyethyl)-cysteine, was reported to have produced no effect on male mouse fertility at a dose of 1,000 mg/kg when administered daily for 5 days by oral intubation, although experimental details and data were not presented. The data indicate that oral exposure to ethylene dibromide induces temporary sterility in male rats. However, further evaluation of the data is not possible because of the lack of experimental details, such

as number of test animals or any data about control animals, that were not given by the authors in the publication.

(c) Metabolism

In 1940, Abreu and Emerson [48] reported that mice subjected to ethylene dibromide vapor at a concentration of 0.75 mM/liter (approximately 18,350 ppm) for 1 hour had an increase in liver inorganic bromide concentrations when compared with controls. From the authors' data, this increase appeared to be highly significant. They concluded that the bromide concentrations found could hardly have caused the liver tissue damage previously thought to be caused by local concentrations of hydrobromic acid [28], and suggested that the tissue damage was the result of the intact molecule itself.

In 1948, Heppel and Porterfield [49] reported that rat liver fractions enzymatically dehalogenated ethylene dibromide. The enzymatic system required activation by cyanide and either glutathione or cysteine.

Mammalian liver extracts catalyze a reaction between ethylene dibromide and glutathione [49-52]. Under the assay conditions used, two moles of bromide ion were released for each mole of glutathione consumed, which suggests that the initial reaction product, S-(beta-bromoethyl)-glutathione, rapidly cyclizes to form an S-substituted thirane with concomitant release of the second bromide ion [6,7,9,11,50,53]. Spontaneous hydrolysis of the S-substituted thirane, which is a highly reactive alkylating agent, would yield S-beta-hydroxyethyl glutathione, a known metabolite of ethylene dibromide [50,51]. Hydrolysis and acetylation of the S-beta-hydroxyethyl glutathione would yield S-(beta-hydroxyethyl)-cysteine and S-(beta-hydroxyethyl) mercapturic acid, which have also been

identified as metabolites of ethylene dibromide [51,52]. Other metabolites of ethylene dibromide may be formed in biologic systems by other metabolic pathways; however, a comprehensive study designed to isolate and identify metabolites is needed.

Further research from Nachtomi's laboratory [50,54] identified a characteristic decrease in the concentration of liver sulfhydryl groups during the 1st hours after administration of 110 mg/kg of ethylene dibromide in male and female rats and in male and female chickens, and a subsequent increase of liver sulfhydryl groups in both species after about 20 hours [54]. This increase probably was caused by the enhanced synthesis of glutathione. There was no appreciable loss of ascorbic acid which suggested to the authors that the extensive formation of peroxides, as reported for the metabolism of carbon tetrachloride, did not occur. In 1970, studies [50] conducted with rat liver homogenates supported the earlier conclusions by Nachtomi et al [51] when S-(beta-hydroxyethyl) glutathione was identified as the major in vitro product. In addition, small amounts of the symmetrical intermediate, S,S'-ethylene-bis(glutathione), were identified. The above two compounds and the sulfoxide of S-(beta-hydroxyethyl)glutathione were identified in the livers of rats intubated with 120 mg/kg of carbon-labeled (14 C) ethylene dibromide, and S-(beta-hydroxyethyl) mercapturic acid was identified in the kidneys. The enzymatic reaction between ethylene dibromide and glutathione was found to occur primarily in the liver, and, to a lesser extent, in the kidneys [50]. The capacity of the liver and kidneys to metabolize ethylene dibromide was estimated to be 1.7 ± 0.02 and 1.4 ± 0.05 μmoles/minute/g of

tissue, respectively. The subsequent degradation of the S-(beta-hydroxyethyl) glutathione and its sulfoxide to S-(beta-hydroxyethyl) mercapturic acid and S-(beta-hydroxyethyl) mercapturic acid sulfoxide was reported to occur primarily in the kidneys and was thought to involve more than one enzyme. These data indicate that the capacity of rat liver and kidney to metabolize ethylene dibromide exceeds the amount of ethylene dibromide inhaled from an atmosphere containing 25 ppm (192.5 mg/cu m) by a factor of about 100. Consequently, under steady-state exposure conditions, the concentrations of ethylene dibromide in tissues should be considerably less than the concentration in the inhaled air, which is about 1.02 μmoles/liter.

Differences have been reported in the abilities of rats and chickens to detoxify ethylene dibromide. Nachtomi et al [55] and Nachtomi and Alumot [8] observed that rats possessed a much greater ability to conjugate glutathione with ethylene dibromide than chicks [55]. In addition, the rat liver formed significant amounts of lipids containing conjugated double bonds as a result of the peroxidation of lipids induced by ethylene dibromide in the liver microsomal supernatant fraction, whereas chicken liver was relatively inactive [8]. Also, the concentration of triglycerides increased significantly in the rat liver, probably as a direct result of the formation of conjugated double bonds discussed above.

In 1970, Edwards and coworkers [9] reported the tissue distribution of radioisotope at various time periods after an ip injection of 40 mg/kg of carbon-labeled (14 C) ethylene dibromide in mice. The data are presented in Table XII-3. One hour after injection, most of the 14C-radioactivity was found in the small intestine, with smaller amounts being

found, in descending order, in the kidneys, liver, blood plasma, whole blood, large intestine, fat, and spleen. After 3 hours, the majority of the radioisotope was present in the large intestine, kidneys, and blood plasma, with smaller amounts being present in the whole blood, liver, small intestine, and spleen. After 24 hours, all tissues were below 1.0% retention of 14C-radioisotope except for the whole blood, blood plasma, stomach, and kidneys. After 1 hour, essentially all of the administered radioisotope was present in the various mouse tissues, whereas only 89% was present after 3 hours and 16% after 24 hours. It is of interest to note that 3.1, 4.4, and 0.66% of the administered radioisotope was detected in the tail of the epididymis 1, 3, and 24 hours after injection, respectively. Smaller amounts of 14C-radioisotope, up to 1.5% at 3 hours, were also present in the testes. The data presented by the authors suggest that ethylene dibromide is rapidly distributed to a wide variety of tissues. Major concentrations of radioisotope accumulated in the liver, kidneys, and digestive tract. There also seems to be a rapid depletion of radioisotope from the tissues, but the residual radioactive material is still widely distributed throughout the body tissues.

In 1976, Plotnick and Conner [10] conducted a similar study of the tissue distribution of carbon-labeled (14 C) ethylene dibromide in guinea pigs. The ethylene dibromide was administered in a single ip injection of 30 mg/kg and tissue samples were collected 4-72 hours after the injection. The data are presented in Tables XII-4 and XII-5. At all intervals studied, the highest concentration of radioactivity derived from ethylene dibromide as μg/g of tissue was found in the kidneys, liver, and adrenal glands. At all time periods studied, the organs containing the highest

percentage of the administered dose were the liver and kidneys. Approximately 66% of the injected dose was excreted in the urine over the 72-hour study, 15% of which was in the first 4 hours, and approximately 3% was excreted in the feces. Plotnick and Conner mentioned that unpublished preliminary studies by their laboratory "strongly suggest that excretion of unchanged ethylene dibromide in the expired air also represents a significant (10-12% of the dose) route of excretion."

From the data of Edwards et al [9] and Plotnick and Conner [10], it seems that a relationship may exist between the tissue distribution and the destructive tissue changes, particularly with respect to the liver and kidney damage, in other animal [27,33] and human [22,25] studies reported previously in this chapter.

Carcinogenic, Mutagenic, and Teratogenic Studies

(a) Carcinogenesis

In 1973, Olson et al [56] reported the results of a preliminary investigation to determine the potential carcinogenic effects of ethylene dibromide in rats and mice of both sexes by oral administration. Further statements by Powers et al [57] and Ward and Habermann [58,59] in abstracts, and by Page (written communication, December 1976) have been used to supplement the preliminary report by Olson et al [56]. Osborne-Mendel rats, 50 males and 50 females, 6 weeks of age, and (C57BL x C3H) F1 mice, 50 males and 50 females, 6 weeks of age, were administered daily doses of ethylene dibromide dissolved in corn oil by gastric intubation. Each rat received either what was thought to be the maximum tolerated dose of 80 mg/kg/day or one-half that dose for 5 days/week, for 16 weeks. At 16

weeks, the 80 mg/kg/day dose was discontinued for 14 weeks because of cumulative toxicity. The 40 mg/kg/day dose continued without interruption for the full 54-week experimental period. At 30 weeks, the 80 mg/kg/day group of rats was placed on a 40 mg/kg/day regimen for the remainder of the experiment. The rats started on the 80 mg/kg dose received a total dosage of 11.2 g/kg of ethylene dibromide during the experiment and those given the 40 mg/kg dose received a total dosage of 10.8 g/kg. Control groups of rats and mice, 20 males and 20 females each, received intubations of corn oil or nothing for the duration of the test.

Initially the only deleterious effect of ethylene dibromide on the rats was a depression in weight gain [56]. As early as 10 weeks after the initiation of the experiment (dose not stated), one squamous cell carcinoma of the stomach was noted in one male and in one female rat. Squamous cell carcinomas of the stomach became more prevalent as the experiment progressed, developing in 83 of 100 male rats and in 70 of 100 female rats intubated with ethylene dibromide. The tumors originated in the forestomach, invaded locally, and eventually metastasized throughout the abdominal cavity. Only one mammary tumor in a female rat was noted in the concurrent corn oil controls; however, in the untreated control group, 6 of 20 male rats and 14 of 20 female rats developed tumors, none of which were squamous cell carcinomas of the stomach. Tumor incidence was 68% in the male rats receiving ethylene dibromide at 80 mg/kg/day versus 98% in those receiving 40 mg/kg/day, and it was 58% in the female rats at 80 mg/kg/day versus 82% at 40 mg/kg/day. Olson et al [56] postulated that this decreased incidence of tumorigenesis may have resulted from the earlier death of the animals receiving the higher dose or because the animals did

not receive ethylene dibromide for a 14-week period between weeks 16 and 30. The induction sequence and results of microscopic examinations were discussed in detail only for the male rats receiving the 40 mg/kg dose, since the authors considered the induction and progression of the tumors to be similar in all experimental groups [59]. All 50 male rats had stomach lesions, although one did not contain a neoplasm. The forestomachs had diffuse squamous cell hyperplasia with many papillomatous projections. Focal areas of invasion were reported from the origin of the carcinomas. Invasion occurred through the stomach wall to the peritoneal cavity, where nodules were reported in 70% of the rats. The metastatic tumors were less differentiated or formed keratin pearls, often accompanied by abcesses or peritonitis. The authors [59] stated that other types of tumors and lesions were also present in a few rats, including mesotheliomas, intestinal tumors, nodular hyperplasias of the liver, and poorly differentiated stomach tumors.

Each group of mice received ethylene dibromide in doses of 60 or 120 mg/kg/day for 13 weeks [56]. After 13 weeks, the doses were increased to 100 and 200 mg/kg/day but were reduced to the original doses after 2 weeks because of toxicity. At 42 weeks, the daily dose of ethylene dibromide for all mice was changed to 60 mg/kg/day. At 42 weeks, one male and one female mouse developed squamous cell carcinomas of the stomach at the higher dose regimen, and three males and two females at the lower regimen. High early mortality was encountered in mice receiving the higher dose (40%). No squamous cell tumors were found in the concurrent controls, although 6 of 40 males and 3 of 40 females developed other types of tumors. Squamous cell carcinomas of the stomach developed in 74% of the males and in 72% of

the females at the terminal killings between weeks 59 and 90 (NP Page, written communication, December 1976).

It seems that ethylene dibromide caused an increased incidence of gastric carcinoma in rats treated by intubation with either 40 mg/kg/day or an ordered combination of 80, 0, and 40 mg/kg/day. Carcinomas were found to originate at the site of administration (the stomach) in both species, to invade locally, and eventually to metastasize throughout the abdomen [56,58,59]. Also, 72 of 145 mice that developed squamous cell carcinomas were diagnosed after week 59, whereas the vehicle controls were killed at week 59.

(b) Mutagenesis

In 1972, Buselmaier et al [60] reported the results of an investigation to determine the potential for ethylene dibromide to induce mutations in the host-mediated assay with Salmonella typhimurium G46 and Serratia marcescens a21 Leu- in NMRI mice and in the in vitro plate test with Salmonella typhimurium G46. Immediately after ip injection of each bacterial strain, 500 mg/kg of ethylene dibromide emulsified in edible oil was injected subcutaneously into one hind leg of six 10- to 12-week-old mice. Three hours after the injections, they were killed and the fluid from the peritoneal cavity was collected for plating to determine the number of mutant colonies induced. In the comparative in vitro plate test with Salmonella typhimurium, a concentration equivalent to the 500 mg/kg dose was applied to a filter paper disc in the center of the plate, and after a 12-hour incubation, the number of mutant colonies were counted.

The investigators [60] found that ethylene dibromide was definitely mutagenic in the host-mediated assay with Salmonella typhimurium G46 and in

vitro with the same organism. The mutation frequencies for Salmonella typhimurium G46 in the host-mediated assay were 0.77×10^{-8} in the control test and 6.23×10^{-8} in the experimental test. The ethylene dibromide-exposed mutation frequency was significantly different ($P < 0.01$) from the control. The in vitro plate test results were also positive. Ethylene dibromide did not induce a significant number of mutants in the host-mediated assay with Serratia marcescens a21 (6.93×10^{-7} for control, 2.43×10^{-7} for ethylene dibromide-exposed). The authors [60] concluded that ethylene dibromide did not require activation through in vivo metabolism to exert its mutagenic effects on Salmonella typhimurium, nor did metabolism of ethylene dibromide sufficiently deactivate its mutagenic potential, since both the host-mediated assay and the in vitro plate tests were positive.

In 1972, Epstein et al [61] published the results of a screening study on the detection of chemical mutagens by a dominant lethal assay in ICR/Ha Swiss mice. Ethylene dibromide was tested along with 173 other industrially important chemicals or chemical mixtures. Ten male mice, 8- to 10-weeks-old, were given 0, 50, or 100 mg/kg of ethylene dibromide for 5 consecutive days by oral administration and were then mated weekly with three different virgin 8- to 10-week-old female mice for 8 consecutive weeks. One male mouse died at the 50 mg/kg dose and two males died at the 100 mg/kg dose during the experiment. Two different groups of seven or nine male mice received single ip injections of 18 or 90 mg/kg of ethylene dibromide, respectively, and were then mated as above. The mated females were killed about the 13th day after presumptive mating and were scored for the number of total implants, live implants, early fetal deaths, and

pregnancies. The experimental animal scores were contrasted to concurrent control scores. The authors included ethylene dibromide in a class of agents which did not meet "any screening criteria for mutagenic effects," although specific data for ethylene dibromide were not presented. No effect of ethylene dibromide on fertility was reported in this paper, which differs from the finding of Edwards et al [9].

In 1973, Clive [62] tested the mutagenic potential of ethylene dibromide on the back mutation frequency of the thymidine kinase locus in a mammalian somatic cell tissue culture derived from mouse lymphoma cells. The L5178Y mouse lymphoma cells were exposed to ethylene dibromide at concentrations of 0.0-3.0 mM in culture media for 2 hours. The exposed cells displayed an induced mutational frequency that was dose related and typical of other alkylating agents, such as methyl methanesulfonate and ethyl methanesulfonate.

Thirteen new mutations for each 10,000 surviving cells were found under these experimental conditions [62]. The frequency of induced mutations generally increased monotonically after exposure of the cells to increasing concentrations of ethylene dibromide. The induced mutation frequency indicated that ethylene dibromide was mutagenic over the entire range of experimental concentrations when plotted against the growth inhibition, although less potent than ethyl methanesulfonate (an induced mutational frequency of about 2/10,000 cells versus one of 7/10,000 cells at 60% growth inhibition, respectively). The induced mutational frequency in L5178Y mouse lymphoma cells corresponded with that of a mutational frequency of over 600 R of X-irradiation at the highest concentration. Clive concluded that ethylene dibromide was mutagenic to the L5178Y mouse

lymphoma cells although not as potent as ethyl methanesulfonate.

In 1974, Meneghini [5] reported the results of an investigation to determine the potential for ethylene dibromide to induce DNA repair synthesis in cultured opossum lymphocytes. Two-year-old opossums, Didelphis virginiana, were used to obtain lymphocytes for culture. Blood was removed from their tails and lymphocyte suspensions were prepared and maintained at 37 C. Samples containing about 5 x 10^6 cells/ml were incubated with various concentrations of ethylene dibromide for 1 hour at 37 C. At the end of this period, the cells were incubated in culture media with 3H-thymidine for 4 hours to allow for DNA radiolabeling to occur. Control cells were processed as above, but without exposure with ethylene dibromide.

At concentrations between 0.001 and 1 mM, ethylene dibromide was very effective in inducing DNA repair in opossum lymphocytes [5]. Repair induction decreased at a concentration of 10 mM of ethylene dibromide, which was presumed to be the result of repair-inhibition phenomena such as those observed for ultraviolet-induced repair. The author indicated that ethylene dibromide was very effective in inducing DNA repair in a manner similar to other alkylating agents, such as methyl methanesulfonate and ethyl methanesulfonate, which were also studied. He stated that only compounds that interact with DNA through covalent bonds induce repair synthesis. Therefore, these data suggest that ethylene dibromide, or its metabolites, may interact via covalent bonding with DNA.

In 1974, Vogel and Chandler [6] measured the possible genetic effects induced by ethylene dibromide in sex-linked lethal tests with fruit flies, Drosophila melanogaster. The adult Berlin K male flies were allowed to

feed on a 0.3-mM ethylene dibromide solution for 3 days before being allowed to mate with two females for 3 days. A sequence of two 3-day brood periods was initiated (broods 1 and 2), followed by one 4-day brood period (brood 3). At the end of each 3-day breeding period, the treated males were transferred to a new vial and were allowed to mate with two new females. The frequencies of recessive lethal mutations in meiotic and postmeiotic germ cells were determined in the offspring of treated and control males. Ethylene dibromide induced significant numbers of recessive lethal mutations in the offspring of all three broods. These were 0.50, 1.49, and 1.30% in broods 1, 2, and 3, respectively, and an average lethal frequency for all broods of 1.10%. The authors concluded that the brood pattern resulting from ethylene dibromide exposure was consistent with the action of chemicals that affect spermatids and spermatocytes because of the higher incidence of recessive lethal mutations in broods 2 and 3. The authors considered ethylene dibromide to be a bifunctional alkylating agent capable of introducing cross-links into large biologic molecules, and to be definitely mutagenic in Drosophila melanogaster.

In 1971, Ames [63] published the results of an experiment to determine the mutagenic potential of ethylene dibromide to the his-G46 and TA 1530 strains of Salmonella typhimurium. A 5-μl (11.0 mg) sample of ethylene dibromide was placed in the center of a petri plate previously seeded with bacteria that were unable to grow because of a deficiency mutation. The number of reverted mutant colonies was measured as an indication of the mutagenic potential. Ames reported that ethylene dibromide was a mutagen when tested in these systems, although experimental data were not reported.

Brem and coworkers [64], in 1974, tested the possible mutagenic and DNA-modifying effects of ethylene dibromide on two bacterial systems, Escherichia coli and Salmonella typhimurium. E coli pol A+ or E coli pol A1- were spread onto the surface of agar plates and were exposed to 10μl (22.0 mg) of ethylene dibromide deposited directly onto a sterile disc centered in the plate. After an 8-hour incubation at 37 C, the diameter of the zone of growth inhibition in each plate from three replications was measured as an indication of the mutagenic potential. Salmonella typhimurium TA 1530, TA 1535, or TA 1538 were exposed to 0.0-11.5 μM of ethylene dibromide in a similar manner except that the plates were incubated in the dark for 54 hours. The number of reverted mutant colonies on each plate was counted and used as an indication of the mutagenic potential.

Ethylene dibromide inhibited the growth of the E coli pol A1- strain preferentially to isogenic pol A+ strain as indicated by zone of inhibition diameters of 20 versus 15 mm, respectively, at concentrations of 10 μl (22.0 mg)/plate [64]. Since the isogenic strains differed only in the structural gene for DNA polymerase A, it was concluded that ethylene dibromide affected cellular DNA in E coli. Ethylene dibromide was found to be mutagenic at 10.0 μM/plate to Salmonella typhimurium TA 1530 (1329 revertants/plate versus 23 revertants/plate for the water control) and to Salmonella typhimurium TA 1535 (1438 revertants/plate versus 26 revertants/plate for the water control), but it was not mutagenic to Salmonella typhimurium TA 1538 (18 revertants/plate versus 19 revertants/plate for the water control). The authors suggested that ethylene dibromide induced mutations resulting from base substitutions, as

indicated by the positive results with TA 1530 and TA 1535, and not by frame shifting, as indicated by the negative results with TA 1538. These data substantiate the earlier results of Buselmaier et al [60] for the induction of back mutations at the his-G46 locus in Salmonella typhimurium.

In 1975, Alper and Ames [65] published the results of a study to determine whether ethylene dibromide or 39 other agents could cause mutants by chromosomal deletions of various lengths in Salmonella typhimurium LT2 and Salmonella typhimurium galE503. Bacteria were poured onto agar plates as suspensions in top agar and were incubated for 8 hours. A few drops of the test compound, 1-5 μl (2.2-11.0 mg), were placed near the edge of the plate immediately after the top agar had solidified. The number of colonies clustered within a 3.5-cm radius of the spot where the compound was applied was used as an index of the extent of mutagenesis. The zone of inhibition for ethylene dibromide was reported as less than 1.5 cm from the application spot, but specific data were not presented. Although no experimental data were given, ethylene dibromide was included in a list of compounds which failed to increase the frequency of deletion mutants by as much as fourfold over that of the control.

In 1974, Sparrow et al [66] investigated the effects of ethylene dibromide vapor on genetic mutagenesis in the interspecific hybrid clone 4430 of Tradescantia plants heterozygous for flower color. Mutation frequencies were determined after doses of X-rays ranging from 0.0 to 432.0 rads at 30 rad/minute, or after dynamic exposures to measured concentrations of gaseous ethylene dibromide ranging from 3.6 to 148.2 ppm (27.72 to 1,141.14 mg/cu m) for 6 hours. Mutation frequencies were obtained by averaging the mutant events from 11-15 days after irradiation,

or 10-15 days after ethylene dibromide exposure.

The frequencies of pink flower mutations in Tradescantia was found to be linear for X-irradiation doses below 6 rads [66]. No linear dose-response relationship was observed above 50 rads, the mutation frequencies at higher doses actually decreasing below those for some lower doses. A straight-line relationship was demonstrated for the entire range of ethylene dibromide concentrations from 3.6 to 148.2 ppm (27.72 to 1,141.14 mg/cu m) for the pink mutant response in clone 4430 when the number of induced mutations was plotted against the concentration of ethylene dibromide. The authors postulated that saturation, or the nonlinear, decreased mutation rates noted for X-irradiation at high doses, was not attained at the 148.2 ppm (1,141.14 mg/cu m) concentration of ethylene dibromide, even though plant damage was very apparent and flower production was considerably reduced.

Sparrow et al [66] concluded that ethylene dibromide was "highly mutagenic" to Tradescantia, eliciting a well-defined dose-response relationship with surface exposures as low as 3.6 ppm (27.72 mg/cu m). NIOSH has calculated the slope of the dose-response curve for the induction of pink mutants for Tradescantia clone 4430 to be 1.3 between 3.6 and 148.2 ppm (27.72 and 1,141.14 mg/cu m), which is consistent with the prediction of the multi-hit theory for mutation induction in this species under these experimental conditions.

Ethylene dibromide has been reported to induce mutations in Neurospora crassa [67,68], but complete experimental details were not given in these abstracts.

(c) Teratogenesis

In 1976, Short et al [69] examined the effects of ethylene dibromide vapor on rats and mice during organogenesis. The production of congenital defects was used as a measure of inherent toxicity. Female Charles River CD rats or female CD-1 mice were caged overnight with proven male breeders; successfully bred females were identified the next morning by the presence of sperm in vaginal smears from rats or of copulation plugs in mice. Pregnant animals were divided into a control group of 18 rats and 17 mice which would receive a normal diet without inhalation exposure to ethylene dibromide, a group of 18 rats and 13 mice to receive 31.6 ppm (243.32 mg/cu m) of ethylene dibromide and a normal diet, and a group of 17 rats and 9 mice to receive a restricted diet without exposure to ethylene dibromide. All groups of animals were housed in the inhalation chambers for 10 consecutive days beginning on day 6 of gestation. During this time, the groups to be given ethylene dibromide were exposed to the vapor at an average concentration of 31.6 ± 1.9 ppm (243.32 ± 14.63 mg/cu m) for 23 hours a day. Rats and mice were killed on gestational day 20 or 18, respectively. Fetuses were surgically removed from the dams, weighed, examined for external anomalies, and fixed for either soft-tissue or skeletal examinations. Ethylene dibromide exposure did not produce mortality in either pregnant rats or mice during the 10-day inhalation period; however, two of nine pregnant mice from the restricted diet group died. Both rats and mice exposed to ethylene dibromide consumed less feed and gained significantly ($P<0.05$) less weight than the controls during the exposure period, although feed consumption and weight gain returned to normal after cessation of the exposure on the 15th day of pregnancy.

Ethylene dibromide-exposed rats consumed about twice as much food daily as did the feed-restricted rats (10.5 ± 0.6 versus 5.0 ± 0.1 g/day, respectively), but only about one-half of that consumed by the controls (10.5 ± 0.6 versus 21.1 ± 0.4 g/day, respectively).

Rats exposed to ethylene dibromide at a concentration of 31.6 ppm (243.32 mg/cu m) had statistically significant decreases ($P<0.05$) when compared with the unexposed controls in the mean numbers of implants/dam (12.4 versus 15.4) and live fetuses/dam (12.2 versus 15.3) [69]. Also, the percentage of viable fetuses decreased slightly and the percentage of early resorptions increased slightly in ethylene dibromide-exposed rats. The number of male fetuses in the live litters also increased slightly in rats receiving ethylene dibromide from 48% males in control rats to 59% males in ethylene dibromide-exposed rats.

The only significant ($P<0.05$) difference in reproductive success found in mice exposed to ethylene dibromide at 31.6 ppm (243.32 mg/cu m) was a decrease in the live fetal weights, which also occurred in the feed-restricted mice [69]. Slight, but not significant, unfavorable changes in the number of implants/dam, viable fetuses, early resorptions, late resorptions, complete resorptions, fetuses/dam, and percentage of live males/litter were also found in both the ethylene dibromide-exposed and feed-restricted mice when compared with the controls.

Specific organogenic anomalies in the fetuses of rats exposed to ethylene dibromide at 31.6 ppm (243.32 mg/cu m) that were significantly different from controls included what the authors termed "hydrocephaly" of the fourth ventricle ($P<0.10$), decreased occurrence of the fourteenth pair of ribs ($P<0.05$), and wavy ribs ($P<0.05$), none of which were significantly

different in the fetuses of the feed-restricted rats when compared with control fetuses [69]. Other noticeable but not significant changes found in the fetuses of rats exposed to ethylene dibromide included limb reduction, "hydrocephaly" of the lateral and third ventricles, hydronephrosis, club foot, incompletely ossified supraoccipital and parietal bones, and fused ribs. The incidence of "hydrocephaly" of the lateral ventricles and the incomplete ossification of the supraoccipital bone were also different in fetuses from the feed-restricted rats and fetuses from the controls.

Fetuses of mice exposed to ethylene dibromide vapor were found to have significant increases in skeletal anomalies when compared with those from control mice [69]. These anomalies included no ossification of the incus bone ($P<0.01$), incomplete ossification of the supraoccipital bone ($P<0.01$), and variations in ossification of the sternabrae ($P<0.05$ or $P<0.01$). Only the variations in the sternabrae ($P<0.10$ or $P<0.05$) were noted in the feed-restricted mice. Other changes in ethylene dibromide-exposed mice included an increase in "hydrocephaly" of the third and fourth ventricles and split sternabrae.

The authors [69] concluded that the above-mentioned effects of ethylene dibromide exposure in mice were most likely attributable to malnourishment rather than to ethylene dibromide exposure. It is apparent from the authors' data that some of the anomalies may be compounded by the presence of ethylene dibromide. In addition, if the malnourished controls and ethylene dibromide-exposed animals had been compared with the nonexposed controls at the same statistical probability levels, only the ethylene dibromide-exposed animals would have been significantly different

from the controls. Skeletal anomalies, in particular the variations in ossification of the incus and supraoccipital bones, were much more prevalent in the ethylene dibromide-exposed mice than in the feed-restricted mice (80-90% of the litters versus 33-50%, respectively). The authors also attributed many of the observed anomalies in the rat dams and fetuses exposed to ethylene dibromide to malnutrition rather than to direct effects of ethylene dibromide exposure. However, they concluded that the increase in the fourth ventricular "hydrocephaly," the reduction in the occurrence of the fourteenth rib, and the increase in the frequency of wavy ribs could be correlated to ethylene dibromide exposure in the rat.

The significance of the effects of malnutrition in rats is not clear, and even though ethylene dibromide-exposed rats consumed less food than did controls, they consumed more food than did the rats in the feed-restricted group. It is apparent, however, that an increase in the incidence of anomalies in fetal rats was induced by exposure to the one concentration of ethylene dibromide, but no dose-response relationship for this action is available.

Correlation of Exposure and Effect

Neither studies that correlate workplace concentrations of ethylene dibromide with observed toxic effects nor any epidemiologic studies have been found. Few reports of human exposure to ethylene dibromide exist. Those that have been reported for occupational [23,24], accidental [22,25], and experimental [24] exposures do not present exact quantitative data concerning the concentrations or durations of exposure; thus, correlation of exposure and effect is exceedingly difficult and further correlation in

humans can be accomplished only partially from qualitative, and often subjective, symptomatic observations. In humans, ethylene dibromide vapor was reported to cause irritation of the eyes [23] and of the respiratory tract [22,23], headaches [23], anorexia [23], swelling of the glands beneath the chin and maxillary angles [23], a generalized body pallor [23], insomnia, dizziness, and vomiting [22]. Contact of the skin with liquid ethylene dibromide caused intense burning pain preceding a generalized hyperemia that developed into blisters [24]. Repeated contact with liquid ethylene dibromide in experiments with one volunteer caused skin sensitization [24].

Kochmann [23] reported that an employee repeatedly exposed to ethylene dibromide vapor during his employment suffered from conjunctival irritation, swelling of the glands in the neck, and a generally poor condition. After recovery, the employee returned to work, but became ill again after being reexposed to ethylene dibromide and reportedly suffered from conjunctivitis, pharyngeal and bronchial irritation, anorexia, headaches, and depression. Improvement was rapid after the employee was removed from exposure.

Marmetschke [22] described an accidental exposure to ethylene dibromide in a female patient who was supposed to be anesthetized by ethyl bromide. Instead, the woman inhaled an unknown portion of the approximately 70 g of ethylene dibromide mistakenly placed on an oronasal mask. She suffered from a burning in the chest, diarrhea, vomiting, restlessness, nervousness, labored breathing, abdominal pain, and uterine hemorrhaging. Death occurred 44 hours after the accidental administration of ethylene dibromide. Autopsy showed signs of upper respiratory tract

irritation, swelling of the pulmonary lymph glands, advanced stages of muscular degeneration in the heart, liver, and kidneys, and hemorrhages in the trachea and along the mediastinum.

Pflesser [24] reported an incident in which a seaman spilled some ethylene dibromide-containing fluid into his boots but did not remove them. Blistering appeared on both feet some hours later, but no vascular or neurogenic disturbances were evident. The author discovered that ethylene dibromide was the active component of the fluid by subjecting himself and volunteers to the fluid and to each individual component. Covering the application site increased the severity of the reaction, with a very painful inflammation developing in 15-20 hours that included reddening, swelling, and blistering. He observed that a moderately strong, painful, local glandular swelling occurred after he had subjected himself to repeated doses of ethylene dibromide on the forearm. Swelling was not limited to the most recent application site, but appeared at all sites previously exposed; Pflesser, therefore, concluded that a sensitization reaction to ethylene dibromide had developed.

Olmstead [25] described the only reported fatality from ethylene dibromide ingestion in a case report of a 43-year-old woman who accidentally ingested about 140 mg/kg of ethylene dibromide. Immediately after swallowing the capsules, she began vomiting, and this recurred periodically until just prior to death. Other symptoms of toxic effects included diarrhea, anuria, tachypnea, marked nervous agitation, abdominal pain, nausea, systolic heart murmurs, sinus tachycardia, and a weak, sporadic pulse. Death occurred 54 hours after ingestion. Autopsy showed lung edema and congestion, reddening of the intestinal mucosa, massive

centrilobular liver necrosis, and damage to the tubular epithelium of the kidneys.

Adverse effects resulting from exposure of experimental animals to ethylene dibromide are similar to those described for human exposures and include ocular, dermal, and respiratory irritation and systemic effects on the liver, kidneys, spleen, circulatory system, and nervous system.

The effects of ethylene dibromide exposure on the eyes have been noted in several animal species. Merzbach [31] discovered that a dog exposed for 1 hour to 1 ml (2.2 g) of ethylene dibromide vaporized into a 100-liter chamber showed signs of ocular irritation during the exposure. Five hours after the exposure ended, a milky-blue corneal opacity developed which became more pronounced and developed into purulent conjunctivitis in both eyes and an ulcer in one eye. Kochmann [23] showed that 100 ppm (770 mg/cu m) of ethylene dibromide for 30 minutes/day produced conjunctivitis in cats exposed to the vapor repeatedly until death occurred at approximately 10 days. Rowe et al [33] reported that instillation of undiluted ezhylene dibromide into the eyes of rabbits caused conjunctival izritation that cleared within 48 hours and left only very slight superficial corneal necrosis which healed completely. Rowe et al [33] also found that a 10% solution of ethylene dibromide in propylene glycol produced a more severe reaction in the eye than did the undiluted material. Moderate conjunctival and corneal irritation developed and persisted for 48 hours; healing was complete within 12 days without corneal scarring. A 1% solution produced an effect similar to that caused by the undiluted material. These reports indicate that ethylene dibromide can cause adverse

ocular effects after exposure to 1 and 10% solutions, as well as to the undiluted material.

The skin of experimental animals is susceptible to penetration and local surface effects resulting from exposure to ethylene dibromide. Thomas and Yant [27] noted that liquid ethylene dibromide at doses of 0.25, 0.50, and 1.0 ml (0.55, 1.1 and 2.2 g)/animal produced marked hyperemia of the small cutaneous blood vessels of the shaved abdomens of rats. All animals died within 6-18 hours after application. Rowe et al [33] found that undiluted ethylene dibromide or a 10% solution in butyl carbitol acetate killed within 24 hours all rabbits to which it was applied dermally; subsequent experiments showed that the dermal LD50 was about 400 mg/kg. Marked erythema and edema were noted when the material was prevented from evaporating from the skin, whereas only slight erythema was noted when evaporation was not inhibited. Undiluted doses of about 210 mg/kg produced a moderate-to-severe erythema, edema, and necrosis of the skin. Similar results were described by Pflesser [24] in human volunteers exposed to 0.5 ml (1.1 g) of liquid ethylene dibromide for 1-30 minutes by placing small quantities on their arms or hands. Confinement produced more extensive erythema and edema surrounding the application site, and, in one case, blistering occurred as a result of continued contact between ethylene dibromide and the skin. These investigations indicate that localized surface effects on the skin, such as erythema, edema, blistering, or necrosis, may occur after contact with either undiluted or 10% solutions of ethylene dibromide. Percutaneous absorption of 10% solutions has also caused death in experimental animals.

Respiratory irritation after single exposures to ethylene dibromide vapor has been reported in guinea pigs at concentrations of 8,000 ppm (61,600 mg/cu m) for 30 minutes, 4,000 ppm (30,800 mg/cu m) for 60 minutes, and 2,000 ppm (15,400 mg/cu m) for 150 minutes [27], and in cats exposed at 100 ppm (770 mg/cu m) for 30 minutes [23]. In cats, three 30-minute exposures at 100 ppm (770 mg/cu m) produced signs of nasal irritation that consisted of a strong reddening of the nasal mucosa [23]. Lucas [28] demonstrated that a 12-minute exposure of a rabbit to ethylene dibromide vapor caused the lungs to become enlarged and filled with a frothy exudate. Merzbach [31] found that 5 ml (11.0 g) of ethylene dibromide allowed to vaporize in a 100-liter chamber produced severe bleeding in the right lung of a dog exposed for 1 hour. Another dog exposed to the vapor of 1 ml (2.2 g) for 1 hour developed severe hyperemia and bronchopneumonic foci in both lungs. Rowe et al [33] showed that death occurring in rats exposed to ethylene dibromide at concentrations of 100-10,000 ppm (770-77,000 mg/cu m) for 0.02-16.0 hours was caused by respiratory or cardiac failure at the higher exposures and by pneumonia at the lower ones. The lungs of animals exposed at these concentrations were congested, edematous, hemorrhagic, and inflamed.

Respiratory irritation has also been noted after the repeated inhalation exposure of cats at 100 ppm (770 mg/cu m) to ethylene dibromide for 30 minutes daily for an average of 10 days. This concentration produced dark red discolorations in the lungs, and the lungs were partially nonfunctional [23]. Rowe et al [33] reported on lethal dose rates in rats exposed to ethylene dibromide for 0.02-16.0 hours at concentrations of 100-10,000 ppm (770-77,000 mg/cu m). Death was caused by respiratory or

cardiac failure at the higher concentrations and by pneumonia at the lower ones. Half of the male rats exposed at 25 ppm (192.5 mg/cu m) for 7 hours/day for 151 exposures in 213 days died during the experiment, primarily from pneumonia and upper respiratory tract infection. Pulmonary infections were also responsible for a 50% mortality in male guinea pigs and a 25% mortality in females exposed at 25 ppm (192.5 mg/cu m) for 7 hours/day for 145 exposures in 205 days [33].

Systemic parenchymal cell damage, particularly in the liver, kidneys, and spleen, develops after single and repeated exposure of experimental animals to ethylene dibromide vapor. Thomas and Yant [27] observed that guinea pigs receiving single exposures of ethylene dibromide vapor at concentrations of 8,000 ppm (61,600 mg/cu m) for 30 minutes, 4,000 ppm (30,800 mg/cu m) for 60 minutes, or 2,000 ppm (15,400 mg/cu m) for 150 minutes had a slight granular degeneration of the parenchymal tissue of the liver. Rabbits exposed to an unknown concentration of ethylene dibromide vapor for 10 or 12 minutes had enlarged livers, with slight-to-moderate diffuse fatty changes evident in the rabbit exposed for 10 minutes [28]. Merzbach [31] published a study detailing similar results in a dog exposed to 1 ml (2.2 g) of vaporized ethylene dibromide in a 100-liter chamber for 1 hour; autopsy showed that a pronounced fatty degeneration of the liver had occurred. Rowe et al [33] observed that rats exposed at 100-10,000 ppm (770-77,000 mg/cu m) of ethylene dibromide for 0.02-16.0 hours developed cloudy swelling, centrilobular fatty degeneration, and necrosis of the liver. Repeated exposure at approximately 100 ppm (770 mg/cu m) for 30 minutes/day for up to 10 days caused incipient fatty degeneration in the liver of cats [23]. According to Rowe et al [33], rats exposed to 100 ppm

(770 mg/cu m) of ethylene dibromide for 7 hours/day for seven exposures in 9 days had a cloudy swelling of the liver, whereas rabbits exposed to the same concentration for three or four exposures had widespread central fatty degeneration and some necrosis of the other cells of the liver. Rowe et al [33] reported that slight central fatty degeneration of the liver developed in guinea pigs repeatedly exposed to 50 ppm (385 mg/cu m) of ethylene dibromide for 7 hours/day for 57 exposures in 80 days and in monkeys repeatedly exposed to the same concentration for 49 times in 70 days.

Renal damage, in the form of pronounced granular degenerative changes in the parenchymal tissues, occurred in guinea pigs exposed to ethylene dibromide at concentrations of 8,000 ppm (61,600 mg/cu m) for 30 minutes, 4,000 ppm (30,800 mg/cu m) for 60 minutes, or 2,000 ppm (15,400 mg/cu m) for 150 minutes [27]. Rowe et al [33] demonstrated that the kidneys of rats exposed at 100-10,000 ppm (770-77,000 mg/cu m) of ethylene dibromide vapor for 0.02-16.0 hours showed slight interstitial congestion and edema, with slight cloudy swelling of the tubular epithelium in some cases. Repeated exposures at approximately 100 ppm (770 mg/cu m) for 30 minutes/day for an average of 10 days in cats produced a swelling and discoloration of the kidneys and possibly a slight degeneration of the tubules [23]. Rowe et al [33] reported that repeated exposure of guinea pigs at 50 ppm (385 mg/cu m) of ethylene dibromide for 7 hours/day for 57 times in 80 days produced a slight interstitial congestion and edematous condition in the kidneys which was accompanied by degeneration of the tubular epithelium. From these data, it can be concluded that renal damage can occur in experimental animals after exposure to single or repeated exposures of ethylene dibromide.

Adverse splenic effects have been seen in experimental animals after exposure to ethylene dibromide. Single exposures of ethylene dibromide at 8,000 ppm (61,600 mg/cu m) for 30 minutes, 4,000 ppm (30,800 mg/cu m) for 60 minutes, or 2,000 ppm (15,400 mg/cu m) for 150 minutes produced a slight granular degeneration of the parenchymal tissue in the spleen of guinea pigs [27]. Rats exposed to 0.25, 0.50, or 1.0 ml (0.55, 1.1, or 2.2 g) of ethylene dibromide applied on the abdomen died within 6-18 hours; autopsy showed that the spleens of these animals were highly congested and edematous [27]. Kochmann [23] noted a slight enlargement of the spleens of cats exposed at 100 ppm (770 mg/cu m) of ethylene dibromide for 30 minutes/day for an average of 10 days. Rowe et al [33] observed that rats receiving seven exposures to ethylene dibromide at 100 ppm (770 mg/cu m), 7 hours/day, for 9 days developed a slight congestion of the spleen and some hemosiderin deposition. The results from these experiments indicate that adverse effects to the spleen can occur after exposure to ethylene dibromide by inhalation or by percutaneous absorption.

Cardiovascular system effects have been noted in experimental animals subjected to single or multiple exposures of ethylene dibromide. Concentrations of 8,000, 4,000, and 2,000 ppm (61,600, 30,800, and 15,400 mg/cu m) of ethylene dibromide for 30, 60, and 150 minutes, respectively, produced a slight granular degeneration of the muscular tissue of the heart and a generalized interstitial edematous degeneration of the endothelial lining in the abdominal vascular system in guinea pigs [27]. Merzbach [31] exposed a dog to the vapor of 5 ml (11.0 g) of ethylene dibromide in a 100-liter chamber for 1 hour and found subendocardial hemorrhages; a dog exposed to 1 ml (2.2 g) of ethylene dibromide under the same conditions

developed a thrombus in the heart. Subsequent in vitro experiments with isolated frog hearts produced deleterious effects on the heart rate, causing cardiac arrest without recovery at 800 ppm [31]. Kochmann [23] postulated that death resulting from exposure of cats and rabbits at 50-100 ppm (385-770 mg/cu m) of ethylene dibromide for up to 22 days was caused by injury to the circulatory system, especially to the heart and blood vessels. Rowe et al [33] also concluded that deaths occurring at the higher concentrations after exposure of rats at 100-10,000 ppm (770-77,000 mg/cu m) of ethylene dibromide for 0.02-16.0 hours were caused by cardiac or respiratory failure.

Few studies have specifically addressed the potential of ethylene dibromide to adversely affect the CNS. Rowe et al [33] reported that CNS depression was observed at the higher concentrations in rats exposed to 100-10,000 ppm (770-77,000 mg/cu m) for 0.02-16.0 hours, but did not further elucidate the nature of the effects. Rowe et al [33] also observed marked CNS depression in rabbits when ethylene dibromide was applied dermally at doses of 210, 300, 650, and 1,100 mg/kg. CNS effects, such as agitation [23], restlessness [31], body tremors [23,31] or unconsciousness [31], have been mentioned in various papers, but not in sufficient detail to enable a conclusive evaluation to be made to determine their validity or relevance.

Carcinogenicity, Mutagenicity, Teratogenicity, and Effects on Reproduction

Quantitative data that indicate a nonhazardous concentration for exposure to ethylene dibromide have not been found.

Abnormalities in the reproductive process have been demonstrated in

bulls [41-43], rats [9], and chickens [44-46]. Newborn, young adult, and mature adult bulls receiving an average of 2 mg/kg/day of ethylene dibromide for various periods in their development exhibited abnormalities in their reproductive systems, including the production of abnormal spermatozoa [41-43], decreased spermatozoic motility [41], and decreased spermatozoic count [41]. Abnormal spermatozoa with various degrees of degeneration and malformations, such as coiled tails, pyriform heads, and tailless spermatozoa, have been reported in semen from experimental bulls receiving ethylene dibromide for 14-16 months [41]. These reproductive abnormalities were reversible after administration of ethylene dibromide was discontinued, but reinstatement produced recurring effects that persisted longer after discontinuing administration again [41]. Experiments with bulls receiving 4 mg/kg on alternate days for 7 or 10 doses showed that the abnormal spermatozoa were present in both the epididymis and testes after 7 doses. After 10 doses, abnormal spermatozoa constituted 67% of the population in the ductus deferens and 77% of the ejaculate, with almost all the abnormal spermatozoa exhibiting tail and acrosomal defects [42]. These spermatozoic abnormalities may lead to a decrease in the fertility of the bulls and might induce sterility if they continued long enough, although reports have not been found that experimentally test this hypothesis. In rats, ip injections of ethylene dibromide at a dose of 10 mg/kg for 5 days produced a decrease in the average live litter size in female rats mated with experimental males [9]. This decrease in fertility occurred during the 3rd and 4th weeks following administration, which corresponds to the maturation cycle of spermatids in the genital tract of rats. This finding, which corresponds with the

findings of the reports of spermiogenic impairment in bulls, supports the postulated sterilizing effect discussed above. It is important to point out that reports have not been found delineating a no-effect level for the reproductive system abnormalities caused by the repeated administration of ethylene dibromide to bulls.

In chickens, the function of the female reproductive system is impaired by ethylene dibromide exposure [44-46], but the male system appears to be unaffected [45]. Cockerels fed 300 ppm of ethylene dibromide for 12 months showed significant growth retardation at 12 weeks of age; however, semen collected between 9 and 12 months did not differ from control semen, and body and testicular weights were comparable with those of controls [45]. Semen collected between 2 and 10 weeks from cockerels fed 300 ppm for 105 days showed no significant differences when compared with control semen with respect to ejaculated volume and spermatozoic motility and concentrations. Subsequent fertility tests at 60 or 105 days in hens gave no significant differences in fertilization rates or hatchability of eggs. These results indicate that the reproductive system in male chickens was not detectably affected by ethylene dibromide under these experimental conditions. This is in contrast to the effects reported in two mammalian species, cattle and rats, in which definite impairments of the male reproductive system have been noted.

Laying hens fed grain containing 10-40 ppm of ethylene dibromide for approximately 4-5 months were found to have a reduction in the weight of eggs laid [44,45]. A cessation of egg laying was observed when hens were fed 180-320 ppm of ethylene dibromide for 46-56 days [44], and a reduction in egg laying was observed when hens were fed 10 mg/day for 2 months [46].

A striking reduction (12 versus 86% in the control) in the fertilization rate was seen in hens fed 100 ppm of ethylene dibromide for 4 weeks; all embryos in the eggs from the experimental group were dead [45]. These results indicate that the female reproductive system in chickens is affected by ethylene dibromide. Although no reports have been found that explore the potential of ethylene dibromide to affect fertility in females of mammalian species, the avian data suggest the potential of ethylene dibromide to impair female fertility. However, until experiments are conducted with mammalian species to assess this potential, extrapolation of the effects seen in chickens to mammals and humans must be uncertain.

There has been only one study concerning the potential of ethylene dibromide to induce cancer in rats and mice [56-59]. In this study, rats and mice were given the maximum tolerated dose (80 mg/kg for rats, 120 mg/kg for mice) and one-half the maximum tolerated dose by daily intubation for 54 or 62 weeks, except when toxicity forced the total discontinuation of administration or reduction of the maximum tolerated dose to that of one-half the maximum tolerated dose during the experiment. The tumors (squamous cell carcinomas), which were first noted during the 10th week of ethylene dibromide administration in rats, originated in the forestomach, invaded locally, and metastasized throughout the abdominal cavity. The fraction of animals with tumors was greater in the male rats than in the females (an average of 83% of the males developed tumors versus 70% of the females), and was greater at the lower dose than at the higher dose in rats at the termination of the experiment at 54 weeks (98 versus 68% in males and 82 versus 58% in females, respectively). The concurrent control populations did not develop squamous cell carcinomas of the stomach. The

fraction of mice that developed squamous cell carcinomas was 74% in males and 72% in females by the termination of the experiment at 90 weeks [57].

The irregularities in the dose regimens of both species, the use of the suggested maximum tolerated dose, and the route of administration do not negate the importance of the fact that ethylene dibromide has induced carcinomas in two mammalian species. The data from this single study indicate that ethylene dibromide is a carcinogen after daily introduction of about one-half the maximum tolerated dose into the stomach of rats and mice for up to 62 weeks.

The mutagenic potential of ethylene dibromide has been established in a wide spectrum of procaryotic and eucaryotic mutational test systems. It has induced mutations in vertebrate cell cultures [62], insects [6], bacteria [60,63,64], plants [66], and fungi [67,68]. It has induced mutations or mutagenic events in a number of experimental test systems, including the recessive lethal test in Drosophila melanogaster [6], the host mediated assay in mice with Salmonella typhimurium [60], and a backward mutation system with mouse lymphoma cells [62]. In addition, positive mutagenic induction has been reported in tests with back mutational systems in certain strains of Salmonella typhimurium [60,63,64]. Ethylene dibromide has also been reported to have given negative results in the dominant lethal test in mice [61] and in the back mutational test system with Serratia marcescens in the host mediated assay [60].

The dominant lethal test conducted in male mice given 50 or 100 mg/kg of ethylene dibromide for 5 consecutive days by oral intubation or given 18 or 90 mg/kg by ip injection did not induce a significant increase in the number of dead implants when compared with controls [61], although specific

data were not presented for evaluation. Buselmaier et al [60] reported that ethylene dibromide was mutagenic in the host mediated assay with Salmonella typhimurium G46 at a dose of 500 mg/kg injected subcutaneously in mice and in vitro with the same organism at an equivalent dose. The mutational frequency for the experimental group was significantly ($P<0.01$) different from the control mutational frequency. The positive results in the in vivo and in vitro tests indicate that metabolic activation is not necessary for ethylene dibromide to exert its mutagenic effects and that metabolic deactivation does not reduce the mutagenic effect. Buselmaier et al [60] reported that a similar in vivo test with Serratia marcescens, also a back mutational test system, was negative; this finding may only indicate that species differences exist in reactions with ethylene dibromide.

In a recessive lethal test with Drosophila melanogaster, Vogel and Chandler [6] reported that 0.3 mM of ethylene dibromide fed to adult males for 3 days induced significant numbers of recessive lethal mutations in the offspring of three successive broods. The brood patterns resulting from ethylene dibromide exposure indicated that ethylene dibromide affected spermatozoic maturation more than spermatozoic formation. The authors considered ethylene dibromide typical of a bifunctional alkylating agent and capable of introducing cross-links into biologic molecules.

A mammalian somatic cell tissue culture of L5178Y mouse lymphoma cells was exposed to 0.0-3.0 mM of ethylene dibromide in culture media [62]. The induced mutagenic frequency was dose related, typical of other alkylating agents tested, and approximately equivalent to a dose of 600 R of X-irradiation at the highest concentration. Meneghini [5] noted that ethylene dibromide very effectively induced DNA repair synthesis in opossum

lymphocyte cell cultures at concentrations between 0.001 and 1 mM but decreased at 10 mM. Only compounds that interact with DNA through covalent bonds induce repair synthesis; therefore, ethylene dibromide is typical of other alkylating agents in forming covalent bonds with DNA.

Bacterial and other plant systems have also been used to show the mutagenic potential of ethylene dibromide. Ames [63] reported that ethylene dibromide induced mutations in two backward mutation test strains of Salmonella typhimurium, his G46 and TA 1530, at a concentration of 5 μl(11.0 mg)/plate. Brem et al [64] observed similar positive results in Salmonella TA1530 and TA1535 at 10 μM/plate. Ethylene dibromide did not induce mutations in the frame shift indicator strain, Salmonella typhimurium TA 1538 [64], indicating that the action exerted by ethylene dibromide in inducing mutations is not through frame shifts during the reading of the genetic code, but rather is through cross-linking and covalent bonding with DNA. Sparrow et al [66] published that ethylene dibromide was highly mutagenic in a plant system, Tradescantia clone 4430. A linear relationship for mutation responses over the range of 3.6-148.2 ppm (27.72-1,141.14 mg/cu m) was observed, with a well-defined dose response occurring with exposures as low as 3.6 ppm (27.72 mg/cu m) for 6 hours.

The ability of ethylene dibromide to induce mutations in a wide variety of test systems is suggestive of its potential to induce mutations in human populations, but there is no evidence available to enable an adequate assessment of the quantitative aspects of the relative risks for human populations. Since ethylene dibromide is a bifunctional alkylating agent, the most plausible basis for the induction of mutations in these

systems is the covalent bonding of ethylene dibromide to the genetic material, DNA. The linear relation between the number of induced mutations and the concentration of ethylene dibromide in Tradescantia [66] is consistent with the idea of covalent bonding between ethylene dibromide and DNA.

Only one study pertaining to the potential of ethylene dibromide to induce fetal anomalies has been found [69]. Fetuses from pregnant mice and rats exposed to approximately 31.6 ppm (243.32 mg/cu m) of ethylene dibromide for 23 hours/day during days 6-15 of gestation were significantly different from fetuses of pregnant control mice and rats. These differences include costal anomalies and hydrocephaly in rat fetuses, and additional anomalies in other ossification processes in mice fetuses. Fetuses from control mice fed a feed-restricted diet during the experiment to simulate malnourishment exhibited some of the effects found in ethylene dibromide-exposed mice fetuses; however, the frequency of the type and number of anomalies formed in the malnourished group was reduced, but the degree of significance was not as great as that found in the ethylene dibromide-exposed mice fetuses. Although it can be argued that the effects produced by ethylene dibromide exposure are similar in part to those produced by malnourishment, the anomalies in both rat and mouse fetuses from ethylene dibromide-exposed dams were significantly different from those produced by malnourishment alone when both were compared with those found in fetuses from control dams. From these data, it is evident that ethylene dibromide caused fetal abnormalities in mice and rats that were not caused by malnourishment alone.

In summary, it is concluded from the data from human and experimental studies that the adverse systemic effects resulting from exposure to ethylene dibromide may include ocular, dermal, and respiratory irritation, in addition to systemic effects on the liver, kidneys, spleen, cardiovascular system, and nervous system. Although dose-response relationships or no-adverse-effect levels have not been established for ethylene dibromide, experimental data from animals strongly suggest that exposure to ethylene dibromide may induce sterility, malformations and heritable damage in offspring, and cancers in these systems.

Summary Tables of Exposure and Effect

The effects of short- and long-term exposures to ethylene dibromide in humans and animals which were presented in detail in Chapter III are summarized in Tables III-2, III-3, III-4, III-5, and III-6. Human data appear in Table III-2, general animal toxicity data appear in Table III-3, carcinogenic animal data appear in Table III-4, mutagenic data appear in Table III-5, and teratogenic and related reproductive data appear in Table III-6.

TABLE III-2

SUMMARY OF EFFECTS OF ETHYLENE DIBROMIDE EXPOSURE IN HUMANS

Route of Administration	Concentration and Duration	Observed Effects	Reference
Respiratory	70 g -	Vomiting, abdominal pain, diarrhea, difficulty in breathing, restlessness, nervousness, dizziness, death by 44 hr	22
"	Unknown	Irritation of conjunctiva, swelling of eyelids and glands under chin	23
Dermal	0.5 ml* 30 min	Painful inflammation, swelling, and blistering of skin	24
"	0.5 ml* 10 min	Heat sensation, slight burning, painful swelling and reddening of skin for next 24 hr	24
"	0.5 ml* 30 min	Swelling, reddening, and itching 30 min later	24
"	55%** several hr	Painful burning of feet with reddening and blisters between toes	24
Oral	140 mg/kg 1 dose	Vomiting, abdominal pain, diarrhea, nausea, anuria, death by 54 hr	25

*Skin was washed with soap and water after the exposure.
**Unknown quantity mixed with gauge fluid.

TABLE III-3

SUMMARY OF EFFECTS OF ETHYLENE DIBROMIDE EXPOSURE IN ANIMALS

Route of Administration	Species	Concentration and Duration	Observed Effects	Reference
Respiratory	Rats	100 ppm 7 hr/d x 7 exposures in 9 d	Weight loss, increased weight of kidneys, lungs, and liver; cloudy swelling of liver and congestion of spleen; lung irritation; blood in stomach; 3/10 deaths	33
"	"	50 ppm 7 hr/d x 63 exposures in 91 d	Increased weight of kidneys, lungs, and liver; decreased weight of testes and spleen	33
"	"	25 ppm 7 hr/d x 151 exposures in 213 d	13/40 deaths	33
"	"	25 ppm 7 hr/d x 13 exposures in 17 d	No adverse effects reported	33
"	Guinea pigs	50 ppm 7 hr/d x 57 exposures in 80 d	Weight loss; decreased rate of growth; congestion and parenchymatous degeneration of kidneys; fatty degeneration of liver	33
"	"	25 ppm 7 hr/d x 145 exposures in 205 d	6/16 deaths because of pulmonary infections	33
"	"	25 ppm 7 hr/d x 13 exposures in 17 d	No adverse effects reported	33

TABLE III-3 (CONTINUED)

SUMMARY OF EFFECTS OF ETHYLENE DIBROMIDE EXPOSURE IN ANIMALS

Route of Administration	Species	Concentration and Duration	Observed Effects	Reference
Respiratory	Rabbits	100 ppm 7 hr/d 2-4 d	Fatty degeneration of liver, 3/4 deaths	33
"	"	50 ppm 7 hr/d x 59 exposures in 84 d	Small increase of liver and kidney weights	33
"	"	25 ppm 7 hr/d x 152 exposures in 214 d	No adverse effects reported	33
"	Monkeys	50 ppm 7 hr/d x 49 exposures in 70 d	Increased weight and slight fatty degeneration of liver	33
"	"	25 ppm 7 hr/d x 156 exposures in 220 d	No adverse effects reported	33
Dermal	Rabbits	1,100-210 mg/kg 24 hr	CNS depression, erythema, edema, and necrosis of skin	33

TABLE III-4

SUMMARY OF CARCINOGENIC EFFECTS
OF ETHYLENE DIBROMIDE IN ANIMALS

Route of Administration	Species	Concentration and Duration	Observed Effects	Reference
Oral	Rats	80 mg/kg/d 5 d/wk x 16 wk, then no dose for 14 wk, then 40 mg/kg/d x 24 wk	Squamous cell carcinomas of the stomach in 68% of males and in 58% of females; total dosage of 11.2 g/kg	56
"	"	40 mg/kg/d 5 d/wk 54 wk	Squamous cell carcinomas of the stomach in 98% of males and in 82% of females; total dosage of 10.8 g/kg	56
"	Mice	120 mg/kg/d 5 d/wk x 13 wk, then 200 mg/kg/d x 2 wk, then 120 mg/kg/d x 27 wk	Squamous cell carcinomas of the stomach in 73%; 40/100 deaths	56
Oral	"	60 mg/kg/d 5 d/wk x 13 wk, then 100 mg/kg/d x 2 wk, then 60 mg/kg/d x 27 wk	Squamous cell carcinomas of the stomach in 73%	56

TABLE III-5

SUMMARY OF MUTAGENIC EFFECTS OF ETHYLENE DIBROMIDE

Species or System	Strain	Dose	Results	Reference
Salmonella typhimurium	G 46 TA 1530	5 µl -	Positive mutagenic response by reversion of growth deficiency	63
"	TA 1530 TA 1535 TA 1538	10 µM x 8 hr	Base substitution mutation for TA 1530 and TA 1535, no frame-shift mutation for TA 1538	64
Escherichia coli	pol A+ pol A-	10 µl x 8 hr	Damage to cellular DNA and growth inhibition of pol A-, preferential to A+	64
Salmonella typhimurium	LT 2 galE503	1-5 µl x 8 hr	No long deletions of chromosomes	65
Salmonella typhimurium and Serratia marcescens	G46 a21	500 mg/kg -	Host mediated assay compared to in vitro indicate no metabolic activity is necessary for ethylene dibromide to exert its mutagenicity	60
Tradescantia	Clone 4430	3.6-148.2 ppm x 6 hr	Well-defined, linear dose-responsive mutagenic effect	66
Mouse lymphoma cells	L5178Y	0.0-3.0 mM -	Mutational frequency equivalent to 600 R of X-irradiation at highest concentration	62
Didelphis virginiana	-	0.001-1 mM x 1 hr	Induction of DNA repair in opossum lymphocytes	5
Drosophila melanogaster	-	0.3mM x 3 d	Induction of significant number of recessive lethal mutations	6
Swiss mice	ICR/Ha	50 or 100 mg/kg	No mutagenic effects reported	61

TABLE III-6

TERATOGENIC AND OTHER REPRODUCTIVE EFFECTS
OF ETHYLENE DIBROMIDE IN ANIMALS

Route of Administration	Species	Concentration and Duration	Observed Effects	Reference
Oral	Bulls	4 mg/kg 10 doses every other day	Abnormal spermatozoa and decreased spermatozoic concentration after administration ended	43, Amir*
"	"	4 mg/kg 10 doses in 21 d or 7 doses in 12 d	Abnormal spermatozoa in testes, epididymis, ductus deferens, and in ejaculate	42
"	"	2 mg/kg/d 4 d-24 mon	Abnormal spermatozoa, decreased spermatozoic density and motility	41
"	Chickens	320-50 ppm 9 wk	Decreased weight and number of eggs, cessation of egg laying after 46-56 d and lasting for several months after exposure ended	44
"	"	300 ppm 3-12 mon	Decreased growth, feed intake, and comb weight; normal fertilization rate and weight gain	45
"	"	100 ppm 4 wk	Decreased egg weight and fertilization rate	45
"	"	200 mg/kg/d 10 d	Anorexia, general depression, liver congestion, crop inflammation, excess pericardial fluid, deaths occurred by 3rd dose	40

TABLE III-6 (CONTINUED)

TERATOGENIC AND OTHER REPRODUCTIVE EFFECTS
OF ETHYLENE DIBROMIDE EXPOSURE ON ANIMALS

Route of Administration	Species	Concentration and Duration	Observed Effects	Reference
Oral	Chickens	40 ppm twice daily 4.5-5 mon	Decreased weight and number of eggs	45
ip	Rats	10 mg/kg/d 5 d	Decreased average size of live litter in females mated after 3 wk of exposure; no litters at 4 wk	9
Respiratory	"	31.6 ppm on d 6-15 of gestation	Decreased number of implants/dam, live fetuses/dam, % of viable fetuses, and % of early resorptions; hydrocephaly of fourth ventricle, decreased incidence of 14th pair of ribs, wavy ribs	69
"	Mice	31.6 ppm on d 6-15 of gestation	Decreased live fetal weights, no ossification of incus, incomplete ossification of supraoccipital, variation in ossification of sternabrae	69

*Adapted from reference 43 and D Amir (written communication, August 1976)