

criteria for a recommended standard....

**OCCUPATIONAL EXPOSURE
TO
ETHYLENE DIBROMIDE**



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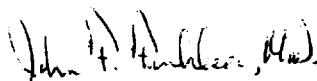
PREFACE

The Occupational Safety and Health Act of 1970 emphasizes the need for standards to protect the health and safety of workers exposed to an ever-increasing number of potential hazards at their workplace. The National Institute for Occupational Safety and Health has projected a formal system of research, with priorities determined on the basis of specified indices, to provide relevant data from which valid criteria for effective standards can be derived. Recommended standards for occupational exposure, which are the result of this work, are based on the health effects of exposure. The Secretary of Labor will weigh these recommendations along with other considerations such as feasibility and means of implementation in developing regulatory standards.

It is intended to present successive reports as research and epidemiologic studies are completed and as sampling and analytical methods are developed. Criteria and standards will be reviewed periodically to ensure continuing protection of the worker.

I am pleased to acknowledge the contributions to this report on ethylene dibromide by members of the NIOSH staff and the valuable constructive comments by the Review Consultants on Ethylene Dibromide, by the ad hoc committees of the Society for Occupational and Environmental Health and the American Industrial Hygiene Association, and by Robert B.

O'Connor, M.D., NIOSH consultant in occupational medicine. The NIOSH recommendations for standards are not necessarily a consensus of all the consultants and professional societies that reviewed this criteria document on ethylene dibromide. A list of Review Consultants appears on page vi.



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The Division of Criteria Documentation and Standards Development, National Institute for Occupational Safety and Health, had primary responsibility for development of the criteria and recommended standard for ethylene dibromide. Gamil Debbas, Ph.D., was the criteria manager for the major development of the document and Jerry LR Chandler, Ph.D., served as criteria manager during the latter stages. SRI International developed the basic information for consideration by NIOSH staff and consultants under contract No. CDC-99-74-31.

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The views expressed and conclusions reached in this document, together with the recommendations for a standard, are those of NIOSH, after review of the evidence and consideration of the comments of reviewers. These views and conclusions are not necessarily those of the consultants, other federal agencies, and professional societies that reviewed the document, or of the contractor.

CRITERIA DOCUMENT:
RECOMMENDATIONS FOR AN OCCUPATIONAL
EXPOSURE STANDARD FOR ETHYLENE DIBROMIDE

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I. RECOMMENDATIONS FOR AN ETHYLENE DIBROMIDE STANDARD

The National Institute for Occupational Safety and Health (NIOSH) recommends that employee exposure to ethylene dibromide in the workplace be controlled by adherence to the following sections. The standard is designed to protect the health and provide for the safety of employees for up to a 10-hour workday, 40-hour workweek, over a working lifetime. Compliance with all sections of the standard should prevent adverse effects of ethylene dibromide on the health of employees and provide for their safety. Techniques recommended in the standard are valid, reproducible, and available to industry and government agencies. Sufficient technology exists to permit compliance with the recommended standard. Although NIOSH considers the workplace environmental limit to be a safe level based on current information, the employer should regard it as the upper boundary of exposure and make every effort to maintain the exposure as low as is technically feasible. The criteria and standard will be subject to review and revision as necessary.

The possible health effects of employees chronically exposed to ethylene dibromide may include the induction of cancers, malformations and heritable changes in offspring, and sterility. Ethylene dibromide may also cause adverse effects to the liver, kidneys, heart, and other internal organs and systems. Direct contact of the skin with ethylene dibromide may induce chemical burns as well as systemic effects, and ingestion of ethylene dibromide may damage internal organ systems, or even be fatal. The alkylation of cellular constituents, including the genetic material, deoxyribonucleic acid (DNA), is the most plausible molecular basis for the

induction of adverse effects after exposure to ethylene dibromide.

These criteria and the recommended standard apply to occupational exposure of workers to the brominated hydrocarbon $\text{BrCH}_2\text{CH}_2\text{Br}$, hereinafter referred to as "ethylene dibromide." Synonyms for ethylene dibromide include ethylene bromide, dibromoethane, sym-dibromoethane, 1,2-dibromoethane, glycol dibromide, and EDB. The major uses of ethylene dibromide are as an additive to leaded gasoline and as a component of fumigants.

"Occupational exposure to ethylene dibromide" is defined as work in any establishment where ethylene dibromide is manufactured, blended, stored, used, handled, or otherwise present. The "action level" is defined as one-half of the recommended workplace exposure concentration designated as a ceiling limit for ethylene dibromide. Exposure to airborne ethylene dibromide at concentrations less than the action level, as determined in accordance with Section 8, will not require adherence to Sections 2, 3, 4(a), or 8(c,d). If exposure to other chemicals also occurs, the employer shall comply with any applicable standard for the other chemicals.

Section 1 - Environmental (Workplace Air)

(a) Concentration

The employer shall control workplace concentrations of ethylene dibromide so that no employee is exposed in his workplace to concentrations greater than 1.0 mg/cu m (0.13 ppm) as a ceiling limit, as determined by a sampling period of 15 minutes.

(b) Sampling and Analysis

Procedures for the collection and analysis of workroom air samples for compliance with the standard shall be as provided in Appendices I and II, or by any methods shown to be at least equivalent in precision, sensitivity, and accuracy to the methods specified.

Section 2 - Medical

Medical surveillance shall be made available to employees as outlined below:

(a) Comprehensive preplacement and annual medical examinations unless a more frequent schedule is indicated by professional medical judgment based on such factors as emergencies, variations in work periods, and the preexisting health status of the individual worker.

(b) These examinations shall include at least:

(1) Comprehensive or interim medical and work histories, with special emphasis directed to disorders of the heart, liver, kidneys, and nervous system.

(2) A comprehensive physical examination, with particular emphasis given to cardiovascular, pulmonary, neurologic, hepatic, and renal systems, and to the skin.

(3) An evaluation of the employee's physical ability to safely wear a negative or positive pressure respirator.

(c) Employees shall be counseled by the physician to ensure that each employee is aware that ethylene dibromide has been shown to induce in experimental animals adverse effects in reproductive processes, including

abnormalities in offspring, mutations, and stomach cancer following direct administration. The relevancy of these findings in animals to male or female employees has not yet been established. They do indicate, however, that both employers and employees should do everything possible to minimize exposures to ethylene dibromide. If a physician becomes aware of any adverse effects on the reproductive system, or cancers in individuals who have been exposed to ethylene dibromide, or any abnormal babies born to parents either or both of whom have been exposed to ethylene dibromide, such information should be forwarded to the Director, National Institute for Occupational Safety and Health, as promptly as possible.

(d) Medical attention shall be provided promptly to all employees suspected of being exposed to ethylene dibromide vapor at or above the action level or to liquid ethylene dibromide. A 48-hour medical observation period for delayed systemic or dermal effects is recommended.

(e) Examinations of current employees shall be performed as soon as practicable after the promulgation of a standard based on these recommendations.

(f) The employer shall maintain medical records for all persons exposed to ethylene dibromide at or above the action level. All medical records, including information on required medical examinations and supporting documents, shall be kept for at least 30 years after the termination of the individual's employment.

(g) The medical representatives of the Secretary of Health, Education, and Welfare, of the Secretary of Labor, of the employee or former employee, and of the employer shall have access to these medical records.

Section 3 - Labeling and Posting

All labels and warning signs shall be printed both in English and in the predominant language of non-English-reading workers. Illiterate workers and workers reading languages other than those used on labels and posted signs shall receive information regarding hazardous areas and shall be informed of the instructions printed on labels and signs.

(a) Labeling

The following warning label shall be affixed in a readily visible location on ethylene dibromide processing or other equipment and on ethylene dibromide storage tanks or containers:

ETHYLENE DIBROMIDE

WARNING!!

CANCER-SUSPECT AGENT

BREATHING VAPOR MAY BE HAZARDOUS TO HEALTH
CAN BE FATAL IF SWALLOWED
HARMFUL IF ABSORBED THROUGH SKIN
CAUSES SEVERE IRRITATION OF SKIN AND EYES

Avoid breathing vapor.
Avoid contact with skin or eyes.
Use only with adequate ventilation.
Keep containers closed when not in use.
Wash thoroughly before eating, drinking, smoking, or using toilet.

First aid: If inhaled, remove to fresh air. Give artificial respiration if needed. Give oxygen if breathing is impaired. Call a physician.

In case of contact, immediately flush eyes or skin with water for at least 15 minutes. Call a physician.

If swallowed, induce vomiting immediately if patient is conscious. Call a physician.

(b) Posting

Areas in which ethylene dibromide is present shall be posted with a sign reading:

ETHYLENE DIBROMIDE

WARNING! CANCER-SUSPECT AGENT

HARMFUL IF INHALED
CAN BE FATAL IF SWALLOWED
HIGHLY IRRITATING TO SKIN AND EYES
HARMFUL IF ABSORBED THROUGH SKIN

Section 4 - Personal Protective Equipment and Clothing

(a) Respiratory Protection

(1) Engineering controls shall be used wherever needed to keep airborne ethylene dibromide concentrations below the recommended occupational exposure limit. Compliance with this limit may be achieved by the use of respirators under the following conditions only:

(A) During the time necessary to install or test the required engineering controls.

(B) For nonroutine operations, such as emergency maintenance or repair activities.

(C) During emergencies when air concentrations of ethylene dibromide may exceed the recommended occupational exposure limit.

(2) When a respirator is permitted by paragraph (a)(1) of this section, it shall be selected and used pursuant to the following requirements:

(A) The employer shall ensure that no employee is exposed to ethylene dibromide because of improper respirator selection, fit, use, or maintenance.

(B) The employer shall establish and enforce a respirator program meeting the requirements of 29 CFR 1910.134 as amended.

(C) The employer shall provide respirators in accordance with Table I-1 and shall ensure that the employee uses the respirator provided when necessary.

(D) Respiratory protective devices described in Table I-1 shall be those approved under the provisions of 30 CFR 11.

(E) Respirators specified for use in higher concentrations of ethylene dibromide may be used in atmospheres of lower concentrations.

(F) The employer shall ensure that chemical cartridges are not used with ethylene dibromide except for evacuation or escape because of the poor warning properties of ethylene dibromide at the recommended occupational exposure limit.

(G) The employer shall ensure that respirators are adequately cleaned and maintained, and that employees are instructed and drilled, at least annually, in the proper use and testing for leakage of respirators assigned to them.

(H) Respirators shall be easily accessible and employees shall be informed of their location.

TABLE I-1

RESPIRATOR SELECTION GUIDE FOR ETHYLENE DIBROMIDE

Concentration	Respirator Type Approved under Provisions of 30 CFR 11
Less than or equal to 10 mg/cu m	Supplied-air respirator with half-mask facepiece operated in demand (positive pressure) mode
Less than or equal to 50 mg/cu m	(1) Supplied-air respirator with full facepiece, helmet, or hood (2) Self-contained breathing appara- tus with full facepiece
Less than or equal to 2,000 mg/cu m	Type C supplied-air respirator with full facepiece operated in pressure- demand or other positive pressure mode or with full facepiece, hood, or hel- met operated in continuous-flow mode
Greater than 2,000 mg/cu m or entry into area of unknown concentration	(1) Self-contained breathing appara- tus with full facepiece operated in pressure-demand or other positive pressure mode (2) Combination respirator that in- cludes Type C supplied-air respirator with full facepiece operated in pres- sure-demand or other positive pressure or continuous-flow mode and auxiliary self-contained breathing apparatus op- erated in pressure-demand or positive pressure mode (3) Supplied-air suits may be neces- sary

(b) Eye Protection

Eye protective devices shall be provided by the employer and used by each employee where contact of ethylene dibromide with the eyes is likely. Chemical safety goggles or plastic face shields (8-inch minimum) with goggles made completely of ethylene dibromide-resistant materials shall be used. Selection, use, and maintenance of eye protective devices shall be in accordance with 29 CFR 1910.133.

(c) Protective Clothing

Protective clothing shall be resistant to the penetration and to the chemical action of ethylene dibromide. Additional protection, including gloves, bib-type aprons, boots, and overshoes, shall be provided for, and worn by, each employee while in any operation that may cause direct contact with liquid ethylene dibromide. Supplied-air hoods or suits resistant to penetration by ethylene dibromide shall be worn when entering confined spaces, such as pits or storage tanks. In situations where heat stress is likely to occur, supplied-air suits, preferably cooled, are recommended. The employer shall ensure that all personal protective clothing is inspected regularly for defects and is maintained in a clean and satisfactory condition by the employee.

Section 5 - Informing Employees of Hazards from Ethylene Dibromide

(a) All new and present employees working where occupational exposure to ethylene dibromide may occur shall be informed orally and in writing of the hazards, relevant signs and symptoms of exposure, appropriate emergency procedures, and proper conditions and precautions concerning safe use and handling of ethylene dibromide. First-aid

procedures shall be included. This information shall be readily available to all employees involved in the manufacture, use, transport, or storage of ethylene dibromide and shall be posted in prominent positions within the workplace.

(b) All employees involved with the manufacture, use, transport, or storage of ethylene dibromide shall be informed that ethylene dibromide has been reported to induce cancer in experimental animals after repeated oral intubation.

(c) Employers shall institute a continuing education program to ensure that all employees have current knowledge of job hazards, maintenance procedures, cleanup methods, emergency procedures, and evacuation procedures. This program should include at least:

- Emergency procedures and drills.
- Instruction in handling spills and leaks.
- Decontamination procedures.
- Location and use of firefighting equipment.
- First-aid procedures, equipment location, and use.
- Rescue procedures.
- Confined space entry procedures.
- Inadequacy of odor as a means of detection.

Records of such training should be kept for inspection by authorized personnel as required. This program shall be held for all employees with occupational exposure to ethylene dibromide at intervals not greater than quarterly, or whenever there is a process change.

(d) Information as required shall be recorded on the "Material Safety Data Sheet" shown in Appendix III, or on a similar form approved by the Occupational Safety and Health Administration, US Department of Labor.

Section 6 - Work Practices

(a) Emergency Procedures

For all work areas where emergencies may occur, the employer shall take all necessary steps to ensure that employees are instructed in and follow the procedures specified below and any others appropriate to the specific operation or process.

(1) Procedures shall include at least prearranged plans for:

(A) Immediate evacuation, transportation, and medical assistance for affected employees; this procedure should include alerting medical treatment facilities of the impending arrival of affected employees.

(B) Designation of medical receiving facilities and names of physicians trained in ethylene dibromide emergency procedures.

(C) Reentry into areas where ethylene dibromide leaks or spills have occurred for cleanup, decontamination, or maintenance purposes.

(2) Evacuation alarm systems shall be provided by the employer.

(3) Personal protective equipment and clothing as specified in Section 4 shall be used by trained personnel essential to emergency operations.

(4) Nonessential employees shall be evacuated from hazardous areas during emergencies. Perimeters of these areas shall be delineated, posted, and secured. The employees in adjacent areas shall be trained in evacuation procedures if these work areas become involved.

(5) Only personnel trained in the emergency procedures and protected against the attendant hazards shall shut off sources of ethylene dibromide, clean up spills, control and repair leaks, and fight fires in ethylene dibromide areas.

(6) Firefighting procedures shall be established for areas where flammable materials are used with ethylene dibromide. Chemical foam, carbon dioxide, or dry chemicals shall be used for fighting fires in areas where ethylene dibromide is present. Proper protective respirators and clothing shall be worn by all personnel in the hazard area until concentrations of airborne ethylene dibromide have been demonstrated by monitoring to be below the recommended occupational exposure limit.

(7) Showers, eyewash fountains, and washroom facilities shall be provided and so located as to be readily accessible to workers in all areas where skin or eye contact with liquid ethylene dibromide is likely. If liquid ethylene dibromide is splashed on the clothing or skin, contaminated clothing shall be promptly removed and the skin washed thoroughly with soap and water for at least 15 minutes. If liquid ethylene dibromide gets into the eyes, they shall be irrigated immediately with copious quantities of running water for at least 15 minutes.

(8) Medical attention shall be provided promptly for any affected worker. Such exposures shall be reported to the immediate supervisor by the affected worker or by a fellow employee.

(b) Control of Airborne Ethylene Dibromide

(1) Suitable engineering controls designed to limit exposure to ethylene dibromide to that prescribed in Section 1(a) shall be used. The use of completely enclosed processes is the recommended method

of control for ethylene dibromide. Local exhaust ventilation may also be effective, used alone or in combination with process enclosure. When a local exhaust ventilation system is used, it shall be designed to prevent the accumulation or recirculation of ventilation control or process air in the workroom, to maintain ethylene dibromide concentrations below the limit of the recommended standard, and to remove ethylene dibromide from the breathing zones of employees. Exhaust systems discharging into outside air must conform with applicable local, state, and federal air pollution regulations. Ventilation systems shall be subjected to regular preventive maintenance and cleaning to ensure effectiveness, which shall be verified by periodic airflow measurements at least every 3 months. Measurements of system efficiency shall also be made immediately by personnel properly attired in specified protective equipment when any change in production, process, or control might result in increased concentrations of airborne ethylene dibromide. Tempered makeup air shall be provided to work areas in which exhaust ventilation is operating.

(2) Forced-draft ventilation systems shall be equipped with remote manual controls and shall be designed to turn off automatically in the event of a fire in the work area.

(c) Handling of Ethylene Dibromide and General Work Practices

(1) Written operating instructions and emergency medical procedures shall be formulated and posted where ethylene dibromide is handled or used.

(2) Prompt medical attention shall be provided if there is known or suspected exposure to ethylene dibromide, whether or not symptoms are present.

(3) The employer shall ensure that safety showers, eyewash fountains, and other emergency equipment is in proper working order through regularly scheduled inspections performed by qualified maintenance personnel.

(4) Ethylene dibromide operating systems shall be inspected daily for signs of leaks by personnel attired in specified protective equipment. All equipment including valves, fittings, and connections shall be checked for tightness and good working order. All newly made connections shall be checked for leaks immediately after ethylene dibromide is introduced by trained personnel attired in prescribed personal protective equipment.

(5) If there is a leak, the leak shall be corrected immediately. Work shall resume normally only after necessary repair or replacement has been completed, the area has been ventilated, and the concentration of ethylene dibromide has been determined by monitoring to be below the recommended occupational exposure limit.

(6) Transportation and use of ethylene dibromide shall comply with all applicable local, state, and federal regulations. Where ethylene dibromide is used as a fumigant, strict adherence to the pesticide container label requirements for application and personal protection shall be followed. Additional standards for pesticide use by agricultural workers can be found in 40 CFR 170.

(7) When ethylene dibromide containers are being moved, or when they are not in use and are disconnected, valve protection covers shall be in place. Containers shall be moved only with the proper

equipment and shall be secured to prevent dropping or loss of control while moving.

(8) Process valves and pumps shall be readily accessible and should not be located in pits and congested areas.

(9) Containers and systems shall be handled and opened with care. Approved protective equipment as specified in Section 4 shall be worn while opening, connecting, and disconnecting ethylene dibromide containers and systems. Adequate ventilation shall be available to prevent exposure to ethylene dibromide when opening containers and systems.

(10) Personnel shall work in teams when ethylene dibromide is first admitted to a system, while repairing leaks, or when entering a confined or enclosed space.

(11) Any odor of ethylene dibromide shall be reported to a responsible authority and an alarm sounded immediately.

(d) Work Areas

(1) Ethylene Dibromide Hazard Areas

A hazard area shall be considered as any space workers may enter that has physical characteristics and sources of ethylene dibromide that could result in air concentrations exceeding the recommended limit. Exits shall be plainly marked and shall open outward. Emergency exit doors shall be conveniently located and shall open into areas which will remain free of contamination in an emergency. At least two separate means of exit shall be provided from each room or building in which ethylene dibromide is stored, handled, or used in quantities that could create a hazard.

(2) Confined or Enclosed Spaces

Entry into confined spaces, such as tanks, pits, process vessels, tank cars, sewers, or tunnels, where there may be limited egress shall be controlled by a permit system. Permits shall be signed by an authorized employer representative certifying that preventive and protective measures have been followed.

Confined spaces which have contained ethylene dibromide shall be thoroughly ventilated to ensure an adequate supply of oxygen, tested for ethylene dibromide and other contaminants, and inspected for compliance with these requirements prior to each entry. Adequate ventilation shall be maintained while workers are in the space. Leakage of ethylene dibromide into the confined space while work is in progress shall be prevented by disconnecting and blanking the ethylene dibromide supply lines. An individual entering confined spaces shall be furnished with appropriate personal protective equipment and protected by a lifeline harness tended by another worker outside the space, who shall also be equipped for entry with approved personal protective equipment and who has contact with a third party. Communication (visual, voice, signal line, telephone, radio, or other suitable means) shall be maintained by the standby person with the employee inside the confined or enclosed space. A third employee, equipped to proceed to the aid of the other two if necessary, shall have general surveillance of their activities.

(e) Storage

(1) Storage facilities shall be designed to contain spills completely within a surrounding dike and to prevent contamination of workroom air.

(2) Storage of ethylene dibromide in the same area as reactive metals, such as aluminum or magnesium, or as liquid ammonia shall be prohibited.

(3) Ethylene dibromide shall be stored in tightly closed containers in a well-ventilated area away from excessive heat and sunlight.

(4) Storage containers shall be periodically inspected for leakage.

(5) Ventilation switches and emergency respiratory equipment shall be located outside storage areas in readily accessible locations which will be free of ethylene dibromide in an emergency.

(f) Spills, Leaks, and Waste Disposal

(1) If ethylene dibromide leaks or is spilled, the following steps shall be taken:

(A) Evacuate all nonessential personnel from the area.

(B) Adequately ventilate the area of the spill or leak to prevent accumulation of the vapor.

(C) If in liquid form, collect spilled material for reclamation or absorb in vermiculite, dry sand, earth, or similar nonreactive material.

(D) If in solid form, collect spilled material in the most convenient and safe manner for reclamation or for disposal.

(2) Personnel entering the spill or leak area shall be furnished with appropriate personal protective equipment. All other personnel shall be excluded from the area.

(3) All wastes and residues containing ethylene dibromide shall be collected in ethylene dibromide-resistant containers and incinerated or buried in such a manner that no ethylene dibromide or toxic decomposition products are released to the environment.

Section 7 - Sanitation Practices

(a) Plant sanitation shall meet the requirements of 29 CFR 1910.141.

(b) Appropriate locker rooms shall be available for changing into required protective clothing in accordance with 29 CFR 1910.141(e). Clothing contaminated with liquid ethylene dibromide shall be immediately removed and placed in a closed container in a well-ventilated area for later disposal or decontamination. Employers shall require personnel who work with ethylene dibromide to shower before leaving the workplace at the end of a workday.

(c) Employers shall ensure that employees who handle ethylene dibromide wash their hands thoroughly with soap and water before eating, smoking, or using toilet facilities.

(d) The storage, dispensing, preparation, and consumption of food, beverages, or tobacco shall be prohibited in ethylene dibromide work areas.

(e) The employer shall ensure that personnel who launder and clean clothing or equipment contaminated with ethylene dibromide are provided adequate personal protective equipment to prevent exposure and shall ensure that these employees are aware of the potential hazards of exposure to ethylene dibromide.

Section 8 - Monitoring and Recordkeeping Requirements

(a) Industrial hygiene surveys shall be made in any workplace where ethylene dibromide is handled, processed, or stored. Records of these surveys, including the basis for concluding that environmental concentrations are below the recommended limit or below the action level, shall be maintained. Surveys shall be repeated every month or whenever a process change is made.

(b) Where exposure concentrations have not been determined, they shall be determined as soon as practicable after the promulgation of a standard based on these recommendations.

(c) Requirements set forth below apply to work areas in which the concentration of ethylene dibromide has been determined to be at or above the action level.

(1) An adequate number of samples shall be collected monthly in accordance with Appendix I for the evaluation of the work environment with respect to the occupational exposure of the employees.

(2) Environmental samples shall be taken when a new process is installed or process changes are made which may cause an increase in environmental concentrations. Significantly increased production, relocation of existing operations, interruption of normal maintenance schedules, or other functions which may increase ethylene dibromide concentrations shall require resampling and analysis.

(3) In all monitoring, samples shall be collected in accordance with the provisions prescribed in Section 1(b).

(4) The minimum number of representative exposure determinations for an operation or process shall be based on variations in

exposures and production schedules and in accordance with the provisions prescribed in Section 1(b).

(5) If initial, periodic, or special evaluations indicate that the recommended limit is exceeded, corrective engineering or other control measures shall be immediately instituted to ensure the safety of employees until a concentration below the recommended occupational exposure limit is achieved. In such cases, sampling of each operation and work location shall be conducted at least weekly until two consecutive employee exposure measurements, taken at least 1 week apart, reveal that the employee is not exposed to ethylene dibromide above the recommended occupational exposure limit. Employers shall notify in writing, within 5 days, every employee who is found to be exposed to ethylene dibromide above the recommended environmental limit.

(d) Employers or their successors shall maintain records which shall include sampling and analytical methods, types of respiratory protection used, concentrations found, and information concerning exposure of employees to ethylene dibromide. Each employee shall have access to data on the employee's own environmental exposures. Pertinent records of occupational accidents and environmental exposures within the workplace shall be kept for at least 30 years after the worker's employment has ended. Records of occupational exposures applicable to an employee should be included in that employee's medical records. The medical representatives of the Secretary of Health, Education, and Welfare, of the Secretary of Labor, of the employee or former employee, and of the employer shall have access to all such records.

II. INTRODUCTION

This report presents the criteria and the recommended standard which were prepared to meet the need for preventing disease or injury arising from exposure to ethylene dibromide. The criteria document fulfills the responsibility of the Secretary of Health, Education, and Welfare under Section 20(a)(3) of the Occupational Safety and Health Act of 1970 to "...develop criteria dealing with toxic materials and harmful physical agents and substances which will describe...exposure levels at which no employee will suffer impaired health or functional capacities or diminished life expectancy as a result of his work experience."

The National Institute for Occupational Safety and Health (NIOSH), after a review of data and consultation with others, has formalized a system for the development of criteria upon which standards can be established to protect the health and provide for the safety of employees exposed to hazardous chemical and physical agents. Any criteria and recommended standard should enable management and labor to develop better engineering controls and more healthful work practices. Simple compliance with the recommended standard should not be used as a final goal.

These criteria for a standard for ethylene dibromide are part of a continuing series of documents developed by NIOSH. The recommended standard applies to the processing, manufacture, use of, or other occupational exposure to ethylene dibromide as applicable under the Occupational Safety and Health Act of 1970. The standard was not designed for the population-at-large, and any extrapolation beyond occupational exposures is not warranted. It is intended to (1) protect against the

development of short- and long-term adverse effects on health from ethylene dibromide exposure, (2) protect against local effects on the skin, (3) be measurable by techniques that are valid, reproducible, and available to industry and government agencies, and (4) be attainable with existing technology.

The development of the criteria for the recommended standard for occupational exposure to ethylene dibromide has demonstrated a need for further research in the following areas: (1) epidemiologic studies of employees exposed to ethylene dibromide, (2) animal studies designed to determine the cumulative effects from dermal contact and inhalation of ethylene dibromide, (3) studies on the mutagenic effects of ethylene dibromide in mammals, and (4) animal studies to investigate the carcinogenic and reproductive effects from ethylene dibromide by dermal absorption and inhalation.

Adherence to the recommended standard for occupational exposure to ethylene dibromide is intended to minimize the potentials for the induction of sterility, carcinogenesis, mutagenesis, or teratogenesis in employees and their offspring from exposure to ethylene dibromide in the workplace. Adherence to the recommended standard is also intended to minimize the hazards from skin penetration and irritation associated with exposure to liquid ethylene dibromide and the delayed and insidious long-term systemic effects resulting from exposure to both liquid and vaporous ethylene dibromide.

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 μ moles/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 $\mu\text{moles/minute/g}$ 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 μ moles/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 14 C-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 ^{14}C -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 ^{14}C -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 $\mu\text{g/g}$ 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 abscesses 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 5×10^6 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- μ l (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 10 μ l (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 μ M 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 μ l (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 μ M/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 μ l (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 ethylene dibromide into the eyes of rabbits caused conjunctival irritation 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 Pflessler [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 spermatzoic maturation more than spermatzoic 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 μ l(11.0 mg)/plate. Brem et al [64] observed similar positive results in *Salmonella* TA1530 and TA1535 at 10 μ M/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 μ l -	Positive mutagenic response by reversion of growth deficiency	63
"	TA 1530 TA 1535 TA 1538	10 μ M 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 μ l x 8 hr	Damage to cellular DNA and growth inhibition of pol A-, preferential to A+	64
Salmonella typhimurium	LT 2 gale503	1-5 μ l 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)

IV. ENVIRONMENTAL DATA AND ENGINEERING CONTROLS

Environmental Concentrations

Little information has been found on concentrations of airborne ethylene dibromide in industrial or ambient air. In 1975, the Environmental Protection Agency (EPA) [19] reported measurements of atmospheric ethylene dibromide concentrations at selected sites near sources which were generally related to gasoline production, distribution, use, and manufacture of ethylene dibromide. Urban locations near major streets were selected in three Western cities to represent worst-case ambient concentrations of ethylene dibromide. Each site was within 200-300 feet of 2 or more gasoline service stations and was located near roadways carrying traffic loads of 25,000-50,000 vehicles/day. At each site, tandem TENAX-GC adsorption tubes with the necessary collection assembly were directed into the wind and positioned 5-6 feet above the ground. The collection assembly consisted of a prefilter of 8 μm mean pore size, a drying tube packed with silica gel, a tandem adsorption train packed with TENAX-GC support material housed in a dry-ice chest, and a vacuum pump controlled by a critical orifice needle to maintain a constant flowrate of 1 liter/minute. Adsorbed ethylene dibromide was extracted from the adsorbent with hexane and the hexane-ethylene dibromide mixture was stored in a freezer until analyzed. The analyses were performed by a gas-liquid chromatograph equipped with an electron capture detector capable of detecting as small a quantity of ethylene dibromide as 1-2 picograms/injection. All samples were initially identified on a Carbowax 20M column and further identified by analysis on a QF-1, DEGS, or OV-101

column, although unequivocal characterization by an independent method of analysis was not done. The results of the urban study, presented below in Table IV-1, suggest that urban workers may be continually exposed to miniscule quantities of ethylene dibromide. The significance of such an exposure is not known at present.

TABLE IV-1

AMBIENT AIR CONCENTRATIONS OF ETHYLENE
DIBROMIDE AT URBAN ROADWAY SITES

City	Concentration	
	$\mu\text{g}/\text{cu m}$	ppb
Phoenix, Arizona	0.069	0.008
Los Angeles, California	0.11	0.013
Seattle, Washington	0.083	0.010

Adapted from reference 19

The concentration of airborne ethylene dibromide on the premises of an oil refinery ranged from 0.23 to 1.65 $\mu\text{g}/\text{cu m}$ (0.00023 to 0.00165 $\text{mg}/\text{cu m}$) at two locations 50-400 feet downwind of a bulk transfer and a tank truckloading operation [19]. Sampling and analytical methods were those described for the urban air study above. Atmospheric concentrations of ethylene dibromide measured at the production facilities of two major

manufacturers were reported to be between maximum values of 90 and 115 $\mu\text{g}/\text{cu m}$ (0.090 and 0.115 $\text{mg}/\text{cu m}$) at downwind locations near the perimeters of the plant boundaries.

In 1975, the EPA [70] estimated the potential emission concentrations of ethylene dibromide from refueling losses and automotive evaporative or exhaust emission. In this report, both the most likely and worst-case emission concentrations were calculated, using the average value of 0.9-1.3 g of ethylene dibromide/gal of automotive fuel (one equivalent of organic bromine is added to gasoline for each two equivalents of lead added). The estimated emission rate for ethylene dibromide by measurement was reported to be 0.000063 g/g lead/gal. The reported losses for entrainment and spillage each were 0.000047 g/g lead/gal. Thus, the total loss because of refueling was reported to be 0.000157 g/g lead/gal. Evaporative losses were calculated to be 0.00014 g/g lead/gal from the automobile fuel tanks. The most likely estimate of loss from the carburetor was 0.0016 g/g lead/gal, whereas the worst case was 0.0043 g/g lead/gal. Exhaust emissions were also calculated, the most likely being 0.0065 g/g lead/gal and the worst case being 0.3 g/g lead/gal. Summarizing the three types of emissions, the total emissions calculated for refueling, evaporation, and exhaust was most likely 0.008397 g/g lead/gal and the worst case, 0.304597 g/g lead/gal. The total emissions can also be estimated directly in terms of ethylene dibromide by expressing the relative percentages of ethylene dibromide/lead in a gallon of gasoline. Usually, a gallon of leaded gasoline contains between 1.9 and 3.0 g of lead/gal [70]. Therefore, the total ethylene dibromide emissions would calculate to 0.02099 g/gal for the most likely situation and to 0.76149 g/gal for the worst-case situation,

assuming an average lead content of 2.5 g/gal [70]. Therefore, NIOSH estimates that between 21 and 761 mg of ethylene dibromide may be released to the environment for every gallon of gasoline sold.

The Dow Chemical Company [71] submitted information from limited studies on the exposure of gas station operators to ethylene dibromide during their work that indicated that these workers were exposed to about 7 ppb (0.054 mg/cu m) while near the gas pump and had an 8-hour time-weighted average (TWA) exposure of about 5 ppb (0.038 mg/cu m). The 8-hour TWA concentration for garage mechanics was 3 ppb (0.023 mg/cu m). No further information or data were given in the submission.

Ethyl Corporation submitted data from personal and area monitoring by job classification for its ethylene dibromide-manufacturing plant at Magnolia, Arkansas, and provided a description of the sampling and analytical methods used [15]. Personal monitoring was conducted with a calibrated, battery-operated pump and charcoal collection tubes. The method for area monitoring was not specified. Carbon disulfide was used to desorb the ethylene dibromide, and the subsequent analyses were conducted in a gas-liquid chromatograph equipped with a flame ionization detector. Sampling times varied from 147 to 397 minutes and actual exposure concentrations ranged from 0.02 to 3.47 ppm (0.154 to 26.72 mg/cu m) of ethylene dibromide. The results of this monitoring are summarized in Table IV-2.

Ethyl Corporation also submitted environmental monitoring results for various worksites within its Magnolia manufacturing plant and within its blending plant at Baton Rouge, Louisiana [15]. Sampling times ranged from 129 to 309 minutes and ethylene dibromide concentrations varied from

nondetectable to 18.25 ppm (140.525 mg/cu m). The results of this survey are presented in Table IV-3. For the most part, the environmental concentrations were lower in the blending plant than in the manufacturing plant, and within each plant, specific worksites had consistently lower concentrations than others. Samples taken at worksites where ethylene dibromide was unloaded, transferred, or stored, or where maintenance of process vessels was being done, were consistently higher than samples taken around the manufacturing or blending equipment itself.

E. I. du Pont de Nemours and Company submitted similar data from its blending facility in Deepwater, New Jersey [15]. The results of this survey are summarized in Tables IV-4 and IV-5.

These environmental and personal monitoring data suggest that current industrial operating procedures may maintain workplace air concentrations of ethylene dibromide substantially below the current federal standard 8-hour TWA concentration of 20 ppm.

TABLE IV-2

ETHYLENE DIBROMIDE CONCENTRATIONS BY
PERSONAL MONITORING AT ETHYL CORPORATION

Job Function	Date	Sample Time (min)	Reported Concentration	
			ppm	mg/cu m
EDB control room operator	01/19/76	319	0.16	1.23
	01/20/76	397	0.69	5.31
	01/21/76	332	0.23	1.77
	01/21/76*	234	0.44	3.39
	01/22/76	313	0.225	1.73
	01/23/76	201	0.42	3.23
Vinyl bromide control room operator	01/19/76	314	0.10	0.77
	01/20/76	270	0.02	0.15
	01/21/76	319	0.14	1.08
	01/21/76*	217	<0.04	<0.31
	01/22/76	352	0.06	0.46
Loaders of EDB or VBr**	01/19/76	250	1.31	10.09
	01/19/76	241	1.64	12.63
	01/20/76	365	0.22	1.69
	01/20/76	204	1.67	12.86
	01/21/76	386	0.39	3.00
	01/21/76	208	0.06	0.46
	01/22/76	293	0.045	0.35
	01/22/76	221	0.04	0.31
	01/23/76	191	1.85	14.24
	01/23/76	180	1.57	12.09
Laboratory technician (Plant chemist)	01/19/76	121	3.47	26.72
	01/20/76	212	0.45	3.46
	01/21/76	147	0.09	0.69
	01/22/76	209	0.13	1.00
Production supervisor	01/22/76	250	0.04	0.31

*Denotes evening shift workers

**Load ethylene dibromide (EDB) or vinyl bromide (VBr), but at different times

Adapted from reference 15

TABLE IV-3

ETHYLENE DIBROMIDE CONCENTRATIONS IN AIR BY
ENVIRONMENTAL MONITORING AT ETHYL CORPORATION

Location	Date	Sample Time (min)	Reported Concentration	
			ppm	mg/cu m
Manufacturing plant				
Reaction building	07/14/75	234	0.59	4.54
Bulk storage	07/15/75	257	3.90	30.03
EDB* load rack	07/16/75	180	0.41	3.16
EDB surge tank	07/16/75	129	0.47	3.62
Top EDB-Br ₂ building	07/17/75	265	0.05	0.38
West of VBr** reactor	01/19/76	257	0.23	1.77
Alumina scrubber area by EDB building	01/20/76	248	18.25	140.52
East of VBr reactor	01/21/76	262	1.64	12.63
EDB storage area	01/21/76	265	1.40	10.78
East of VBr reflux pump (by stairs)	01/22/76	299	0.08	0.62
Portapak purge area	01/22/76	309	-	-
EDB surge (make) tank	01/23/76	208	4.81	37.04
Blending plant				
Load rack	04/01/75	240	0.56	4.31
"	04/18/75	274	0.21	1.62
"	07/23/75	275	0.10	0.77
Pump house	04/18/75	268	0.30	2.31
"	07/23/75	270	0.03	0.23
1st floor - blender tank	07/23/75	255	0.02	0.15
1st floor - blenders	07/09/75	247	<0.02	<0.15
2nd floor - blenders	07/09/75	249	0.04	0.31
3rd floor - EDB weigh tank	07/09/75	254	<0.03	<0.23
3rd floor - EDB weigh tank	07/23/75	253	0.04	0.31

*Ethylene dibromide

**Vinyl bromide

Adapted from reference 15

TABLE IV-4

ETHYLENE DIBROMIDE CONCENTRATIONS
BY PERSONAL MONITORING AT
E. I. DU PONT DE NEMOURS AND COMPANY

Job Description	Date	8-hr TWA Concentration	
		ppm	mg/cu m
Shipping and receiving	07/10/75	<0.065	<0.50
	07/11/75	0.130	1.00
	07/14/75	<0.065	<0.50
	07/17/75	0.256	1.97
	01/27/76	<0.004	<0.03
	01/28/76	0.033	0.25
	01/29/76	<0.004	<0.03
	01/30/76	0.579	4.45
	01/31/76	<0.004	<0.03
Laboratory technician	02/24/75	<0.007	<0.05
Blender operator	11/05/75	<0.114	<0.88

Adapted from reference 15

TABLE IV-5

ETHYLENE DIBROMIDE CONCENTRATIONS IN AIR BY
ENVIRONMENTAL MONITORING AT E. I. DU PONT DE NEMOURS AND COMPANY

Site	Date	Sample Time (min)	Reported Concentration	
			ppm	mg/cu m
Top of tank car	11/21/74	2.7	3.068	23.60(A)
Unloading tank car	11/05/74	5.0	0.061	0.47(B)
	11/05/74	247.0	0.075	0.58(B)
	11/05/74	2.0	<0.001	<0.01(B)
Between blenders 3 & 4	08/12/74	-	<0.001	<0.01(C)
EDB* pumphouse	11/05/75	330.0	<0.046	<0.35
Chainer transfer	11/05/75	180.0	<0.118	<0.91
Top EDB tank	11/06/75	290.0	1.109	8.53(D)
Top of TS-8**	11/10/75	485.0	<0.034	<0.26
Compound bulk 5 feet above ground	11/10/75	490.0	<0.048	<0.37

*Ethylene dibromide

**Tank storage unit 8

(A) Breathing zone of workers

(B) 18 inches above ground during hookup, emptying, and disconnecting, respectively

(C) Spot sample

(D) 2 cars unloaded into tank during sampling period

Adapted from reference 15

Sampling and Analytical Methods

(a) Sampling

Most analytical methods are dependent on the reproducibility and effectiveness of the adsorption of ethylene dibromide by the different collection media and the subsequent desorption, chemical treatment, or extraction efficiencies. Air samples are normally collected and transported to a laboratory where chemical treatment or desorption and analysis occur at a later time.

Liquid absorption media, such as a 1:1 mixture of monoethanolamine and dioxane, have been used as trapping solutions for ethylene dibromide [72,73]. This medium decomposes ethylene dibromide and many other brominated organic contaminants to inorganic bromide and, as such, is not specific for ethylene dibromide.

Porous polymer beads have been used as a collection medium for chlorinated and brominated hydrocarbons [74]. This procedure has not been tested specifically for ethylene dibromide but has been used successfully for closely related halogenated hydrocarbons; thus, it seems feasible that ethylene dibromide could also be collected by this method. The same column is used for sample collection and gas-liquid chromatographic analysis, but only one analysis can be made on each sample.

Silica gel has been used as a collection medium for ethylene dibromide [75]. One advantage of using a solid adsorbent is that sample loss cannot occur from spillage during sampling or in transit for analysis. However, silica gel is a polar adsorbent and shows pronounced selectivity in adsorbing polar molecules, particularly water [76]. Studies with silica gel tubes indicated that water vapor could displace organic molecules

during a normal sampling operation [77]. Although similar studies have not been conducted for ethylene dibromide, it is reasonable to assume that similar displacement of significant quantities of ethylene dibromide by water vapor could occur.

Activated charcoal has been used as an adsorbent in conjunction with gas-liquid chromatography [78,79]. Charcoal is an excellent collecting medium because of its nonpolarity and its affinity for organic vapors and gases. As such, water vapor does not readily displace organic molecules as is the case with silica gel. However, adsorption and desorption efficiencies may vary with different batches of charcoal; therefore, it is necessary to determine the desorption efficiency for each new batch of charcoal. Charcoal tubes containing as much as 600 mg of activated charcoal are commercially available [1].

In the past several years, direct-reading instruments and devices have been developed which make continuous or "on-the-spot" monitoring of halogenated hydrocarbons, including ethylene dibromide, feasible. These devices, when properly calibrated and used within their performance characteristics and limitations, can be helpful in monitoring airborne halogenated hydrocarbons [80]. Colorimetric indicator tubes are available from at least three sources [81-83] which provide semiquantitative measurement in the range of 1-200 ppm, although no detector tubes have been certified as yet by NIOSH for response to ethylene dibromide. The use of infrared light absorption at a wavelength of 8.4 μm [84] is claimed to detect airborne ethylene dibromide at a concentration of 0.1 ppm. These direct-reading instruments are portable and also may be used as part of a multipoint sampling system for continuous, unattended monitors.

Other sampling devices used for the collection of organic solvents and halogenated hydrocarbons may be adaptable to ethylene dibromide collection. These include sampling bottles [85], bubblers [86], and plastic bags [87,88]. No specific tests have been reported on the use of plastic bags for sampling ethylene dibromide. However, studies by Stewart et al [89], Smith and Pierce [90], and Calibrated Instruments, Inc, [91] indicate that halogenated hydrocarbons, such as vinyl chloride, dichloromethane, perchloroethylene, and trichloroethylene, may be successfully collected in plastic bags made of Saran, aluminized Scotch Pak, and Mylar. Sampling bags are commercially available with capacities ranging from approximately 0.5-96 liters. Although plastic bags have the disadvantage of being bulky when handled or transported to the analytical laboratory, they have the advantage of enabling samples of the collected air to be injected directly into the gas-liquid chromatograph, thus eliminating the need for sorbent materials, such as activated charcoal, alumina, and silica gel. Before actual field use can be evaluated, controlled laboratory experiments must be conducted to determine the effectiveness and practicality of these procedures.

(b) Analysis

Chemical analysis has been used to estimate ethylene dibromide concentrations in the collection media. Aeration has been used by Peterson et al [75] to desorb ethylene dibromide from the silica gel; pyrolysis of the free ethylene dibromide produced bromine and hydrobromic acid. These products were collected in a second absorption solution of 1% sodium carbonate and 1% sodium formate in deionized water and quantitated by titrimetric analysis of the inorganic bromide present. Aman et al [32]

estimated the airborne ethylene dibromide concentration by absorbing the free ethylene dibromide with 1% sodium hydroxide and by quantitating the hypobromite formed iodometrically. Dumas [92] determined airborne ethylene dibromide concentrations by coulometric titration of sodium bromide after absorption of ethylene dibromide in methanolic sodium hydroxide and digestion by boiling under reflux for 15 minutes on a steam bath. These methods, like most chemical methods, require somewhat bulky apparatus and are not specific for ethylene dibromide since any aliphatic brominated compound will interfere and be included in the quantitative estimate.

Physicochemical methods have been developed or adapted to identify and quantitate concentrations of airborne ethylene dibromide. Christie et al [93] modified a refrigerant leak-detector lamp to detect organic halogen compounds, including ethylene dibromide, in the atmosphere at a minimum concentration of 5 ppm. The procedure is dependent on visual estimation of the intensity of a flame color in response to the vapor concentration and is not specific for ethylene dibromide.

Flame chemiluminescence has been reported by Crider [94] to detect ethylene dibromide concentrations in air of 0.03 ppm. This method has not been tested on mixtures of halogenated hydrocarbons, and it is too early in its development to enable prediction of the practicality of the method for area or personal monitoring in an occupational environment.

Other physicochemical methods have been developed for halogen compounds, but they have not been tested for identification and quantitation of ethylene dibromide. One of these methods, based on nitrogen enhancement of an AC spark, has been used for continuous measurement of halogenated compounds in field situations [95]. The

instrument is lightweight, portable, fast-responding, and reportedly capable of detecting halogenated hydrocarbons in the ppm range. However, until testing with ethylene dibromide has been conducted, the feasibility and reliability of this instrument is not known.

In recent years, gas-liquid chromatography has become the most prevalent method for the detection and analysis of organic materials [1,96-101]. Direct analysis of airborne ethylene dibromide has been reported to result in measurement of amounts as small as 0.002 $\mu\text{g}/\text{cu m}$ (0.000002 $\text{mg}/\text{cu m}$) [96]. Gas samples were drawn into a gas flask and subsequently injected directly into a gas-liquid chromatograph equipped with a hydrogen flame ionization detector system. This method has not been tested for ethylene dibromide personal monitoring in an occupational environment. However, it should be easily adaptable to surveying the workplace environment on a periodic basis should the need arise.

The combination of charcoal tube sampling followed by desorption and gas-liquid chromatographic analysis [78] has been developed and used in the occupational environment [15]. These procedures involve the use of charcoal tubes for sampling of the breathing zone, with subsequent benzene-methanol (99:1) desorption and analysis by a gas-liquid chromatograph equipped with an electron capture detector (S Tucker, written communication, March 1977). The quantitation range of the detector (40-800 monograms/sample solution) allows this method to be used to measure air concentrations of ethylene dibromide ranging from 1.6 $\mu\text{g}/\text{cu m}$ (0.0002 ppm) to concentrations above 16 $\text{mg}/\text{cu m}$ (2 ppm). The relative standard deviation for this analytical method in the range of 203-2,370 nanograms/charcoal tube sample is 0.070. The advantages of this combined

method are that the sampling device is small, portable, and involves no liquids, and the analytical method is sensitive, rapid, and subject to minimal interference. These NIOSH recommended sampling and analytical methods are described in detail in Appendices I and II.

Engineering Controls

Engineering design and work practices for operations with ethylene dibromide should be oriented toward minimizing the vapor concentrations and toward preventing skin and eye contact with liquid ethylene dibromide. The achievement of these goals can be accomplished by the use of a properly constructed and maintained closed-system operation with appropriate safety precautions. Closed-system operations are effective only when the integrity of the system is maintained by frequent inspection for, and prompt repair of, any leaks. Where closed systems cannot be adequately designed and effectively used, local exhaust ventilation systems must be provided to direct vapor away from employees and to prevent the recirculation of exhaust air. Guidance for designing a local exhaust ventilation system can be found in Industrial Ventilation--A Manual of Recommended Practice [102], or more recent revisions, and in Fundamentals Governing the Design and Operation of Local Exhaust Systems, ANSI Z9.2-1971 [103]. Ventilation systems of this type will require regular inspection and maintenance to ensure effective operation. These regularly scheduled inspections should include face velocity measurements of the collecting hood, inspection of the air mover and collector, and measurements of workroom air concentrations.

V. WORK PRACTICES

Strict adherence to stringent and detailed work practices is required to prevent hazardous occupational exposure to ethylene dibromide. The potential hazards from exposure to ethylene dibromide which determine to a large extent the nature of necessary work practices are: (1) the delayed and insidious onset of symptoms, (2) an odor threshold which is not adequate to provide warning of dangerous concentrations, (3) its irritating and penetrating effects on the skin, and (4) its potential for causing cancer, mutations, sterility, and fetal anomalies.

The principal method for manufacturing ethylene dibromide is the bromination of ethylene [1]. Small quantities of vinyl bromide, ethyl bromide, and ethyl chlorobromide may be formed as impurities in the production of ethylene dibromide [15], and caution must be taken to avoid exposure to these substances as well.

Ethylene dibromide is nonflammable and nonexplosive at ordinary temperatures, but since it can be decomposed to toxic and corrosive compounds, such as hydrobromic acid, by contact with open flames or red-hot surfaces [1], it should be appropriately stored and handled to prevent such contacts. Special precautions are necessary for maintenance and emergency repair work, such as welding, cutting, or any spark- and flame-generating operations. Furthermore, it is recommended that smoking be prohibited in workplace areas where ethylene dibromide is manufactured, handled, blended, or stored. Since decomposition may occur [16] and highly toxic aldehyde vapors and acid gases may be emitted [104], ethylene dibromide must be

protected from direct light and excessive temperature. Ethylene dibromide reacts rapidly with certain metals, such as aluminum and magnesium, to form combustible and explosive organometallic compounds [104] and with liquid ammonia [16]. Therefore, it is recommended that reasonable precautions be taken to keep ethylene dibromide separated from these materials.

Engineering controls should be used to keep the concentration of airborne ethylene dibromide below the recommended occupational exposure limit. Ethylene dibromide should be used only in a closed system. Proper ventilation, consisting of forced-draft exhaust systems and tempered makeup air systems, should be used to minimize employee exposure to ethylene dibromide in the workplace, and to aid in reducing the extent of exposure in such routine operations as systems maintenance, routine cleanup, and daily sanitation practices. Design principles for all exhaust and ventilation systems should be in accordance with common engineering practices [102,103]. Portable exhaust systems should be used to reduce the concentrations of airborne ethylene dibromide in such situations as cleanup of small leaks and spills, line and vessel entry, and emergency decontamination. Periodic inspections by trained personnel should be made to determine the proper functioning of exhaust and ventilation systems by measuring airflow, static pressure, and leakage.

Untrained or nonessential personnel should be restricted from entering areas where ethylene dibromide is manufactured, handled, blended, or stored. Storage areas where ethylene dibromide is kept in large quantities, such as tanks and tank cars, should be diked with a system of sufficient volume to prevent contamination of surrounding areas in the event of a leak. Drainage within the diverting dikes should be channeled

to a collection area for reclamation or disposal. Floors in the ethylene dibromide work areas should be constructed of ethylene dibromide-resistant materials and sealed or made impervious to ethylene dibromide to prevent adsorption, in the event of a spill or leak, and subsequent delayed release to the atmosphere. Periodic scheduled inspections should be made to ensure the proper functioning of dikes, collection systems, and floors. Any malfunctions should be immediately corrected.

All containers used to transport, hold, or process ethylene dibromide should be made of ethylene dibromide-resistant materials, such as lined steel or stainless steel, and should be periodically inspected for signs of wear, corrosion, or leaks by manual and instrumental means. All valves, pipes, and seals used in pumping ethylene dibromide from processing areas to storage areas or transportation loading sites should be made of ethylene dibromide-resistant materials and should be periodically inspected for possible leaks, weak points, or signs of wear.

Ethylene dibromide is a severe eye and skin irritant in humans (G Ter Haar, written communication, January 1977) and can be absorbed through the intact skin of animals in quantities sufficient to present an imminent hazard [33]. In view of this, the use of personal protective equipment, including nylon-impregnated neoprene gloves [105], ethylene dibromide-resistant and fire-retardant clothing, rubber boots or overshoes, bib-type aprons, and chemical safety goggles is recommended when contact by liquid ethylene dibromide with the skin and eyes is possible. In addition, it is recommended that proper respiratory protection be worn when entering an area where the concentration of the vapor of ethylene dibromide may be greater than the recommended occupational exposure limit. Clothing should

be immediately removed if it becomes contaminated, and the skin of the exposed area should be thoroughly washed with water.

Personnel working with ethylene dibromide must be instructed in emergency procedures and participate in periodic, simulated emergency drills. All personnel not involved in the specified emergency operations must be immediately evacuated from the area. Special training sessions must be held and written emergency procedures must be updated periodically. These should include the location, use, and maintenance of first-aid, firefighting, and decontamination equipment.

To prevent the adverse effects caused by ethylene dibromide, it is recommended that exposure to ethylene dibromide vapor or liquid be kept at a minimum. Routine visual and functional inspections must be made by trained personnel to ensure that processes in which ethylene dibromide is used are completely closed. If leaks or spills occur in the processing, handling, or storage of ethylene dibromide, these must be promptly corrected, regardless of the ethylene dibromide concentration in the environment. It is recommended that nonessential personnel be evacuated from the immediate area where a leak or spill has occurred until decontamination of the area is complete. If employees must withdraw samples from a process involving the use of ethylene dibromide, an impervious suit, including gloves, boots, and air-supplied hood, should be worn. An effective exhaust system for trapping any ethylene dibromide vapor may be used in place of the air-supplied hood. Any waste or residues containing ethylene dibromide should be incinerated, buried, or otherwise disposed of so that no ethylene dibromide is released into the environment. Applicable local, state, and federal regulations should be followed. Air

exhausted from ethylene dibromide workplace areas must be decontaminated by incineration, chemical treatment, or other effective means so that the release of ethylene dibromide into the environment will be minimized.

Safety showers and eyewash fountains should be located in or near areas where ethylene dibromide exposures are likely to occur and should be properly maintained. It is recommended that employees immediately remove contaminated clothing, flush all affected skin surfaces with water for at least 15 minutes, and then obtain medical attention.

Since ethylene dibromide is toxic when ingested and has caused death in a woman after ingestion [25], it is recommended that handwashing facilities, soap, and water be made available to the employees. As a good hygiene practice, it is recommended that employees wash their hands before consuming beverages or food, using tobacco, or using toilet facilities. The employer should provide lunchroom facilities physically separated from the ethylene dibromide work areas.

It is recommended that any contaminated article of personal protective equipment be discarded or, if feasible, decontaminated with soap and water. It is also recommended that employer-supplied clothing be worn while working with ethylene dibromide and that the employer arrange to have this clothing laundered daily and properly maintained. The employer should inform the launderers of the possible hazard of coming into contact with contaminated clothing and advise him on safe methods of handling such material. The employer should provide separate locker and change facilities for work and street clothes. As a good hygiene practice, it is recommended that shower facilities be provided for the employees and that

they be required to shower before leaving the workplace at the end of the work shift.

Since ethylene dibromide is a component of some insecticidal fumigants and conventional work practice guidelines are inappropriate to protect agricultural workers from the hazards of exposure, it is recommended that the label precautions on such pesticides be followed and stringently adhered to. These label requirements usually specify allowable time limits before a fumigated space or area may be reentered and safe practices for the application of the particular fumigant mixture. Specific requirements of worker protection standards for agricultural pesticides can be found in 40 CFR 170.

In summary, precautions should be exercised with ethylene dibromide to prevent serious consequences which may result from ingestion, inhalation, and skin or eye contact. Processes in which ethylene dibromide is used in large quantities should be carried out in closed systems. Well-designed hoods, ventilation systems, and exhaust systems should be used to maintain concentrations below those specified by this standard. Personal protective equipment and clothing should be worn by employees engaged in the manufacture, handling, or blending of ethylene dibromide. It is important that employees be informed of the hazards associated with ethylene dibromide before job placement and whenever changes are made in any process that may alter their exposure. Appropriate posters and labels should be displayed. The US Department of Labor form OSHA-20, "Material Safety Data Sheet," or a similar OSHA-approved form, should be filled out and posted. All employees in the ethylene dibromide exposure area should know where the safety sheet is posted. Safety showers, eyewash fountains,

and fire extinguishers should be located in areas where ethylene dibromide exposures are likely to occur, and employees should be instructed in their proper use and maintenance.

Records of maintenance schedules, written work practices, emergency procedures, storage locations and quantities of ethylene dibromide present in each location, employee accidents, and employee exposures should be kept and readily accessible to employees and management.

The safe handling of ethylene dibromide depends to a great extent on the effectiveness of employee education, proper safety instructions, intelligent supervision, and the use of proper and safe equipment. The education and training of employees to work safely and to use the personal protective equipment are the responsibility of management. Training classes for both new and current employees should be conducted frequently to maintain a high degree of safety in working with ethylene dibromide.

VI. DEVELOPMENT OF STANDARD

Basis for Previous Standards

In 1953, the American Conference of Governmental Industrial Hygienists (ACGIH) adopted a threshold limit value (TLV) of 25 ppm as an 8-hour TWA concentration for 1,2-dibromoethane (ethylene dibromide) [106]. No specific basis for this TLV has been found. In 1954, the ACGIH [107] changed the name to ethylene dibromide in the official TLV list, and, in 1956, they added the value of 190 mg/cu m to the official entry [108]. In 1962, the ACGIH [109] restored 1,2-dibromoethane as the principal designation of the compound and reported in the Documentation of the Threshold Limit Values of Substances in Workroom Air that the TLV of 25 ppm as a TWA concentration was based on the report of Rowe et al [33]. Rowe et al [33] reported that ethylene dibromide was readily absorbed through the intact skin of rabbits and from the gastrointestinal tract of rats, mice, guinea pigs, chickens, and rabbits. Ethylene dibromide vapor caused CNS depression, pulmonary irritation, and liver and kidney damage in rats and guinea pigs after single exposures. Rats, guinea pigs, monkeys, and rabbits tolerated repeated exposures of ethylene dibromide vapor at 25 ppm for 7 hours/day, 5 days/week, for about 6 months without adverse effects, but these species did not tolerate well a similar exposure at 50 ppm.

In 1965, the ACGIH [110] recommended that special emphasis be given to the potential for skin absorption of 1,2-dibromoethane by adding the designation "skin" after the name in the TLV list. Such a notation refers to the potential contribution to overall exposure by the dermal route, including mucous membranes and eyes, either by airborne, or more particularly, by direct contact with ethylene dibromide. This designation

was intended to indicate that measures for the prevention of absorption from skin and mucous membranes were necessary if the TLV was to be successful in limiting occupational exposure to a safe level. In 1965, the ACGIH [110] also recommended that the TLV of 25 ppm as a TWA concentration for ethylene dibromide be tentatively changed to a ceiling limit of 25 ppm. The basis [111] for this limit was primarily the report by Rowe et al [33], discussed previously, and the paper by Lucas [28]. Lucas [28] observed that single 10- to 12-minute exposures of rabbits to a concentration of ethylene dibromide vapor sufficient to produce anesthesia resulted in rapid breathing, phonation, and death within 15-18 hours. The shift of the TLV from a TWA value to a ceiling limit was made final in 1967.

In 1971, the ACGIH [112] recommended changing the ceiling limit of 25 ppm to an 8-hour TWA concentration of 20 ppm (145 mg/cu m). The 1971 Documentation of the Threshold Limit Values for Substances in Workroom Air [113] cited several studies with no particular emphasis on how the limit was set. These reports included Rowe et al [33], Lucas [28], Kochmann [23], Olmstead [25], Rowe et al [38], and McCollister et al [37], which are discussed in Chapter III. Presumably, the study by Rowe et al [33] was the principal basis on which the new limit was set. The proposed value of 20 ppm as a TWA concentration was adopted in 1973 [114]. The basis for this change as stated in the 1974 supplement of the 1971 Documentation [113], did not differ from that stated in 1971.

In 1976, the ACGIH [115] added to the TWA value a tentative short-term exposure limit (STEL) of 30 ppm (220 mg/cu m), which is defined as a maximal concentration to which workers can be exposed for a period up to 15 minutes continuously. No more than 4 such excursions are permitted each day, with at least 60 minutes between successive exposure periods. Also,

the daily TLV-TWA is not to be exceeded.

Florida, Mississippi, Pennsylvania, and South Carolina have adopted a TWA concentration of 25 ppm (190 mg/cu m) as their environmental limit for ethylene dibromide [116].

In Finland, the German Democratic Republic, and Yugoslavia, the maximum allowable concentration (MAC) for the workplace environment is 190 mg/cu m (25 ppm) [116]. In the Rumanian Socialist Republic, 200 mg/cu m (approximately 26 ppm) of ethylene dibromide is the maximum concentration allowed in the occupational environment, whereas in Poland, the MAC allowed for ethylene dibromide is 100 mg/cu m (13 ppm) [116]. Although the USSR does not currently recommend a standard for ethylene dibromide, the US recommendation of 145 mg/cu m (20 ppm) is stated to be inadmissibly high. The 1976 edition of the Handbook for Chemists, Engineers and Physicians [117] states that, in all likelihood, the permissible concentration should be on the same order as the Russian MAC for dichloroethane (12.5 ppm) or lower [117]. No bases for these standards have been found.

The current federal standard (29 CFR 1910.1000) for occupational exposure to ethylene dibromide is 20 ppm as an 8-hour TWA limit, with an acceptable ceiling concentration of 30 ppm. A maximum peak above the acceptable ceiling concentration for an 8-hour work shift of 50 ppm not to exceed 5 minutes (Federal Register 40:103, May 28, 1975) is also permitted. This standard was adopted from the American National Standards Institute (ANSI) recommendation Z37.31-1970 [3], which was based on the reports of Rowe et al [33] and Olmstead [25], and the summary presentation by Irish [4], which includes synopses of several other reports [23,24,27,33] discussed in Chapter III.

Basis for the Recommended Standard

(a) Environmental (Workplace Air)

Employees exposed to ethylene dibromide vapor may suffer deleterious effects which may be delayed in their onset. Single short-term exposures to sublethal concentrations have resulted in conjunctival irritation, glandular swelling, and a generally poor condition, while repeated exposures to sublethal concentrations have resulted in conjunctivitis, pharyngeal and bronchial irritation, anorexia, headaches, and depression [23]. Exposure to a lethal inhaled dose of ethylene dibromide vapor derived from some unknown portion of an estimated 70 g of the liquid applied to an anesthesia mask produced nervousness, vomiting, diarrhea, abdominal pain, upper respiratory irritation, degeneration of the parenchymal tissue in the heart, liver, and kidneys, and hemorrhaging into the trachea, blood vessels, and along the sternum, culminating in death about 44 hours after the exposure [22].

Ethylene dibromide in humans produces blistering when the liquid is allowed to remain in contact with the skin for more than 10 minutes and can cause death when ingested. Volunteers subjected to dermal contact with ethylene dibromide for up to 10 minutes, developed burning pain and reddening of the skin; blistering occurred after prolonged contact [24]. Repeated dermal exposure caused a skin sensitization to ethylene dibromide in one of the volunteers. Ingestion of about 4.5 ml (140 mg/kg) of ethylene dibromide caused the death of a 43-year-old woman [25]. Prior to death 54 hours after ingestion, she developed diarrhea, vomiting, anuria, tachypnea, nervous agitation, abdominal pain, systolic heart murmurs, sinus tachycardia, and an arrhythmic, sporadic pulse. Autopsy results indicated edema and congestion of the lungs, intestinal mucosal erythema, massive

centrilobular liver necrosis, and tubular epithelial damage in the kidneys.

Animal experiments confirm and extend the clinical observations of the effects reported in humans that have been exposed to ethylene dibromide. A dog exposed to 1 ml of vaporized ethylene dibromide (a calculated concentration of about 2,830 ppm or 21,790 mg/cu m) for 1 hour showed signs of ocular irritation, including corneal opacity, that developed into purulent conjunctivitis in both eyes and ulceration of one cornea [31]. Conjunctivitis also developed in cats exposed to 100 ppm (770 mg/cu m) of ethylene dibromide for 30 minutes/day for approximately 10 days [23], and conjunctival irritation was noted in rabbits after instillation of undiluted, 10%, or 1% solutions of ethylene dibromide [33]. The irritation subsided within 2-12 days in the rabbits without causing corneal scarring. Marked hyperemia of the cutaneous blood vessels surrounding the application site was found after 0.25, 0.50, or 1.0 ml (0.55, 1.1, or 2.2 g) of ethylene dibromide was applied to the abdomen of rats [27]. Rabbits responded similarly when undiluted or 10% solutions of ethylene dibromide were applied to the abdomen [33]. Application of 210 mg/kg of the undiluted chemical caused marked erythema, edema, and necrosis of the skin.

Respiratory tract irritation caused by ethylene dibromide vapor has been seen in guinea pigs after single exposures at 2,000 ppm (15,400 mg/cu m) for 150 minutes [27], and for cats after exposures as low as 100 ppm (770 mg/cu m) for 30 minutes [23]. A strong reddening of the nasal mucosa was observed after three 30-minute exposures at 100 ppm (770 mg/cu m) [23]. A dog exposed to 5 ml (11.0 g) of vaporized ethylene dibromide for 1 hour in a 100-liter chamber developed severe bleeding in the right lung, and another dog exposed to 1 ml (2.2 g) for 1 hour had severe hyperemia and bronchopneumonic foci in both lungs [31]. The lungs of rats

exposed to ethylene dibromide at concentrations between 100 and 10,000 ppm (770 and 77,000 mg/cu m) for 0.02-16.0 hours were congested, edematous, hemorrhagic, and inflamed [33].

Repeated exposure of cats to concentrations of ethylene dibromide of 100 ppm (770 mg/cu m) for 30 minutes daily for an average of 10 days caused discoloration and partial dysfunction of the lungs [23]. Pulmonary infection, probably a secondary result of the irritation of the lungs by ethylene dibromide, was responsible for a 50% mortality in male rats and guinea pigs and a 25% mortality in female guinea pigs exposed repeatedly to 25 ppm (192.5 mg/cu m) of ethylene dibromide for 7 hours/day, 5 days/week, for about 6 months [33]. Similar mortality was not observed in the control group exposed in a chamber ventilated with clean air, although high mortality was observed in the control animals simply maintained in the colony.

Other systemic damage also occurred in animals after single and repeated exposures to ethylene dibromide vapor. Guinea pigs exposed at concentrations of ethylene dibromide of 2,000 ppm (15,400 mg/cu m) for 150 minutes developed a pronounced granular degeneration of the parenchymal tissue of the kidneys and a slight degeneration of the parenchymal tissue of the liver, spleen, and heart [27]. Rats exposed at concentrations between 100 and 10,000 ppm (770 and 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, cloudy swelling and a slight interstitial congestion and edema of the kidneys, and CNS depression at the higher concentrations [33].

Repeated inhalation exposures of rats for 7 days and rabbits for 3-4 days at 100 ppm (770 mg/cu m) of ethylene dibromide for 7 hours/day

produced slight congestion of the spleens of rats, cloudy swelling and a slight leukocytic infiltration in the livers of rats, and widespread central fatty degeneration and some necrosis in the livers of rabbits [33]. Exposure of guinea pigs and monkeys at 50 ppm (285 mg/cu m) of ethylene dibromide for 7 hours/day, 5 days/week, for 70-80 days caused only slight central fatty degeneration of the liver [33]. Cats exposed at 100 ppm (770 mg/cu m) of ethylene dibromide for 30 minutes/day for about 10 days had enlarged spleens. Death resulted from circulatory system damage to the heart and vessels [23].

CNS effects, such as agitation, restlessness, body tremors, or unconsciousness, are caused by exposure to ethylene dibromide [23,29-31], but they have not been sufficiently described to permit an adequate evaluation or quantitation of their relevance or validity.

The information provided by the few reports on humans [22-25] and such experimental animal data as those given in the following references [23,27,31,33] indicates that many effects produced by ethylene dibromide on humans and animals are similar and differ only in magnitude. Respiratory tract irritation, damage to the liver, kidneys, spleen, and lungs, irritation of the skin and eyes, and gastrointestinal disturbances are the predominant effects of ethylene dibromide exposure. Since systemic effects may occur from ingestion, inhalation, or dermal contact with ethylene dibromide, NIOSH recommends that work practices be used to minimize employee exposure to ethylene dibromide liquid or vapor through inhalation, body or eye contact, or ingestion.

Mammalian studies indicate that reproductive abnormalities, including antifertility [9] and spermatozoic anomalies [41-43], occur from exposure to ethylene dibromide. One report [9] indicated that five ip injections of

10 mg/kg given to male rats produced a decrease in fertility only during the 3rd and 4th weeks after injection. Since spermatids require about 3-4 weeks to mature into spermatozoa in the rat, the evidence indicates that ethylene dibromide affects the development of the spermatids that were present at the time of injection. This is further supported by the return of normal fertility 5 weeks after the injection. Three studies with bulls [41-43] that received an average daily dose of 2 mg/kg of ethylene dibromide for various periods during their development indicated that the abnormalities expressed in their reproductive systems were the result of the action of ethylene dibromide on the maturation step in the spermatogenic cycle and not a direct action of ethylene dibromide on the germinal tissue. Discontinuance of administration of ethylene dibromide resulted in reversal of the impairment, and the bulls produced normal semen and spermatozoa until ethylene dibromide administration was begun again in one of the studies [41]. In another experiment [42], production of abnormal spermatozoa occurred with a dose as small as 4 mg/kg given on alternate days for seven doses. These spermatozoic abnormalities would greatly reduce the fertility, even if they did not cause total sterility. Although these effects were from the ingestion of ethylene dibromide and not from inhalation or dermal contact, they indicate that a hazard, including decreased fertility and even temporary sterility, may result from inhalation or percutaneous absorption of ethylene dibromide.

One study, reported by several authors [56-59], was conducted to determine the carcinogenic properties of ethylene dibromide. Rats and mice given daily oral doses by gavage of ethylene dibromide at 40 and 60 mg/kg, respectively, for 52-64 weeks developed squamous cell carcinomas in the stomach. These carcinomas invaded locally and metastasized throughout the

abdominal cavity. Male and female rats developed stomach carcinomas as early as 10 weeks after the start of administration of ethylene dibromide. The carcinomas became more prevalent as the daily doses of ethylene dibromide were continued, and the final percentage of male rats with tumors after administration of 40 mg/kg/day was 98% after termination of the experiment at 54 weeks. Male rats were more susceptible to tumorigenesis than female rats; 80% of all the males in the study developed tumors versus 38% of all the females. The concurrent control populations did not develop squamous cell carcinomas. More than 70% of all the mice were reported to have developed squamous cell carcinomas of the stomach by the termination of the experiment at 62 weeks. Since the numbers of tumors induced by nearly equivalent quantities of ethylene dibromide administered at different dose rates and on different schedules were substantially different, the experimental data are not consistent with a single-hit model of cancer induction for ethylene dibromide after administration by gastric tube in the rat. However, these data suggest that repeated ingestion of ethylene dibromide by humans may result in gastric carcinomas. At the present time, the induction of cancers by ethylene dibromide by other routes of exposure has not been demonstrated.

The mutagenic potential of ethylene dibromide is well established in both animal and plant systems. It induces mutations in vertebrate cell cultures [62], insects [6], bacteria [60,63,64], plants [66], and fungi [67,68].

One study with *Drosophila melanogaster* [6] showed that ethylene dibromide induced a significant number of recessive lethal mutations in three successive broods of offspring. The adult males fed 0.3 mM of ethylene dibromide for 3 days produced subsequent brood patterns indicative

of impaired spermatozoic maturation rather than of impaired formation. Studies with mouse lymphoma cells [62] indicated that a dose-related effect, typical of those of other alkylating agents tested, existed over the range of 0.0-3.0 mM ethylene dibromide. The effect of the highest concentration was approximately equal to that of a dose of 600 R of X-irradiation.

A study in a host-mediated assay system [60] with *Salmonella typhimurium* G46 in mice suggested that ethylene dibromide was mutagenic at a dose of 500 mg/kg. A second part of this study [60] showed that an equivalent dose produced a positive mutagenic effect on *Salmonella typhimurium* G46 in vitro, indicating that ethylene dibromide did not require metabolic activation and was not deactivated by metabolism. Similar positive mutagenic results occurred in *Salmonella typhimurium* TA 1530 at 5 μ l (11.0 mg)/plate [63] or 10 μ M/plate [64] and in *Salmonella typhimurium* TA 1535 at 10 μ M/plate [64]. A linear relationship for mutation responses occurred over the range of 3.6-148.2 ppm (27.72-1,141.14 mg/cu m) in *Tradescantia* clone 4430 [66], exhibiting a well-defined dose response with single exposures to as little as 3.6 ppm (27.72 mg/cu m) of ethylene dibromide for 6 hours.

Several studies [5,6,64] suggest that the mechanism of mutagenic activity of ethylene dibromide is based on its ability to alkylate, or covalently bond to, DNA in the exposed cells. Ethylene dibromide is a bifunctional alkylating agent capable of introducing cross-links into biologic materials [6] by displacement of the two reactive bromine atoms by reacting with amine, sulfhydryl, carboxy or other electron-donating groups. Ethylene dibromide, or its metabolites, has interacted with DNA through covalent bonds to induce DNA repair synthesis in opossum lymphocyte cells

[5]. Another indication of ethylene dibromide's ability to alkylate DNA is that ethylene dibromide is mutagenic in *Salmonella typhimurium* TA 1530 and TA 1535, both transitional mutational systems [64]. These data [5,6,64] suggest strongly that the most plausible chemical basis for the mutagenic activity of ethylene dibromide in procaryotic and eucaryotic organisms is its alkylation of cellular constituents such as DNA. This broad biologic reactivity suggests that ethylene dibromide may be capable of increasing spontaneous mutation rates in humans. However, the quantitative aspects of this potential have not been determined. Since the process of induction of mutations is a stochastic, virtually irreversible process, any increased frequency of mutation in exposed populations would accumulate as a function of the total absorbed dose of ethylene dibromide. Therefore, an adequate assessment of the importance of the plant and submammalian animal data in extrapolating to the concentrations of airborne ethylene dibromide present in the workplace environment is difficult, but the widespread mutagenic activity of ethylene dibromide does give cause for concern about damage to the genetic mechanisms in employees working with it.

The teratogenic effects found in a rat and mouse study [69] involved brain and costal anomalies in the offspring of dams exposed to ethylene dibromide. Pregnant rats and mice were exposed at a concentration of about 32 ppm of ethylene dibromide for 23 hours/day on days 6-16 of gestation. Some of the effects were attributed to malnourishment, but the abnormalities in the ethylene dibromide-exposed rats and mice were significantly different, both qualitatively and quantitatively, from those in the nonexposed controls; some of these abnormalities did not appear in mice fed a restricted amount of the normal diet whereas others appeared in both the restricted and the control mice with about the same incidences.

These data suggest that ethylene dibromide causes fetal anomalies in mice and rats that are not caused by malnourishment alone. Since inhalation is one of the major routes of exposure for the employee, these data suggest also that the babies of female employees may be subject to increased risks of developmental defects if their mothers are exposed to ethylene dibromide in the workplace during the critical phases of pregnancy.

The total risk to the health of employees exposed to ethylene dibromide is the result of the compounded risks from carcinogenicity, mutagenicity, teratogenicity, sterility, and damage to the kidneys, liver, spleen, respiratory tract, central nervous system, circulatory system, skin, and eyes. Although no comprehensive epidemiologic studies have been conducted to assess adequately these risks in the industrial environment, evidence of their existence in experimental animal systems or in isolated human exposures to ethylene dibromide has been discussed above and in Chapter III. Experimentation conducted with animal models, as outlined above, generally supports the findings observed in the limited number of human exposures.

Concern for employee health requires that the probability of the occurrence of long-term effects of ethylene dibromide be minimized. The preliminary report available to NIOSH from a review of the mortality experience of 161 employees of one manufacturer [26,71] may indicate that employees exposed to unknown concentrations of ethylene dibromide from industrial processes operating under the current federal standard of 153 mg/cu m (20 ppm) are not suffering measurable long-term effects. The authors (26), while recognizing the limitations posed by the small study group and the variety of toxic agents to which the employees may have been exposed stated, however, that an indication of increased mortality due to

ethylene dibromide exposure may have existed in one of the plants. An individual exposed to 153 mg/cu m (20 ppm) of ethylene dibromide for 40-46 weeks/year with a calculated minute volume of about 10 liters/minute would inhale approximately 6,700 g during 40 years of occupational exposure, which is about 8.7 times the lifetime oral doses (10.8 and 11.2 g/kg) that induced stomach cancer in the rat. In addition, the extensive experimental evidence on the induction of adverse effects in lower species [5-7,9,33,41-43,56-60,62,66,69] and the formation of stable covalent bonds between ethylene dibromide and cellular constituents [7,11] indicate that the occupational exposure limit should be lowered to decrease the potential hazard to employees.

Because of the intrinsic, stochastic, and virtually irreversible character of the chemical reactions that initiate the carcinogenic and mutagenic processes, the risk of adverse effects is a function of the rate of absorption and the total absorbed dose. Consequently, this risk can be reduced to any value necessary to protect the employees by decreasing both the absorption rate (to prevent saturation of the enzymatic detoxification mechanisms) and the total lifetime dose (to reduce the probability of deleterious stochastic processes, such as carcinogenesis and mutagenesis, from occurring).

The unusual complexity of the dynamics of the cellular response mechanisms to ethylene dibromide intoxication is emphasized by the dose-rate effect relationship observed for induction of gastric neoplasms. Daily intragastric doses of 40 and 60 mg/kg induced tumors in rats and mice within 10 weeks [56], whereas daily 7-hour inhalation exposures of about 49 mg/kg did not result in the observation of tumors even after 30 weeks [33]. The data suggest that one of the major factors in the development of gastric

carcinomas is direct contact between the mucosal cells and ethylene dibromide, and that a major difference in tumor induction may exist between the two routes of exposure. However, the animals used in the inhalation study were not maintained until the end of their normal lifespan; therefore, a direct comparison between the final incidences of tumors initiated by the two routes of exposure cannot be made.

A plausible pharmacokinetic explanation for the observed differences exists. Since the rat's capacity to metabolize ethylene dibromide [50] exceeds the rate of absorption (at 25 ppm) by a factor of at least 100, the concentration of ethylene dibromide in tissues should be considerably less than that in the air, which is about 1.02 μ moles/liter at 25 ppm [33]. The concentration of ethylene dibromide in corn oil used in the intubation study ranged between 0.066 and 0.132 moles/liter. Consequently, the mucosal cells of the stomach may have been exposed for short periods to concentrations of ethylene dibromide up to 100,000 times that to which lung tissue would be exposed during inhalation exposures at 25 ppm (192.5 mg/cu m). The saturation of enzymatically catalyzed detoxification and repair mechanisms may occur in the stomach tissues, resulting in an amplification of the tissue damage, which otherwise may be minimal. These pharmacokinetic considerations are consistent with the experimental results of Rowe et al [33], where the product of the concentration and duration of exposure to produce 50% mortality among the exposed rats was found to be approximately constant for higher concentrations of ethylene dibromide but not for lower ones. The evidence indicates that tissue detoxication and repair mechanisms exist, but that they cannot negate completely the intrinsic capacity of ethylene dibromide to alkylate cellular constituents. Because of this, exposure to ethylene dibromide has potentials for the

induction of adverse effects by both local and systemic actions. Reducing the concentration of ethylene dibromide in the air should optimize the protection to the organism afforded by cellular detoxication and repair mechanisms, thus minimizing the accumulation of ethylene dibromide in tissues and decreasing the subsequent cellular damage to a negligible level.

In summary, the limited reports of effects on humans along with experimental animal data indicate that the adverse effects which have been observed from exposure to ethylene dibromide are similar and that differences are primarily ones of magnitude. Epidemiologic evidence of adverse effects in workers is confined to a single mortality study which was hampered by small cohort size, mixed exposures, and minimal findings which were not sufficient to adequately identify a worker problem. The findings are regarded as little more than preliminary observations; nevertheless, increased deaths from malignancies cannot be ruled out and seem to have been most apparent in employees having 6 or more years exposure in ethylene dibromide operations. Available animal data indicate reversible reproductive abnormalities manifested as abnormal development of spermatozoa in young bulls and decreased fertility in rats at a time which indicated interference with the normal development of spermatids into mature spermatozoa. Carcinomas of the stomach invading into the abdominal cavity have been produced in mice and rats administered ethylene dibromide by direct intubation into the stomach. Cancers resulting from ethylene dibromide administration have not been demonstrable by any other route of administration. A major factor in the development of gastric carcinoma in the rats and mice is considered to be the direct contact between mucosal cells and concentrated ethylene dibromide in a quantity which exceeded the ability of the tissues to handle the chemical. Mutagenic effects from

ethylene dibromide have been demonstrated in microbes, plants, insects, and mammalian cells in vitro, presumably due to its ability to alkylate, or covalently bond, to DNA in a cross-linking mechanism by virtue of its bifunctional structure. Although this potential has not been demonstrated in humans, the broad biologic activity of ethylene dibromide warrants concern about possible damage to genetic mechanisms in employees exposed to it. Teratogenic effects which seem to be due to maternal exposure to ethylene dibromide have been confined to brain and costal anomalies in fetal mice and rats, but malnourishment as a contributing factor cannot be discredited unequivocally.

Although it is not possible at this time to state categorically an exposure concentration at which ethylene dibromide may be regarded to be completely without risk, NIOSH considers that the recommended occupational exposure limit should be substantially lower than the current federal standard of 20 ppm as an 8-hour TWA limit, 30 ppm ceiling. The recommended occupational exposure limit for ethylene dibromide, at the least, should be reduced sufficiently to keep the total lifetime dose well below the cumulative doses shown to be hazardous in animal experiments. In considering the alkylating capability of ethylene dibromide and the potential adverse effects on the organism which may result, especially at the subcellular level, NIOSH recommends that the occupational exposure limit for ethylene dibromide be reduced to a ceiling concentration of 1.0 mg/cu m (0.13 ppm) for any 15-minute sampling period. This represents a reduction to one-two-hundred and thirtieth of the current federal ceiling limit for ethylene dibromide and is a level at which an employee would inhale a maximum of about 686 mg/kg of ethylene dibromide during a 40-year working lifetime, which is substantially below that total dose known to induce adverse effects in experimental animals. It is believed that so

long as care is taken to prevent entrance of any appreciable amount of ethylene dibromide into the digestive tract, adherence to this exposure limit will protect against acute adverse effects and will reduce the potential long-term effects to a negligible level. NIOSH concludes that reduction to a ceiling concentration of 1.0 mg/cu m (0.13 ppm) will protect employees from acute illness resulting from exposure to ethylene dibromide and will reduce markedly, and perhaps remove entirely, any hazard of adverse effects on health from long-term exposure to this chemical.

It is recognized that many employees work with solid or liquid forms of ethylene dibromide in situations where there may be contact with the substance, resulting in dermal, ocular, or systemic effects. Consequently, appropriate work practices, training, and other protective measures should be required regardless of concentrations of airborne ethylene dibromide. Therefore, occupational exposure to ethylene dibromide has been defined as work in an area where ethylene dibromide is manufactured, blended, stored, used, handled, or otherwise present. The action level is defined as one-half the recommended occupational exposure limit, thereby delineating those work situations which do not require the expenditure of resources for environmental monitoring and associated recordkeeping.

(b) Sampling and Analysis

The technology is currently available to sample and analyze ethylene dibromide at the recommended occupational exposure limit to allow institution of the proper engineering controls. As discussed in Chapter IV and presented in greater detail in Appendices I and II, a charcoal tube method is recommended for personal breathing zone sampling of airborne ethylene dibromide. Gas-liquid chromatography is recommended for analyzing the trapped ethylene dibromide.

(c) Medical Surveillance and Recordkeeping

Several human [22-25] and animal [23,27,31,33] studies reported that exposure to ethylene dibromide vapor or liquid produced skin, eye, or respiratory irritation, CNS disorders, systemic damage in the liver, kidneys, spleen, lungs, and heart, and death. Thus, a medical surveillance program should include preplacement and periodic medical examinations that give attention to the nervous system, skin, eyes, lungs, kidneys, spleen, liver, and circulatory system. Medical attention should be provided for employees accidentally exposed to ethylene dibromide. Because ethylene dibromide induces cancer and mutations in experimental animals, it is recommended that all medical and other pertinent records involving ethylene dibromide exposure be kept for 30 years after termination of employment. This will allow enough time for future detection of chronic sequelae which may be related to the employee's known occupational exposure. Because of the possibilities of impaired development of fetuses and of the induction of sterility in men, employees of reproductive age must be counseled to minimize exposure to ethylene dibromide.

(d) Personal Protective Equipment and Clothing

Dermal [24,33] and ocular [23,33] contact with liquid ethylene dibromide induces irritation of the skin and eyes in humans and animals. Therefore, care must be exercised to ensure adequate protection against contact with ethylene dibromide. Personal protective clothing, including ocular protective devices and impervious work clothes, should be available and worn where exposure to ethylene dibromide is likely. Work practices that prevent skin and eye contact must be followed. Showers and eyewash fountains must be available for immediate use if accidental contact occurs.

(e) Informing Employees of Hazards

A continuing education program is an important part of a preventive hygiene program for employees occupationally exposed to hazardous materials

such as ethylene dibromide. Properly trained persons should periodically apprise employees of possible sources of ethylene dibromide exposure, the adverse health effects associated with such exposure, the engineering and work practice controls in use and being planned to limit exposure, and the environmental and medical monitoring procedures used to check on control procedures and on the health status of employees. Personnel occupationally exposed to ethylene dibromide must be warned and advised of the adverse effects of accidental exposure and must be informed of the signs and symptoms of the disorders. Employees should be warned that the onset of these symptoms may be delayed. If skin or eye contact occurs, the affected areas should be immediately flushed with copious amounts of water and examined by a physician.

(f) Work Practices

Because ethylene dibromide can produce death from ingestion [25] and has been found to induce gastric cancers in experimental animals [56], it is recommended that food storage, handling, dispensing, and eating be prohibited in ethylene dibromide work areas regardless of the air concentrations. In addition, it is recommended that employees who work in an ethylene dibromide area thoroughly wash their hands before eating, smoking, or using toilet facilities.

Engineering controls must be used whenever possible to control concentrations of airborne ethylene dibromide within the recommended occupational exposure limit. Where ethylene dibromide is present, a closed system of control should be used. During the time required to install adequate controls and equipment, to make process changes, to perform routine maintenance operations, or to make emergency repairs, exposure to ethylene dibromide can be minimized by the use of respirators and protective clothing. However, respirators should not be used as a substitute for proper engineering controls for normal operations.

VII. RESEARCH NEEDS

Research is needed in the following areas to provide a better scientific basis for the recommended occupational health standard.

(a) Epidemiologic Study

A limited examination of the health records of employees at the Dow Chemical Company did not reveal any adverse health effects associated with exposure to ethylene dibromide [71]. In the absence of any published data, it is not possible to critically evaluate the basis of this conclusion or to estimate the significance of any effects which may have been induced by ethylene dibromide. A retrospective cohort study of a working population exposed primarily to ethylene dibromide for a long duration should provide valuable information. Such a study should also address the effects of alcohol consumption, smoking habits, and obesity on the assessment of occupational hazards and risks. A study of a large population, such as gasoline station operators, chronically exposed to very low but measurable concentrations of ethylene dibromide should be given consideration.

A group of manufacturers and users has informed NIOSH that they are currently planning to conduct an epidemiologic study of employees with a history of exposure to ethylene dibromide.

(b) Carcinogenic Study

One carcinogenic study [56] has been found. However, because the route of exposure was by gastric intubation and the dosages were quite large, they do not provide a substantive basis for estimating the risk for human populations exposed to low concentrations of ethylene dibromide throughout their working lifetime. Properly designed and performed studies

should be conducted on at least two mammalian species by inhalation and dermal absorption over a range of doses to further determine the risk of neoplastic induction by ethylene dibromide at concentrations and dosages approaching the recommended environmental limit. In addition, studies should be conducted to determine the cocarcinogenic or promotion potential of ethylene dibromide with substances with which it is commonly used, such as with ethylene dichloride, tetraethyl lead, and tetramethyl lead, or with carbon tetrachloride, ethylene dichloride, and carbon disulfide.

(c) Mutagenic Effect

This effect must be systematically investigated in greater detail with respect to dose, time, and route of exposure in both lower organisms and mammals. Animal tests using a variety of doses, schedules, and routes of administration should be performed to further elucidate the mutagenic potential of ethylene dibromide. Specific locus tests, heritable translocations, and multigeneration studies should be considered. Animals should also be tested to determine whether ethylene dibromide has any cytogenic effects. Experiments designed to either establish or refute the general applicability of the linear dose-response relationship for mutation induction found in *Tradescantia* [66] should be conducted in mammalian and submammalian species for both point mutations and chromosomal aberrations.

(d) Teratogenic and Related Reproductive Effects

Terata have occurred in the offspring of mammals as a result of exposure to ethylene dibromide vapor [69], and definite impairment of the reproductive system has occurred in mammals and avians as a result of ingesting ethylene dibromide [41,42,44,45]. Definitive experiments are needed with exposure concentrations approaching the recommended

environmental limit to determine the effects of these small concentrations of airborne ethylene dibromide on the reproductive processes in a variety of mammalian species, such as dogs or monkeys. Additional studies are needed to determine whether sufficient quantities of ethylene dibromide can be absorbed through the skin to produce abnormal reproductive effects.

(e) Kidney and Liver Function Studies

The impairment of kidney and liver functions as a result of ethylene dibromide exposure has occurred in animals and in humans. As yet, there is no evidence that functional damage occurs in workers exposed to ethylene dibromide. Since a portion of a working population exposed primarily to ethylene dibromide can easily be identified, kidney and liver function tests should be given periodically to see whether any changes are occurring as a result of occupational exposure to ethylene dibromide.

(f) Skin Sensitization

Ethylene dibromide has been implicated in one study [24] on humans as being a skin sensitizer. However, the data presented in this study are far from complete or unequivocal. Additional information on the degree and character of skin sensitization of humans is highly desirable.

(g) Biologic Monitoring

Studies should be conducted to determine the feasibility of using body fluids, such as blood or urine, as the basis of a method for biologic monitoring of workers that are occupationally exposed to ethylene dibromide.

(h) Long-term Animal Exposure Studies

Long-term exposure of several animal species at a variety of concentrations of ethylene dibromide vapor approaching the recommended environmental limit is needed. These studies should simulate occupational exposure conditions of 8-10 hours/day, 4-5 days/week, for at least 18-24 months and the animals maintained until the end of their natural life. These studies should be properly designed and performed to allow for assessment of general body parameters, biochemical/physiologic parameters, and gross or microscopic examinations of involved organs including at least the liver, lungs, spleen, kidneys, CNS, and circulatory system.

In addition, repeated long-term experiments should be performed to determine the effects of ethylene dibromide absorption through the skin. Similar schedules and experimental designs as those for inhalation studies should be followed.

The National Cancer Institute has informed NIOSH that a long-term experiment to study the possible carcinogenic effects from the inhalation of ethylene dibromide is presently being conducted.

(i) Metabolism and Distribution

The pathways of metabolic transformation, distribution, and elimination of ethylene dibromide as a function of the dose rate and route of administration in mammals have not been adequately investigated. It is critical to determine the fraction of the dose that is converted into harmless metabolites and the dependence of the magnitude of this fraction on the dose rate. Both in vivo and in vitro studies should be conducted to determine the pathways. It is also essential to determine the

concentration at which partial impairment of the detoxification mechanisms begin to occur.

(j) Excretion in Biologic Fluids

Several studies [9,10] have indicated that ethylene dibromide, or its metabolites, is widely circulated throughout the body and remains widely distributed in the body tissues for a considerable time. These studies also indicated that ethylene dibromide, or its metabolites, is excreted in the urine and eliminated in the feces. No studies have been conducted to determine whether ethylene dibromide is excreted intact in the milk of lactating mammals. It is imperative to determine whether ethylene dibromide is excreted in the milk of mammals and, if so, at what concentrations and for how long.

(k) Electroencephalographic (EEG) Studies

The reports of possible CNS effects in animals [23,31,33] and the broad chemical reactivity of ethylene dibromide toward all classes of cellular nucleophiles suggest a careful study of CNS function by noninvasive techniques in human populations exposed to ethylene dibromide. A thorough study of EEG patterns may provide useful information.

(1) Personal Protective Equipment

Materials impervious to ethylene dibromide should be identified for use in protective clothing, boots, gloves, and air-supplied hoods. Materials chemically resistant to ethylene dibromide should be identified for use in waste containers, drainage channels, diverting dikes, and floors.

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IX. APPENDIX I

METHOD FOR SAMPLING ETHYLENE DIBROMIDE IN AIR

The following sampling method is adapted from Method No. S104 of the Physical and Chemical Analysis Branch of NIOSH [78, and S Tucker, written communication, March 1977].

Atmospheric Sampling

Collect breathing zone or personal samples representative of the individual employee's exposure. At the time of sample collection, record a description of sampling location, equipment used, time and rate of sampling, total sample volume, temperature, atmospheric pressure, relative humidity, and any other pertinent information. Collect enough samples to permit calculation of an exposure for every operation or location in which there is exposure to ethylene dibromide.

(a) Equipment

The sampling train consists of a charcoal tube and a vacuum pump.

(1) Charcoal tubes: Glass tubes, with both ends flame-sealed, 7-cm long, with a 6-mm OD and a 4-mm ID, containing two sections of 20/40 mesh activated charcoal separated by a 2-mm portion of polyurethane foam. The activated charcoal is prepared from coconut shells and is fired at 600 C prior to packing. The primary section contains 100 mg of charcoal, the backup section, 50 mg. A 3-mm portion of polyurethane foam is placed between the outlet end of the tube and the backup section. A plug of silylated glass wool is placed in front of the primary section.

The pressure drop across the tube when in use must be less than 1 inch of mercury at a flowrate of 1 liter/minute. Tubes with the above specifications are commercially available.

(2) Pump: A battery-operated pump, complete with clip for attachment to the employee's belt, capable of operation at 200 ml/minute or less with a controlled accuracy of $\pm 5\%$.

(b) Calibration

The accurate calibration of a sampling pump is essential for the correct interpretation of the volume sampled. The frequency of calibration is dependent on the use, care, and handling to which the pump is subjected. Pumps should also be recalibrated if they have been misused or if they have just been repaired or received from a manufacturer. If the pump receives hard usage, more frequent calibration may be necessary. Maintenance and calibration should be performed on a regular schedule and records of these should be kept.

Ordinarily, pumps should be calibrated in the laboratory both before they are used in the field and after they have been used to collect a large number of field samples. The accuracy of calibration is dependent on the type of instrument used as a reference. The choice of calibration instrument will depend largely on where the calibration is to be performed. For laboratory testing, a soapbubble meter is recommended, although other standard calibrating instruments can be used. The actual setups will be similar for all instruments.

Instructions for calibration with the soapbubble meter follow. If another calibration device is selected, equivalent procedures should be used. The calibration setup for personal sampling pumps with a charcoal

tube is shown in Figure XII-1. Since the flowrate given by a pump is dependent on the pressure drop across the sampling device, in this case a charcoal tube, the pump must be calibrated while operating with a representative charcoal tube in line.

(1) Check the voltage of the pump battery with a voltmeter to ensure adequate voltage for calibration. Charge the battery if necessary.

(2) Break the tips of a charcoal tube to produce openings of at least 2 mm in diameter.

(3) Assemble the sampling train as shown in Figure XII-1.

(4) Turn on the pump and moisten the inside of the soapbubble meter by immersing the buret in the soap solution. Draw bubbles up the inside until they are able to travel the entire buret length without bursting.

(5) Adjust the pump flowmeter to provide the desired flowrate.

(6) Check the water manometer to ensure that the pressure drop across the sampling train does not exceed 2.5 inches of water at 200 ml/minute.

(7) Start a soapbubble up the buret and measure with a stopwatch the time it takes the bubble to move from one calibration mark to another.

(8) Repeat the procedure in (7) above at least three times, average the results, and calculate the flowrate by dividing the volume between the preselected marks by the time required for the soapbubble to traverse the distance. If, for the pump being calibrated, the volume of

air sampled is calculated as the product of the number of strokes times a stroke factor (given in units of volume/stroke), the stroke factor is the quotient of the volume between the two preselected marks divided by the number of strokes.

(9) Data for the calibration include the volume measured, elapsed time or number of strokes of the pump, pressure drop, air temperature, atmospheric pressure, serial number of the pump, date, and name of the person performing the calibration.

(c) Sampling Procedure

(1) Break both ends of the charcoal tube to provide openings of at least 2 mm, which is half the ID of the tube. A smaller opening causes a limiting orifice effect which reduces the flow through the tube. The smaller section of charcoal in the tube is used as a backup section and therefore is placed nearest the sampling pump. Use tubing to connect the back of the tube to the pump, but tubing must never be put in front of the charcoal tube. The tube is supported in a vertical position in the employee's breathing zone.

(2) Sample a maximum of 25 liters of air at a flowrate of 200 ml/minute. For the determination of ceiling concentrations, the sampling time is 15 minutes.

(3) Measure and record the temperature and pressure of the atmosphere being sampled.

(4) Treat at least one charcoal tube in the same manner as the sample tubes (break, seal, and ship), except draw no air through it. This tube serves as a blank.

(5) Immediately after samples are collected, cap the charcoal tubes with plastic caps. Do not use rubber caps. To minimize breakage during transport, pack capped tubes tightly in a shipping container.

(6) Along with collected samples, send reference samples of the suspected compounds in a glass container capped with a teflon-lined cap. Do not transport these bulk liquid samples in the same container with the collected charcoal tubes.

(7) Low levels of 1,2-dibromoethane cannot be stored on charcoal at ambient temperatures for long periods of time. Therefore, if the analysis cannot be performed within 16-24 hours after sampling has been completed, the samples must be stored at -25°C or below. Refrigerated samples may be stored for two weeks.

(8) For shipment to the laboratory, the samples are packed firmly in an insulated container cooled with dry ice.

(9) If appropriate, a sample of the bulk material in a glass container with a teflon-lined cap is prepared and shipped to the laboratory in a separate container.

X. APPENDIX II

ANALYTICAL METHOD FOR ETHYLENE DIBROMIDE

The following analytical method is adapted from Method No. 260 of the Physical and Chemical Analysis Branch of NIOSH [78, and S Tucker, written communication, March 1977].

Principle of the Method

Ethylene dibromide vapor trapped on charcoal from a known volume of air is desorbed with a 99:1 mixture of benzene-methanol (v/v). A portion of the desorbed sample is injected into a gas-liquid chromatograph equipped with an electron-capture detector. The area of the resulting peak is determined and compared with those obtained from injection of standards.

Range and Sensitivity

This method was used to analyze ethylene dibromide over the range of 1.6 $\mu\text{g}/\text{cu m}$ to 16 $\text{mg}/\text{cu m}$ (0.0002-2 ppm) at an atmospheric temperature and pressure of 25 C and 738 mmHg for a 25-liter air sample [78, and S Tucker, written communication, March 1977]. Under the conditions of sample size (25 liters), the probable range of this method is 40-800 nanograms/sample solution at a detector sensitivity that gives nearly full deflection on the strip chart recorder for a 1-mg sample. The method may be capable of measuring much smaller amounts if the desorption efficiency is adequate and

if a photon-ionization detector is used on the chromatograph. Desorption efficiency must be determined over the range used.

The upper limit of the range of the method is dependent on the adsorptive capacity of the charcoal tube. This capacity varies with the concentrations of ethylene dibromide and other substances in the air. The first section of the charcoal tube held at least 21.4 mg of ethylene dibromide when a test atmosphere containing 446 mg/cu m of ethylene dibromide in dry air was sampled at 200 ml/minute for 240 minutes; at that time, the concentration of ethylene dibromide in the effluent was less than 2% of that in the influent. If a particular atmosphere is suspected of containing a large amount of contaminant, a smaller sampling volume should be taken.

Interferences

Compounds which have about the same retention time as ethylene dibromide and which are detected by the electron capture detector will interfere with the analysis. This type of interference can be overcome by changing the operating conditions of the instrument, usually by modifying the column, the column temperature, or both.

Ethylene dibromide will not be efficiently trapped when the amount of water vapor in the air is so great that condensation occurs in the trapping media. Lesser amounts of water vapor in the air may severely decrease the breakthrough volume. When interfering compounds are known or suspected to be present in the air, such information including their suspected identities could be transmitted with the sample.

Precision and Accuracy

The relative standard deviation for the combined analytical and sampling method in the prescribed range of 203-2,370 nanograms/charcoal tube was 0.070.

Advantages and Disadvantages of the Method

This method uses a sampling device that is small, portable, and involves no liquids. Interferences are minimal and can usually be eliminated by altering chromatographic conditions. Analysis of the charcoal tubes can be accomplished rapidly. Simultaneous analysis of two or more compounds suspected of being present in the same sample can usually be accomplished by simply changing chromatographic conditions.

One disadvantage of the method is that the amount of sample which can be collected by this method is limited by the weight of ethylene dibromide which the tube will hold before breakthrough. When the sample value obtained for the backup section of charcoal exceeds 25% of that found in the front section, the possibility of appreciable sample loss exists. Recoveries of ethylene dibromide from charcoal are decreased upon storage of the charcoal tube samples at room temperature, particularly with lesser amounts of analyte. The use of an internal standard is required in order to attain good precision. Other organic compounds in high concentrations may displace ethylene dibromide from the charcoal. High humidity may decrease the absorptive efficiency and capacity of the charcoal. The precision of the method is limited by the reproducibility of the pressure drop across the charcoal tube. This drop will affect the flowrate and the volume of air sampled, since the pump is usually calibrated for one tube only.

Apparatus

(a) Gas-liquid chromatograph equipped with an electron capture detector. A glass tube filled with 20/40 mesh activated charcoal should be attached to the exit port in order to trap the ethylene dibromide in the effluent gas stream.

(b) Gas chromatography column. 1.8 m x 4 mm (ID) constructed from borosilicate glass and packed with 3% OV-210 on 80/100 Gas Chrom Q. Other columns which achieve the desired separation may be used.

(c) A mechanical or electronic integrator or a recorder for determining peak area.

(d) Small glass-stoppered test tubes or equivalent.

(e) A 10- μ l syringe and other conveniently sized syringes for preparation of the standards.

(f) Pipets, 10.0 ml and 1.0 ml.

(g) Volumetric flasks, 10-ml capacity for standard dilutions.

Reagents

(a) Benzene, pesticide quality.

(b) Methanol, pesticide quality.

(c) Ethylene dibromide of known purity.

(d) An appropriate internal standard, such as 1,1,2,2,-tetrachloroethane or 1,2 dibromopropane.

(e) A solution of internal standard in 99:1 benzene-methanol (v/v).

(f) 99:1 Benzene-methanol (v/v).

Analysis of Samples

All glassware used for the laboratory analysis should be washed in detergent and rinsed with tap and distilled water.

(a) Preparation: Score each charcoal tube, including the blank from field samples, with a file and break open in front of the first section of charcoal. Remove and discard the glass wool. Transfer the charcoal in the first (larger) section to a small stoppered test tube. Remove and discard the separating section of foam and transfer the second section of charcoal to another test tube. Analyze the two charcoal sections separately.

(b) Desorption: Prior to analysis, pipet 10.0 ml of benzene-methanol (99:1) into each test tube to desorb the ethylene dibromide from the charcoal. For the internal standard method, a 0.2% solution of n-pentadecane in benzene-methanol (99:1) is used for desorption. Sample vials should be capped after adding solvent to minimize volatilization. Desorption is complete in 30 minutes if the sample is stirred occasionally. Recoveries of the analyte from charcoal decrease with increasing storage times at room temperature. The decreases are more pronounced at lower levels of analyte. The recoveries after storage are adequate, however, when the charcoal tube samples are refrigerated. If analyses cannot be performed within 3-5 hours after sampling, the charcoal tube samples must be stored at -25 C. The refrigerated samples may be stored for up to two weeks.

BENZENE CAN CAUSE SERIOUS BLOOD ABNORMALITIES INCLUDING LEUKEMIA. EXTREME CAUTION MUST BE EXERCISED AT ALL TIMES WHEN USING BENZENE BECAUSE OF ITS HIGH TOXICITY AND FLAMMABILITY. ALL WORK WITH BENZENE MUST BE PERFORMED UNDER AN EXHAUST HOOD.

(c) Typical gas-liquid chromatographic operating conditions for analysis:

- | | | |
|-----|---------------------------------|-------------------------------------|
| (1) | Nitrogen carrier gas flow: | 35ml/min |
| (2) | Inlet temperature: | 175 C |
| (3) | Detector temperature: | 315 C |
| (4) | Column temperature: | 50 C |
| (5) | (63Ni) detector operating mode: | DC at 90 to 95%
standing current |

(d) Injection: The first step in the analysis is the injection of the sample into the gas-liquid chromatograph. Employ the solvent flush injection technique. This eliminates difficulties arising from blowback or distillation within the syringe needle, thus increasing the accuracy and reproducibility of the injected sample volume. First, flush the 10.0- μ l syringe with solvent several times to wet the barrel and plunger, then draw 3.0 μ l of solvent into the syringe. Next, remove the needle from the benzene-methanol (99:1) and pull the plunger back about 0.2 μ l to separate the solvent flush from the sample with an air pocket to be used as a marker. Immerse the needle in the sample and withdraw a 5.0- μ l portion, taking into consideration the volume of the needle since the sample in the needle will be completely injected. After the needle is removed from the sample and prior to injection into the gas-liquid chromatograph, pull the plunger back a short distance to minimize sample evaporation from the tip. Make duplicate injections of each sample and of the standard. No more than a 3% difference between the peak areas of the similar injections should be accepted. Automatic sample injectors may be used if shown to give reproducibility at least as good as the solvent flush injection technique. In this case, injections of 2 μ l are satisfactory.

(e) Measurement of area: The areas of the sample peaks are measured by electronic integration or some other suitable method of area measurement. Preliminary sample results are read from a standard curve prepared as outlined below.

(f) The approximate retention times of ethylene dibromide and two potential internal standards under the GC conditions described in this method are:

Compound	Retention Time	B.P.
Ethylene dibromide	2.2 min	131 C
1,2-Dibromopropane	2.9 min	140 C
1,1,2,2-Tetrachloroethane	4.1 min	146 C

Determination of Desorption Efficiency

The desorption efficiency of a particular compound can vary from one laboratory to another and also from one batch of charcoal to another. Thus, it is necessary to determine at least once the percentage of ethylene dibromide that is removed in the desorption process. Repeat this procedure for each new batch of charcoal used.

Place the same amount of activated charcoal as in the first section of the sampling tube (100 mg) into a 5-cm, 4-mm ID glass tube; flame seal at one end. This charcoal must be from the same batch as that used in sampling and can be obtained from unused charcoal tubes. Cap the open end with Parafilm or equivalent. Inject a known amount of n-heptane solution containing 55 mg/ml of ethylene dibromide directly into the activated charcoal with a microliter syringe and recap the tube with more Parafilm or equivalent. The amount injected is equivalent to that present in a 1-liter sample at the selected level.

Prepare at least six tubes in this manner for each concentration and allow to stand overnight or longer to ensure complete adsorption of the ethylene dibromide on the charcoal. These six tubes are referred to as the "desorption samples." Treat a parallel blank tube in the same manner, except add no ethylene dibromide to it. Desorb and analyze the desorption samples and blank tubes in exactly the same manner as the sampling tube described for unknown air samples.

Prepare two or three standards by injecting the same volume of ethylene dibromide into 10.0 ml of the 99:1 benzene-methanol mixture (containing 0.2% n-pentadecane if the internal standard method is used) with the same syringe used in the preparation of the desorption samples.

These are analyzed with the desorption samples.

The desorption efficiency equals the difference between the average peak area of the desorption samples and that of the blank divided by the average peak area of the standards, or:

$$\text{desorption efficiency} = \frac{\text{area of sample} - \text{area of blank}}{\text{area of standard}}$$

The desorption efficiency is dependent on the amount of ethylene dibromide collected on the charcoal. Plot the desorption efficiency versus the weight of ethylene dibromide found. This curve is used to correct for adsorption losses when sample concentrations are calculated.

Calibration and Standards

It is convenient to express the concentration of standards in terms of mg ethylene dibromide/ml of benzene-methanol because samples are desorbed in 10 ml of benzene-methanol. Use the density of ethylene dibromide to convert milligrams into microliters for easy measurement with a microliter syringe. Prepare a series of standards varying in concentration over the range of interest and then analyze them under the same gas-liquid chromatographic conditions and during the same time period as the unknown samples. To minimize error, inject 10 times the desired weight of ethylene dibromide into 10 times the desired volume of benzene-methanol. Prepare standard curves by plotting concentration in mg/ml versus peak area.

In the case of the internal standard method, prepare standard curves by plotting concentration in mg/ml versus the ratio of the peak areas of ethylene dibromide to n-pentadecane.

Calculations

Read the weight in milligrams of ethylene dibromide corresponding to the total peak area from the standard curve. No volume corrections are needed because the standard curve is based on mg ethylene dibromide/ml of benzene-methanol and the volume of sample injected is identical to the volume of the standards injected.

Make corrections for the blank from the field sampling for each sample by subtracting the amounts of ethylene dibromide found on the front and back sections of the blank from the amounts found in the respective sections of the sample:

$$\text{corrected amount} = \text{amount on sample} - \text{amount on blank}$$

Add the corrected amounts present in the front and in the backup sections of the same sample tube to determine the total amount of ethylene dibromide in the sample. Divide this total amount by the desorption efficiency to obtain the adjusted total amount of ethylene dibromide in the sample:

$$\text{adjusted total amount} = \frac{\text{total amount}}{\text{desorption efficiency}}$$

The concentration of ethylene dibromide in the air sampled, expressed in mg/cu m, is given by the quotient of the adjusted amount in mg divided by the volume of air sampled in cu m:

$$\text{concentration (mg/cu m)} = \frac{\text{adjusted amount (mg)}}{\text{volume (cu m)}}$$

Another method of expressing concentration is ppm:

$$\text{concentration (ppm)} = \text{concentration (mg/cu m)} \times \frac{24.45}{\text{MW}} \times \frac{760}{P} \times \frac{(T + 273)}{298}$$

where:

24.45 = molar volume (liter/mole) at 25 C and 760 mmHg

760 = standard atmospheric pressure (mmHg)

P = pressure (mmHg) of air sampled

T = temperature (degrees C) of air sampled

MW = molecular weight of ethylene dibromide (g/mole)

298 = standard atmospheric temperature (degrees K)

X. APPENDIX III
MATERIAL SAFETY DATA SHEET

The following items of information which are applicable to a specific product or material shall be provided in the appropriate block of the Material Safety Data Sheet (MSDS).

The product designation is inserted in the block in the upper left corner of the first page to facilitate filing and retrieval. Print in upper case letters as large as possible. It should be printed to read upright with the sheet turned sideways. The product designation is that name or code designation which appears on the label, or by which the product is sold or known by employees. The relative numerical hazard ratings and key statements are those determined by the rules in Chapter V, Part B, of the NIOSH publication, An Identification System for Occupationally Hazardous Materials. The company identification may be printed in the upper right corner if desired.

(a) Section I. Product Identification

The manufacturer's name, address, and regular and emergency telephone numbers (including area code) are inserted in the appropriate blocks of Section I. The company listed should be a source of detailed backup information on the hazards of the material(s) covered by the MSDS. The listing of suppliers or wholesale distributors is discouraged. The trade name should be the product designation or common name associated with the material. The synonyms are those commonly used for the product, especially formal chemical nomenclature. Every known chemical designation or

competitor's trade name need not be listed.

(b) Section II. Hazardous Ingredients

The "materials" listed in Section II shall be those substances which are part of the hazardous product covered by the MSDS and individually meet any of the criteria defining a hazardous material. Thus, one component of a multicomponent product might be listed because of its toxicity, another component because of its flammability, while a third component could be included both for its toxicity and its reactivity. Note that a MSDS for a single component product must have the name of the material repeated in this section to avoid giving the impression that there are no hazardous ingredients.

Chemical substances should be listed according to their complete name derived from a recognized system of nomenclature. Where possible, avoid using common names and general class names such as "aromatic amine," "safety solvent," or "aliphatic hydrocarbon" when the specific name is known.

The "%" may be the approximate percentage by weight or volume (indicate basis) which each hazardous ingredient of the mixture bears to the whole mixture. This may be indicated as a range or maximum amount, ie, "10-40% vol" or "10% max wt" to avoid disclosure of trade secrets.

Toxic hazard data shall be stated in terms of concentration, mode of exposure or test, and animal used, eg, "100 ppm LC50-rat," "25 mg/kg LD50-skin-rabbit," "75 ppm LC man," or "permissible exposure from 29 CFR 1910.1000," or, if not available, from other sources of publications such as the American Conference of Governmental Industrial Hygienists or the American National Standards Institute Inc. Flashpoint, shock sensitivity

or similar descriptive data may be used to indicate flammability, reactivity, or similar hazardous properties of the material.

(c) Section III. Physical Data

The data in Section III should be for the total mixture and should include the boiling point and melting point in degrees Fahrenheit (Celsius in parentheses); vapor pressure, in conventional millimeters of mercury (mmHg); vapor density of gas or vapor (air = 1); solubility in water, in parts/hundred parts of water by weight; specific gravity (water = 1); percent volatiles (indicated if by weight or volume) at 70 degrees Fahrenheit (21.1 degrees Celsius); evaporation rate for liquids or sublimable solids, relative to butyl acetate; and appearance and odor. These data are useful for the control of toxic substances. Boiling point, vapor density, percent volatiles, vapor pressure, and evaporation are useful for designing proper ventilation equipment. This information is also useful for design and deployment of adequate fire and spill containment equipment. The appearance and odor may facilitate identification of substances stored in improperly marked containers, or when spilled.

(d) Section IV. Fire and Explosion Data

Section IV should contain complete fire and explosion data for the product, including flashpoint and autoignition temperature in degrees Fahrenheit (Celsius in parentheses); flammable limits, in percent by volume in air; suitable extinguishing media or materials; special firefighting procedures; and unusual fire and explosion hazard information. If the product presents no fire hazard, insert "NO FIRE HAZARD" on the line labeled "Extinguishing Media."

(e) Section V. Health Hazard Information

The "Health Hazard Data" should be a combined estimate of the hazard of the total product. This can be expressed as a TWA concentration, as a permissible exposure, or by some other indication of an acceptable standard. Other data are acceptable, such as lowest LD50 if multiple components are involved.

Under "Routes of Exposure," comments in each category should reflect the potential hazard from absorption by the route in question. Comments should indicate the severity of the effect and the basis for the statement if possible. The basis might be animal studies, analogy with similar products, or human experiences. Comments such as "yes" or "possible" are not helpful. Typical comments might be:

Skin Contact--single short contact, no adverse effects likely; prolonged or repeated contact, possibly mild irritation.

Eye Contact--some pain and mild transient irritation; no corneal scarring.

"Emergency and First Aid Procedures" should be written in lay language and should primarily represent first-aid treatment that could be provided by paramedical personnel or individuals trained in first aid.

Information in the "Notes to Physician" section should include any special medical information which would be of assistance to an attending physician including required or recommended preplacement and periodic medical examinations, diagnostic procedures, and medical management of overexposed employees.

(f) Section VI. Reactivity Data

The comments in Section VI relate to safe storage and handling of hazardous, unstable substances. It is particularly important to highlight instability or incompatibility to common substances or circumstances, such as water, direct sunlight, steel or copper piping, acids, alkalies, etc. "Hazardous Decomposition Products" shall include those products released under fire conditions. It must also include dangerous products produced by aging, such as peroxides in the case of some ethers. Where applicable, shelf life should also be indicated.

(g) Section VII. Spill or Leak Procedures

Detailed procedures for cleanup and disposal should be listed with emphasis on precautions to be taken to protect employees assigned to cleanup detail. Specific neutralizing chemicals or procedures should be described in detail. Disposal methods should be explicit including proper labeling of containers holding residues and ultimate disposal methods such as "sanitary landfill," or "incineration." Warnings such as "comply with local, state, and federal antipollution ordinances" are proper but not sufficient. Specific procedures shall be identified.

(h) Section VIII. Special Protection Information

Section VIII requires specific information. Statements such as "Yes," "No," or "If necessary" are not informative. Ventilation requirements should be specific as to type and preferred methods. Respirators shall be specified as to type and NIOSH or US Bureau of Mines approval class, ie, "Supplied air," "Organic vapor canister," etc. Protective equipment must be specified as to type and materials of construction.

(i) Section IX. Special Precautions

"Precautionary Statements" shall consist of the label statements selected for use on the container or placard. Additional information on any aspect of safety or health not covered in other sections should be inserted in Section IX. The lower block can contain references to published guides or in-house procedures for handling and storage. Department of Transportation markings and classifications and other freight, handling, or storage requirements and environmental controls can be noted.

(j) Signature and Filing

Finally, the name and address of the responsible person who completed the MSDS and the date of completion are entered. This will facilitate correction of errors and identify a source of additional information.

The MSDS shall be filed in a location readily accessible to employees exposed to the hazardous substance. The MSDS can be used as a training aid and basis for discussion during safety meetings and training of new employees. It should assist management by directing attention to the need for specific control engineering, work practices, and protective measures to ensure safe handling and use of the material. It will aid the safety and health staff in planning a safe and healthful work environment and in suggesting appropriate emergency procedures and sources of help in the event of harmful exposure of employees.

--

MATERIAL SAFETY DATA SHEET

I PRODUCT IDENTIFICATION		
MANUFACTURER'S NAME	REGULAR TELEPHONE NO EMERGENCY TELEPHONE NO	
ADDRESS		
TRADE NAME		
SYNONYMS		
II HAZARDOUS INGREDIENTS		
MATERIAL OR COMPONENT	%	HAZARD DATA
III PHYSICAL DATA		
BOILING POINT, 760 MM HG		MELTING POINT
SPECIFIC GRAVITY (H ₂ O=1)		VAPOR PRESSURE
VAPOR DENSITY (AIR=1)		SOLUBILITY IN H ₂ O, % BY WT
% VOLATILES BY VOL		EVAPORATION RATE (BUTYL ACETATE=1)
APPEARANCE AND ODOR		

IV FIRE AND EXPLOSION DATA				
FLASH POINT (TEST METHOD)		AUTOIGNITION TEMPERATURE		
FLAMMABLE LIMITS IN AIR, % BY VOL.		LOWER		UPPER
EXTINGUISHING MEDIA				
SPECIAL FIRE FIGHTING PROCEDURES				
UNUSUAL FIRE AND EXPLOSION HAZARD				
V HEALTH HAZARD INFORMATION				
HEALTH HAZARD DATA				
ROUTES OF EXPOSURE				
INHALATION				
SKIN CONTACT				
SKIN ABSORPTION				
EYE CONTACT				
INGESTION				
EFFECTS OF OVEREXPOSURE				
ACUTE OVEREXPOSURE				
CHRONIC OVEREXPOSURE				
EMERGENCY AND FIRST AID PROCEDURES				
EYES				
SKIN				
INHALATION				
INGESTION				
NOTES TO PHYSICIAN				

VI REACTIVITY DATA
CONDITIONS CONTRIBUTING TO INSTABILITY
INCOMPATIBILITY
HAZARDOUS DECOMPOSITION PRODUCTS
CONDITIONS CONTRIBUTING TO HAZARDOUS POLYMERIZATION
VII SPILL OR LEAK PROCEDURES
STEPS TO BE TAKEN IF MATERIAL IS RELEASED OR SPILLED
NEUTRALIZING CHEMICALS
WASTE DISPOSAL METHOD
VIII SPECIAL PROTECTION INFORMATION
VENTILATION REQUIREMENTS
SPECIFIC PERSONAL PROTECTIVE EQUIPMENT RESPIRATORY (SPECIFY IN DETAIL)
EYE
GLOVES
OTHER CLOTHING AND EQUIPMENT

IX SPECIAL PRECAUTIONS

PRECAUTIONARY
STATEMENTS

OTHER HANDLING AND
STORAGE REQUIREMENTS

PREPARED BY _____

ADDRESS _____

DATE _____

XII. TABLES AND FIGURE

TABLE XII-1

CHEMICAL AND PHYSICAL PROPERTIES OF ETHYLENE DIBROMIDE

Formula	CH ₂ BrCH ₂ Br
Formula weight	187.88
Melting point (C)	9.6
Boiling point (C)	131.4
Vapor density (air=1)	6.5
Vapor pressure (mmHg at 25 C)	12.0
Firepoint	None
Heat of vaporization (cal/g at 25 C)	+53
Flashpoint	None
Flammability	Nonflammable
Viscosity (centipoise at 20 C)	1.65
Density (g/ml at 20 C)	2.18
Density of saturated air (air=1)	1.08
Concentration of saturated air (by volume at 25 C)	1.3%
Solubility:	
in ethanol	Soluble
in ethyl ether	Soluble
in water	0.43 g/100 g water at 30 C
Conversion factors (760 mmHg at 25 C)	1 mg/liter=1 g/cu m=130 ppm 1 ppm=7.68 mg/cu m=7.68 µg/liter

Adapted from references 1,3,4,118

TABLE XII-2

OCCUPATIONS WITH POTENTIAL EXPOSURES TO ETHYLENE DIBROMIDE

Antiknock compound makers	Lead scavenger makers
Cabbage growers	Motor fuel workers
Celluloid makers	Nematode controllers
Corn growers	Oil processors
Drug makers	Organic chemical synthesizers
Ethylene dibromide workers	Resin makers
Fat processors	Seed corn maggot controllers
Fire extinguisher makers	Soil fumigators
Fruit fumigators	Termite controllers
Fumigant workers	Tetraethyl lead makers
Gasoline blenders	Waterproofing makers
Grain elevator workers	Waxmakers
Grain fumigators	Wood insect controllers
Gum processors	Wool reclaimers

Adapted from reference 21

TABLE XII-3

CONCENTRATIONS* OF RADIOACTIVITY IN MOUSE TISSUES
AFTER INJECTION OF 40 MG/KG IP OF ¹⁴C-ETHYLENE DIBROMIDE

Tissue	1 hr	3 hr	24 hr
Bone (whole femur)	1.4	2.1	0.5
Brain	0.2	0.6	0.1
Tail of epididymis	3.1	4.4	0.7
Fat	4.9	2.4	0.3
Heart	1.1	1.1	0.2
Large intestine + contents	5.3	15.0	0.3
Small intestine + contents	34.0	5.8	0.4
Kidney	13.0	12.0	1.0
Liver	12.0	6.6	0.4
Lung	2.5	3.3	0.4
Muscle (gastrocnemius)	0.9	1.0	0.1
Skin	1.2	2.1	0.3
Spleen	4.1	4.7	0.6
Stomach + contents	1.7	4.6	1.0
Testis	1.1	1.5	0.2
Whole blood	7.2	7.4	6.2
Plasma	12.0	12.0	2.6
Residue of animal	1.3	2.4	0.2

*Results expressed as % administered dose/g wet tissue.

Adapted from reference 9

TABLE XII-4

CONCENTRATIONS* OF RADIOACTIVITY IN MALE GUINEA PIG TISSUES
AFTER ADMINISTRATION OF 30 MG/KG IP OF ¹⁴C-ETHYLENE DIBROMIDE

Tissue	Hours after Administration					
	4	8	12	24	48	72
Kidney	286.64	236.52	147.79	73.45	14.66	10.47
Liver	129.03	104.91	78.39	38.07	16.20	15.58
Adrenal	60.73	60.82	26.56	28.63	13.24	10.43
Pancreas	35.04	36.81	28.85	18.68	7.52	6.01
Spleen	15.79	13.96	15.83	14.89	8.92	6.95
Heart	14.01	15.63	11.59	9.51	4.13	3.23
Lung	20.93	19.00	17.76	15.43	8.08	5.83
Testis	10.68	10.72	9.08	8.34	5.05	3.98
Brain	6.19	7.62	6.75	6.47	3.70	2.51
Fat**	21.39	7.86	8.72	3.19	3.05	2.07
Muscle	5.53	4.97	4.58	4.15	2.91	2.16
Blood	9.97	3.40	5.78	4.59	3.36	2.77

*Values represent mean levels in $\mu\text{g/g}$ of tissue or $\mu\text{g/ml}$ of fluid of duplicate determinations on three animals at each interval.

**Suprarenal fat

Adapted from reference 10

TABLE XII-5

RETENTION* OF RADIOACTIVITY IN MALE GUINEA PIG TISSUES
AFTER INJECTION OF 30 MG/KG IP OF ¹⁴C-ETHYLENE DIBROMIDE

Tissue	Hours after Administration					
	4	8	12	24	48	72
Liver	16.29	13.65	10.50	4.72	2.12	2.16
Kidney	6.00	5.69	3.31	1.64	0.31	0.24
Stomach + contents	1.14	0.52	0.62	0.38	0.18	0.18
Lung	0.35	0.38	0.37	0.24	0.12	0.10
Pancreas	0.31	0.36	0.33	0.20	0.07	0.06
Testis	0.16	0.17	0.12	0.12	0.07	0.06
Heart	0.13	0.16	0.12	0.10	0.04	0.03
Brain	0.12	0.16	0.14	0.13	0.07	0.05
Adrenal	0.09	0.10	0.04	0.03	0.01	0.02
Spleen	0.07	0.06	0.07	0.08	0.03	0.02

*Values represent the mean % of the administered dose of duplicate determinations on three animals at each interval.

Adapted from reference 10

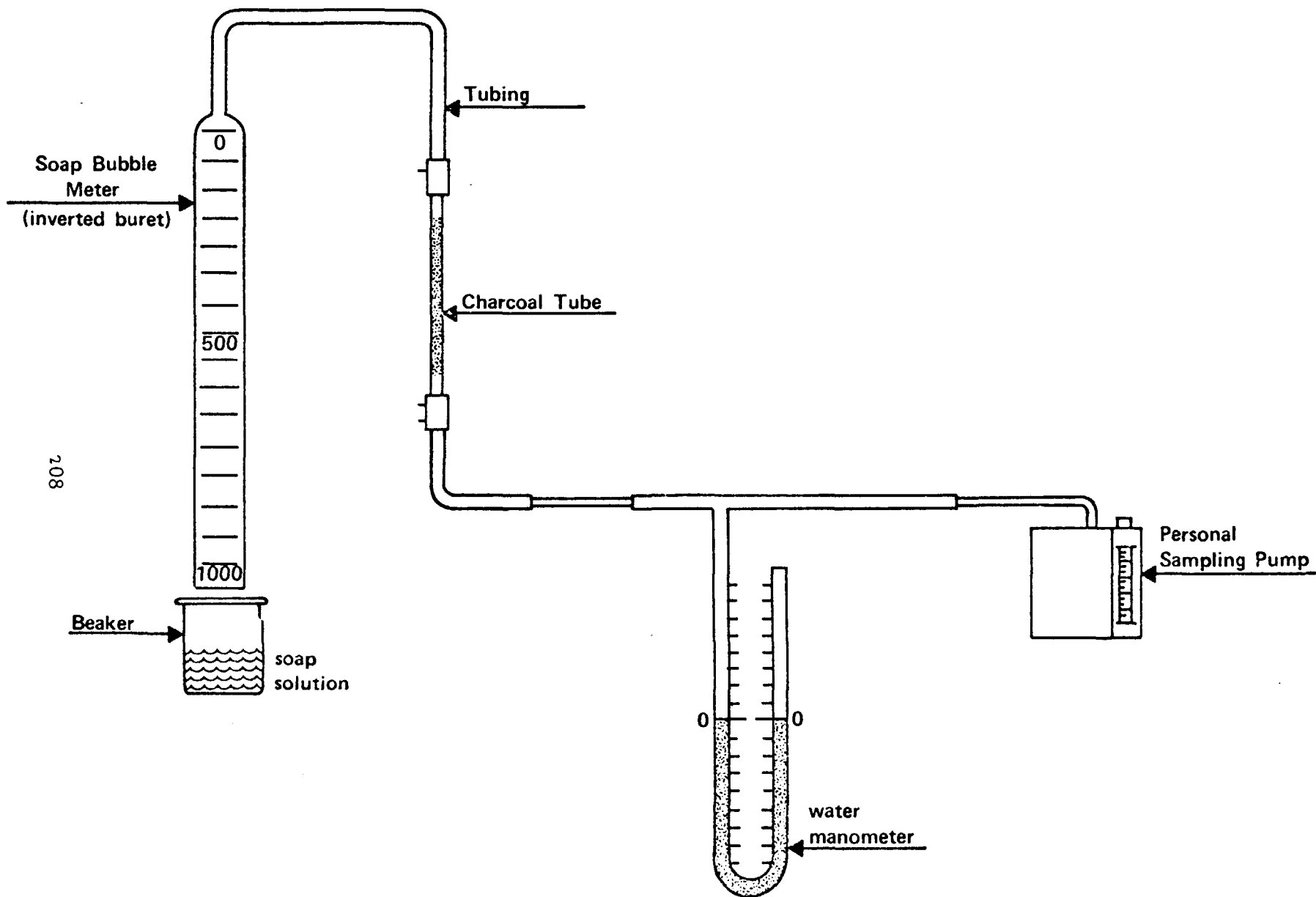


FIGURE XII-1. CALIBRATION SETUP FOR PERSONAL SAMPLING PUMP WITH CHARCOAL TUBE

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PUBLIC HEALTH SERVICE
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NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH
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