REVIEW AND EVALUATION ⸤F RECENT SCIENTIFIC LITERATURE
RELEVANT TO AN OCCUPATIONAL STANDARD
FOR ETHYLENE DIBROMIDE

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I. Introduction

This report is designed to present a critical review and evaluation of the recent scientific literature concerning hazards associated with exposure to ethylene dibromide (1,2-dibromoethane). It is intended to provide a review and evaluation for the existing NIOSH criteria document for ethylene dibromide (1) by considering published and unpublished research results that have appeared since the preparation of the original document. The report will focus on those research results presenting new information which may impact on the recommendations in the criteria document and supply NIOSH with information to assist in determining the need to revise the recommendations for occupational health standards contained in the criteria document.

Ethylene dibromide is primarily used as a fumigant for ground pest control, and as an anti-knock ingredient in gasoline mixtures. Harmful effects to workers exposed to ethylene dibromide include severe acute respiratory damage, central nervous system depression, and severe vomiting upon inhalation of vapors. Prolonged contact with the skin may result in erythema, blistering, and ulceration with reactions delayed for 24-48 hours. The vapor is also irritating to mucous membranes and the eyes.

NIOSH has recommended a 1 mg/m^3 (0.13 ppm) ceiling environmental exposure limit for ethylene dibromide. Research needs identified by NIOSH included the need for additional human studies such as epidemiology surveys, liver and kidney function tests, and biological monitoring among exposed workers. Additional information in experimental animals as to the carcinogenic, mutagenic, and teratogenic effects of ethylene dibromide were recommended.

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In assembling this review pertinent new information about ethylene dibromide was sought relating to the areas of human and animal toxicology, analytical and sampling methods, and work practices and engineering controls. Identification of relevant articles and documents was accomplished through a search of scientific literature using both computerized and manual searching of various data bases and published secondary bibliographic sources.

Data is presented that strongly confirms the previously recognized threat of carcinogenesis that may accompany such other well known toxic effects of ethylene dibromide as mutagenesis, reproductive abnormalities, and development effects. Information will be presented concerning a serious toxic interaction between inhaled ethylene dibromide and ingested disulfiram. Deficiencies in present knowledge concerning the effects of exposure to ethylene dibromide will be noted, and directions for future research will be outlined. This report does not present recommendations concerning exposure limits, work practices, or analytical methods; it is intended, rather, to supplement the body of information contained in the original criteria document by considering recent publications having significance to occupational health.

II. Summary and Conclusions

Environmental health aspects of ethylene dibromide toxicity are confined to occupational exposures to liquid or vapors. The toxic effects of ethylene dibromide have been well documented by NIOSH (1,2) and by Proctor and Hughes (3).

Mittleman (8), White and McAllister (10), Going and Spigarelli (11), and Rumsey and Tanita (12), have presented various data detailing exposure levels for a variety of job descriptions in a number of occupational settings where ethylene dibromide is used as a fumigant. The USEPA (13) has reviewed a number of incidents where exposure to ethylene dibromide resulted in the occurrence of adverse health effects including on fatality after the ingestion of an unspecified amount of the chemical. Exposure data is generally scant for each of the incidents reported. Johns (16), Polkis and Burkett (17), McDermott and Killany (18) have commented on the role of ethylene dibromide associated with exposure to leaded gasoline vapors. McDermott and Killiny (18) calculated an exposure level of 0.08 ppm (0.61 mg/m^3) ethylene dibromide in an airborne gasoline concentration of 500 ppm. It was assumed that exposure to 300 ppm gasoline was an effective Threshold Limit Value. Ramsey et al. (20) of the Dow Chemical Company have compared the incidence of cancers predicted by the Carcinogen Assessment Group carcinogenesis model to the observed cancer data provided by Ott and Scharnweber (21). The CAG model tends to dramatically over estimate the carcinogenic risk associated with occupational exposures to ethylene dibromide.

Weisbuger (23) and the NCI (24) have identified ethylene dibromide as a potent stomach carcinogen of orally administered ethylene dibromide at toxic doses. The doses employed during the study resulted in excessive numbers of early deaths in orally treated animals. Deaths were generally attributed to tumor metastasis with a high degree of squamous cell carcinoma of the stomach being reported. Deaths among control animals were unusually higher than expected.

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Rosenkranz (26) has provided additional evidence of ethylene dibromide's ability to induce base substitutions in standard Salmonella mutagenicity assays at levels of 2-12 μmole per plate. Schairer et al. (32), Nauman et al. (33), Nauman (34), Ma et al. (35), Sparrow and Schairer (36), and Scott (37), have detailed studies demonstrating the mutagenic properties of ethylene dibromide in the vascular plant Tradescantia. Schairer et al. (32) reports that concentrations as low as 1 ppm are significantly mutagenic after 6 hours exposure.

Short et al. (40), Short (41), and Minor et al. (42) have indicated that ethylene dibromide expects a weak teratogenic response in rats and mice. Severe fetotoxic effects are realized only at concentrations producing moderate maternal toxicity.

Watanabe et al. (45,46) of the Dow Chemical Company have detailed the pharmacokinetics of inhaled doses of ethylene dibromide. Urinary excretion was the major route of elimination of radioactivity following inhalation of [^{14}C] ethylene dibromide. Shih (47) and Plotnick and Conner (49) have demonstrated that levels of radioactivity are greatest in the kidney and liver following intraperitoneal injection of ethylene dibromide.

NIOSH (5), Stein et al. (50), Plotnick (51), Yodaiken (52), and El-Lawri (53) have reported that a serious toxic interaction exists between ethylene dibromide and disulfiram. Simultaneous exposure to 20 ppm of inhaled ethylene dibromide and a diet containing 0.05% disulfiram is fatal to more than 90% of experimental animals tested. Plotnick (51) and Wong (54) have described preliminary findings of an increased carcinogenic risk associated with similar exposures. Hepatocellular, kidney, mesentery, and spleen tumors are reported to develop as early as six months after simultaneous exposure to disulfiram and ethylene dibromide.

The USEPA (13) reports that incidents involving adverse health effects on persons exposed to ethylene dibromide generally result from negligent handling, labeling, or use of the chemical. Rumsey et al. (12) have suggested various improvements in engineering control to reduce background concentration of ethylene dibromide in occupational settings. Improved ventillation processes, maintenance checks of equipment, and protective clothing and materials were cited.

III. Human Studies

The USEPA (6) has published a rebuttable presumption against the registration and continued registration of ethylene dibromide. Most of the relevant information on the toxicity of ethylene dibromide to both experimental and domestic animals and pertinent human exposure data have been reviewed.

Leinster et al. (7) have determined ambient levels of ethylene dibromide in the range of 0.001 to 0.17 $\mu g/m^3$ in London air, and levels of 1.2 to 1.8 $\mu g/m^3$ in garage areas.

Mittleman (8) has extensively reviewed exposure levels of ethylene dibromide in a number of occupational processes. The average exposure to ethylene dibromide of workers in several work phases is summarized as follows:

Source of Exposure	Average Exposure (Range) (mg/kg/yr)	Study Location
Soil Fumigation (injection)		
professional applicator	11 (1.8–27)	Great Lakes Chemical Co.
farmers	2.2 (0.4–5.5)	
professional applicator	8.8	Dow Chemical Company
farmer	1.8	
Florida Citrus Fumigation		Wahneta, Florida
Fumigation Station		
corridor operator	4.8 (–6.0)*	
outdoor operator	11 (–15)*	
truckers	0.44 (–0.53)*	
Warehouse workers		
inside (forklift)	287	
outside (forklift)	87 (–95)*	
Spot Treatment		
Grain Storage		
Bark Treatment	No Data Available	
Termite Treatment		

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*No lower limit reported.

No attempt was made to examine adverse health effects among the workplaces investigated. During the course of the measurement of airborne ethylene dibromide levels, however, incidents of misuse and poor work practices were witnessed (i.e., use during temperature inversion, inappropriate cleaning of clogged equipment, non-use of respirators, inadequate cleaning of spills).

Beusch and Severn (9) have estimated that exposure to ethylene dibromide from soil fumigation operations would result in an annual inhaled dose of 3 - 40 mg/kg for professional applicators and 0.7 - 10 mg/kg for farmers applying ethylene dibromide as a soil fumigant themselves.

White and McAllister (10) have calculated potential human exposures of ethylene dibromide resulting from soil fumigation operation. The findings are summarized below:

		Average Concentration (mg/m^3)			
Application Rate (lbs/acre)	Sampling Duration (hrs)	Adjacent Untreated Field[a]	Treated Field[a]	Breathing Zone of Application	Amount Inhaled[b] (mg/kg/day)
135	7.5	0.375	3.325	3.187	0.6
84.3	7.0	0.075	0.712	4.850	1.0
31.5	6.5	not detected	-----	0.500	0.1

[a] Measurement 12 inches above ground.
[b] Assuming breathing rate of 1.83 m^3/hr/8 hr day, 70 kg man, and all ethylene dibromide is absorbed.

Assuming a breathing rate of 1.2 m^3/hr for light activity, a body weight of 70 kg, an exposure duration of 8 hr/day for 250 days, and complete absorption of inhaled ethylene dibromide, Going and Spigarelli (11) have estimated

potential inhalation exposures at a Fort Pierce, Florida citrus fumigation

center as outlined below:

Sample Location	Exposure ($\mu g/m^3$)	Potential Dose (mg/kg/day)
Office	3,100	0.425
Corridor	376	0.052
Exit Driveway	0.73	0.001
1/8 mile south of site	20.3	0.004

Going and Spigarelli (11) have also determined ethylene dibromide concentrations

at various other locations as outlined in the following summary:

Location	Type of Sample (Number of Sites)	Concentration ± S.D.
Continental Oil Co. Ponca City, OK.	Air (20)	0.009 ± 0.003[a]
	Soil (6)	<2[b]
	Dust (6)	<60[c]
	Water (2)	0.113[d]
Mobil Oil Co., Paulsboro, N.J.	Air (13)	0.120 ± 0.039[a]
	Soil (13)	<2[b]
	Dust (8)	<60[c]
Retail Gas Stations		
Phoenix, Ariz.	Air (18)	0.320 ± 0.087[a]
Los Angeles, Calif.	Air (14)	0.111 ± 0.031[a]
Camden, N.J.	Air (3)	0.449 ± 0.058[a]
High Traffic Area		
Phoenix, Ariz.	Air (10)	0.365 ± 0.047[a]
Los Angeles, Calif.	Air (9)	0.124 ± 0.013[a]
Suburban Area		
Kansas City, Mo.	Air (1)	0.060[a]
Rural Area		
Maryville, Mo.	Air (2)	0.071[a]

[a] mg/m^3 (adjusted)
[b] $\mu g/kg$
[c] $pg/cm^2/hr$
[d] $\mu g/l$

Rumsey and Tanita (12) have prepared a comprehensive industrial hygiene survey at two manufacturing and two user facilities of ethylene dibromide. A total of seventeen different job classifications were examined, involving more than 70 workers. Personal and area samples of ethylene dibromide were obtained at various work sites. Plants A and B were described as manufacturers of ethylene dibromide, while Plants C and D were users of the chemical, producing different anti-knock gasoline mixtures. Descriptions of workers activities, occupational titles and job descriptions were presented. Results of exposure data are presented in Table 1. Short-term exposures were demonstrated to be high as 23.4 ppm.

Ethylene dibromide exposure concentrations were highest in those processes involving the collection of quality-control samples and in loading and unloading operations. While these reports do not address the health effects of workers employed at these several locations, they do present detailed exposure data that could be used in further studies describing the health effects on workers exposed to ethylene dibromide.

The USEPA (13) has reviewed those accidental exposures voluntarily recorded in which ethylene dibromide alone or in combination with other chemicals was involved.

Sixteen episodes of ethylene dibromide exposure resulting in adverse health effects in humans were reported for 10-year period from 1967 to 1976. Of these 16 episodes 10 involved exposure to ethylene dibromide alone, while the remainder involved exposure to ethylene dibromide in combination with other chemicals. The 10 episodes citing adverse health effects resulting from exposure to only ethylene dibromide included eight occupational exposures and

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Table 1. Eight-Hour Time-Weighted Exposures to Ethylene-Dibromide by Job Classification at Four Plant Locations (A, B, C, and D) from Rumsey and Tanita (12)

Job Category & Classification	A number[a]	A TWA[b]	B number	B TWA	C number	C TWA	D number	D TWA
Supervisory								
Crewleader	--	----	4	0.04–0.95	--	----	---	----
Shift Superintendent	--	----	--	----	4	0.001–0.004	--	----
Control Room Operator								
Panel Board Operator	4	<0.00006–0.14	--	----	--	----	--	----
Control Room Operator	--	--	4	0.003–0.16	--	---	--	----
Blend Operator	--	--	--	----	4	0.004–1.52	12	0.001–0.009
Reactor Operator	--	--	--	----	---	----	4	0.001–0.003
Surveillance								
Surveillance Technician	8	<0.00002–1.60	--	----	--	----	--	----

[a] Sample size of specific job at appropriate location
[b] Median 8-hour time weighted average concentration range in ppm

Table 1. Eight-Hour Time-Weighted Exposures to Ethylene-Dibromide by Job Classification
At Four Plant Locations (A, B, C, and D) from Rumsey and Tanita (12) (Cont'd)

Job Category & Classification	A		B		C		D	
	number[a]	TWA[b]	number	TWA	number	TWA	number	TWA
Product Loading								
Loader	--	--	3	0.05 0.62	--	---	--	----
Material Handler	--	--	--	---	--	1	--	---
Drum Loader	--	--	--	---	--		16	0.008- 0.018
Raw Material Handler	--	---	--	---	--	---	1	0.082- 0.027
Peripheral Operators								
Laboratory Technician	4	<0.00002- 0.57	1	0.01- 0.08	8	0.002- 0.012	'	0.0001- 0.0005
Brine Field Technician	4	<0.00002- 0.03	--	---	--	---	--	---
Compound Bulk Operator	--	---	--	----	--	---	--	0.001- 0.008
Total	20		12		17		35	

two domestic accidental ingestion of the chemical, one child and one fatal case involving an elderly man.

Seven episodes of environmental contamination were reported during the same time interval. All of these reported contamination were results of occupational accidents, and did not involve adverse effects to humans. The following table summarizes episodes involving exposure to ethylene dibromide, citing the entity affected and site of occurrence:

Entity Affected	Site of Occurrence	Number of Episodes
Humans	Agriculture	7
	Loading Dock	2
	Warehouse	1
	Nut Processing	1
	Commercial Pest Control Operation	1
	Unspecified Job Site	1
	Unspecified Industry	1
	Home (Domestic)	2
	Subtotal	16
Environment	Transportation	5
	Loading Dock	1
	Chemical Company	1
	Subtotal	7
	Total Number of Episodes	23

The following symptoms related to dermal contact were described: erythema, dermatitis, blistering, and chemical burns. Exposure to fumes or vapors resulted in wheezing and chest pain. One man (79 years) accidentally ingested an unspecified amount of an 83% ethylene dibromide solution (repackaged in an unlabeled bottle). Death ensued in less than four hours.

Exposure to ethylene dibromide in combination with other fumigants produced symptoms of: erythema, lacrimation, burning of the lungs, coughing,

headache, nausea, vomiting, loss of consciousness, and severe liver and kidney damage. Cases of simultaneous exposure involved ethylene dibromide in combination with such chemicals as: methyl bromide (2), ethylene dichloride (6), carbon tetrachloride (6), carbon bisulfide (2), chloropirim (1), paraquat (1), and trichloronitromethane (1).[*]

In most occupational accidents that resulted in exposure to ethylene dibromide, failure to read provided labeling information, lack of adequate protective clothing and/or use of respirators, and improper use of fumigation equipment were causative agents implicated in the accidental exposures.

The data included in this report does not contain any description of airborne concentrations of ethylene dibromide to which workers were exposed, or amount ingested in accidental exposures. However levels must be considered high enough to produce symptoms rapidly and severely enough to warrant medical attention for most cases. The results of these case summaries leads to the need for greater safety in the handling of ethylene dibromide in occupational settings, and the prudent use, storage, and labeling of chemicals in the home.

Kristoffersson (14) has reported that a strong toxic effect was exerted on human lymphocytes exposed to various gasoline additives including ethylene dibromide. No chromosome aberrations were observed. No additional information was available from this symposia abstract.

White and Lybarger (15) have conducted a health hazard evaluation determination report on the Houston Chemical Company in Beaumont, Texas. Particular attention was paid to reproductive effects associated with exposure to ethylene dibromide. Medical histories obtained included questions on

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[*]The number of known cases of simultaneous exposure to chemical and to ethylene dibromide is in parentheses.

fertility, sexual dysfunction, sterility, and signs of feminization. Serum gonadtropins and testosterone levels were analyzed. Environmental air sampling determinations (charcoal tube sample analyzed via gas chromatography mass spectrophotometry) of 25 breathing zones indicated that, in general, employees were not exposed to detectable concentrations of ethylene dibromide. No lower limit of detection was provided. A single reading of 0.88 mg/m^3 was obtained at an operator's desk in a blending area. Medical surveys did not indicate that an immediate health hazard was associated with exposure to ethylene dibromide; however, the laboratory evaluation did reveal certain abnormalities that warrant further investigation.

No record of abnormal physical signs or symptomatic complaints from workers exposed to ethylene dibromide was obtained (i.e., changes in facial hair or body hair, decreased muscle mass, testicular atrophy, decreased libido, impotence, or gynecomastia).

The study population consisted of 27 workers aged 26-46 years (mean = 35.2) exposed for 1.5 - 18.5 years (mean = 9.6) to ethylene dibromide, and 9 controls aged 23-50 years (mean = 38) employed at the facility for 0.5-21 years (mean = 9.6).

Laboratory results are presented below as mean values ± standard deviations:

	Number	Sperm Count (10^6/ml)	LH (milliIU/ml)	FSH (milliIU/ml)	Testosterone (ng/dl)
Exposed Workers	22	69+59.8	22.8+11.3	14.2+7.9	527.2+214.0
Unexposed Controls	9	81+24.9	13.0+ 3.6	9.6+4.9	644.8+745.9
Prior, but not current EDB exposure	2	13, 31	12, 15	11.3, 17	330, 530
Significance		---	0.02>P>0.01	0.5>P>0.1	0.5>P>0.1

*Medians and ranges for sperm counts among each group are: 54.0, 4.6-217 for exposed workers and 81, 23-102, 10^6/ml for unexposed controls.

A statistically significant reduction in luteinizing hormone (LH) in exposed workers was seen. Varying degrees of reduction in sperm counts were observed in workers exposed to ethylene dibromide. This study showed results similar to those seen following exposure to 1,2-dibromo-3-chloropropane. The results demonstrated that ethylene dibromide may be involved in potential testicular toxicity. Limited exposure information and great degrees of statistical deviation in sperm counts do not provide strong conclusive evidence in accepting the premise that observed trends were induced by ethylene dibromide _per se_.

Since ethylene dibromide is used as an additive in leaded gasoline, exposure would be likely for those workers involved in the handling and storage of large amounts of gasoline. Johns (16) has reviewed atmospheric sources of ethylene dibromide, and reports that because of the relatively low vapor pressure of the chemical and its concentrations of approximately 0.05 percent by volume in gasoline, it presents a small risk to the general population. Habitual "gasoline sniffers" may have increased exposure to ethylene dibromide. Poklis and Burkett (17) have presented a brief review concerning the effect of gasoline sniffing, and have indicated that ethylene dibromide may contribute to the hazards associated with the handling of leaded gasoline, based on clinical evidence (i.e., liver and renal damage, neurasthetic symptoms) associated with repeated inhalation of gasoline vapors.

McDermott and Killiany (18) have reported concentrations of 80-150 ppm by volume of ethylene dibromide in liquid leaded gasoline, and calculated an airborne concentration of 0.08 ppm (0.61 mg/m^3) ethylene dibromide with 500 ppm of airborne gasoline. Mean gasoline Threshold Limit Values (TLV) calculated

from 95 individual samples was 306 ppm, and ethylene dibromide levels were too
low for measurement. The authors state that negligible exposures to leaded
gasoline additives would result as long as the total gasoline vapor exposure
was maintained low enough to prevent acute hydrocarbon intoxication. Upon
calculating Threshold Limit Values (TLV) for 95 vapor samples containing 23
separate components, a mean TLV of 300 ppm gasoline was obtained (306 ± 43.4)
as an exposure limit to effectively protect workers. This figure represents
a gasoline vapor concentration similar to that generated during tank truck
loading.

The IARC [19] offers a brief review of the toxic effects of ethylene
dibromide. Essentially the same data as that used for the NIOSH [1] criteria
document is covered.

Ramsey et al. [20] of the Toxicology Research Laboratory of Dow Chemical
Company have compared the incidence of cancer predicted by the Carcinogen
Assessment Group of the United States Environmental Protection Agency (CAG
carcinogenesis model) with that observed among a group of workers employed
in ethylene dibromide production. The CAG carcinogenesis model estimates
the carcinogenic risk, where the probability (p) of tumor occurrence is
given by:

$$p(x,t) = 1 - \exp(-\beta x t^{\gamma}),$$

where x is the exposure to ethylene dibromide (mg/kg/day over the entire
lifetime), t is the time span for which the risk is estimated (expressed as
a fraction of total lifetime), and β and γ are factors reflecting the
potency of the carcinogen and time to first tumor formation, both determined
experimentally.

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The epidemiological data to which this model was compared was provided by Ott and Scharnweber (21), and Cook (22) from Dow Chemical production facilities. The results of this epidemiology study were reviewed in the NIOSH criteria document (1). The probability of tumor formation for each individual was then calculated as:

$$p = 1-e[-(31.73)(\text{daily dose})(250/365)(\text{years exposed/age})(\text{age}/70)6.95]$$

The observed and predicted number of neoplastic lesions resulting from exposure to ethylene dibromide is as follows:

| | | | Number of malignant neoplasms | |
| | | | Predicted[a] | |
Location	Number of Employees	Observed	3.0 ppm[b]	0.9 ppm[b]
Michigan	57	5	32	19
Texas	99	3[c]	53	35
Total	156	8	85	54

[a] Number of neoplasms above normal background predicted with the CAG model.
[b] Assumed atmospheric TWA concentration of ethylene dibromide.
[c] Includes one employee with metastatic lymph node carcinoma, where cause of death was arteriosclerotic heart disease.

The failure of the CAG carcinogenic model has been attributed to one or more of the following factors:

1. Limited number of employees examined in the original epidemiology study,

2. Portion of remaining population may contain yet undetected cancers, and,

3. Basis of the model arose from carcinogenic bioassay in rats, utilizing homogeneous population, high concentrations, and orally administered dosing as compared to a heterogeneous human population, exposed to low concentrations of ethylene dibromide vapors.

Cautionary comments concerning the use of predictive models in estimating hazards associated with pollutant exposure were presented. It was concluded that, in this particular instance, the CAG one-hit model for the probability of tumor formation appears to highly exaggerate carcinogenic risk estimates of ethylene dibromide in humans.

Researchers at Dow Chemical have prepared a manuscript for publication. The paper represents a detailed analysis of the data reported by Ott and Scharnweber (21). No information as to acceptance for publication has been obtained from Dow Chemical.

IV. Animal Studies

Johns (16) USEPA (6), and IARC (19) have reviewed the toxic effects of ethylene dibromide in experimental animals.

A. Carcinogenicity

Early reports of an increased occurrence of carcinogenic effects of ethylene dibromide in rats and mice of both sexes following oral administration have been confirmed by the National Cancer Institute (4).

Weisburger (23) has reported on the carcinogenic effect of ethylene dibromide in Osborne-Mendel rats, and B6C3F1 hybrid mice.

Male and female rats and mice in groups of 50 animals each were administered ethylene dibromide by gavage for 5 consecutive days per week for 78 weeks. Maximum dose level for rats was 40 mg/kg, while mice received a maximum dose level of 120 mg/kg. Exposure to ethylene dibromide led to an increase in the occurrence of squamous cell carcinomas of the stomach with many metastases in both sexes of both species.

Final analyses of this bioassay have been prepared by the National Cancer Institute (22). Summarized results and statistical evaluation for orally-treated male and female Osborne-Mendel rats and B6C3F1 mice are presented in Tables 2 and 3.

Early developing squamous cell carcinomas of the stomach were observed in both species at both the high and low doses, but not in control animals. Early deaths were generally attributed to tumor metastasis. Increased hepatocarcinoma was observed in rats, in particular among the high dose females. Hemangiosarcoma was increased in male rats, and respiratory cancers were found in both sexes of mice.

Table 2. Analyses of Incidence of Tumors in Osborne-Mendel Rats Treated with Ethylene Dibromide

Tumor/Site	Males			Females		
	Control[a]	Low	High	Control[a]	Low	High
Number tested	20	50	50	20	50	50
Dose[b]	--	40	80	--	40	80
Observation Period:						
Treated	49	47	34	61	57	44
Untreated	14	2	15	2	4	17
T.W.A. [c] Dosage	0	38	41	0	37	39
Termination (weeks)	49 & 63	49	49	63	61	61
% Survival of						
Termination	--[d]	38	10	--[d]	4	2
Hemangiosarcoma	0	11/50	4/50	0	1/49	3/48
Spleen:						
Frequency	0.00	0.22	0.08	0.00	0.02	0.06
P values[e]	N.S.	0.017	N.S.	N.S.	N.S.	N.S.
Weeks to first						
observed tumor		31	26		38	42
Hepatocellular Car-						
cinoma/Neoplastic	0	3/50	2/50	0	1/47	5/48
Nodule-Liver						
Frequency	0.00	0.06	0.04	0.00	0.02	0.10
P values	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Weeks to first						
observed tumor						
Squamous-Cell	0	45/50	33/50	0	40/50	29/50
Carcinoma-Stomach						
Frequency	0.00	0.90	0.66	0.00	0.80	0.58
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Weeks to first						
observed tumor		31	15		12	12
Hemartoma or Mixed	0	2/49	4/50	--	--	--
Malignant-Kidney						
Frequency	0.00	0.04	0.08	--	--	--
P Value	N.S.	N.S.	N.S.	--	--	--
Weeks to first						
observed tumor		37	41	--	--	--

Tumor/Site	Males			Females		
	Control[a]	Low	High	Control[a]	Low	High
Follicular-Cell Ademona or Carcinoma- Thyroid	0	5/50	8/49	--	--	--
Frequency	0.00	0.10	0.16	--	--	--
P Value	N.S.	N.S.	N.S.	--	--	--
Weeks to first observed tumor		44	15	--	--	--
Cortical Adenoma or Cortical Carcinoma - adrenal gland	--	--	--	0/20	0/44	4/45
Frequency	--	--	--	0.00	0.00	0.09
P Value	--	--	--	N.S.	N.S.	N.S.
Weeks to first observed tumor	--	--	--			47
Adenoma or Adeno- carcinoma-mammary gland	--	--	--	1/20	0/50	2/50
Frequency	--	--	--	0.05	0.00	0.04
P Value	--	--	--	N.S.	N.S.	N.S.
Weeks to first observed tumor	--	--	--	0	0	3
Adenoma or Fibro- adenoma-mammary gland	--	--	--	0/20	0/50	3/50
Frequency	--	--	--	0.00	0.00	0.04
P Value	--	--	--	N.S.	N.S.	N.S.
Weeks to first observed tumor	--	--	--			33

[a] Corn oil vehicle control, same schedule of administration as
experimentally treated animals.
[b] Dose as mg ethylene dibromide per kg body weight.
[c] Time-Weighted Average Dose
[d] No spontaneous deaths among vehicle controls were reported.
[e] Probability level Cochran-Armitage test in control group; Fishe exact
test for comparison of treated to control group. N.S. = not significant

Table 3. Analyses of Incidence of Tumors in B6C3F1 Hybrid Mice Treated with Ethylene Dibromide

Tumor/Site	Males				Females			
	Untreated Control[a]	Vehicle Control[b]	Treatment Low	Treatment High	Untreated Control[a]	Vehicle Control[b]	Treatment Low	Treatment High
Number tested	20	20	50	50	20	20	50	50
Dose[c]	--	--	60	120	--	--	60	120
Observation Period:								
Treated (wks)	0	53	53	34	0	53	53	53
Untreated (wks)	78	6	25	24	90	7	37	25
T.W.A[d] Dosage (mg/kg) over weeks of test period	0	0	62	107	0	0	62	107
Termination (weeks)	78	59	78	78	90	78	90	78
% Survival at termination	30[e]	90	40[e]	20[e]	95[f]	--	56[f]	16[f]
Leukemia or Malignant Lymphoma-Hematopoietic	3/30	0/20	1/50	2/49	5/20	0/20	1/48	0
Frequency	0.15	0.00	0.02	0.04	0.25	0.00	0.02 *	0.00 *
P Value[g]	N.S.	N.S.	N.S.	N.S.	<0.001	N.S.	0.007 N	<0.001 P
Weeks to first observed tumor	76		72	59	90		85	
Alveolar/Bronchiolar Adenoma-Lungs	0/18	0/20	4/45	10/47	0/20	0/20	10/43	6/46
Frequency	0.00	0.00	0.09	0.21 *	0.00	0.00	0.23 *	0.13
P Value	0.011	0.009	N.S.	0.029 **			0.015 **	
				0.021	N.S.	N.S.	0.015	N.S.
Weeks to first observed tumor			58	26			50	49
Squamous Cell-Carcinoma-Stomach	0/20	0/20	45/50	29/49	0/20	0/20	46/49	28/50
Frequency	0.00	0.00	0.90 *	0.59 *	0.00	0.00	0.94 *	0.56 *
P Value	0.003	0.003	<0.001 **	<0.001 **	0.011	0.011	<0.01 **	<0.001 **
			<0.001	<0.001			<0.001	<0.001
Weeks to first observed tumor			24	26			40	34

Table 3. Analyses of Incidence of Tumors in B6C3F1 Hybrid Mice Treated with Ethylene Dibromide (Cont'd)

	Males				Females			
			Treatment				Treatment	
Tumor/Site	Untreated Control[a]	Vehicle Control[b]	Low	High	Untreated Control[a]	Vehicle Control[b]	Low	High
Squamous Cell Papilloma or Squamous Cell Carcinoma-Stomach	0/20	0/20	45/50	31/49	--	--	--	--
Frequency	0.00	0.00	0.90 *	0.63 *	--	--	--	--
P Value	0.001	0.001	<0.001 ** <0.001	<0.001 * <0.001	--	--	--	--
Weeks to first observed tumor			24	26	--	--	--	--
Adenocarcinoma-Mammary	--	--	--	--	0/20	0/20	3/48	1/50
Frequency	--	--	--	--	0.00	0.00	0.06	0.02
P value	--	--	--	--	N.S.	N.S.	N.S.	N.S.
Weeks to first observed tumor	--	--	--	--			82	54
Endometrial Stromal Polypluterus	--	--	--	--	1/20	0/20	1/38	1/44
Frequency	--	--	--	--	0.05	0.00	0.03	0.07
P Value	--	--	--	--	N.S.	N.S.	N.S.	N.S.
Week to first observed tumor	--	--	--	--	90		90	67

[a] Untreated controls received no ethylene dibromide or corn-oil.
[b] Vehicle controls received corn-oil at the same intubation schedule as experimental animals.
[c] Dose as mg ethylene dibromide per kg body weight.
[d] Time-Weighted Average Dose.
[e] Survival rates for male mice were given at 58 weeks. No data for number of animals surviving to termination were provided.
[f] Survival rates for female mice were given at 78 weeks. No data for number of animals surviving to termination were provided.
[g] Probability level for Cochran-Armitage test for incidence of tumors in control group; or Fisher exact test for comparison of a treated group to untreated controls (*) or Vehicle Controls (**). N.S. = not significant.

The toxicity of ethylene dibromide in both rats and mice is detailed
in Section IV.H. Mortalities in treated and control groups were elevated.
Early deaths among treated animals were associated with various lesions of the
forestomach. Increased death rates among male rat and mouse control groups
was not detailed. A higher than expected death rate among control animals
may indicate a flaw in the experimental design of this bioassay. Detailed
morbidity data on the untreated control groups for rats was not included. An
apparent sexual dimorphism in the susceptibility of male rats to stomach cancer
may be influenced by the male control data. Early termination of the vehicle
control males did not allow for an prolonged survivorship analysis of this
group. The data may indicate that males may have been predisposed to a greater
sensitivity than females. Interpretation of this data should include the
realization of excessive mortalities and a lack of morbidity data for control
groups. The maximum tolerated dose employed in this bioassay appears to have
been overestimated, due to the large numbers of early deaths in ethylene
dibromide treated animals. The delayed toxic response observed early in the
study, however, was attributed to the incidence of tumors in the ethylene
dibromide treated groups.

The NCI (22) has prepared a brief review of these findings for release
to various reporting media.

The Data Evaluation/Risk Assessment subgroup of the Clearinghouse on
Environmental Carcinogens (25) has reviewed the findings of the NCI (4) bioassay
of 1,2-dibromoethane for carcinogenicity. In that analysis of the study, it
was noted that selection of doses resulted in excessive numbers of early deaths
among treated animals, and that survival among control animals was poor. The
appropriateness of an oral route of administration is questioned, since human

exposures are generally due to inhalation of the chemical. Despite these apparent shortcomings in experimental design, the NCI study results effectively demonstrate the carcinogenicity of ethylene dibromide.

B. Mutagenicity

Recent literature further demonstrates the mutagenic potential of ethylene dibromide in microbial, mammalian, and plant assays. Rosenkranz (26) has investigated the relative mutagenicity and bacteriocidal properties of various halogenated alkanes including ethylene dibromide. The order of reactivities for 10 µl of test compound inhibiting the growth of E. coli. pol A+/pol A- system were as follows: 1,1,2,2-tetrabromethane>1,1-dibromoethane >1,1,2,2-tetrachloroethane>1,2-dibromoethane>1,5-dibromopentane>1,2-dibromo-2-methyl-propane>1-bromo-2-chloroethane>1,2-dichloroethane. In standard Salmonella assays, the mutagenicity of 10 µmole of the same series of chemicals was: 1,2-dibromoethane = 1,5-dibromopentane>1,2-dibromo-2-methylpropane>1-bromo-2-chloroethane>1,1,2,2-tetrachloroethane = 1,1-dibromoethane. Tetrabromoethane was negative in the standard assay. The procedure involved the deposition of the chemical onto filter discs rather than directly onto the agar surface. The plot of number of revertant colonies per plate versus concentration of chemical graphically demonstrated a linear response for concentrations between 2-12 µmole per plate. Mutants were observed using TA1530 and TA1535 strains in the S. typhimurium assay, but not in strain TA1538, indicating that dibromoethane induces base substitutions but not frame shift mutations. These results confirm the previously reported mutagenicity of ethylene dibromide.

Several recent review articles have appeared that include information concerning the mutagenicity of ethylene dibromide and chemically related compounds. These reviews contribute no new experimental data concerning the

interpretation of various mutagenicity assays. Various methodologies and techniques are presented regarding the routine screening of potential environmental mutagens.

McCann and Ames (27) have reviewed the interpretation of the _Salmonella_-microsome assay mutagenicity as a predictive test for carcinogenicity. A variety of chemicals including ethylene dibromide were analyzed. Blum and Ames (28) have also reviewed the mutagenicity of ethylene dibromide and related halogenated hydrocarbons used as flame retardants. Fishbein (29) offers an extensive review of halogenated aliphatic derivatives including ethylene dibromide. Bignami et al. (30) have presented data concerning structure/activity relationships among various pesticides by the use of _Salmonella_ _typhimurium_ and _Aspergillus_ _nidulans_ systems. Their findings indicated that unspecified amounts of ethylene dibromide were definite mutagens in strains TA100 and TA1535 in _S._ _typhimurium_ assays and in _A._ _nidulans_ where point mutations and somatic segregation were observed.

Rannug et al. (31) have demonstrated that ethylene dibromide provides a stronger mutagenic effect than does 1,2-dichloroethane in a _Salmonella_ _typhimurium_ TA1535 assay system. An unspecified level of ethylene dibromide was activated by an NADPH-independent process, using post-mitochondrial liver fraction (S-9).

Researchers have postulated the use of the plant _Tradescantia_ (Spiderwort) genetic system for monitoring the mutagenic properties of ionizing radiation and chemical mutagens. The following reports have examined the mutagenicity of ethylene dibromide in higher plant forms. The data presented demonstrate a high degree of sensitivity of this plant assay to several chemical mutagens including ethylene dibromide. While there is presently

inadequate evidence for extrapolation of plant mutagenicity to human health hazards, this assay deserves attention as a possible indicator of potential health hazards associated with exposure to chemical mutagens or suspected mutagens.

Schairer et al. (32) have reviewed the use of Tradescantia as a means of exploring mutagenicity of environmental pollutants. Ethylene dibromide, as well as 22 other chemicals was reviewed for its mutagenic effects on Tradescantia. The formation of pink mutation was as follows:

Exposure Time (hr)	Minimum Concentration (ppm)	Number Hairs Scored	Number Pink Mutants	Pink event ± SE per 100 hairs (minus control)	Statistical Significance
6	1	258×10^3	1088	0.118 ± 0.027	1%
144	0.14	148×10^3	1119	0.315 ± 0.035	1%

These results indicate that ethylene dibromide is a potent mutagen in this test.

The efficiency of this system when used in the analysis of cytogentic effects and somatic mutations was discussed; it was suggested that this assay be included as part of the battery of tests providing evidence of potential health hazards resulting from exposure to environmental pollutants.

Nauman et al. (33) and Nauman (34) have reported that gaseous ethylene dibromide concentrations ranging from 3.6 to 222.9 ppm (27.6 - 1776 mg/1) for 6 hours exposure induced pink somatic cell mutations in stamen hair cells of stable clones 02, 0106, and 4430 of Tradescantia. The results of comparisons among X-rays, ethyl methane sulfate, and ethylene dibromide indicate that, in the test system employed, ethylene dibromide can be as effective a mutagen as X-rays. The 4430 Tradescantia clone employed was relatively resistant to X-

rays but more susceptible to ethylene dibromide. The sensitivity of the Tradescantia assay to chemical mutagens was discussed, and the assay was suggested as a reliable test to determine mutagenicity of chemicals.

Ma et al. (35) have reported further investigations of mutagenic activity induced by ethylene dibromide in Tradescantia. Tradescantia 4430 clones were exposed for 6 hours to varying concentrations of ethylene dibromide (3.6 to 148.2 ppm; 27.6 to 1139 mg/m^3). The mutagenicity of ethylene dibromide for the induction of micronuclei in tetrads of microsporogenes followed a dose-response curve similar to that reported by Nauman et al. (33) for ethylene dibromide induction of pink stamen hair mutations; however, the induction of micronuclei in tetrads was a much more sensitive indication of mutagenic activity than was formation of pink stamen hairs. The advantages of scoring micronuclei as opposed to scoring chromosome aberrations was discussed as a more efficient means of testing pollutant effects on eukaryotic chromosomes.

Sparrow and Schairer (36) have demonstrated that Tradescantia grown in atmospheres loaded with ozone, sulfur, nitrogen dioxide, or nitrous oxide (unspecified amounts) increase the mutagenic effect of ethylene dibromide. This data indicates the potential of enhanced toxic interaction of ethylene dibromide with other environmental contaminants. One particular short coming of the Tradescantia assay, may be the inability to accurately score mutation frequencies induced by exposure to higher concentrations of chemicals due to foliar damage to the plants.

Scott (37) has reported increased mutagenicity in the methionine system of Aspergillus nidulans and the tryptophan system of E. coli after metabolic activation of ethylene dibromide by Tradescantia clone 4430.

The inactivation and genetic reversion of E. coli WP2 try⁻ after treatment with ethylene dibromide alone or in the presence of a Tradescantia hybrid clone 4430 extract was as follows:

	Experiment Number	Fraction Surviving ± S.E.	Mutation Frequency per 10^6 surviving cells
Ethylene Dibromide	1	0.0497 ± 0.003	10.8 ± 3.8[b]
	2	0.0112 ± 0.0001	13.4 ± 5.1[b]
Ethylene Dibromide plus Tradescantia extract	1	0.0021 ± 0.0002	372.0 ± 74.0[b]
	2	0.0175 ± 0.001	175.0 ± 44.0[b]
DMSO (control)	1	1.02 ± 0.07	0.04 ± 0.03
	2	0.89 ± 0.09	1.11 ± 0.17
DMSO + Tradescantia (control)	1	0.97 ± 0.06	0.22 ± 0.08
	2	0.89 ± 0.09	1.34 ± 0.20
Tradescantia (control)	1	0.99 ± 0.06	0.26 ± 0.08
	2	1.020 ± 0.056	1.50 ± 0.24
Buffer (control)	1	1	0.06 ± 0.01
	2	1	0.94 ± 0.13

In a similar manner, the effect of ethylene dibromide on inactivation and genetic reversion by suppressor mutation at various loci of a meth G ℓ strain of Aspergillus conidia was observed. These results are summarized in Table 4.

A high boiling point impurity (not specified) in the technical grade ethylene dibromide used might be the cause of the observed effects in both the E. coli and the Aspergillus assays. The residue remaining after distillation exerts a greater inactivation and mutagenicity than the final distillate. In addition, as the age of the distillate increases, the "genetic effectiveness" of ethylene dibromide increases. A 0-1 day old ethylene dibromide solution produced a fraction surviving in the E. coli assay of 0.59 ± 0.03 and a

Table 4. Effects of Ethylene Dibromide on Survival and Mutagenicity in
A. conidia as reported by Scott (37)

		Fraction Surviving ± S.E.	Mutation frequency per 10^6 Survivors			
			Class A[a]	Class B[b]	Class C[c]	A+B+C
Ethylene Dibromide (1)		0.48 ± 0.04	53.6 ± 8.2	16.2 ± 3.7	6.8 ± 2.0	77.0 ± 10.8
(2)		0.53 ± 0.03	86.8 ± 9.9	12.4 ± 3.1	7.37 ± 2.3	106 ± 11.0
EDB + Tradescantia (1)		0.33 ± 0.03	120.0 ± 11.0	30.7 ± 5.0	12.2 ± 3.0	163.0 ± 13.0
(2)		0.27 ± 0.02	258.0 ± 24.0	28.2 ± 5.4	10.1 ± 1.3	296 ± 27
DMSO (control) (1)		1.07 ± 0.19	8.8 ± 1.4	1.7 ± 1.1	0.38 ± 0.27	10.8 ± 1.5
(2)		1.13 ± 0.16	13.5 ± 4.1	1.2 ± 0.7	1.2 ± 0.7	15.5 ± 4.3
DMSO + Tradescantia (control) (1)		0.88 ± 0.16	6.7 ± 1.3	1.6 ± 0.6	0.47 ± 0.33	8.8 ± 1.6
(2)		1.05 ± 0.11	13.1 ± 2.4	0.80 ± 0.85	0.80 ± 0.85	15.5 ± 2.6
Tradescantia (control (1)		0.89 ± 0.16	4.1 ± 1.0	2.1 ± 0.7	0.46 ± 0.33	6.7 ± 1.3
(2)		0.01 ± 0.06	7.14 ± 1.7	0.71 ± 0.51	0.71 ± 0.51	7.9 ± 2.5
Buffer (control) (1)		1	4.9 ± 1.3	2.2 ± 0.8	0.41 ± 0.30	7.6 ± 1.8
(2)		1	5.3 ± 1.7	0.0	0.38 ± 0.38	5.7 ± 1.8

[a] Methionine revertant classified as large green colonies.
[b] Methionine revertant classified as heavily pigmented brown colonies.
[c] Methionine revertant classified as small green colony with white hyaline edge.

mutation frequency of 3.7 $\pm$ 1.1 per 10^6 survivors. These parameters were
measured again with 2 or 3 day old solutions of 0.049 $\pm$ 0.003 and 0.0112 $\pm$
0.0001 (fraction surviving) and 10.8 $\pm$ 3.8 and 13.4 $\pm$ 5.1 mutation per 10^6
survivors. This may indicate the formation of a breakdown or polymerization
compound. It was postulated that ethylene dibromide is converted to a mutagen
by two reactions; one reaction involves an _in vitro_ breakdown product of an
unknown nature, while the other involves metabolic activation. These results
also contribute to confirming the previously reported mutagenic effects of
ethylene dibromide.

Kristoffersson (14) was unable to produce chromosome breaks in _Allium_
roots exposed to ethylene dibromide, and no induction of lysis in _E. coli_
K39(λ) was found at the unspecified concentrations employed.

C.   Reproductive Effects

Amir and Lavon (38) have further examined the effects of protein
changes in epididymal and ejaculated spermatozoa in ten Israeli-Friesian bulls
orally treated with 10 doses of 4 mg ethylene dibromide per kg of body weight
on alternate days. Only one of four young bulls (15-18 months) developed
spermatozoa with abnormally shaped heads, while the other three showed no
differences from control animals. Older bulls (4 1/2 - 5 1/2 years) all de-
veloped abnormally shaped spermatozoa in ejaculate samples collected 6 to 9
days after the end of treatment. By 9 to 13 days after treatment, degenerative
changes, similar to those previously reported in the NIOSH criteria document (1),
developed. The treatment with ethylene dibromide did not significantly change
the total nitrogen, lipoprotein, or total amino acid content of epididymal and
ejaculated spermatozoa. The dry weight of epididymal sperm in the caput showed
a 2-fold decrease from 3340 $\pm$ 107 μg/10^8 sperm before treatment to 1494 $\pm$

137 μg/10^8 sperm after treatment. Decreases in sperm weight, sperm number, and total nitrogen of ejaculated sperm were also observed, but were not significant at $p<0.05$. Significant changes in amino acid composition were: increased isoleucine and tyrosine in caput epididymidis spermatozoa; increased arginine and glycine in cauda epididiymidis spermatozoa; and a decrease of proline in ejaculated sperm. Only in ejaculated spermatozoa were significiant changes of lipoproteins reported (increased half-cystine and tyrosino, decreased threonine, serine, isoleucine, and glutamic acid).

Amir et al. (39) have examined DNA and protein changes in six 22-23 month old Fresian bulls orally treated with 10 doses of 4 mg ethylene dibromide per kg body weight. Sperm concentrations were significantly reduced from control minimals between 9 and 23 days after the last dose. The occurrence of spermatozoa with abnormally shaped heads increased from 5.1 $\pm$ 4.0 percent on day 0 to 98.9 $\pm$ 0.7 percent by day 13, and steadily decreased to 13.4 $\pm$ 3.7 percent by day 30 after the last dose. Significant decreases in u.v.-determined DNA content of both epididymal and ejaculated sperm were shown in treated bulls, and sperm-head urea was significantly reduced, this decrease was observed 6 days after the last treatment in the epididymal sperm and 9 days after treatment in ejaculated sperm. Again, the results confirm evidence previously cited by NIOSH (1).

D. Developmental Effects

Short et al. (40), Short (41) and Minor et al. (42) have investigated the teratogenic potential of exposure to 32 ppm (246 mg/m^3) ethylene dibromide for 23 hrs per day inhaled by pregnant Charles River CD rats and CD-1 mice during the 6th to 15th day of gestation. No significant mortality in exposed

females was noted, however concentrations that produced producing adverse on maternal welfare also affected fetal development. Weight gains were less for exposed adult females as compared to controls and feed consumption was reduced. Short (41) notes that significant effects of ethylene dibromide exposure during organogenesis in both rats and mice were: 1) reduced number of inplants per dam (15.4 ± 0.3 to 12.4 ± 0.9, p<0.05) and reduced numbers of live fetuses per dam (15.3 ± 0.3 to 12.2 ± 0.9 p, <0.05) in rats; and 2) a reduction in fetal weights (1.24 ± 0.03 to 0.93 ± 0.06 gm) in mice. There were trends toward an increased number of resorptions in both species, but the number was not statistically different from control data. Teratogenic response of rat fetuses included significant increases in hydrocephaly, formation of a fourteenth pair of ribs, and occurrence of wavy ribs. The occurrence of various anomalies in murine fetuses exposed to ethylene dibromide in utero included hydrocephaly of the third and fourth ventricles, and skeletal abnormalities including incomplete or lack of ossification of the supraoccipital incus, and strenabrae. These anomalies were observed with similar frequencies in mice on a restricted diet intake. For mice, the observed defects could be attributed to malnutrition rather than to ethylene dibromide exposure per se. The restriction of food intake during pregnancy may obscure toxic effects of ethylene dibromide exposure to fetuses (i.e, fetal weights, live fetuses), and should be taken into account when analyzing these data.

Minor et al. (42) demonstrated greater maternal and fetal toxicity in pregnant rats exposed to 80 ppm (615 mg/m^3) and in pregnant mice exposed to 38 or 80 ppm (230 or 615 mg/m^3). Only those concentrations of ethylene dibromide that seriously affected maternal welfare produced morphological changes in exposed dams' fetuses.

These studies indicate that inhaled doses of ethylene dibromide exert a weak teratogenic response in rats and mice, and that severe fetotoxic effects are realized at concentrations producing a moderately severe toxic response in exposed mothers.

E. Hepatic Effects

Nachtomi and Sarma (43) have examined repair mechanisms of rat liver DNA damaged by ethylene dibromide. Male Wistar rats were intubated with labeled [^{14}C] ethylene dibromide (5 mg/100 g to 22 mg/100 g), and liver DNA was labeled with thymidine-[methyl-^{3}H] injected during peak DNA synthesis after a partial hepatectomy. Upon termination, livers were excised and liver DNA measurements were made. The incorporation of radioactivity into various compartments after administration of [^{14}C] ethylene dibromide was highest in cytoplasmic protein fractions, followed, in decreasing order, by incorporation into microsomal protein, nuclear protein cytoplasmic RNA, ribosomal RNA, and nuclear RNA. Administration of ethylene dibromide caused a slower sedimentation of rat liver DNA in alkaline sucrose gradients. This observation was apparent 2 hours after administration of a 22 mg/100 g dose and 4 hours after a 7.5 mg/100 g dose. The slow sedimentation of rat liver DNA was not dramatically affected by increasing doses of ethylene dibromide above 7.5 mg/100 g, indicating the possibility of the liver's inability to metabolize higher concentrations in the time intervals examined. Within two hours after administration, ethylene dibromide produced a significant slowing of sedimentation of liver DNA and exerted its greatest effect on lowering hepatic soluble sulfhydryl groups. Liver DNA damage induced by a dose of 7.5 mg/100 g was significantly repaired by 17.5 hours and almost entirely repaired by 96 hours after administration. Observed slower sedimentation of

liver DNA was hypothesized to be a result of DNA strand breaks resulting from alkylation following free radical production. This data indicates that ethylene dibromide induces in vivo chemical and physical lesions in liver DNA, and interacts with proteins and RNA.

The mitogenic effect of ethylene dibromide administered by gavage to non-fasted Wistar rats at doses of 7.5 or 10 mg/100 kg body weight has been examined by Nachtomi and Farber (44). DNA synthesis in liver cells was measured by [^{3}H]-methyl thymidine incorporation and confirmed by autoradiographic analysis. Increased liver weights and increased DNA content were obtained 18 hours after dosing. Maximum increase of liver DNA (24-34%) was seen by 36 hours, with peak specific activity 10-fold that of controls at 24 hours. A dose-effect relationship was found with a 6-fold increase in the incorporation of labeled thymidine in rat liver DNA and a 9-fold increase with the higher dose. At concentrations of 7.5 to 10 mg/100 g, increased proliferation of hepatocytes was observed without any indication of the proliferation of cells into the liver. The proliferation of hepatocytes stimulated by exposure to ethylene dibromide is similar to that seen in regenerating liver cells after partial hepatectomy. Concentrations of 15 to 22 mg/100 kg ethylene dibromide proved lethal, with evidence of liver necrosis at 30 hours. The relationship between other mitogenic effects at low doses and necrosis at higher doses was discussed in terms of possible imbalances in the Ca$^+$ content of the liver and alterations of the plasma membrane, endoplasmic reticulum, or mitochondrial membrane.

These studies confirm previously cited information concerning the hepatotoxicity of ethylene dibromide by considering the chemical's effect on DNA synthesis and repair mechanism in damaged livers.

F.   Uptake, Distribution, Metabolism and Excretion

Watanabe et al. (45,46) of Dow Chemical's Toxicology Research Laboratory have examined in detail the fate of ethylene dibromide in male Sprague-Dawley rats following the inhalation of 99% pure [^{14}C] ethylene dibromide at concentrations of 7, 25, and 75 ppm. The routes and rates of elimination were followed for 48 hours after exposure. Urinary excretion was the major route of the elimination of radioactivity, and appeared to be independent of exposure concentration. The excretion of the radioactivity in the urine accounted for nearly 80% of the recovered label. Rates of excretion over a 48-hour period appear to fit first order rate constants, producing half-lives of 5.1, 5.5, and 5.6 hours for each of the three respective exposures of 7, 25, and 75 ppm. Tissue distribution of radioactivity was highest in the kidneys and livers. The total amount of ethylene dibromide metabolized and the extent of macromolecular binding showed a dose-dependent difference, with proportionately more ethylene dibromide being metabolized at 7 ppm exposure than at 25 or 50 ppm. Hepatic, non-protein sulfhydryl content (in particular GSH) of experimental rats was decreased significantly from that of controls 6 hours after exposure to 25 or 75 ppm.

In subsequent tests of 3 rats per group blood levels of ethylene dibromide during and following exposure to 78 ppm for 6 hours reached a steady state of 0.78 µg/ml within the first hour. Clearance of ethylene dibromide during the first hour following exposure produced a first order rate constant corresponding to a 18.7-minute half-life in blood. Exposure to 6 ppm for 7 hours required a longer period to reach steady state with blood concentrations of 0.067 µg/ml being observed after 6 hours exposures. Clearance of ethylene

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dibromide was not determined because no ethylene dibromide was detected in blood samples 15 minutes after exposure.

Profiles of urinary radioactivity, separated by high pressure liquid chromatography, corresponded to co-eluted standards of N-acetyl-S-(2-hydroxyethyl) cysteine and thiodiglycolic acid as the second and third of the four observed peaks. It was postulated that the first peak may be cyclized to form N-acetyl-S-(2-hydroxyethyl)cysteine which hydrolyzed on storage; therefore, the first two peaks appear to be cysteine conjugates of ethylene dibromide.

The researchers speculated that dose-dependent mechanisms of pulmonary biotransformation caused the detoxification of ethylene dibromide to be more efficient at low exposure concentrations.

Shih (47) has examined tissue distribution of [^{14}C] ethylene dibromide in rats and mice intraperitoneally treated. At one to three hours after injection, radioactivity is concentrated in the liver, kidneys, and small intestine. After 24 hours, radioactivity remains in the liver and kidneys and is irreversibly bound to RNA, DNA, and protein. Glutathione-S-transferase and TPNH are presumably involved in the detoxification of ethylene dibromide.

Hill et al. (48) elaborates on the molecular binding and metabolism of ethylene dibromide in male rats given single intraperitoneal injections of 4.2 μmol (1,2-^{14}C) 1,2-dibromoethane (30.4 μCi), 24 hours prior to sacrifice. The amount of bound radioactivity for protein, DNA, and RNA was greatest for kidney and liver. Macromolecules of the brain, heart, and muscle contained little radioactivity, while the lungs, testis, stomach, and large and small intestine were intermediate in their accumulation of radioactivity.

An assay employing 3.2 mM glutathione, 20 mM [^{14}C]-1,2-dibromowthane (30 mCi), 4.2 mM EDTA, 40 mM trischloride butter, and dialyzed rat liver

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supernatant confirmed that ethylene dibromide is a substrate for glutathione S-transferase. The optimum pH for the liver transferase reactions was 8.2 Glutathione S-transferase was present in several rat tissues, but the highest activity was reported for the liver and kidney. The enzyme was also present in lung, testes, spleen and heart.

In rat liver microsomes enzymatic activity lead to the irreversible binding of radioactivity from the labeled ethylene dibromide. This reaction was induced by phenobarbital, but not induced by benz(a)anthracene. NADPH was necessary for the activation of both induced and noninduced reactions.

The enzymatic metabolism of ethylene dibromide progressed rapidly at an estimated rate of 17 μmole per miunte. The hepatoxicity of ethylene dibromide may be related to the depletion of liver glutathione. This depletion of glutathione is followed by the binding of ethylene dibromide or its metabolites to macromolecules. Mass spectral analysis and thin layer chromatography have identified bromoacetaldehyde as a microsomal metabolite metabolite of ethylene dibromide.

These results present further evidence of the activated involvement of ethylene dibromide and its metabolites with macromolecules. Such bio-chemical interactions may help in understanding the mechanisms involved in the carcinogenic and mutagenic processes induced by the chemical. Additional in-sight into the biological activity of ethylene dibromide would be gained by a study, where experimental animals would be pre-treated with phenobarbital, and examination of the enzymatic activities with several various doses of the test chemical.

Plotnick and Conner (49) have examined the tissue distribution of Radioactivity of [^{14}C] ethylene dibromide in guinea pigs. Male Hartley guinea pigs were intraperitoneally injected with a 30 mk/kg solution of labeled eth,lene dibromide. Animals were sacrificed at 4, 8, 12, 24, 48, and 72 hours, and tissue samples were examined for radioactive content. Organs containing the greatest concentration of radioactivity per weight were: kidneys, liver, adrenals, pancreas, and spleen. The distribution of radioactivity, represented as μg/g tissue and percent of dose (± Standard Error of Mean), over the time interval of 72 hours is presented in Table 5.

Cumulative urinary excretion of labeled chemical, expressed as percent of dose, was reported as:

Time (hrs)	% Dose
4	14.9 ± 1.0
8	26.3 ± 10.1
12	43.3 ± 8.1
24	46.0 ± 4.8
48	54.3 ± 3.4
72	65.9 ± 4.6

Fecal excretion accounted for less than 3% of the dose at 72 hours. Preliminary findings indicate that 10-12% of the dose is expired as unchanged ethylene dibromide by eight hours after administration. Peak level in the stomach (potential site of tumor formation) was 1.14 ± 0.44% mg/gm at 4 hours, decreasing steadily throughout the course of the study.

G.   Synergistic Effects

NIOSH has issued a Current Intelligence Bulletin (5) reporting the toxic interaction of ethylene dibromide with disulfiram. Stein et al. (50) have made a similar statement concerning the joint action of ethylene dibromide and disulfiram. Additional warnings concerning this potentially serious inter-action have been presented by Plotnick (51) and Yodaiken (52).

Table 5. Tissue Distribution of Radioactivity of [^{14}C]Ethylene Dibromide in Guinea Pigs Administered 30 mg/kg (ip) at 4 to 72 hours, Plotnick and Conner (49).

Organ	4 hr.	8 hr.	12 hr.	24 hr.	48 hr.	72 hr.
Kidneys	286.64 + 18.74* (16.29 + 2.42)	236.52 + 24.03 (13.65 + 0.39)	147.79 + 10.64 (10.50 + 2.13)	73.45 + 14.06 (4.72 + 0.21)	14.66 + 0.085 (2.12 + 0.07)	10.47 + 0.74 (2.16 + 0.21)
Liver	129.03 + 14.94 (6.00 + 0.04)	104.91 + 7.92 (5.69 + 0.43)	78.39 + 12.99 (3.31 + 0.17)	38.07 + 3.47 (1.64 + 0.45)	16.20 + 1.060 (0.31 + 0.01)	15.58 + 0.48 (0.24 + 0.02)
Adrenals	60.73 + 10.74 (0.09 + 0.02)	60.82 + 8.25 (0.10 + 0.04)	26.56 + 2.88 (0.04 + 0.01)	28.63 + 3.68 (0.03 + 0.01)	13.24 + 1.55 (0.01 + 0.01)	10.43 + 1.10 (0.02 + 0.61)
Pancreas	35.04 + 5.84 (0.31 + 0.10)	36.81 + 3.22 (0.36 + 0.06)	28.85 + 1.08 (0.33 + 0.02)	18.68 + 1.09 (0.20 + 0.03)	7.52 + 0.20 (0.07 + 0.01)	6.01 + 0.04 (0.06 + 0.01)
Spleen	15.79 + 2.80 (0.07 + 0.01)	13.96 + 0.91 (0.06 + 0.01)	15.83 + 0.06 (0.07 + 0.01)	14.89 + 0.25 (0.08 + 0.02)	8.92 + 0.04 (0.03 + 0.08)	6.95 + 0.64 (0.02 + 0.01)

*mg/m tissue

El-hawri (53) has reported that the toxic action of ethylene dibromide (50 mg/kg P.O.) was enhanced by the action of disulfiram (200 mg/kg P.O.), diethyldithiocarbamate (500 mg/kg P.O.), thiram (200 m.g./kg P.O.), or carbon disulfide (100 mg/kg P.O.) administered 1-24 hours before ethylene dibromide dosing. Combined dosing resulted in increased levels of the plasma transaminases, glutamate-oxalacetate transferase (GOT) and glutamate-pyruvate transaminase (GPT), and blood urinary nitrogen (BUN) levels. It was postulated that this group of compounds interferes with the detoxification of ethylene dibromide by either the mixed function oxidase system or the aldehyde dehydrogenase system.

Wong et al. (54) have reported the results of an 18-month carcino-genicity study in Sprague-Dawley rats of both sexes exposed to an inhaled dose of 20 ppm ethylene dibromide with and without dietary exposure to 0.05% di-sulfiram. Rats were exposed to ethylene dibromide for 5 days a week for 7 hours a day. Treatment with disulfiram alone or in combination with ethylene dibromide resulted in depressed body weight gains and decreased food consumption. High mortality rates were reported for the combined dose groups. Specific mortality data was not available in this meeting abstract. Among the tumors occurring were: hepatocellular tumors, hemangiosarcoma of the liver, spleen, mesentery, and kidneys. In the combined dose group, tumors developed as early as 6 months. Further evaluation of this data is pending completion of the study.

NIOSH (5) has summarized the mortality data of concurrent studies pertaining to the joint interaction of disulfiram and ethylene dibromide. The

mortality data available is as follows:

Group	Treatment	Numbers of Deaths/Number Treated Male	Female
Untreated	Filtered air; control diet	0/48	3/48
Disulfiram only	Filtered air diet containing 0.05% disulfiram by weight	3/48	2/48
Ethylene dibromide only	Inhalation of 20 ppm EDB 6 hrs per day 5 days per week control diet	15/48	9/48
Ethylene dibromide and Disulfiram	Inhalation of 20 ppm EDB 6 hrs/ day, 5 day/week; diet containing 0.05% disulfiram	45/48	47/48

Plotnick (51) has reported on the preliminary findings of increased incidence of tumor in 48 rats of both sexes exposed to both 20 ppm ethylene dibromide in filtered air and 0.05% disulfiram in standard rat diet. Histopathologic examination of the ethylene dibromide/disulfiram group is incomplete. The partial data is tabulated below:

Tumor-Site		Number Tumors/Number Examined Male	Female
Hemangiosarcoma	– Liver	3/14	6/15
	– Spleen	3/14	4/16
	– Omentum	4/14	5/16
	– Kidney	2/14	1/16
Adenocarcinoma	– Mammary	----	7/15

Tubular atrophy of the testes was noted in 12 of 13 examined males. Mortality and morbidity in groups exposed to ethylene dibromide or disulfiram alone have not been noted. Publication of these results is pending completion of the study.

The Midwest Research Institute (Kansas City, Missouri) studies partially reported by NIOSH (5), Plotnick (51) El-Lawri (53), and Wong et al. (54) point to increased carcinogenic risk and toxic risk following joint exposure to ethylene dibromide and disulfiram.

H.   General Toxicity

In order to design a dose regimen for a bioassay of the carcino-
genicity of ethylene dibromide in rats and mice, the NCI (22) determined the
maximum tolerated dose for both sexes of both species. Rats and mice were
exposed to 40, 63, 100, 163, and 250 mg/kg ethylene dibromide by gavage for 5
consecutive days per week for 6 weeks, then observed for a 2-week period. At
63 mg/kg, no increased mortality among treated animals was noted and mean body
weights were within 10 percent of controls. At dosages of 100 mg/kg, one rat
of each sex had died by eight weeks and mean body weights were 75% and 82% that
of controls for males and females respectively. The maximum tolerated dose
for rats was determined to be 80 mg/kg. All male mice treated with 159 mg/kg
or less survived the testing period; dosages of 100 and 251 mg/kg/day resulting
in one and two mortalities, respecitvely, in females. At 159 mg/kg, female
mean body weights were greater than controls. Mean body weights of male mice
at 63 and 159 mg/kg were 71 and 91 percent of control weights, respectively.
The maximum tolerated dose of 120 mg/kg was established for male and female
mice.

At the selected maximum tolerated doses, the carcinogenicity bioassay
conducted by the NCI (22) was to have lasted for 110 weeks. An excessive
number of deaths in both the low and high dose groups of rats was observed
(P<0.001, Tarone test for positive association between dosage and accelerated
mortality), however, and the study was terminated at 49 weeks for male rats
and at 61 weeks for female rats. The mouse study was also terminated early,
at 78 weeks, because of the excessive number of deaths, noted among mice
treated with ethylene dibromide. Reduced weight gains in treated animals

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reflected their poor health at the time the studies terminated.   Various

degrees of weight depression were noted among all treated animals.   Alopecia

was observed among treated mice in week 7 of treatment.

Yodaiken (52) has criticized this bioassay for its choice of treat-

ment levels of maximum or one-half maximum tolerated dose and its use of

gavage to administer treatment.

Excessive mortalities in male control groups have been dicussed

in a previous section (IV.A).

V. Work Practices and Engineering Controls

No information pertaining to engineering controls was available to supplement that presented in the NIOSH criteria document (1); however, in light of recent evidence confirming the potential mutagenicity and carcinogenicity of ethylene dibromide, it may be prudent to closely adhere to the requirements detailed in the criteria document concerning ventilation and enclosure of production operations.

Programs designed to educate those working with ethylene dibromide in production processes or in its application as a fumigant, pesticide, or gasoline additive should stringently emphasize the toxicity of the chemical; labeling of ethylene dibromide should reflect the hazards known to be associated with its use. The USEPA (13) has reported that most cases of accidental exposure to ethylene dibromide that required medical observation were the result of inadequate labeling of the chemical or misreading of labels by users, improper use of protective clothing and equipment, or improper handling of application equipment in pesticidal and fumigation processes.

Preliminary experimental data concerning the toxic interaction between ethylene dibromide and disulfiram, recently reviewed by NIOSH (5), strongly suggests an increased risk of carcinogenesis and lethality in rats exposed to both chemicals. Disulfiram, also known as Alcophobin Antabuse, is used in alcohol-control programs. The clinical significance of the interaction between these two chemicals in man is unknown. The number of persons receiving disulfiram therapy who are also exposed to ethylene dibromide can be assumed to be small; however, clinicians prescribing disulfiram as a treatment for alcoholism control should be made aware of this potentially toxic interaction. The

advisability of disulfiram therapy for workers occupationally exposed to ethylene dibromide should be determined by the appropriate medical personnel.

Rumsey et al. (12) have demonstrated in a comprehensive industrial hygiene survey that existing engineering controls in four plants (two manufacturer and two user) were adequate in maintaining ethylene dibromide concentrations within the 20 ppm 8-hour TWA standards; however, none of the four plants surveyed complied with the reassessed NIOSH ceiling limit of 0.13 ppm (1 mg/m^3) as determined by a 15-minute sampling period. The findings show that existing controls must be improved in order to reduce background concentrations of ethylene dibromide in process areas where routine work assignments are carried out.

In manufact. ring facilities, certain job classifications were shown to have made major contributions to the workers' overall exposure. In user facilities, work assignments involving the handling of ethylene dibromide were associated with exposures that would exceed a 0.13 ppm ceiling. Various improvements were suggested including:

- engineering controls at specific activities/sites
    . local exhaust ventilation at specific tasks
    . re-evaluate facilities' emission sources,
      pumps, valves
    . evaluate potential equipment leaks

- maintenance and worker control processes
    . periodic maintenance schedules of equipment
      parts, especially parts prone to wear
    . use of color dyes in anti-knock blending operations
      to more readily identify leaks
    . provide additional protective equipment and additional
      training in the handling of ethylene dibromide

Sansone and Tewari (55) have examined the penetrations of protective clothing materials by several potential carcinogens, including ethylene dibromide. Materials commonly used in the manufacture of protective

clothing that were examined in this study included:

> 0.4 mm natural rubber
> 0.4 mm neoprene
> 0.5 mm mixture of natural rubber and neoprene
> 0.2 mm polyvinyl chloride (PVC)
> 0.4 mm polyvinyl alcohol (PVA)
> 0.4 mm nitrile (co-polymer of 1,3-butadiene and Acrylonitrile)

Ethylene dibromide (97.4% Fisher Scientific Co.) was allowed to come into contact with the material(s) while penetration was measured by gas chromatography. The ranges of penetrant concentrations for ethylene dibromide, expressed as volume percent, after 5 and 240 minutes of observation are as follows:

		Concentration	
Material	5 minutes	240 minutes	
PVA	$1.3 \times 10^{-6} - 3.7 \times 10^{-5}$	$1.3 \times 10^{-3} - 9.8 \times 10^{-3}$	
Nitrile	$2.3 \times 10^{-6} - 5.6 \times 10^{-5}$	15.5 -27.5	

The results of this study indicate that none of the materials tested was suitable for use with the chemicals tested (1,2-dibromo-3-chloropropane, ethylene dibromide, and acrylonitrile). It was recommended that gloves and other protective clothing should be discarded immediately after becoming contaminated.

VI.   Sampling and Analytical Methods

The determination of ethylene dibromide (EDB) concentration in workplace
environments requires the monitoring of work areas or of personnel for levels
of exposure. The selection of a method depends on its intended purpose; although
some, but not all, area monitoring methods, can be used with slight modifications,
for personnel monitoring. Other important criteria to consider in the selection
of an monitoring method selection the method's its ability to provide interference-
free EDB determination, reasonably high recovery of EDB, and the required
sensitivity and reproducibility. Cost-effectiveness should also be considered
in selecting a particular method.

The process of monitoring of EDB concentration in air can be divided into
two phases: sampling and analysis. Normally, the sample is collected at the
site and then transported to a laboratory for analysis. The various sampling
and analytical methods used for EDB determination are discussed below. It
should be mentioned that direct reading instruments are used occasionally for
continuous "on-the-spot" monitoring of EDB in the air. This method, which
requires no separate sampling and analytical procedures will be discussed
separately.

A.   Sampling

Current sampling procedure for EDB involves a variety of techniques.
In the grab sampling method, gas bags or evacuated bulbs or bottles can be used
for collection of air samples containing EDB (56,57,58). Such sampling bags,
bulbs, or bottles are commercially available. Although the samples collected
by grab sampling methods have the advantage of requiring no further treatment
prior to analysis, the disadvantages include inconvenient handling and transpor-
tation of samples, surface adsorption of chemical, poor sensitivity in analysis

resulting from inability to concentrate EDB, and inability to collect integrated samples. Because of these disadvantages, the grab sampling methods are not currently used for monitoring EDB concentration in air.

In the cold-trapping method, the contaminant is collected by freezing it in a cold trap. This method, although used for other halogenated hydrocarbons, (59,60) is rarely used for EDB sampling. The method suffers from various drawbacks including inconvenience during handling, transportation, and analysis. Icing problems resulting in clogging may inhibit sample flow during collection.

Sampling methods that use solvent trapping of EDB have been used by several workers in the past. In one method, a mixture of potassium hydroxide, potassium chromate, and ethanol was used for trapping EDB in air samples (61). A cooled mixture of acetone-isooctane can be used to trap EDB in air samples (62). The use of n-pentane as a gas-trapping solvent for EDB has been suggested by Berck (63). Other liquid-trapping solutions, such as a mixture of 1:1 mono-ethanolamine and dioxane have been used also (64,65). The first and last solvent trapping-media decompose EDB and many other brominated organic contaminants to inorganic bromide, and are, therefore, not specific for EDB. Other disadvantages of solution trapping include the danger of evaporative loss of solvent during collection, prevented either by cooling or by other kinds of supervision, and inconvenience during handling and transportation of samples. The analytical procedure for EDB collected by this method may also be more time-consuming than that used on samples collected by other methods. The solution trapping method cannot be used for personnel monitoring of EDB in a workplace atmosphere.

The collection of EDB from air using solid sorbents is a promising method. A number of solid sorbents have been used for this purpose. Silica

gel was used as a collection medium in early investigations (66.67). This sorbent shows pronounced selectivity in adsorbing polar molecules: consequently, absorption of moisture remains a problem with this method. Studies with silica gel tubes indicated that water vapor could replace organic molecules during a normal sampling operation (68).

A few authors used Tenax-GC as a solid sorbent for the collection of EDB. Russel (69) used stainless steel sampling tubes packed with Tenax-GC for collection of EDB from air samples. Both the detection limit and the recovery of EDB from sampling tubes stored for several days after sampling was reported to be excellent. Although this author (69) also determined the breakthrough volume, he did not report the recovery of EDB from the collection method. Going and Long (70) concluded from their experiments with Tenax-GC tubes as sorbent for EDB that, at ambient temperature, EDB migrates down and out of the tube, this method was, therefore, ineffective for long-term air sampling of EDB. When Tenax-GC traps cooled in dry-ice were used, however, the researchers reported a recovery of 94% after two hexane extractions of the resin. The Tenax-GC system at dry-ice temperature has the disadvantage that water vapor tends to freeze in the tubes and restrict the air flow.

EDB was determined in air samples by adsorption of the compound onto a short column of Chromosorb-102 (71). The breakthrough for this column was also determined. Analysis of these columns indicated a combined recovery efficiency of over 95% for both trapping and elution of EDB, even at picogram levels. Freal et al. (72) used sampling tubes containing Chromosorb-101 and determined the average combined trapping and elution efficiency to be 85%. The interfering effect of biphenyl used for fungal-growth prevention was evaluated in this study and was found to be negligible.

Many authors have used activated charcoal as an adsorbent for collecting EDB in air. Presently, the activated charcoal method is recommended by NIOSH for EDB collection (73,74). Going and Spigarelli (75) evaluated the charcoal adsorption method and found that the recovery efficiency is dependent on the method of charcoal activation. Reactivating the charcoal by heating under a stream of nitrogen gave an average recovery of nearly 50% (75). Partial deactivation with benzene showed an average recovery of 67%, with a standard deviation of 10% (75). Freal et al. (72), on the other hand, reported an average recovery of 95% with charcoal, and found no measurable effect with bipheryl as an interferant. The adsorption and desorption efficiencies may vary with different batches of charcoal; therefore, it is imperative to determine these values for each new batch of charcoal. Furthermore, the variation in pressure drop across the charcoal-containing tubes will affect the flow rate and cause an error in the volume measurement unless the flow rates are measured for each individual tube with an inert gas.

The advantages of the solid adsorbent method over the other collection methods are numerous. Solid adsorbents that are used at ambient temperature are convenient for the purpose of handling, transportation, and storage. This method can be used for both area and personnel monitoring of EDB. The method can also be used for simultaneous collection of multiple contaminants present in the air. Other advantages of this method include its ability to concentrate the contaminant during sample collection, to collect integrated samples, and to simplify the subsequent chemical analysis. The main disadvantage of the method is that water vapor and other contaminants may cause saturation of the column, resulting in the loss of the compound of interest, regardless of the breakthrough volume.

B. Analysis

The method of sample treatment prior to identification and quantification of EDB depends largely on the method of sample collection. Grab samples collected in bags or bottles are commonly analyzed without any pretreatment; analysis is preferred by injecting an aliquot directly into a GC system. With a GC-FID system, it was possible to measure 2 mg/m^3 of EDB in air (76). Thus, the grab sampling method may not be adequate to monitor EDB concentrations in air for two reasons. First, the method is not sensitive enough to detect EDB concentration in the range required by the NIOSH recommended standard (74); second, this method does not allow the specified integrated sampling necessary to determine the ceiling limit (74).

When EDB samples are collected by solvent trapping, the sample treatment involves conversion of organic bromide to inorganic bromide. There are two ways this conversion can be made. EDB is readily converted to inorganic bromide in an oxygen combustion (Schönigger) flask (62) or in a quartz pyrolysis tube (63). Alternatively, hydrolytic debromination of EDB can be used to produce inorganic bromide (61,64,65). The inorganic bromide obtained by either method can then be measured with a bromide-specific ion probe (77) or by potentiometric, argentometric, Volhand, or coulometric titration (78). A six-fold increase in sensitivity can be achieved by oxidizing bromide to bromate by the Kolthoff-Yutzy method, followed by ampherometric (63) or iodometric titration (63). Another method utilizes a sensitive colorimetric test for the determination of inorganic bromide. Additional methods for inorganic bromide determination include fluorimetric (79), gas chromatographic (80), polarographic (81), cathodic stripping voltammetry (82),

neutron activation (83), flame photometric (84), x-ray fluorescence (85), and molecular emission cavity analysis (62). A review of the various earlier methods available for EDB determination is given by Getzendaner (86).

Solvent trapping does not require that EDB be decomposed to inorganic bromide for its determination. Undecomposed EDB can also be determined by GLC (87), and X-ray fluorescence spectroscopy (88).

Although neutron activation and x-ray fluorescence analysis are specific for bromine at trace levels, they are time-consuming expensive, and do not distinguish EDB from other organic bromides. Those methods which are based on titrimetry have the advantage of being less costly than techniques using high energy spectrometry, but they are not as sensitive and may not distinguish the various organic bromides. Although the molecular emission cavity analysis is a relatively simple and sensitive technique, the method is not selective among the various bromine-containing organic contaminants.

Pretreatment of EDB samples collected by solid sorbent is usually done by two methods. In one method, the adsorbent column containing EDB is heat desorbed, and the EDB is determined either by GC-FID (69) or GC-ECD (71). The advantages of the heat desorption method are quantitative recovery and high sensitivity because the samples do not require any other pretreatment or dilution. This main disadvantage of the method is that only a single analysis of the contaminant can be made.

In the second method, EDB is desorbed from the solid sorbents by means of a solvent, or a mixture of solvents, and identified and quantified by GC-ECD. The recovery of adsorbed EDB depends on both the nature of solvent(s) and the method of extraction. With Chromosorb-101, Freal et al. (72), using

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hexane as the solvent and a mechanical mixer for extraction, reported a 85%
recovery of EDB. The detection limit based on GC method was reported to be
0.12 ppb for a 15 minute sampling duration at a rate of 2 1/min. When charcoal
was used as the adsorbent and methanol in benzene as the desorbing medium, the
same authors (72) reported 95% recovery of EDB. On the other hand, Going and
Spigarelli (75) found a 45% and 52% average recovery of EDB under manual
shaking and soxhlet extraction respectively, with benzene as the solvent.
Deactivation of charcoal (72) raised the average recovery to 67%. These
authors also determined that benzene was more effective in EDB removal than was
hexane. Three sequential manual extractions with benzene gave higher recovery
than soxhlet extraction.

Going and Long (70) determined the extraction of EDB from Tenax-GC tubes
with hexane and reported 76% and 18% recovery by first and second extractions
respectively. The analysis of hexane extract containing EDB was done by GC-ECD
and the method could detect 1 to 2 pgm of EDB per injection.

A number of liquid phases have been satisfactorily used for the gas
chromatographic separation of EDB. Some of these are OV-17 (69), didecyl
phthalate (75), Carbowax 20M (71,75), OV-225 (75), OV-101 (70), a mixture of
OV-17 and OV-210 (72), Apiezon L (89), QF-1 (70), DEGS (70), Ucon (90), XE-60
(76), and Proapak QS (76). With the three liquid phases tested, Going and
Spigarelli (75) found no interference from either a number of chlorinated
compounds or one brominated compound resembling EDB in structure. These authors
(75) also concluded that hexane and benzene were compatible with electron
capture detection, but carbon disulfide gave a broad peak that interfered with
EDB detection.

C. Continuous On-Site Monitoring of EDB

In the past several years, portable, direct-reading instruments have been developed which make continuous on-site monitoring of EDB feasible (91). The use of infrared light adsorption at a wavelength of 8.4 μm (92) is claimed to detect airborne EDB at a concentration of 0.1 ppm. Portable gas chromatographic instruments are also available for this purpose (93). However, online halide dectors (93,94) was found to be more suitable than GC or IR analyzers for continuous monitoring of EDB concentration during fumigation and aeration processes involving this fumigant. Although these continuous monitoring instruments are useful as a safety check in areas containing high levels of EDB, these instruments are not adequate for routine monitoring of workplace concentrations of EDB because of their higher detection limit, inaccuracy in the recorded values, and inability to monitor integrated samples.

D. Recommendation for Future Research

The NIOSH recommended method (74) using charcoal adsorption and methanol-benzene desorption of EDB for GLC-ECD identification and quantification, has the required capability to determine EDB concentrations much lower than the ceiling limit value of 0.13 ppm (74). Depending on the extent of activation/deactivation, however, the desorption efficiency with methanol-benzene may vary substantially. The recoveries of EDB from charcoal decrease with increasing storage times at room temperature; therefore, samples should be stored at -25°C. In addition, it is preferable to avoid the use of benzene, a suspected carcinogen, during the desorption process.

The precision of the charcoal tube method is limited by the reproducibility of the pressure drop across the charcoal tube. To avoid all these problems, adsorption tubes packed with Chromosorb-102 are recommended. It has

been determined by Leinster et al. (71) that the flow rate setting is independent of the collector media pressure drop characteristics, which is essential in field use. Replacing charcoal with Chromosorb-102 will permit the use of hexane for desorbing EDB, thereby avoiding the use of benzene. The problem of moisture trapping is expected to be less with Chromosorb-102 than with charcoal. Although the recovery efficiency of sorbed EDB from Chromosorb-102 with hexane is not known, it is expected to be high (72) since Chromosorb-102 does not irreversibly adsorb samples analyte like charcoal.

In a recent workshop on halocarbon measurements, it was discovered that interlaboratory calibrations done with multiple dilutions of pure material were uncertain by as much as a factor of two (95). The errors were maximum at ppt levels. Therefore, permeation tubes should be used to obtain primary EDB standards; permeation tubes made from TFE-Teflon may be satisfactory for this purpose (96). Such validation of sampling and analytical protocol done with permeation tubes as a primary standard has already been reported (71).

VII. Research Needs

The data base of knowledge concerning the toxic effects of ethylene dibromide remains relatively unchanged from that collected by NIOSH (1). While a bulk of the literature reviewed in the present report confirms the toxic effects associated with exposure to ethylene dibromide, existing knowledge concerning the environmental health aspects of exposure to ethylene dibromide remains incomplete. The research needs described in the NIOSH (1) criteria document remain themselves relatively unchanged. The list below recommends research projects that would help to supply information presently lacking about exposure to ethylene dibromide.

1) **Evaluation of carcinogenicity of ethylene dibromide in vivo** - To confirm the carcinogenicity of ethylene dibromide, various bioassays in experimental animals should be performed. Experimental designs should consider major routes of occupational exposure (i.e., inhalation, percutaneous contact).

2) **Evaluation of carcinogenicity of ethylene dibromide in vitro** - To confirm the carcinogenicity of ethylene dibromide, various systems could be employed, such as early passage hamster embryo cells, baby hamster kidney cells, C3H10T 1/2 mouse fibroblasts, and several organ culture systems. Results from such tests would provide important data to support the results of the NCI bioassay (25) and the data derived from mutagenicity studies.

3) **Evaluation of mutagenicity of ethylene dibromide in vitro and in vivo** - Studies are needed to provide evidence of potential risk in this area. In particular, valid test systems that can be used with confidence are necessary. For the detection of gene mutations, the use of mammalian somatic cells in culture (with and without metabolic activation) should be employed. Chromosomal aberrations should be measured by in vivo cytogenetic tests in animals, dominant lethal effects in rodents, and heritable translocation tests in rodents. Primary DNA damage should be detected using tests for unscheduled DNA repair synthesis and sister chromatid exchange in mammalian cells (with and without metabolic activation), DNA repair in bacteria, and mitotic recombination and/or gene conversion in yeast.

4) <u>Cytogenetic testing of workers having high occupational exposures to ethylene dibromide</u> - Analyses should be made for chromosomal aberrations in cultured lymphocytes taken from exposed workers. These determinations are felt by many scientists to provide an indication of increased cancer risk and potential for transmission of birth defects and mutations. The use of somatic cells to predict a mutagenic effect that may occur in germinal cells is not entirely valid. Nevertheless, numerous examples of the positive correlation between a chemical's ability to produce chromosome aberrations in somatic cells and its carcinogenic/mutagenic activity indicate that cytogenetic data should be carefully evaluated. Moreover, the widely held view that cancer arises as a result of a somatic mutation emphasizes the need for cytogenic testing.

5) <u>Conduct of occupational epidemiologic studies to provide morbidity and mortality data regarding chronic ethylene dibromide exposure</u> - Cancer as a biologic endpoint should be of primary interest, but chronic neurological, respiratory, hepatic, and renal disease must also be carefully evaluated. Whenever possible, information should be obtained regarding current and past employment, clinical symptoms, smoking histories, and other health information such as genetic factors.

6) <u>Conduct of further subchronic and chronic inhalation exposure studies in animals using various dose levels</u> - These studies should be designed to detect dose-response relationships and ascertain the reversibility of treatment-induced damage. Included in the experimental protocols should be means to confirm the previously observed carcinogenic effects, and also to detect reproductive effects, teratogenic effects, and threshold dose levels for non-neoplastic responses.

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