PESTICIDE TOXICITY

Toxicity Study of a Grain Fumigant (Dowfume EB-5)

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Dowfume EB-5 is a liquid fumigant mixture used primarily for the control of insects in stored grain. The purpose of this investigation was to provide toxicological information for the evaluation of health hazards associated with the manufacture, handling, and use of this product. When given in single oral doses to four different species of laboratory animals, LD_{50} values ranging from 0.28 to 0.78 gram per kg. were obtained. Livestock were fed freshly fumigated, unaerated grain without apparent adverse effect. Direct contact with the eyes caused pain and conjunctival irritation, but no lasting injury. Extensive prolonged contact with the skin resulted in burns and possibly absorption of toxic amounts. Because vapor concentrations dangerous to life are readily attainable, single inhalation exposures of rats were utilized in order to measure capacity to kill and to cause injury without death. These results enable the recommendation of protective measures and precautions that will allow the safe handling and use of Dowfume EB-5.

DOWFUME EB-5 is a fumigant mixture used primarily for the control of insects in stored grain. It consists of 63.6% carbon tetrachloride, 29.2% ethylene dichloride, and 7.2% ethylene dibromide by weight. The material studied was obtained from commercial stock ready for distribution and its composition was checked by infrared absorption to be certain it was representative.

The purpose of this study was to provide toxicological information which would allow an evaluation of the hazards to health of persons who handle and use the subject material and to livestock which might be fed recently treated grain.

Because a grain fumigant generally is applied infrequently, a health hazard due to chronic exposure to the material will seldom be encountered except perhaps among a few pest control operators. Accordingly, only the effects of single exposures were determined on laboratory animals. As this fumigant may be used on feed stored for consumption by livestock, the response of farm animals fed freshly fumigated grain for periods of several days' duration also was studied.

Extensive toxicological information is available on the separate constituents (*2,7,8*) as well as on Dowfume EB-15, a so-called "spot fumigant" (*1*), which contains the same constituents in a different proportion. Extensive experience with these individual materials as industrial chemicals and solvents assisted in the evaluation of the possible health hazards presented by the use of the mixture.

Oral Administration

Laboratory Animals

Procedure. The toxicity of EB-5 when administered in single oral doses to white rats, guinea pigs, rabbits, and chicks was determined. The rats, guinea pigs, and rabbits were mature young adult animals from the stock colonies of this laboratory. The chicks were New Hampshire Reds purchased from a commercial hatchery and were approximately 3 weeks of age when treated.

Aliquots of a 10% solution of Dowfume EB-5 in corn oil were emulsified in about 2 ml. of a 5 to 10% acacia solution and fed by intubation. All surviving animals were observed for about 2 weeks or until they had fully recovered from any loss of weight and were gaining normally.

Results. The acute oral LD_{50}'s with their 19/20 confidence limits were calculated for each species by the method described by Litchfield and Wilcoxon (*6*). The results of the statistical treatment of the dosage-response data are presented in Table I. Of the four species tested, rabbits and guinea pigs appear to be the most sensitive, whereas rats and chicks appear to be the least sensitive.

Heifer

In a check to determine whether the acute oral toxicity for cattle was appreciably different from that found for the various species tested in the laboratory, a heifer weighing about 300 kg. was given a single dose of 50 mg. per kg. by capsule. Symptoms of slight illness characterized by a slight temperature (+1°), lack of appetite, decreased peristalsis, and laziness were apparent to some extent for about 48 hours after treatment. The animal appeared to be entirely normal thereafter.

These observations, while far from conclusive, suggest that cattle are similar to ordinary laboratory animals in susceptibility to this fumigant mixture.

Eye Contact

Rabbits

Procedure. In this study the rabbits' eyes first were examined by using fluorescein staining to ensure that they were normal, special attention being paid to the lens and the iris, as well as to the surface of the cornea. About 4 hours after this preliminary check, one drop of the test material was introduced into each eye. In about 30 seconds one eye was flushed for 3 minutes with copious amounts of running water, after which both eyes were stained and observed for injury as before. Examinations were repeated after 2, 24, and 48 hours and for longer periods if necessary, to determine the degree of injury and the course of the healing period.

Results. The undiluted material promptly caused moderate pain and conjunctival irritation which cleared within a few days without evidence of

residual injury. Prompt washing immediately after contact markedly reduced the amount of irritation observed.

Ten per cent solution of Dowfume EB-5 in propylene glycol produced a more severe response than did the undiluted material. Slight pain was apparent immediately; moderate conjunctival irritation and corneal injury developed within 2 hours and persisted for 48 hours before these effects began to subside. Again prompt washing helped to reduce the intensity of the injury. In all cases, however, final healing was complete and no permanent impairment of vision resulted.

When a 1% solution in propylene glycol was tested, it was very slightly painful and irritating to the conjunctival membranes but did not cause any corneal injury. All evidence of adverse effect disappeared within a few days.

Skin Contact

Rabbits

Procedure. The effects of the fumigant upon the skin were determined by applying the undiluted material to the open face of the ear and by bandaging a compress wet with the material onto the shaven belly.

The method employed to evaluate the hazard from absorption through the skin was essentially that of Draize *et al.* (*4*), except that the impervious sleeves were covered by heavy cloth bandages and the animals were allowed the freedom of their cages for the 24-hour exposure period rather than being confined in stocks.

Results. Repeated contact upon the surfaces of the skin resulted in some slight irritation characterized by mild erythema and exfoliation. When the material was under a bandage, however, rather severe local burns developed. These were characterized by intense erythema, edema, blistering, and some denaturation.

The number of rabbits treated with measured amounts of Dowfume EB-5 and the number dying as a result of the applications are summarized in Table II. In all cases the material produced a moderate to severe effect upon the skin. Erythema, edema, and necrosis were always present. Healing with scar formation was the rule.

Vapor Inhalation Studies

Rats

Procedure. The experimental procedures were essentially those described (*8*). Only mature, young adult, albino rats from the stock colony of this laboratory were used in the vapor inhalation experiments. The liquid fumigant was metered at a constant rate and volatilized completely into the flow of air entering the 154-liter glass exposure chamber. Vapor concentrations were checked by combustion and determination of total halogen by the Volhard titration. Periodic analyses indicated that the vapor concentration was maintained within ±10% of the desired concentration.

Vapor concentrations were expressed as parts per million by volume calculated on the basis of an "average" molecular weight of 134 for the mixed vapors. The calculation of the average molecular weight was based on the mole fractions of the constituents of EB-5.

Animals that received single exposures were observed as to their behavior, body weight changes, and time of death. Survivors were observed for 2 to 3 weeks or until it was certain that they had fully recovered from the effects of the exposure as judged by gross appearance, behavior, and recovery of body weight.

Special matched groups of male rats were exposed to determine the nature of the toxic effects as indicated by the gross response of the animals, the type of organic injury to be expected from excessive single exposures, and the single exposures of maximum intensity not causing any evidence of toxic injury.

Mortality. The total number of rats used and the number that died from each exposure are listed in Table III. From these data, the exposures expected to cause 0.01, 50, and 99.99% mortality of rats were determined according to the method described by Litchfield and Wilcoxon (*6*). These points were plotted on log-log ordinates (Figure 1) and the lines *AB*, *XY*, and *CD* drawn to represent the intensities of single exposure which can be expected to produce death in essentially all rats ($LD_{99.99}$), 50% (LD_{50}), and essentially no rats ($LD_{0.01}$). The average factors for determining 19/20 confidence limits were 1.05 for the LD_{50} points and 1.38 for the points representing the two extremities.

Gross Response. When exposures were prolonged sufficiently, all concentrations studied produced drowsiness and unsteadiness or weakness, but none produced deep anesthesia. Deaths usually occurred within 4 days.

Organic Injury. Special matched groups of 10 male rats each were killed for examination 1 and 4 days following exposures that produced 20 to 60% mortality. The concentrations and exposure times used were: 9720 p.p.m. for 0.35 hour, 2450 p.p.m. for 1.35 hours, and 920 p.p.m. for 4.5 hours. A similar matched group of male rats was used as unexposed controls for comparative purposes.

Table II. Mortality of Rabbits Caused by Dowfume EB-5 Absorbed Through Intact Skin

Dowfume EB-5 Applied, G./Kg.	Period of Contact, Hours	Total No. of Rabbits	No. That Died
0.3	24	5	0
0.5	24	5	1
0.7	24	10	1
1.0	24	5	1
1.7	24	4	4

The exposed animals uniformly showed a moderately severe organic injury in both liver and kidney and a very slight organic injury in the lung. Injury in the liver consisted of a typical central fatty degeneration with some necrosis 1 day after exposure. There was definite evidence of regeneration in the livers from animals sacrificed 4 days following these exposures. Examination of the kidneys revealed congestion, hemorrhage, and degeneration of the tubular epithelium with hemoglobin and hyaline cast formation. The injury in the kidney was more severe after 4 days than after 1 day. There was also an increase in the absolute and relative weights of the liver and kidney.

Exposures Having No Adverse Effects. In order to determine the maximum intensities of single exposure without detectable adverse effect, special matched groups of 10 male rats each were killed and examined 20 to 24 hours following single exposures of decreasing intensity until groups were obtained without any detectable injury. Similar matched groups of male rats were used as unexposed controls for comparative purposes. The most sensitive criteria of injury consisted of a slight increase in the weight of the liver and slight histopathological changes in this organ.

The results of this experiment are

Table I. Acute Oral Toxicity of Dowfume EB-5 Administered to Laboratory Animals

Species	Sex	Total No. of Animals Used	LD_{50} (19/20 Confidence Limits), G./Kg.	Slope Function[a]
Rats	Male	75	0.78 (0.69–0.89)	1.14
	Female	70	0.55 (0.46–0.66)	1.49
Guinea pigs	Male	69	0.28 (0.25–0.32)	1.24
	Female	70	0.36 (0.32–0.41)	1.21
Rabbits	Mixed	69	0.29 (0.22–0.38)	1.35
Chicks	Unknown	36	0.78 (0.49–1.23)	1.99

[a] Fold change in dosage required to produce one unit standard deviation change in response along the line.

summarized in Table IV. From these data, line *EF*, Figure 1, was drawn to represent the most severe single exposures without adverse effect in the rat.

Feeding of Fumigated Grain to Livestock

As it seemed possible that farmers or others might feed freshly fumigated or poorly aerated grain to livestock, some evaluation of the hazards of such procedure was desired.

The following experiments were undertaken with this objective in mind.

Procedure for Fumigation. Grain (shelled corn or wheat) for experimental feeding was fumigated in 55-gallon drums equipped with gas-tight lids. At the end of specified fumigation periods samples were taken from the top 6 inches of grain and quickly sealed in tightly closed containers for transmittal to the analytical laboratory and to the feeding stations without the occurrence of appreciable aeration.

Chickens. Two experiments were conducted. In both, a group of 24 chickens weighing about 2.5 pounds each were divided into four equal groups, each to receive untreated corn, untreated wheat, treated corn, or treated wheat. All birds were maintained on similar untreated feed until they became accustomed to such feed before the experimental feeding was begun. All birds were starved 24 hours before the experimental feedings in order to ensure the rapid consumption of the treated grain. The experimental groups were maintained for 5 days on their respective diets.

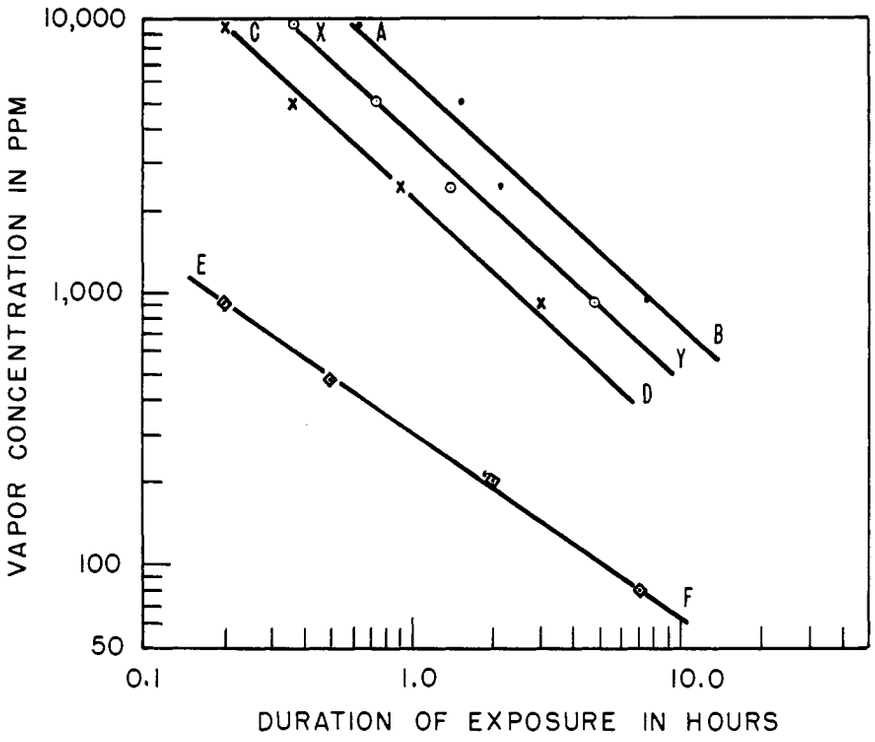


Figure 1. Vapor toxicity of Dowfume EB-5

AB. Intensities of single exposures expected to produce 99.99% mortality of rats
XY. Single exposures expected to produce 50.0% mortality
CD. Single exposures expected to produce 0.01% mortality
EF. Most severe single exposures not causing detectable adverse effects in rats

In the first experiment, the corn and wheat were fumigated at the rate of 4 gallons of EB-5 per 1000 bushels for 42 to 44 hours (see Table V, drum 2A). The second experiment was essentially a repeat of the first experiment, except that the grain was treated for 10 days instead of about 2 days (see Table V, drum 3A).

As judged by food consumption, weight gains, and general appearance, there was no evidence during or after either experiment to indicate that the feeding of the freshly treated corn or wheat had any adverse effects upon the chickens.

Swine. Eight litter mate hogs weighing from 67 to 79 pounds each were divided into two matched groups of four animals each, one group to serve as controls and the other as experimental animals. All were fed untreated whole corn and slop for several days before the experimental feeding began, in order to accustom them to the diet. All were starved for 24 hours before experimental feedings were begun.

The four animals in the experimental group were fed unaerated corn from drum 2 (see Table V) for 4 days without apparent ill effects. After a 3-day rest the same animals were fed the corn from drum 3 (see Table V) for 4 days, without apparent ill effect. After another 3-day rest, the same group was fed the twice fumigated corn from drum 4 (see Table V) for 4 days, again without evidence of adverse effect.

The untreated corn from drums 1 and 5 was used for the control group and for the experimental group during the 3-day rest periods.

Throughout the test period the ex-

Table III. Summary of Mortality Data for Single Exposures of Male Rats to Dowfume EB-5 Vapor

Vapor Concentration		Duration of Exposure,		
P.p.m.	Mg./l. (oz./1000 cu. ft.)	Hours	No. of Rats Used	No. That Died
9720	53.3	0.50	20	20
		0.40	20	18
		0.35	20	6
		0.25	20	1
		0.20	20	0
5040	27.6	1.00	20	20
		0.85	20	15
		0.75	20	13
		0.63	20	3
		0.50	20	1
		0.40	20	0
2450	13.4	2.00	20	20
		1.50	20	17
		1.35	20	4
		1.20	20	4
		1.00	19	0
920	5.0	7.0	20	20
		6.0	20	19
		5.0	20	16
		4.5	20	7
		4.0	18	1
		3.0	19	0
480	2.6	6.0	20	1

perimental group actually gained more weight than the control group and appeared to be in excellent health at all times.

Table IV. Single Exposures to Dowfume EB-5 Vapor Having No Adverse Effects on Male Rats

Vapor Concentration		Hours of Exposure	
P.p.m.	Mg./l.	Without adverse effect	With adverse effect
920	5.0	0.2	0.3
480	2.6	0.5	0.6
200	1.1	...	2.0
80	0.4	7.0	10.0

Cattle. Three heifers weighing from 650 to 750 pounds each were obtained from a stock dealer and kept for 2 weeks before experimental feeding began. During this period the animals were fed all the untreated shelled corn and alfalfa hay they wanted. Clinical observations including pulse rate, temperature, peristaltic activity, and general appearance were made daily by a veterinarian during the pre-experimental period of 2 weeks and the experimental period of 1 week.

During the experimental period all animals received hay and water *ad libitum* and as much corn as they would eat during an 8-hour period.

One animal received unaerated corn from drum 8 fumigated at the rate of 5 gallons of EB-5 per 1000 bushels for 7.5 days (see Table V). This animal consumed an average of 8.8 pounds per day and gave no indication of dislike for the grain, even though it smelled strongly of the fumigant, nor did she exhibit any evidence of adverse physiological effects at any time.

Another animal received unaerated corn from drum 10 fumigated at the rate of 10 gallons per 1000 bushels for 7.5 days (see Table V). This grain smelled very strongly of the fumigant and the animal refused to eat it until it had aerated for perhaps 1 to 3 hours. However, she consumed an average of 6.7 pounds per day during the experimental period without exhibiting any adverse effects.

The other animal received only untreated corn from drum 5 (see Table V) and consumed an average of 9 pounds per day.

All cows were held for a week after the experimental period and none showed any evidence of intoxication.

Significance of Results

Ingestion The results of studies on four species of laboratory animals (Table I) and one heifer show that Dowfume EB-5 is moderate in acute oral toxicity and that there are no marked differences in susceptibility between the species tested.

In the ordinary handling and usage of this product, there would appear to be no danger of ingesting toxic amounts. On the other hand, the accidental or willful swallowing of substantial quantities (perhaps of the order of 0.5 ounce) might jeopardize the life of an average adult human subject.

Eye Contact The studies on rabbits show that while undiluted EB-5 is not likely to cause permanent injury to the eye, appreciable pain and soreness lasting for several days may be expected. Strong solutions (10% or more) may cause more serious injury than the undiluted material. Weak solutions (1%) appear to be much less irritating. Eye protection adequate to prevent splashes into the eyes is recommended for those handling EB-5. If the eyes become contaminated, they should be flushed immediately with copious amounts of flowing water for at least 15 minutes. Medical attention then should be obtained.

Skin Contact When confined to the skin of rabbits, the mixture produced rather severe burns. However, on the uncovered skin where evaporation was uninhibited, it caused only mild irritation upon repeated contact; thus, occasional short exposures would not be expected to cause appreciable effects. EB-5 penetrates the intact skin and toxic amounts of the material might be absorbed as the result of an unusually severe exposure. In handling this fumigant, care should be exercised to prevent prolonged or frequent contacts with the liquid. It is particularly important to prevent contamination of any covering of the skin, such as shoes, gloves, or clothing. In case of contact, contaminated clothing, including shoes, should be removed immediately and not worn again until absolutely free of all odor of the fumigant. Contaminated skin should be cleansed promptly with soap and water. Medical attention should be obtained for any irritations or injuries that may develop.

Vapor Inhalation The experimental work with rats subjected to single exposures (Figure 1) shows that the vapors are moderately toxic if inhaled. Gross observation and histopathological examination of the rats exposed to the fumigant vapor revealed a type of action similar to that observed with the separate constituents. At high concentrations there was depression of the central nervous system (anesthesia). Appropriate exposures re-

Table V. Results of Analyses of Grain Fumigated with Dowfume EB-5[a]

Drum No.	Treatment (EB-5) Gal./1000 bu.	Days	Analytical Results, P.P.M. Total Br Corn	Total Br Wheat	Org. Br Corn	Org. Br Wheat	Ethylene Dibromide[b] Corn	Ethylene Dibromide[b] Wheat	Total Cl Corn	Total Cl Wheat
1A	0	0	0	0	7	7	8	8	320	320
			1	1	2	0	2	0	230	350
2A	4	1.8	61	136	26	132	31	155	423	489
			79	138	38	113	45	133	515	479
3A	4	10	64	103	20	75	24	88	442	374
			54	73	27	78	32	92	586	447
1	0	0	3		1		1			
2	4	1	53		28		33			
3	4	7	57		46		54			
4	4	7								
and then	4 for another	7	87		73.5		87			
5	0	0	0		1		1			
8	5	7.5	51		32		37[c]			
10	10	7.5	81		97		114[c]			

[a] Except as indicated, samples were taken at end of fumigation period from approximately top 6 inches of grain and without aeration.
[b] Calculated from organic bromide.
[c] Samples taken for analysis at end of feeding period (7 days after fumigation). Some aeration occurred from opening containers each day.

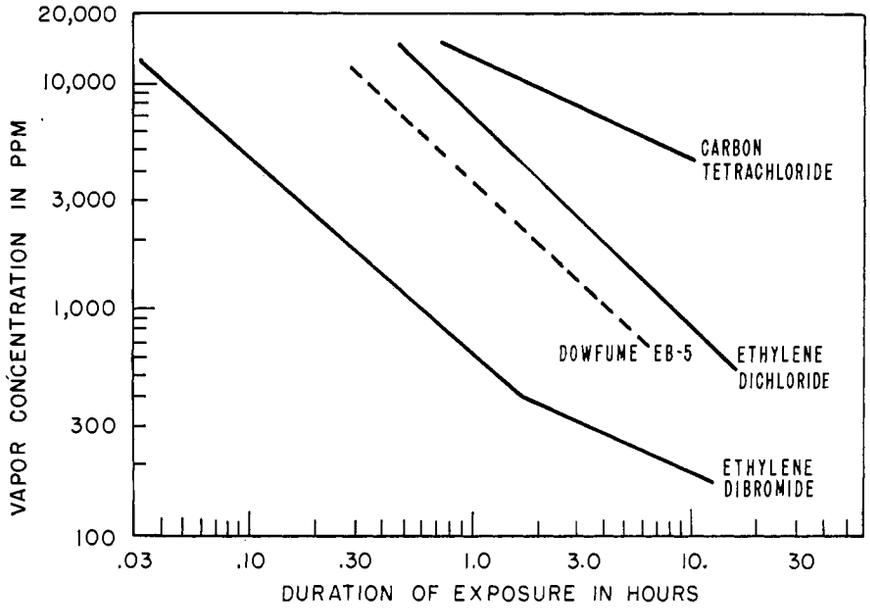


Figure 2. Vapor toxicity of Dowfume EB-5 and constituents

Intensities of single exposures producing 50% mortality to rats

sulted in an injury to the liver and kidneys similar to that produced by ethylene dichloride (*8*) and ethylene dibromide (*7*), and injury to the liver similar to that produced by carbon tetrachloride (*2*). The over-all similarity to the toxic effects observed with the constituents and with Dowfume EB-15 (*1*) is to be emphasized.

In Figure 2, data are presented for a quantitative comparison of the vapor toxicity of EB-5 and its constituents. The position of the line for carbon tetrachloride is an approximation; all the other lines were drawn by inspection through points calculated by the method of Litchfield and Wilcoxon using data such as those shown in Table III. On the basis of single exposures expected to produce mortality in 50% of rats tested, the vapor of EB-5 is slightly more toxic than that of ethylene dichloride and considerably less toxic than that of ethylene dibromide.

A different comparison of quantitative data is given in Figure 3, wherein exposures of maximum intensity without detectable injury to rats are presented. In this chart line *EF* from Figure 1 is compared with similar lines derived from studies of the individual constituents (*2,7,8*). The close proximity of the lines representing EB-5 and its major constituent, carbon tetrachloride, indicates that the capacity of EB-5 to cause organic injury, typified by slight changes in weight and histopathology of the liver, is similar to that of carbon tetrachloride, and that its chronic toxicity will be high and similar to that of carbon tetrachloride.

In evaluating the possible health hazards presented by exposure of human subjects to the vapor of Dowfume EB-5, the extensive experience from the industrial and solvent uses of the separate constituents may also be utilized. Thus, the measure of toxicity shown in Figure 3 affords a basis for evaluating the significance of workmen's exposure. The line representing the most severe single exposures to carbon tetrachloride without detectable injury to rats seems to be well verified for human subjects on the basis of general experience in industrial use and specific experiments with human subjects (*3,5*). Such a degree of verification is not available for ethylene dichloride and ethylene dibromide. A general industrial experience with these materials, however, indicates that the measures for exposures of several hours' duration shown for them cannot be greatly in error. Thus, it may be concluded that the line given in Figure 3 for Dowfume EB-5 (*EF*, Figure 1) may be used with little probability of error by the industrial hygienist for evaluating single exposures of workmen. This standard must be used as an absolute maximum; any single exposure must have an intensity definitely below those represented by the line to be considered to be without adverse effect.

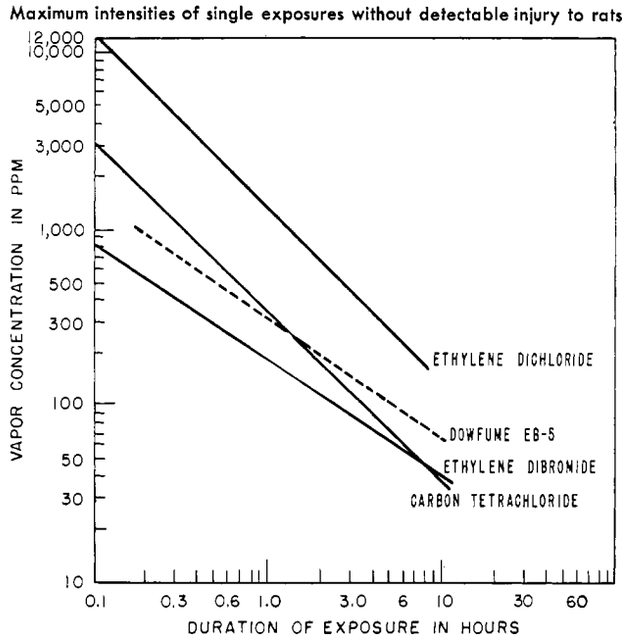
The hazard from breathing acutely dangerous amounts of Dowfume EB-5 vapor probably is not so great as the toxicity measures might indicate. Vapor concentrations below those which are dangerous to life upon short exposure have a definite, and perhaps sickening, odor. While this property of the vapor may not be sufficiently disagreeable to drive a person out of an acutely dangerous atmosphere, it certainly will warn of the presence of the vapor.

Although studies of chronic effects of vapor have not been conducted directly with EB-5, the constituents of this product have been extensively studied (*2,7,8*). The results of these studies have shown that carbon tetrachloride and ethylene dibromide are similar in capacity to cause injury and that both are appreciably more potent in this respect than ethylene dichloride. Therefore, as carbon tetrachloride appears in much larger proportions than ethylene dibromide, it is believed that if the vapor concentration of EB-5 is controlled to the limits safe for repeated exposures to carbon tetrachloride, the vapor concentrations of the other constituents will automatically be controlled to safe limits.

Procedures for the handling and use of EB-5 as a grain fumigant must ensure that exposures to vapor are maintained at safe levels. If it is necessary for persons to enter an area contaminated with EB-5 vapor—during the application to grain stored within a building, for example—a gas mask must be worn. In most instances, a full-face gas mask with a black canister for organic vapors will be suitable. Canisters should be replaced every 30 minutes, or oftener if any

Figure 3. Vapor toxicity of Dowfume EB-5 and constituents

Maximum intensities of single exposures without detectable injury to rats



odors are detected coming through the mask. Special precautions should be taken to prevent unsuspecting persons from entering a building where a fumigation is taking place and even during the aeration period, until all odors of the fumigant have vanished.

If a person should be overcome from vapors or feel ill from inhaling them, he should be removed to fresh air and kept warm. Medical attention should be obtained promptly.

Feeding of Livestock The experiments with animals indicate that the feeding of grain freshly fumigated with Dowfume EB-5 at the dosages used constituted no hazard to life for chickens, hogs, or cattle. Actually, the freshly fumigated wheat and corn used in these tests were taken from the tops of the drums where the concentration of fumigant (particularly ethylene dibromide) is the highest. All samples gave off a strong, definite odor of the fumigant immediately prior to feeding. In the commercial use of EB-5, it is recommended that fumigated grain not be fed to livestock until aeration has been adequate to remove all odor of the fumigant, thus assuring an even greater margin of safety.

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INSECTICIDE RESIDUES

Simplified Method of Estimating DDT Residues

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Nitrated DDT reacts with isopropylamine in benzene solution to give an intense yellow color which is more stable than the color similarly produced by alcoholic potassium hydroxide. Traces of DDT may be estimated by the use of this reaction. It has advantages where the work must be done under primitive laboratory conditions.

A SIMPLE METHOD of estimating DDT deposits on leaves or slides placed in the field during spraying trials was required for estimating efficiency of deposition of spray material and the gradual loss of the deposits from leaf surfaces. It was essential that the method should be rapid, capable of use where full laboratory facilities are not available, and sufficiently simple to be used by semiskilled assistants.

Of the two methods in use at present, the Schechter-Haller method (*2*) has the required sensitivity, but is tedious when used on many samples. The Alessandrini method (*1*), although simple, has the serious drawback that the color produced fades rapidly and must be estimated immediately. The blue color shows a red fluorescence which, under certain conditions, confuses the color matching. No suitable color standards have yet been found and the technique requires a spectrophotometer.

A modification of the Alessandrini method has, therefore, been attempted, substituting an amine for alcoholic potassium hydroxide in the production of a color with the tetranitro derivative of DDT. A number of amines were tried and it was found that the rate of development of color was in the order primary > secondary > tertiary; with primary amines the color develops instantaneously. Isopropylamine was chosen because of its availability and lower volatility than the lower members of the series. It gives an intense yellow compound soluble in benzene, with a single absorption peak at 3500 A. The reaction has not been investigated, but is presumably the replacement of chlorine by amine in a manner analogous to the reaction of picryl chloride and amines.

Although the color is much more stable, even in the presence of strong alkalies, than that developed by potassium hydroxide, it tends to redden appreciably after several hours, and permanent color standards cannot, therefore, be made up using the isopropylamine compound. However, aqueous solutions of potassium dichromate can be prepared at various dilutions to give tolerably good permanent color standards. No independent investigation was made of the effect of other insecticides, because the method was required for assessment.

It is intended to test this method under semifield conditions in the tropics, using native assistants. The method is expected to be as quick to use as the Alessandrini method and sensitive down to 0.05 mg. of DDT, and between 0.1 and 1.0 mg. the results are reproducible to the nearest 0.1 mg. For deposits of 1 to 10 mg., the color is diluted 1 to 10 with benzene, giving results reproducible to the nearest milligram.

The stability of this yellow color gives great advantage, as by successive dilutions the range of the estimation can be extended to cover 0.05 to 100 mg.

Method of Estimating DDT

Wipe the slide or leaf surface gently with a clean cotton swab washed with ether. Wash the cotton with ether through a funnel into a clean test tube;