LIMITING CONCENTRATIONS FOR FUMIGATION EFFICIENCY IN THE CONTROL OF INSECT PESTS

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INTRODUCTION: Fumigation is a control method often carried out at short notice in an emergency when a heavy infestation is noticed or when quarantine regulations demand during transit in or out of ports. It is an expensive method of control, largely because of the cost of immobilising transport or working machinery at factories, mills, docks, etc. The event of a fumigation failure may therefore be financially no less than catastrophic and over the years this has justified a high input on research into improving fumigation efficacy, i.e., keeping an infested commodity in a toxic atmosphere for long enough to kill all pests present. This paper investigates what constitutes a toxic atmosphere for two fumigants, methyl bromide and phosphine, and how to estimate the time required for action. Fumigation efficiency is here defined as an economy of dosage, measured as a product of concentration and time, in achieving control.

For most fumigants, the concentration-time (CT) product has proved a useful expression of dosage as usually long exposures at low concentrations give comparable results to shorter exposures at higher concentrations, i.e., mg/l x hr (combined units mg hr/l) approximates to k, a constant for mortality. With phosphine, this principle is not expressed clearly because of the enormous variations in insect tolerance as development proceeds from egg to adult (Table I), some stages appearing to be immune for short

Table I
CT PRODUCTS (mg h/l) for 100% KILL OF LIFE STAGES OF E.*cautella* (2-DAY EXPOSURES FOR PH_3)

	EGG	LARVA	PUPA	ADULT
CH_3Br	26	31	50	< 15
PH_3	77	1.4	2.6	< 0.7

periods. These stages can be killed only if the exposure time is long enough to allow them to develop to a stage of susceptibility (1). Hence for phosphine mortality is often related more closely to exposure time than to CT product when a mixture of stages or a natural population is fumigated. If the variations due to insect

development can be eliminated, however, by choosing a stage not undergoing rapid change, then, even for phosphine, a relationship of C x T approximating to k can be discerned and fumigant efficiency assessed. The test insect chosen for this study was the diapausing larva of the warehouse moth *Ephestia elutella*, a stage persisting for months in nature and with a low metabolic rate at moderate temperatures.

For all fumigants, the CT principle breaks down for very low concentrations or too short exposure periods because at these levels some individuals survive CT products normally lethal to them. The present work investigates such limits for methyl bromide and phosphine and describes some factors influencing them. All tests were conducted in 1700l. steel fumigation chambers fitted with ports for the transfer of insect material, and gas samples were taken using evacuated glass flasks at intervals throughout each experiment (2).

LIMITING CONCENTRATIONS FOR EFFICIENT ACTION OF METHYL BROMIDE

A pilot series of tests over the concentration range of 0.6 to 20 mg/l at 20°C subjected diapausing larvae to three standard CT products which at non-extreme concentrations were expected to give about 25%, 50%, and 70% kill. Estimated CT products for 50% kill (LD 50) indicated that maximum efficiency was obtained with concentrations of 3-15 mg/l and that outside this range LD 50 values increased (2). At 20 mg/l and an exposure range of 2-4 2-4-1/2 hr the loss of efficiency was small but at 14.6 mg/l kill was recorded at all. It was decided to investigate the effect of temperature on the concentration threshold, a factor heretofore largely ignored, and six concentrations between 0.9 and 6.0 mg/l were tested at 15 and 25°C.

Some lower concentrations proved less toxic at 25° than at 15°C (Figure 1). The reason for this reversal of the usual order of tolerance was twofold. Firstly there was a higher threshold for efficient action at 25° than at 15°C, some individuals at 25°C surviving higher than expected CT products at 2.7 and 1.9 mg/l. Secondly at and below the threshold, the rate of loss of efficiency (i.e. rate of increase in LD values) as concentration was lowered was not constant at 25°C, being at first gradual (4.0 - 1.9 mg/l) and then rapid (<1.9 mg/l). In general, observations at the LD 50 revealed smaller changes and at 25°C indicated a somewhat lower threshold level than at the LD 99. This was because although 2.7 mg/l was too low to be effective against a few individuals, for most individuals both 2.7 and 1.9 mg/l were still effective. Thus in Figure 1 the 15 and 25 C lines cross between 2.7 and 1.9 mg/l at the LD 99 and between 1.9 and 1.3 mg/l at the LD 50. For practical purposes the threshold identified at the LD 50 is an underestimate of the lowest concentration for efficient action, and better results are obtained by consideration of an extreme mortality level. All members of a population need to be controlled for fumigation to be viable.

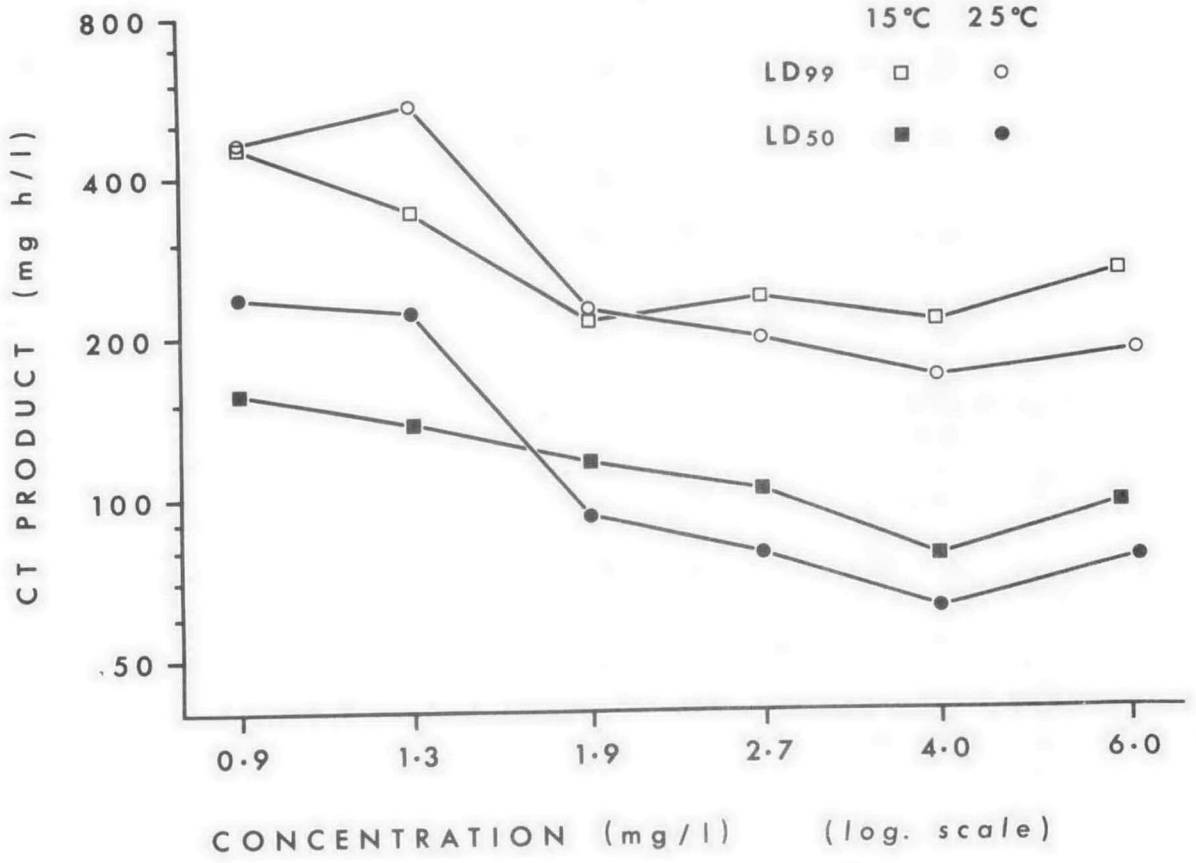

Figure 1 Variation of LD 50 and LD 99 values with temperature, testing six low methyl bromide concentrations against diapausing larvae of *Ephestia elutella*.

Another aspect which has received little attention is the effect of exposure to a high (well above threshold) concentration on the subsequent tolerance to low concentration, i.e., a fumigation with a fall in concentration. This is, of course, the form of the exposure in most practical fumigations, and knowledge of the efficiency threshold for the 'tail' of the concentration curve is necessary for meaningful decisions on their proposed duration. The recommendation to extend a fumigation without regassing would thus depend on the concentration level and its rate of fall-off, with a defined terminal concentration in mind.

In laboratory tests, two concentrations were established in separate chambers at 25°C. Larvae were exposed firstly at 12 mg/l, a concentration well above the threshold range but lower than many occurring in practice, and were then quickly transferred to a selected low concentration in the second chamber. The exposure at high concentration was initially set at a length expected to achieve no more than 10-20% kill. A slight depression of the concentration threshold was discernible, concentrations down to 2.8 mg/l retaining full efficiency (Table II). More recently a

TABLE II
CT PRODUCTS FOR LD 50 and LD 99 OF DIAPAUSE LARVAE EXPOSED AT 25°C TO LOW CH_3Br CONCENTRATIONS AFTER 12 mg/l for 3-1/2 HOURS

CONCN.(mg/l):		4.1 ± 0.1	2.8	1.9	1.2 ± 0.1
EXPOSED 3½ hr AT 12 mg/l	LD 50	62	74	100	147
	LD 99	144	151	275	431
EXPOSED AT LOW CONCENTRATION ONLY	LD 50	62	79	92	220
	LD 99	166	198	225	544

second series of fumigations has been conducted in which exposure at 12 mg/l was extended to give an expected kill of about 70% (CT product 90 mg hr/l). The latest results from this series indicate a concentration threshold of about half the value obtained at 25°C without exposure to high concentration. Thus the higher the level of kill, the lower the concentration that can be tolerated by survivors.

THE EFFICIENCY OF PHOSPHINE OVER A WIDE RANGE OF CONCENTRATIONS at 20°C. In the first series of tests a concentration range of 0.02 to 1.4 mg/l was examined using three standard CT products as described for methyl bromide (2). Estimates of LD 50 gave no evidence of a fall-off in efficiency at the lower concentrations tested; indeed the lowest concentration of all gave the lowest LD 50. At higher concentrations it was clear that efficiency decreased markedly, the LD 50 at 0.75 mg/l being much higher than at 0.27 mg/l. However LD 50 values for 0.75 and 1.0 mg/l were obtained only by extrapolation and no significant kill at all was obtained at 1.4 mg/l at which all exposures were less than 6 hr. Additional data were thus needed to clarify results. Further examination of the effect of low concentrations was also needed because a previous test with a gradually falling concentration had identified a threshold near 0.03 mg/l. In this test, very little increase in kill was obtained after the concentration fell to this level (Figure 2).

To obtain a more complete picture of the concentration limits for efficient action of phosphine, larvae were fumigated at 20°C at concentrations of 0.014-4.0 mg/l and exposure periods of 3-1/2 hr to 43 days, giving a CT product range of 3.5-56 mg h/l. Examining firstly the effect of low concentrations it is evident that a loss of fumigant efficiency occurs below 0.04 mg/l, as judged by an increase in the CT product for 90 and 99% kill (Figure 3, Table III). However, this loss of efficiency is not evident at the LD 50 and hence remained undetected in the pilot test series. At higher concentrations (0.5 mg/l and above) fumigant efficiency declined to such a marked extent that LD 99 values had to be calculated by extrapolation beyond the tested exposure ranges. This deterioration in fumigant efficiency was also evident at the LD 90, but not at the LD 50 at 0.7 mg/l and below. Concentrations of 0.5 mg/l and above are described by Winks (3) as narcotic and it is possible that from this level upwards a few individuals become narcotised and survive for longer than expected, causing curvature of probit lines and increasing the LD 99 rather than the LD 50 in a linear fit.

A further series of six tests was conducted at 1.5-6 mg/l, each test having eight exposures from 3-1/2 hr to 40 hr. Single larvae survived the 40 hr exposure at 1.5 and 2.0 mg/l, but in the other four tests complete kill was obtained in both 40 and 28 hr exposures. The LD 99 values calculated were similar to their projected counterparts in the earlier series, but indicated that above 2 mg/l the whole population was narcotised and mortality was dependent solely on duration of exposure (Table IV). The time for 50% kill (LT 50) at these concentrations was about 9 hr and the LT 99 about 29 hr.

For phosphine, then, four phases of efficiency can be distinguished as concentration is increased from very low to very high levels. Firstly, there is the below-threshold region up to

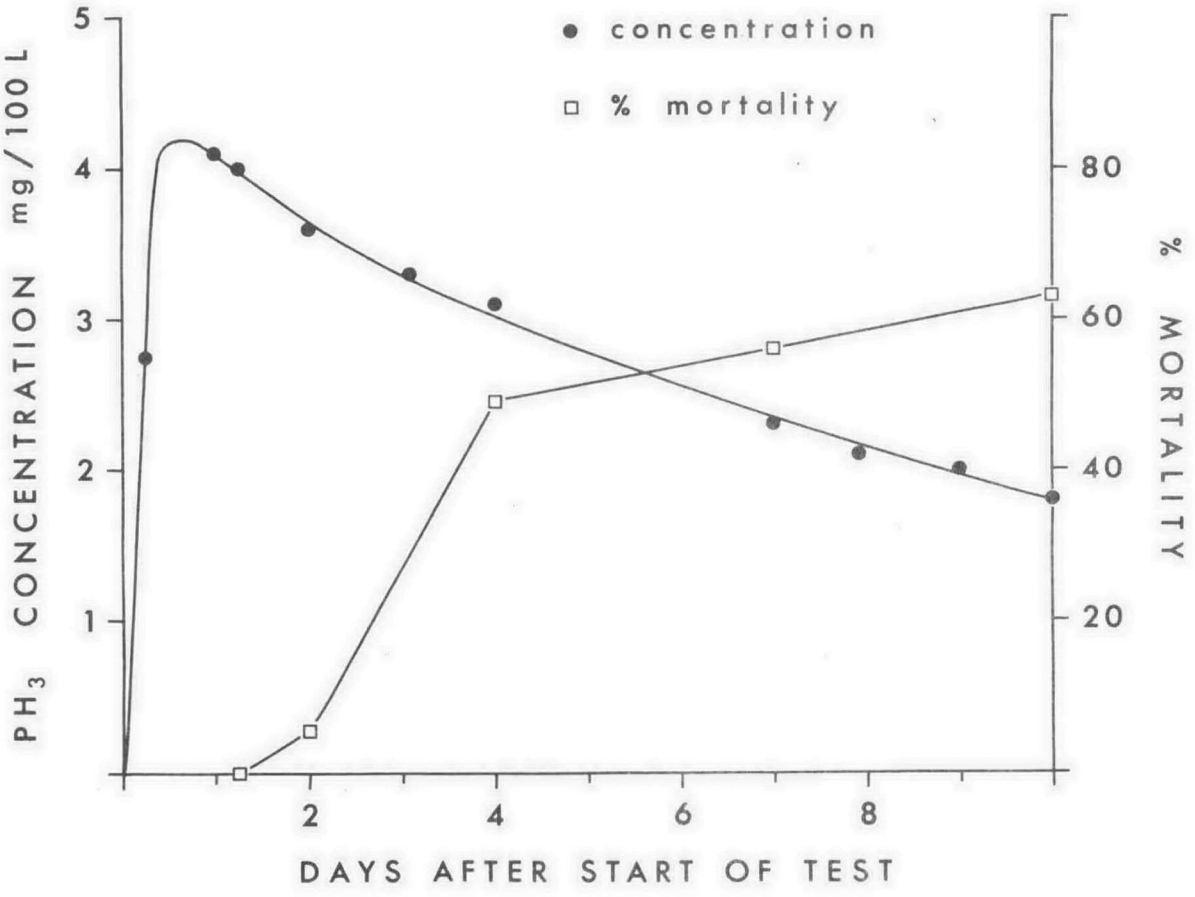

Figure 2. Effect of a low and falling concentration of phosphine on the mortality of diapausing larvae of *Ephestia elutella* at 25°C

0.04 mg/l with some individuals surviving disproportionally long periods and reducing efficiency. Between 0.04 and 0.35 mg/l the action of phosphine is highly efficient and the CT principle holds as shown by the linear portion of Fig. 3. The slope of this

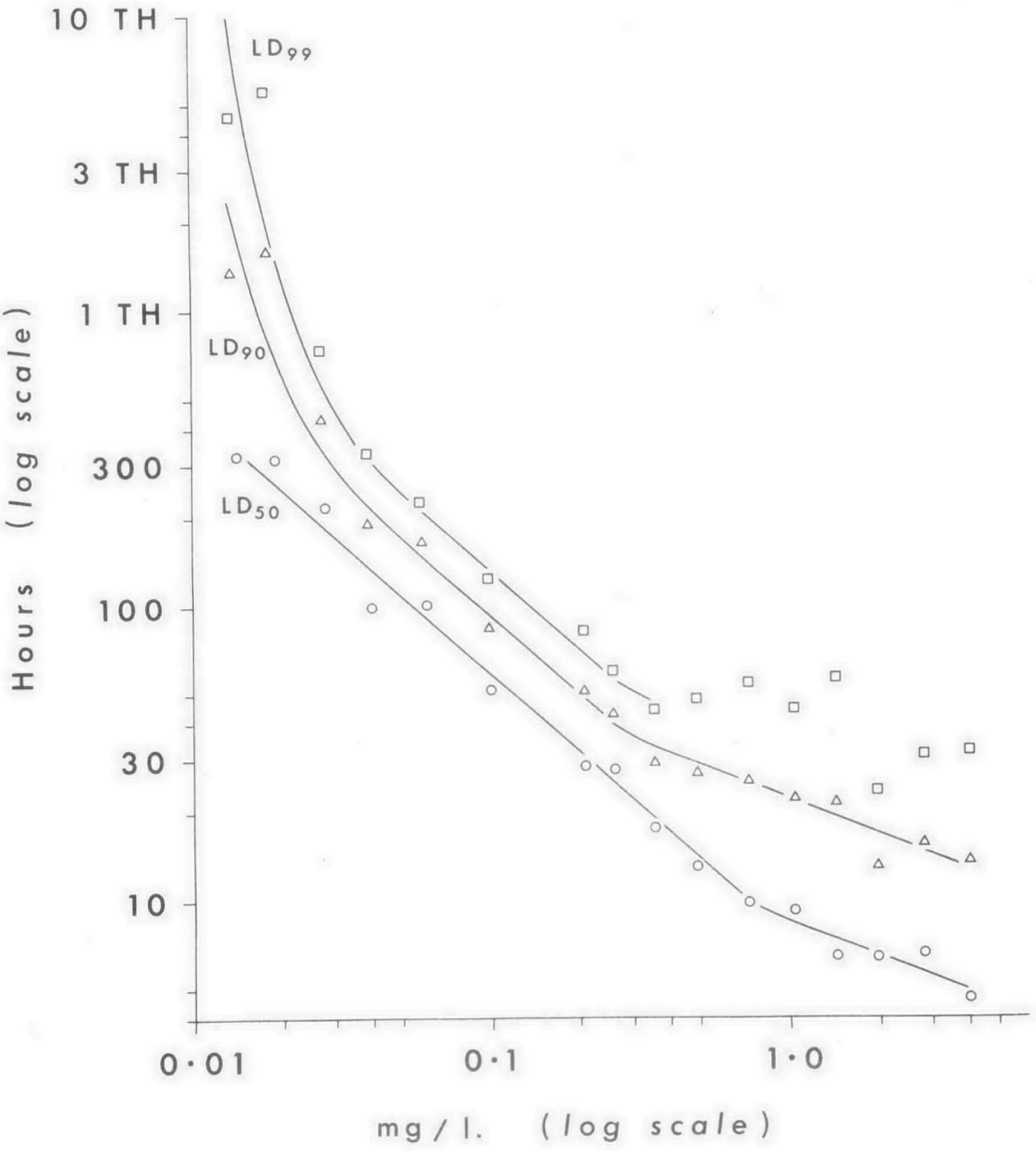

Figure 3. The relationship between time and concentration of phosphine for mortality of *Ephestia elutella* diapausing larvae fumigated at 20°C

TABLE III. TOXICITY OF LOW CONCENTRATIONS OF PHOSPHINE TO *E. elutella* DIAPAUSE LARVAE (AT 20°C)

CONCN. (mg/l)	LD 50 (mg h/l)	LD 99 (mg h/l)
0.014	4.5	65
0.019	6.1	103
0.027	6.1	20
0.039	4.0	12
0.058	6.0	13

TABLE IV. EXPOSURE TIME FOR LD 50 and LD 99 of DIAPAUSE LARVAE IN HIGH DOSE PHOSPHINE TESTS AT 20°C

CONCENTRATION mg/l	LD 50 (hr)	LD 99 (hr)
5.8	9	29
4.1	9	29
2.8	8	25
2.3	9	29
2.0	16	50
1.5	13	44

linear portion is -0.9, indicating a slight time bias in the CT relationship ($C^{0.9} T = K$). At 0.04-0.10 mg/l, a CT product of 14 mg h/l is adequate for 100% kill of the test insects.

Above 0.35 mg/l the efficiency of phosphine declines, but increases in concentration up to 2 mg/l continue to shorten the exposure required for 100% kill. A heavily time-biased CT relationship (approximately to $C^{0.4} T = K$) may still operate over this concentration range. Lastly, for concentrations above 2 mg/l, mortality is determined by time of exposure only, individuals varying in their capacity to survive in a narcotised state.

As temperature has already been shown to influence the minimum effective concentration with methyl bromide, some pilot tests have been conducted on the effect of temperature on the minimum exposure for 99% kill with phosphine. Again (Table V)

TABLE V PERCENTAGE KILL OF *Ephestia elutella* DIAPAUSING LARVAE IN HIGH DOSE PHOSPHINE TESTS

EXPOSURE (HOURS)	2.0 mg/l		4.1 mg/l	
	15°C	20°C	15°C	20°C
3.5	37	23	63	33
5	56	46	80	68
7	89	50	79	79
10	89	54	89	75
14	93	78	98	95
20	98	90	100	98
28	100	93	100	100
40	100	98	100	100

a reversal is seen in the order of tolerance at higher and lower temperatures, tests at 2 and 4 mg/l proving more toxic at 15°C than at 20°C. LT 99 values calculated for the two concentrations at 15°C are similar (22-23 hr) indicating that time alone was governing mortality. The minimum effective exposure period for complete control, like the LT 99, is likely to be shorter at 15°C than at 20°C.

DISCUSSION AND CONCLUSIONS: For methyl bromide the maximum concentration range for high fumigation efficiency in normal practice is about 2-20 mg/l, but concentrations up to 4 mg/l may prove ineffective against certain species in warm conditions. Very low concentrations are more toxic at low temperatures than at higher ones, and are more effective when following exposure to higher concentrations.

For phosphine, a CT relationship exists over a restricted concentration range but is usually observed by insect development. In general, all concentrations above a very low minimum (0.03 mg/l for *Ephestia elutella* diapausing larvae at 20°C) are effective, but no advantage is gained in terms of extra kill by increases above 2 mg/l. However, because insect development largely controls mortality, with phosphine long exposures are always required to span tolerant stages and achieve control, especially at lower temperatures, and high initial doses may be required to ensure that gas is present at the end of the exposure. High concentrations may induce a state of narcosis in insects exposed but the period so survived is relatively short, especially at lower temperatures.

Against this background, factors contributing to the development of resistance can be viewed more clearly. The worst situation for long term efficacy is persistent exposure of an insect population to low concentrations at high temperature. With methyl bromide the biggest danger is when highly sorptive commodities such as oilseed cakes and fishmeal are treated as these can lower the free-space concentration to threshold levels. The minimum effective concentration increases with temperature, implying that for a given temperature rise the rate of detoxification increases more rapidly than the rate of uptake. This being so, individuals surviving marginal treatments at higher temperatures will be the fastest detoxifiers. At lower temperatures, individuals with slow rates of uptake may prevail equally well. Hence the danger of low dosage levels at high temperature is the rapid selection for resistance based on detoxification. It is fortuitous, perhaps, that warm rearing conditions have been used in laboratory selection studies on resistance. For resistance based on slow uptake, however, a different approach may be required.

Because of the temperature effect, it can be seen that fumigant resistance is more likely to appear and cause problems in tropical rather than temperate zones. With a fumigant like phosphine the possibility of narcosis provides an additional resistance mechanism. Selection for this type of resistance is most likely to occur by repeated exposure of a population to high dosages for short periods, again at high temperature. Fumigation of leaky containers or railway trucks in the propics provide examples of how this combination of conditions can occur in practice.

At present resistance to fumigants has not become a serious economic problem. Some resistance to phosphine in short exposures was reported in the 1973 Global Survey (4) but all strains that have been tested with longer exposure periods have shown more normal tolerance. The threat of future increases in tolerance cannot, however, be ignored.

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