PROBLEMS IN THE LABORATORY INVESTIGATION
OF THE TOXICITY OF PHOSPHINE TO STORED
PRODUCT INSECTS

R. W. HOWE
Pest Infestation Control Laboratory, Slough, Berks., England

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Abstract—The principles to be observed in determining the toxicity of fumigants to stored product insects by means of laboratory experiments, and the circumstances in which these must be modified for phosphine are discussed. After assessing the published literature two kinds of experiment are recommended, to imitate the levels of concentration and exposures used in practice and to limit the exposure period to 8 or 24 hr. For the latter exposures higher concentrations than are used in practice would be required and it might not be possible to achieve complete mortality of test samples. However, this technique should separate the tolerant and susceptible stages of the life cycle and reveal any stages of intermediate susceptibility. The maximum duration of the developmental period of the tolerant stages would represent the minimum exposure period for phosphine desirable in practice provided all the individuals in a tolerant stage developed and reached a susceptible one.

INTRODUCTION

The use of fumigants is increasing where quarantine measures have been introduced, and in conditions where infestation may pose a hazard to the safe storage of food. Thus a fumigant such as phosphine, which can be applied simply as a tablet, pellet or powder and leaves little residue in the treated produce has much to commend it. Wherever application is not too laborious, and provided that no unsuspected hazards are discovered and a relatively long fumigation period is not inconvenient, phosphine is likely to increase in use.

Another reason for an increase of use of fumigants is the spread of insects resistant to insecticides. A residual insecticide can only kill those insects that come into contact with a treated surface. Many insects will acquire only a sub-lethal dose and their offspring may be still harder to kill with this insecticide. There is less opportunity for an insect to avoid a toxic dose of a fumigant, especially one like phosphine that penetrates well. If the operator can ensure that a toxic dose is efficiently applied there should be no survivors. If there are survivors of a fumigation, we can choose whether or not we subject them to further fumigation and so risk developing resistance whereas once a persistent residual insecticide has been applied, it remains part of the environment for a considerable time. We must ensure that fumigations are done properly to avoid this problem.

There are numerous accounts of the successful practical use of phosphine (Rai et al., 1963) but on some occasions insects or mites have survived (Sinha et al., 1967). We must not rely too heavily on the apparent successes of practical fumigations since a critical and exhaustive assessment of such trials is difficult. Even laboratory data from experiments with phosphine
are not very consistent. It is well worthwhile surveying the published literature to consider
the objectives of the laboratory studies reported and the assumptions implicit in the ap-
proaches made by various authors. Because the problems of investigating phosphine appear
superficially to differ in several ways from those of other fumigants, I will start with a brief
outline of the underlying principles of the laboratory study of the toxicity of a typical
fumigant, methyl bromide, to stored product insects. These have already been
discussed by Monro (1969) with some emphasis on the practical aspects of fumigation. I
will then search for an explanation for the erratic inconsistencies of reported results for
phosphine and try to propose a method of experiment that will give consistent results. To
this end a selection of published papers must be reviewed in an effort to determine whether
the sources of variability lie in the techniques used, in the insects, or in the poison.

REMARKS ON A TYPICAL FUMIGANT

Laboratory experiments on the toxicity of fumigant gases to insects are usually performed
with direct practical objectives or with theoretical objectives needed to elucidate practical
aims. The most usual practical objective is to determine the lowest dosage of fumigant that
can be relied upon to kill every individual in the insect population. The theoretical aims are
usually comparative, to discover whether one species is more or less susceptible than another
to a particular fumigant, how susceptibility changes as the insect develops and the adult
ages, or how tolerance is influenced by the physical environment or by the methods of
handling the insects. For such theoretical work the LD$_{50}$, the dose that kills exactly half the
-treated population, is a convenient basis for comparison. It can usually be determined with
reasonable accuracy and almost always more accurately than any other selected percentage
mortality, but it has little practical utility.

Requirements for basic studies

The eventual purpose of amassing basic knowledge about the toxicity of a fumigant to an
insect, however, is to understand the practical control of populations of the species. With
the longer established fumigants such as methyl bromide, it is usually possible to gain
enough information from fairly simple laboratory experiments to judge the dosages needed
in practice, but it is impossible to do so with a given statistical precision. Not only do we
need to know the amount of gas required per litre of air to kill all the insects at the environ-
mental temperature, but we have to estimate the amount of gas absorbed by the product,
the probable losses by leakage and the probable unevenness of gas distribution in the treated
stack. The concentrations of gas present at various points in a fumigated bulk can be
measured but this is seldom possible at enough points or as a routine.

The underlying assumptions of experiments to determine the LD$_{50}$ are given fully by
Finney (1971). The most important of these is that the tolerance curve representing the
mortality of an insect population plotted against the logarithm of the dosage of poison,
whether expressed as concentration, time or their product, should be normally distributed.
For this to be likely, the insect population must be of a homogeneous age and the duration
of the exposure to the fumigant must be short relative to the rate of change of tolerance.
This tolerance of an insect to a fumigant changes as the insect develops from one stage to
another and also as it grows within each stage. Although susceptibility continues to change
as the adult ages, the rate of change is probably slowest in the adult stage. Unfortunately,
eggs and pupae are usually the most difficult stages to kill by fumigation, so for practical
success we must determine the dosages needed to kill these two stages and we cannot limit ourselves to the more accurate experiments possible with adults. With methyl bromide, exposures at practical dose levels need not be carried beyond 8 hr even though for convenience fumigations are often longer in practice. Experiments to determine and compare the toxicity of fumigants like methyl bromide can, therefore, be completed during a working day and the susceptibility of the insects is not likely to change much during the period of the experiment.

If the assumption that the tolerance of the insects to the fumigant is normally distributed is true, then the mortality at a dose, expressed as a percentage, can be transformed to a probit and the curve relating this probit \(y\) to the logarithm of the dosage applied \(x\) will be a straight line,

\[
y = a + b \log x.
\]

In experiments, there are two ways of varying dosages to determine the position of such a probit line. The samples of insects can be exposed either for a fixed period of time to a series of concentrations or to a fixed concentration for a range of times. If both concentration \(C\) and time \(T\) are varied, a probit plane,

\[
y = a + b_1 \log C + b_2 \log T,
\]

can be fitted. When the probit method is valid the mortality will increase with dosage. If the dose is varied by changing the time at a single concentration, decreases of mortality with increase of dose can be explained as random variation. If the concentration is varied for fixed exposures, however, there may be a real discontinuity or a curvature in the probit line if the gas narcotizes the insects or in some other way depresses the metabolism so that it protects them from poisoning.

The choice between the two approaches is largely a matter of convenience. From the literature it seems that varying the concentration is the method most often used but at the Pest Infestation Control Laboratory we vary the exposure period because we have large chambers from which cages containing insects can be removed without a significant loss of gas. However, the London, Ontario Laboratory which also has large chambers usually uses a fixed exposure period.

An alternative transformation for percentage mortality is the logit (ASHTON, 1972). In some ways this is simpler for people without access to a computer because it does not require an iterative solution.

The concentration-time principle

With established fumigants such as methyl bromide, it has long been known that Haber's rule applies sufficiently well to be of use in practice. According to this rule, in a specified environment, the dose required to give a certain level of mortality can be expressed as a product of the time (usually in hours) and the gas concentration (usually in mg/l) which is approximately the same over a wide range of times and concentrations. That is to say, in the formula given above for the probit plane, the coefficients of both \(C\) and \(T\) are the same. The rule is known to have a threshold for both concentration and time below which it fails to apply (WHITNEY and WALKDEN, 1961). For methyl bromide, the lowest concentration at which the rule applies is about 2.5 mg/l, but from 3 mg/l upwards to about 15 mg/l, the
CT product required to give and LD_{50} falls gradually rather than remaining exactly constant (see also ESTES, 1965). This is unimportant in practice, however, because the errors it causes to the determination of the practical dosage are much less than the compensatory additions required to allow for leakage, adsorption and other sources of fumigant loss.

The experimental worker in the laboratory, therefore, has a wide range of concentrations available to enable him to conduct a satisfactory experiment within a working day of 8 hr. The aim of each experiment is to cover a range of concentrations or times such that the middle dose causes about 50 per cent mortality and the rest are spaced equally. For estimates of the LD_{50} the range of mortality should be at least 20–80 per cent and not more than 10–90 per cent. Dosages killing none of the test insects are rather wasteful, but for practical purposes an extra dosage level causing a complete kill is a useful guide to the practical dose required for an efficient fumigation.

In the past the numbers of different dosage levels used by many workers in fumigation experiments to estimate the position of the probit line representing the dosage mortality relationship has been too small, often too few insects were used in each sample and too few replicates made at each dosage level, but in much recent work, adequate numbers have been used throughout. I think that at least seven points are needed; one may be used to aim at a complete kill with the remainder balanced around the LD_{50}. It is not too difficult to meet these requirements using a single concentration and varying the exposure time on a logarithmic scale, but it imposes a great deal of labour where a period is fixed and several concentrations of fumigant are used. With seven or more adequately spaced points, it is usually possible to form a reasonable opinion as to whether they fall on a straight line or on a smooth curve, even when there is considerable random variation.

Factors affecting toxicity in laboratory experiments

MONRO (1969) points out that fumigant toxicity to an insect species may be influenced by temperature, humidity and food. In practice, this is partly attributable to changes in the amount of gas sorbed, this usually being enhanced by low temperature or high humidity. However, over the range of temperature that can reasonably be used fumigants are most toxic at the higher temperatures, some 2–4 times more at 25°C than at 10°C (BROWN, 1954). Thus fumigations at low temperatures require high doses and may still fail or may be unnecessary if the low temperature itself is fatal to the insect. Although humidity is not known to influence toxicity directly, it is clearly desirable to try to perform laboratory experiments in a controlled constant environment, holding the insects both before and after fumigation in these conditions. This is not always possible and the variations of result when temperatures must be changed during an experiment are dauntingly complex (PRADHAN and GOVINDAN, 1954).

In addition to the differences caused by sorption if foodstuffs are used in fumigation experiments, there are claims that the food on which the insect developed also influences its susceptibility. Thus MURTHY and SRIVASTAVA (1971) claim up to two-fold differences for a number of fumigants against adult Callosobruchus maculatus (F.) bred on various pulses and PUNJ (1970) estimates an increase approaching five-fold in the susceptibility of Trogoderma granarium larvae bred on groundnuts to ethylene dibromide compared with those bred on Phaseolus mungo. Yet Punj found the larvae bred on groundnuts were more tolerant to methyl bromide than those bred on any of his other nine foodstuffs.

It is difficult, however, to rely on the results of experiments with developing stages because
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changes of the order of at least 50-fold occur during the life cycle (HOWE and HOLE, 1966). Adult beetles are the most frequently used test stage, but about twice the adult dose is needed to achieve a comparable mortality of pupae of certain ages. Larvae may be used for species in which the adults are delicate or are short-lived, and for these toxicity may be related to size. If the susceptibility of the fumigant per unit weight remains constant, then each successive larval instar will be more difficult to control. Whilst pupae are almost always the most tolerant individuals, the much lighter eggs are sometimes nearly as tolerant and occasionally more so.

The selection of insects for experiments therefore, requires great care.

The end point for mortality

After test insects have been exposed to a fumigant, we must decide whether or not they have been killed, and since poisoned insects may not die quickly, this decision may have to be postponed for one or two weeks. A suitable date for making this decision can often be found by examining fumigated and control insects at frequent and regular intervals, possibly daily, and separating each sample into the two groups, living and dead. If there is a period during which no insects in any group die or recover, then it is sufficient to select a convenient point during this period as the standard time after fumigation to examine the insects in subsequent experiments. Sometimes, although for each dosage there is an interval in which there are no deaths, this interval is earlier for high dosages than for low ones. In these circumstances, no single standard time is suitable and several inspections of treated insects must be accepted as a routine and the mortality at each dosage determined from the stable period. In the worst circumstances, insects die sporadically more or less continuously and there is no definitive end point. Given computer facilities, it is possible to calculate a probit line for every examination, but I prefer to allow the treated populations to reproduce and count the individuals in the next generation (HOWE and HOLE, 1967). HEWLETT (1974) discusses in detail the interpretation of mortality data where the time to death is important and points out that the probit lines may be curved if the wrong time of examination is selected.

It is always advisable to prolong experiments by at least one life-history stage, because the lethal influence of a poison may be delayed. Doomed eggs may hatch into larvae that will not pupate whereas the true survivors will eventually yield healthy adults. Affected pupae may produce sterile adults that die quickly and treated adults may lay very few eggs that may be normal or infertile. This extension of the experiments may impose a burden but it should always be considered.

For experiments with immature weevils or bruchids, it is usually most convenient to allow the surviving individuals to emerge from the seed. It is, however, possible to dissect the seeds to look for the survivors, but this will not always give a reliable result for the reasons mentioned above.

The practical value of the CT product

In the laboratory the median lethal dose can be expressed as a concentration (LC50) if a fixed time is used, as a time (LT50) if a fixed concentration is used, or as a LD50 using the CT product for either. To decide the dose required in practice we need some value for LD100, which theoretically is not possible, and then to add allowances for loss of fumigant by leakage and sorption on material, and hence for a concentration of fumigant that changes as the fumigation proceeds. Thus the concentration of fumigant must be measured along
with temperature at many places in the course of fumigation. The values so obtained can be plotted on a graph against time and a concentration curve drawn and from this by determining the area under the curve, the CT product at each point of measurement estimated. These estimates of CT product are usually satisfactory for judging the efficacy of a fumigation against those species for which we have laboratory data for toxicity, providing that the threshold concentration is reached quickly. At this Laboratory, REYNOLDS et al. (1964) carried out two series of experiments in which concentrations of 1.5 and 6 and of 6 and 1.5 mg/l of methyl bromide were applied successively. When the low concentration was applied at the end of the experiment, it contributed fully to the total CT product, whereas that applied at the beginning caused no increase of mortality above that caused by the high concentration alone.

SPECIAL PROBLEMS ARISING WITH PHOSPHINE

Phosphine is not a new fumigant, but in spite of many convenient features and energetic marketing over the past 20 yr its use has not increased as much as I expected. One important reason leading operators to choose an alternative fumigant is the long period of exposure needed to ensure success in the cooler technical advanced regions of the world. Thus it cannot be fitted readily, like methyl bromide, into a rapid conveyor-belt system of fumigation.

At the same time laboratory experiments appear to yield contradictory results. Thus while QURESHI et al. (1965) reported that some of the susceptible adult stages of *Sitophilus granarius* (L.) survived concentrations of more than 20 mg/l, REYNOLDS et al. (1967) found that the much more tolerant egg and pupal stages of this species could be killed by a concentration as low as 0.01 mg/l. It is evident that biochemical work to find out exactly how this poison kills the insects is desirable, but meanwhile the literature should be examined to find clues as to the cause of these differences.

Laboratory work at the Pest Infestation Control Laboratory on the toxicity of phosphine produced from 'Phostoxin' (a commercial preparation of aluminium phosphide) to insects (REYNOLDS et al., 1967) was undertaken after field work had demonstrated the need for exposures of at least 5 days (HESELTINE and THOMPSON, 1957) so exposures of the order of 1–16 days have been almost consistently used in laboratory assays. The concentrations used, also, have been in the range measured in the course of practical fumigations, namely from 4 mg/l down to 0.01 mg/l, and most of the recent work has been in the lower part of this range (BELL and GLANVILLE, 1973). One consequence of the use of long exposure periods is that probit or logit methods are often invalid, but an advantage is that the experiments are relevant to practical control. Much of the work performed at the Pest Infestation Control Laboratory by Miss B. D. Hole has measured the exposure time from the introduction of the tablets or pellets of 'Phostoxin' and so includes the period during which phosphine is generated. She has collected much useful data as yet unpublished over a temperature range of 15°–30°C for a wide range of species.

Many other workers have accepted the need for exposure periods of at least 4 days (LINDGREN et al., 1958; OZER, 1961; MORI and KAWAMOTO, 1966; VINCENT and LINDGREN, 1972a) and all these workers have tested concentrations above 4 mg/l without killing all the insects fumigated. CHILDS et al. (1973) using a maximum concentration of 500 ppm (under 0.8 mg/l) killed most individuals of *Lasioderma serricorne* (F.) in 6 days or less at 15°C or higher.

Some of the experiments carried out at the London, Ontario, Laboratory (BOND and
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MONRO, 1961; QURESHI et al., 1965; ROBINSON and BOND, 1970), at Winnipeg (BARKER, 1969b) and at other laboratories (MUTHU et al., 1970; BANG and TELFORD, 1966) have been designed to complete experiments during a working day, and have usually included dosages well above 4 mg/l, even as high as 64 mg/l (JALIL et al., 1970). BARKER (1969a) used high concentrations of 8 mg/l or above for 20-40 hr.

Some authors appear to have accepted the validity of the CT principle and quote the time, concentration or CT needed to give a prescribed level of mortality and sometimes the slope of the probit line but give the range for only one variable. Thus MORI et al. (1969), MUTHU et al. (1971), RAJAK and HEWLETT (1971) and SATO et al. (1973), all quote the gas concentration used but not the periods of exposure. On the other hand, LINDGREN and VINCENT (1966), VINCENT and LINDGREN (1972b), BASKARAN and MOOKHERJEE (1971) and MURPHY and SRIVASTAVA (1971) quote exposure times but not gas concentrations. MUTHU (1973) states neither explicitly.

SATO et al. (1973) found that the CT principle held at low concentrations of up to 100 ppm (approximately 0.15 mg/l) for a 50 per cent knockdown of adult Callosobruchus chinensis (L.). BELL and GLANVILLE (1973) found that the CT principle held approximately for the LD$_{50}$ for exposures above about 15 hr and concentrations below about 0.32 mg/l for larvae of Ephesia elutella (Hb.) in diapause. Many papers, however, mention or illustrate that the slope of the dosage-mortality probit lines decreases above 90 per cent mortality. These include OZER (1961), QURESHI et al. (1965), LINDGREN and VINCENT (1966), BARKER (1969b) and VINCENT and LINDGREN (1972a).

MONRO et al. (1972) threw new light on this matter when they examined the possibility of inducing resistance to phosphine by laboratory selection. Their initial mortality curve for Sitophilus granarius as usual flattens above 90 per cent mortality but the curves for resistant populations flatten at a lower mortality and become very steep at 90 per cent mortality. HOWE and HOLE (1967) observed this kind of change of slope for methyl bromide and attributed it to heterogeneity of susceptibility to the fumigant. Such a flattening may well be a sign of potential phosphine resistance.

The published results of laboratory tests with phosphine give data for 26 species of stored product beetles, five moths, three mites, a cockroach and the housefly, but because of the diverse approaches already described these need careful interpretation. To date, the most reliable facts have been elucidated by the Ontario Laboratory. BOND et al. (1967) demonstrated that phosphine was not toxic to adult Sitophilus granarius in the absence of oxygen, and BOND et al. (1969) repeated the experiment with this species and with adults of Tribolium confusum Duv., Periplaneta americana (L.) and larvae of Tenebrio molitor L. The exposure periods were necessarily short so as not to kill the insects by oxygen deprivation but there was a greater uptake of phosphine by the beetles and irreversible phosphine poisoning of the cockroach in the presence of oxygen. Tribolium confusum absorbed more phosphine than Sitophilus granarius and did so more quickly. ROBINSON and BOND (1970) investigated further the uptake of phosphine by adult Tribolium confusum using radio-active phosphine and found that this species absorbed it quickly and proportionately more rapidly at higher concentrations. BOND and UPTITIS (1973) claim that a partitioned dose of phosphine applied 4 times at 2-3 day intervals is more effective than a single equivalent large dose against Tribolium confusum and Sitophilus granarius adults. A concentration that kills none if applied once for 5 hr, kills over 99 per cent when repeated 3 more times on alternate days. The Tribolium appeared able to repair the damage if allowed 10 days between fumigations but the weevils recovered less well. Larvae of Tenebriodes mauritanicus L. were also
vulnerable to a second fumigation for 5 hr within 10 days of the first, but results are complicated by the delay of death of many larvae for some weeks, by the failure of some still alive to pupate and by deformities of pupae and adults similar to those caused by exposure of prepupae to minimum threshold temperatures (HOwE, 1962).

Relative susceptibility of developmental stages and adults

The most complete comparison of the relative susceptibility of the different immature stages of a single species was reported by HowE (1973) for Sitophilus granarius. He obtained groups of age known to within 24 hr and exposed every age group up to 36-days-old at 25°C to 4 mg/l of phosphine for periods up to 8 hr, to 1 mg/l for periods from 8 up to 46 hr, and to 0.5 mg/l from 4 up to 128 hr. The first adults normally emerge from the wheat kernel on the 37th day in these conditions and adults of a similarly restricted age were exposed to 0.01 mg/l. The most tolerant stages were the egg and the pupa, larvae and adults being very susceptible. At 1 mg/l all eggs were killed in 32 hr but at 0.5 mg/l and below some eggs survived exposures of 120 hr by which time all should have reached the larval stage even if they had not yet hatched. Pupae were even more tolerant, a few surviving the longest exposure used at 4 and 1 mg/l, but all were killed by 90 hr at 0.5 mg/l. At 0.1 and 0.05 mg/l, 5 and 8 day exposures were necessary to kill all pupae.

Eggs were tolerant up to four days old and became more susceptible when the larva had developed. Pupae were most tolerant in the middle of the stage, at about 32-days-old, and adults gradually became more susceptible as they got older. Mori et al. (1969) using cultures laid over 24 hr also demonstrated an increase of the tolerance of immature stages of Sitophilus zeamais Motsch. as they aged from 19 to 31 days. OzEr (1961) apparently found the eggs of S. granarius susceptible and those of S. oryzae (L.) tolerant. Larvae and adults of both species were susceptible and the stages he described as pre-pupa, pupae and pre-adult were tolerant. Lindgren and Vincent (1966) reported that eggs of both these species were tolerant and pupae slightly more so in 24 hr exposures, whilst Mori and Kawamoto (1966) recorded survival of both these stages of S. granarius and S. zeamais for 4–5 days when exposed to concentrations up to 8 mg/l. Lindgren et al. (1958) found that pupae of S. granarius were a little more tolerant than those of S. oryzae.

No other species has been investigated in so much detail, but where several stages have been investigated the egg was more tolerant than the adult or larval stages and usually more tolerant than the pupa, and the pupa was usually but not always more tolerant than the larva or adult. Thus a tolerant egg stage has been recorded for Tribolium confusum (Lindgren and Vincent, 1966) and T. castaneum (Hbst.) (Muthu, 1973), AttagenuS megatalona (F.) and three species of Trogoderma (Vincent and Lindgren, 1972a), Lasioderma serricorne (Childs et al., 1973), Oryzaephilus surinamensis (L), four species of Nitidulid beetles and the moths EphseSta ffigulilella Gregson and Plodia interpunctella Hb. (Vincent and Lindgren, 1972b), the moth Corcycra cephaleonica St. and the bruchid beetle Callosobruchus chinensis (Muthu, 1973). The pupa was more tolerant than the egg only in Oryzaephilus surinamensis (Vincent and Lindgren, 1972b) and Callosobruchus chinensis (Muthu, 1973), and in some conditions for Lasioderma serricorne (Childs et al., 1973) but was more susceptible than larvae, presumably fully grown, in the Dermestids (Vincent and Lindgren, 1972a).

Bang and Telford (1966) who did not examine eggs found that pupae were more tolerant than larvae or adults in Tribolium confusum and T. castaneum. These authors investigated the later stages of the life cycle of T. confusum in some detail, obtaining insects of age known
Laboratory investigation of phosphine to within 24 hr and fumigating them in age groups from 20 to 29-days-old at 29°C. They found that the age group 23–25 to be much the most tolerant. They erroneously infer that the fumigation accelerates development of the survivors. They failed to realize that in their younger age groups it is the older larvae or pupae that survive and in their older groups that it is the younger ones that survive.

Temperature coefficient

It is clear from the literature that phosphine is more toxic at higher temperatures. SATO et al. (1973) related toxicity directly to oxygen consumption by showing that the CT product needed to give a 50 per cent knockdown multiplied by the oxygen consumption of Callosobruchus chinensis adults was constant from 20° to 40°C, that is from a temperature near to the developmental minimum to one which is lethal in a few hours. They also found that the phosphine uptake needed to give this 50 per cent knockdown was constant over this temperature range.

Four other workers have experimented at temperatures over a range from 5 to 32°C, i.e. from well below the minimum for development to just below or just above the optimum for development according to species. All four papers deal with the immature stages as well as the adults. Three of them restrict the exposure to fumigant to 4 or 5 days and hence suffer little control mortality, but do not achieve complete mortality of all stages of all species by fumigation. At 5°C CHILDS et al. (1973) needed to expose eggs of Lasioderma serricorne to 500 ppm (0.7 mg/l) of phosphine for 10 days to achieve 100 per cent mortality, but by then 93 per cent of controls had died from exposure to cold. At 15°C, a 6-day fumigation killed all eggs and only 10 per cent of the untreated eggs died, although this temperature also, is below the developmental minimum. At 5°C, some pupae survived a 20-day fumigation, but none survived a 6-day fumigation at 15°C. LINDGREN et al. (1958) used 4 treatments involving two concentrations for 3 and 4 days, against several species at 10 and 21°C. With Trogoderma granarium Everts, they had survivors at 10°C among the eggs for all treatments and among larvae for three treatments but had none at 21°C. With Sitophilus granarius and S. oryzae, they had a few more survivors at the lower temperature than at the higher one. OZER (1961) working with these same two weevils, recorded less than 3 per cent of survivors for any exposure at 21°C or above but the lethal exposure period became shorter at the higher temperatures. At 16°C there were survivors from the longest exposure to the highest dosage used. MORI and KAWAMOTO (1966) claimed that there was a similar temperature effect for S. granarius and Callosobruchus chinensis but none for Sitophilus zeamais. With this last species, there is some evidence in their tables that temperature influences toxicity, but mortality is very high for most treatments. Thus, eggs seem to have survived a 24 hr treatment better at 15° than at either 5 or 25°C but for a 48 hr exposure they survived best at 5°C. Clearly we must expect the metabolic processes to differ between temperatures at which development is rapid, slow or impossible and for the toxic action of fumigants to differ also.

It is probable that temperature influences the toxicity of phosphine to mites in a similar way. The table presented by JALIL et al. (1970) for 5 hr exposures at three temperatures, 2°, 18° and 24°C appears to substantiate this hypothesis, but, on inspection it is evident that the highest mortality of adult Caloglyphus berlesei (Michael) caused by phosphine in their experiments was 50 per cent at 24°C and that even fewer eggs were killed. Although more Tyrophagus putrescentiae (Schr.) were killed, neither adult mortality at 2°C nor egg
mortality at any temperature reached 90 per cent. Barker (1969a) worked with this latter species at 19° and 24°C. Since he used a higher concentration at the lower temperature and obtained very similar estimates for the periods required to give 50 and 95 per cent mortality, his results confirm that phosphine is more toxic at higher temperatures.

Relative susceptibility of species

Because of the wide variety of gas concentrations, of exposure periods and of life-cycle stages of the experimental organisms used by the various experimenters, a precise comparison of the susceptibility of species is not possible, but it seems probable that weevils, dermestids, moths and mites are among the species most difficult to kill with phosphine.

This opinion is based mainly on papers in which several species are tested under the same experimental conditions but the apparent higher tolerance of weevils may possibly be attributed to the more frequent inclusion of the egg and pupal stages in experiments with them. Thus Bang and Telford (1966) estimated an LD₉₀ only slightly higher for adult Sitophilus granarius than for adults of Cryptolestes pusillus (Schön), Oryzaephilus surinamensis, Tribolium castaneum and T. confusum, and Lindgren and Vincent (1966) recorded little difference of LD₉₀ between adults of Sitophilus granarius, S. oryzae, Acanthoscelides obtectus (Say), Gnathocerus cornutus (F), Lasioderma serricorne, Oryzaephilus surinamensis, Rhyzopertha dominica (F), Stegobium paniceum (L.) and Tribolium confusum. On the other hand, Bond and Monro (1961) needed a much higher concentration to achieve a mortality of 99 per cent for Sitophilus granarius than for Tribolium confusum and Bond et al. (1969) related a similar observation to phosphine uptake. The importance of the developmental stage is stressed by the fact that Bang and Telford (1966) estimate the LD₉₀ for pupae of T. castaneum to be some 4 times higher than that for adult weevils. The literature does not make valid comparisons possible, but from work at this Laboratory I conclude that weevils are amongst the more tolerant species to phosphine.

Whenever a range of species is tested, the larvae of Trogoderma species are amongst the more tolerant. Thus Bang and Telford (1966) record an LC₉₀ for T. variabile Baillon that is some 3 times higher than that of pupae of Tribolium castaneum, the next most tolerant in their range of species. Similarly Lindgren and Vincent (1966) recorded values of LC₅₀ and LC₉₀ some 4 times higher for larvae of Trogoderma sternale Jayne than for larvae of Plodia interpunctella and adults and larvae of several beetles. Lindgren et al. (1958) found that eggs and larvae of Trogoderma granarium were tolerant of 3-day exposures at 50% r.h. but only 1 per cent of larvae survived 4 days, so that they were less tolerant than the weevil pupae. Baskaran and Mookherjee (1971) found eggs and Muthu (1973) larvae of this species were tolerant to 24 hr exposures to phosphine, but the latter found moth eggs much more tolerant. Vincent and Lindgren (1972a) fumigating for 24 hr at 21°C found that some eggs of T. gladrum Herbst, T. sternale and T. variabile survived at 6 mg/l and some eggs of Attagenus megatoma (F) survived at 2 mg/l.

As just mentioned, Muthu (1973) reported values of LD₉₀ for the eggs of Ephestia cautella Walk. and Corcyra cephalonica of more than 10 and 20 times that for Trogoderma granarium larvae. Values for the pupae of the latter moth were some 7 times higher than for pupae of Tribolium castaneum and close to that for eggs of this species. Vincent and Lindgren (1972b) found eggs of Plodia interpunctella were tolerant to 24 hr exposures at 27°C. the LC₉₀ being 1.9 mg/l, a value 6 times above that recorded for pupae of the species 14 times that for eggs of Ephestia figulilella and 10 times that for the most tolerant beetle
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egg *Carpophilus mutilatus* Er. tested by them. At 20°C using 0.75 mg/l, for nearly 10 hr *Bell* and *Glanville* (1973) killed only 20 per cent of larvae of *Ephestia elutella* in diapause. Using 0.035 mg/l for 160 hr and 0.02 mg/l for 280 hr they killed 60–80 per cent.

There is no published work directly comparing mites with insects and two of the papers on mites use very short exposure periods, but the predicted values of the LD₉₀ reported for a 5 hr exposure by *Jalil et al.* (1970) are exceptionally high. Also *Barker* (1969b) working with *Cryptolestes* spp. recorded mortalities of 70 per cent and more for 5 hr exposures to phosphine in the concentration range 0.4 to 6.4 mg/l, but needed *Barker* (1969a) exposures of 22 hr at 8 mg/l to record 50 per cent mortality of *Tyrophagus putrescentiae*.

**The design of experiments**

It is evident that we should at this stage think more deeply about how toxicity experiments should be done. Clearly there is a need for those that test the exposure periods and concentrations used in practice, over a range of temperatures and aimed at killing all the test insects of all developmental stages. The paper by *Childs et al.* (1973) on *Lasioderma serricorne* is an example of an excellent piece of work of this kind. There is also real value in work aimed towards elucidating how phosphine kills (e.g. *Robinson* and *Bond*, 1970) and at assessing the risk that serious resistance to phosphine might evolve (*Monro et al.*, 1972). The current unsolved problem, however, concerns the evaluation of toxicity in the laboratory and until this is resolved, I doubt if there is any real prospect of comparing the subtle influences of the food on which the insects were bred as attempted by *Baskaran* and *Mookherjee* (1971) and by *Murthy* and *Srivastava* (1971).

The values to be determined are realistic gas concentrations and exposure periods and an appropriate level of mortality of the test insect. The aim is to start with samples of insects with a statistically normally distributed tolerance. To do this we must first have some knowledge of the change of tolerance between stages and between ages in those stages, and then we must watch for genetically based heterogeneity. The use of adult insects for toxicity experiments makes the first of these requirements relatively unimportant and the last relatively easy. Even for phosphine, the change of adult tolerance with age is not large, so that experiments using exposures that last several days may be analysed legitimately using probit lines. At 25°C, *Howe* (1973) working with the young tolerant adults of *Sitophilus granarius*, recorded only one survivor from thousands exposed to a dose of 1 mg/l applied for 8 hr, and to one of 0.01 mg/l applied for 4 days. These doses, therefore, ought to kill the adult insects of most species and any survivors may well be suspected of being resistant. The age span of other stages, however, must be very carefully selected. It is not sufficient just to collect eggs, larvae or pupae because of rapid changes of tolerance within these stages. For eggs and pupae, one reason for such a change is that these become pharate larvae and adults before their external appearances change, but both are also stages of development during which the biochemistry of the metabolism alters within hours. Larvae not only increase immensely in size but pass through phases of feeding, fasting and moulting. Since some of these metabolic phases may last only a few hours, if we are to elucidate the influence of the gas on these phases we must use short periods of exposure to fumigants and brief age spans. However, there is a practical limitation of the span that can be conveniently achieved. To get eggs, the adults may need a period up to 24 hr for laying. For larvae and pupae, the problem varies with species, but the collection of freshly hatched larvae and freshly formed
Figs. 1. Mortality of eggs of *Sitophilus granarius* of different ages caused by 24 hr exposures to 4 nominal concentrates of phosphine (■ 0–1 days old; ○, 1–2; ▲, 2–3; ▼, 3–4; ▲, 4–5; at start of fumigation). Each age group was replicated daily for ten days, and the gas concentration fell slightly over this period. Symbols represent means of mortality and of CT products. Where the change in mortality with daily gas concentration can be represented by a line of significant slope, these are drawn.

...pupae is usually laborious. Nevertheless, the homogeneity requirement restricts both the collection and the exposure periods to 24 hr and preferably to 8 hr.

The restriction of the exposure period in this way along with the limitation of the developmental span will govern the gas concentrations that are worth examination. Thus Howe (1973) working with eggs of *Sitophilus granarius* and using 24 hr exposures found that 0.2 mg/l of phosphine killed hardly any eggs under 4 days old and almost all those 5–6 days old (Fig. 1). The concentration range 0.05 to 0.4 mg/l is quite a useful one for the older eggs but a higher range of concentration is required for the younger ones. This work was appropriate for its purpose because exposures up to 5 days were used and the eggs developed into the older susceptible stages during this time. But it does not reveal what concentration is needed to kill young eggs, nor indeed, if they can be killed at all if any of their metabolism is anaerobic (Bond et al., 1969). This latter point is of great importance in temperate countries if insects are fumigated at temperatures at which the eggs develop very slowly. For phosphine not only must we try to determine the dose required to eradicate the least susceptible stages of development as we do with fumigants like methyl bromide but we must also study the more susceptible stages and judge the probability that these stages will be reached during a fumigation. Knowledge of the concentration needed for a fixed period of exposure required to cause some stipulated level of mortality in successive age groups would provide a useful clue to the time needed for a resistant stage to develop into a susceptible one and hence, if a high level of mortality is chosen, of a practical exposure period.

The desired level of mortality is not easy to select but because there are numerous references to the ability of part of the population, sometimes as much as 30 per cent, to survive a
very high concentration of gas (Barker, 1969b), I would augment the LD$_{50}$ for each age group with the LD$_{95}$ or 99. If there is no curvature of the probit lines, but these are not parallel, various comparisons are possible in any event, since these lines must cross somewhere and at the dosage where they cross the mortalities would be equal. The slope of the probit lines is a measure of the homogeneity of susceptibility to fumigant, and this might well vary with stage or the metabolic processes involved. Marked changes of slope in one line imply resistance in the population and I have already remarked upon the lowering of the nearly horizontal section of the probit line for the population used by Monro et al. (1972) from the region of 90 per cent mortality in the parent population to about 20–40 per cent in the selected population. I think it is essential that the scale of work should be such that a 1 per cent survival or less is easily discovered.

In evaluating the significance of resistance, the question of whether or not we concentrate on the most or least susceptible stages again becomes important. As a rule, we can tolerate adult resistance which is the most easily detected because resistant adults are unlikely to be more difficult to kill than normal eggs. But if the eggs of resistant adults are also resistant, it becomes essential to know their susceptibility at the time of hatching. In particular, the susceptibility of pupae becomes important. In experiments with weevils the occasional survivor is noted both for high concentrations and for long exposures. It is impossible to guess whether these are resistant individuals or happen to be at a stage of development that helps them to survive when others die.

At this stage of knowledge, the most useful laboratory assessment of the toxicity of phosphine to stored-products insects should include the following features.

- It should be on a large enough scale to record survivals of less than 1 per cent and include a lethal concentration.
- It should cover the entire life-cycle with the developmental stages in spans of not more than 24 hr, and with the adult age known to within 2 days over the first month and to a week for older adults.
- The maximum exposure period should not exceed 24 hr and preferably not 8 hr, but should be accompanied by measurement of uptake of fumigant.
- Sufficient exposures for each concentration or concentrations for each exposure period or both should be used to judge whether or not the probit line is curved or has a break and hence if there is some risk that resistant strains will be selected by fumigation.
- This pattern should make it possible to determine whether or not it is worth trying to kill the less susceptible strains or to wait for them to develop to a more susceptible stage, and thus when near developmental threshold, whether phosphine fumigation is likely to succeed. It will not help at lower temperatures where insects are not developing or for insects in diapause which must be examined in a straightforward way.

No work published to date has met all these requirements. Meeting them might involve some experiments using unrealistically high concentrations of gas, Jalil et al. (1970) claiming to have used 64 mg/l. Clearly the spontaneous combustion limit of 1.79% by volume in air must not be approached but I would expect it to be possible to combine the results to yield a practical dose exposure combination. For instance, in the work with weevil eggs by Howe (1973) mentioned above, the mortality for each concentration among eggs removed at 6 days old after fumigation for 1–5 days was approximately identical irrespective of the period of exposure, except for those of 24 hr at the lower concentrations. This was equally true for eggs removed at 5 days old. Further work to discover why certain individuals survive must eventually entail biochemical work.
REFERENCES


POSTSCRIPT

Nakakita et al. (1974) have attempted to relate the toxicity of phosphine to the respiration of insects. They fumigated adults of Sitophilus zeamais for 12 hr at a range of phosphine concentrations from 5 to 20,000 ppm (rather less than 0.01 to about 30 mg/l) and recorded the mortality, and in parallel experiments measured respiration during and after fumigation. Like so many others mentioned above they found a high mortality at 75 ppm (0.12 mg/l) which did not increase at higher concentrations. They observed narcosis at the high concentrations. The rate of respiration always fell during fumigation and partially recovered afterwards. The fall was quicker at the higher concentrations for which oxygen consumption approached zero.

Unfortunately the figures quoted for mortality were those recorded 24 hr after the termination of fumigation, although there was always further mortality rising to above 90 per cent by 10 days after treatment. Also the authors assume that phosphine uptake is proportional both to oxygen consumption and to phosphine concentration without establishing this hypothesis. Phosphine uptake was estimated by Bond et al. (1960) and by Bond and Wing (1973) from the depletion of the gas from fumigation flasks. Neither measured oxygen consumption, but both found the phosphine uptake over a fixed period increased, though not proportionally, with the concentration. This kind of result is consistent with the Nakakita hypothesis. Robinson and Bond (1970), however, measured the phosphine uptake of insect homogenates using radio-active phosphine and their figures are not consistent with the hypothesis. For exposures of 5 hr a 23-fold increase of concentration increased phosphine uptake 40 times which implies a greater rather than a lesser oxygen consumption at the higher concentration. Of course, live insects may behave differently from homogenates, dead insects may absorb phosphine and we do not know how accurately phosphine uptake can be measured. Nevertheless, I suggest that the maximum product of phosphine concentration and oxygen consumption gives a good guide to the most effective concentration of this fumigant.