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CONTROL OF GRAIN PESTS WITH PHOSPHINE AT TEMPERATURES BELOW 10°C

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by

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ABSTRACT

Tests were conducted on the toxicity of phosphine to all stages of 5 species of stored grain beetles at 5, 7.5 and 10°C. Two strains of each species were tested, one a laboratory stock and the other of relatively recent acquisition from the field. *Ahasverus advena, Oryzaephilus surinamensis* and the laboratory strain of *Tribolium castaneum* were all killed by the relatively low dosage of 0.1 mg/l phosphine held for 4 days at all temperatures tested. The field strain of *T. castaneum*, which proved to be phosphine resistant, and the two strains of *Cryptolestes ferrugineus*, required an 8-day exposure at 0.3 mg/l, or a 12-day exposure at 0.1 mg/l for control. The eggs and younger larvae of all species were adversely affected by the cold.

Older stages of *Sitophilus granarius* were highly tolerant both of cold and exposure to phosphine, and survived 15-day exposures to 0.64-1.35 mg/l phosphine at 10°C, and even longer exposures at 5 and 7.5°C. As a result fumigation can only be recommended below 10°C in the absence of this species.

Adults were generally more susceptible to phosphine than immature stages, although this difference tended to reduce as temperatures were lowered. Times to end-point mortality were protracted and some individuals recovered from exposure after three days of incubation at 25°C. For phosphine resistant strains differences in tolerance between adult and immature stages are likely to be reduced at all temperatures and the presence of resistance will affect dosage recommendations.

OBJECTIVES

1. To asses the tolerance of important beetle pests of stored grain to phosphine at 10 °C and below.

2. To use the data so gathered to design a dosage schedule in terms of dosage rate and exposure period for the control of pests at various temperatures on floor stored bulk grain.

INTRODUCTION

Grain is a valuable commodity which is prone to infestation by beetles and mites. Newly harvested grain, if cooled and placed in a clean infestation-free store, should remain in good condition for a considerable period of time (Chakrabarti, 1994). However, in the UK bulk grain is stored in many different types of structures from purpose built stores to machinery sheds. Infestations can occur easily in poorly cleaned premises, where residues and pockets of infested grain from previous harvests can infest the new harvest. The general transport and movement of grain within the trade presents similar risks. Low levels of infestation can often remain undetected for some time, but under warm moist conditions insect pests can multiply readily forming thriving infestations, and spoiling the commodity through the creation of warm, humid areas. These 'hot spots' also allow fungal infections to flourish which in turn create habitats for mould-feeding insects such as the foreign grain beetle, *Ahasverus advena* (Waltl). The result is a vicious circle of damage and spoilage.

Almost four million tonnes of wheat and barley were exported from the UK from July 1993 to June 1994 (HGCA, 1994). This comes at a time when more effective and accurate sampling techniques within the grain industry have led to the frequent detection of insect pests. Three major pest species are commonly encountered as adults wandering on grain even at temperatures below 10^{o}C, these being the beetles the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.), the rust-red grain beetle, *Cryptolestes ferrugineus* (Stephens) and the granary weevil, *Sitophilus granarius* (L.). Two other beetles which may be seen are the rust-red flour beetle, *Tribolium castaneum* (Herbst) and *A. advena.* Detection of one or more of these species can ultimately lead to the rejection of consignments, or buyers imposing heavy penalties by charging for fumigations (Chakrabarti, 1994). Once infestations have been identified, often the only course of action is to recommend fumigation with phosphine gas. This is the sole method currently available for treating a whole bulk *in situ* (Mills et al., 1990) since the withdrawal of the liquid fumigants once commonly used. Phosphine is a highly toxic gas liberated from commercially obtainable aluminium or magnesium phosphide preparations in the presence of moisture. These are available in a variety sizes from 0.6g pellets which liberate 0.2g gas to 3400g bag blankets which liberate over 1kg of gas. Phosphine is released over a number of days depending upon the temperature within the structure.

In the UK there are several factors which can contribute to the success or failure of such bulk fumigations :

Leakage - Many storage structures are not designed or constructed with fumigation in mind. In some only the grain surface can be sheeted as there is no space between the bulk and the walls of the building. Even with a well-sheeted grain bulk, gas diffuses easily through aeration ducts, cracks in walls and by permeation through the sheet itself. In addition, smaller bulks of grain (below 1000 tonnes) have increasingly large surface-to- volume ratios, which permit a greater percentage loss of fumigant per unit time (Bell *et al.*, 1991).

Poor distribution - Uneven placement of formulations, especially surface treatments, can lead to high concentrations of gas in some areas, and low concentrations in others due to poor penetration, particularly where the grain is deep and temperatures are low.

Pest identification - Correct identification of the insect pest is imperative because there is a broad spectrum of tolerance to phosphine among different species, as reflected in the current UK fumigation schedule (Anon., 1984).

Phosphine toxicity - In general, long exposures to low concentrations have been found to be more effective than short exposures to high concentrations (Reynolds *et al.*, 1967; Howe, 1973; Hole *et al.*, 1976; Bell, 1979). This is because longer exposures allow time for the naturally tolerant stages of the life cycle, the pupae and eggs, to develop into more susceptible stages, namely adults and larvae.

Phosphine resistance - Resistance is known in all the species in the present study with the exception of *A. advena* which has not hitherto been investigated. A particular concern is the importation of products from overseas where there is a higher incidence of resistance. During 1993-1994 in excess of 1,600,000 tonnes of wheat was imported into the UK (HGCA, 1994).

Temperature - Low temperatures cause a slow release of phosphine from metal phosphide formulations. In poorly-sealed structures this can result in a significant loss of gas before it has a toxic effect on the pest. Hole *et al.* (1976) concluded that many insect species were more tolerant to phosphine at lower temperatures and that at 15^{o}C and below effective control could only be achieved by arranging long exposure periods. Due to the lack of information available on the control of insects below 10^{o}C, fumigation schedules do not make recommendations for treatment at

such temperatures. Currently high doses are applied to increase chances of success, and there is some concern that phosphine residue levels will exceed the maximum residue limit (MRL) of 100 μg/kg. However, the cold-susceptibility of some species may permit a reduction in the dosage applied with an efficient dosing and gas distribution system, particularly if the infestation consists of adults only because of the effect of cold on immature stages. Hence, any risk of exceeding MRLs could be avoided without loss of efficacy and low temperature treatments in the UK could be recommended with confidence and seen to be effective.

The current programme was undertaken to investigate the effect of phosphine at temperatures down to 5°C on beetles commonly occurring in bulk grain.

METHODS

Insects

The five species of insect used in the fumigation programme were all common beetle pests of grain and other cereals: *A. advena, C. ferrugineus*, *O. surinamensis, S. granarius* and *T. castaneum* .

For each species two strains were tested, one laboratory-bred strain known to be susceptible to commonly-used contact insecticides and fumigants, and one 'field' strain recently collected from a natural infestation and put into culture. The beetles used in the experiments had all been through a quarantine procedure (see below), had been found susceptible to phosphine by the FAO resistance test (Anon., 1975) and were established in regular culture within CSL by the start of the programme with the exception of the field stock of *T.castaneum*. This was collected from Wells, Somerset, at the start of the programme and was subjected to quarantine procedures to ensure that it was free of disease and mites.

Quarantine procedure

To obtain eggs of all of the species, other than *S. granarius,* approximately 200-300 adults were placed into a 750 ml glass jar containing a thin layer of whole-wheat flour which had previously been passed through a 100 mesh sieve. The jar was incubated at 25°C, 70% relative humidity (rh) for five days, after which the contents were sieved to remove the adult beetles. The remaining flour was passed through an 80 - 90 mesh sieve to separate out the newly laid eggs. The separated eggs were carefully placed into a small conical flask, and 20 - 30 mls of 0.5% Zephiran (benzalkonium chloride) was added. The flask was shaken carefully and the solution decanted off, taking care to retain the eggs in the flask. This washing process was repeated followed by a rinse

with distilled water. The eggs and a small amount of water were poured into a small funnel containing a cone of filter paper. The eggs were again gently rinsed with distilled water and after draining the filter paper was removed and transferred to the main laboratory. Once trimmed, to allow insertion into a freshly prepared food jar, the paper was left to dry and then placed egg-side down on the food. The resulting culture was left to develop at 25°C, 60% rh for several generations before use.
For *S. granarius,* adults were allowed to oviposit on grain which was treated with the Zephiran solution , rinsed and dried thoroughly in a similar manner to the free eggs.

Preparation of test insects

Insects for fumigation were reared at 25°C, 60% rh with the exception of *A. advena* and *C. ferrugineus.* For these 25°C, 70% rh, and 30°C, 60%rh respectively were used to accelerate their life cycles in line with the other species. All the strains tested were bred on food mixtures specially selected to permit rapid development and optimum yield (Table 1). The food media, with the exception of the brewers' yeast, were sterilised before use for 16 hours in a hot air oven set at 70°C.

Preliminary tests

Before embarking on the 'main body' of tests, it was necessary to perform 'preliminary' ranging tests on each species. In each test, both laboratory and field strains were tested at 10°C and 5°C, 60% rh. For each test two cultures were prepared for each strain, by adding one hundred adult beetles to 750 ml glass jars, each containing a precise quantity of previously prepared food mixture. The seeded cultures were then placed at their respective breeding temperatures for a period of between two and three weeks. After this the 'original' adults were removed and discarded. The remaining 'infested' food mixture was divided into ten equal portions and placed into smaller 120 ml glass jars to provide five cultures for each of the two test temperatures.

On the same day that the original adults were removed and discarded, a second set of cultures was prepared for each strain in the same manner as the first. Thus the life-cycle had been divided into two equal halves, the first set providing older stages (predominantly pupae and older larvae), and the second set providing younger stages (predominantly younger larvae and eggs). All cultures were incubated until a few days prior to the proposed test, at which time the younger cultures were sieved to remove the adult insects and divided into smaller jars in the same manner as the older cultures.

In addition to the immature stages a number of adults were included in some of the tests. All test cultures were simultaneously conditioned down in temperature in steps

of 5°C for periods of 24 hours until they reached 15°C. Here they remained for 48 hours before being placed directly at the test temperature, where they were conditioned for a further 48 hours before testing.

All preliminary tests were performed in 6 litre calibrated glass desiccators fitted with a stainless steel mesh platform and a magnetic stirrer. For each test temperature, a culture of each strain and life cycle stage was presented for each of four exposures and the control. A separate desiccator was used for each exposure period. Once sealed, the desiccators were dosed with a precise quantity of 86% phosphine gas previously prepared using a method similar to that described in Anon. (1975) (Fig.1) using a gas-tight syringe according to the calculation:

Volume dosed =

$$\frac{a \times \text{desired concentration (mg/l)} \times \text{desiccator volume (l)} \times b \times 1000 \times 1000 \times 100}{c \times 1000 \times d \times e}$$

Where: a = 298°K (25°C in degrees Kelvin)
b = 22.414 l (Gram molecular volume)
c = 273°K (Absolute temperature in degrees Kelvin)
d = 33.9977g (Gram molecular weight of phosphine)
e = 86 (Purity of phosphine i.e. 86%)

e.g. desired concentration (mg/l) X desiccator volume (l) X 836.81 = Volume dosed (µl)

Once dosed, the contents of the desiccator were mixed well using the magnetic stirrer, after which the desiccators were transferred to a controlled temperature room held at the test temperature. Control cultures were held in a similar desiccator which was left undosed in the same room as the treated cultures. During each exposure period the concentration of phosphine in the desiccator was measured by analysing samples using a gas chromatograph fitted with a flame photometric detector (FPD). The concentrations achieved were used to calculate the concentration x time product (CTP), expressed as mg h/l for each exposure period.

After the allotted exposure period the desiccator was opened and 'aired' in a fume-cupboard. When all four treated cultures had been aired, these and the control cultures

were returned to 25°C, 60% rh (25°C, 70% rh for *A. advena* samples) in steps of 5°C. Each sample was examined on a weekly basis to assess survival.

Main tests

A total of 20 tests was carried out on immature stages and adult insects at 5, 7.5 and 10°C at concentrations ranging from 0.05 - 1.62 mg/l. Test cultures were prepared in a similar manner to the preliminary tests by dividing the insect life-cycles into two equal halves. For each strain in each test a total of seven exposure periods was planned, in addition to controls at the test temperature and 25°C, with three replicates per exposure. Cultures were set up by adding 50 adult beetles to 350 ml glass jars containing a precise quantity of the selected food mixture (Table 1). The seeded cultures were sealed, by means of double filter paper tops secured with molten paraffin wax, and placed at their breeding temperatures until the original adults were removed. At this point, unlike in the preliminary tests, there was no need to subdivide the cultures so they were returned to incubate until just prior to the test. When the adults were removed from the 'older' cultures, fresh adults were added to the set of 'younger' cultures. A few days prior to the test the adult beetles were removed from the 'younger' cultures before gradually acclimatising all the material (with the exception of the 25°C controls) simultaneously to the test temperature as for the preliminary tests.

The adult insects to be fumigated were prepared by adding 50 beetles to 120 ml jars, each containing a small spoonful of food mixture. Each jar was sealed by means of a nylon top secured in place by an aluminium screw ring. As with the immature stages a total of seven exposure periods and two sets of controls were included in each test, with three replicates of each. The same acclimatisation regime was used for the immature stages.

Fumigation procedure

Due to the large number of jars available for testing, fumigations were performed in 1700 litre stainless steel chambers. Each chamber was fitted with a port 15 cm in diameter, and a row of ports 2 cm in diameter. A specially designed polythene sleeve was attached to the internal surface of the chamber surrounding the large port, through which the sleeve protruded. By means of a bag attachment, it was thus possible to transfer jars into and out of the chamber with minimal loss of gas. The chamber was sealed after each transfer by means of a steel plate bolted onto a rubber gasket.

Chambers were dosed for each test by adding pellets of a commercial aluminium phosphide preparation. Each pellet weighed 0.6 g, and was capable of releasing 0.2g

of gaseous phosphine. In the presence of a small quantity of water the aluminium phosphide decomposed to produce phosphine gas according to the equation:

$$AlP + 3H_2O \longrightarrow Al(OH)_3 + PH_3$$

Due to the low temperatures chosen for the experiments the release of phosphine from the pellets took several days. Therefore the chambers were dosed at least five days prior to the fumigation to allow complete decomposition of the pellets. All material to be fumigated was clearly labelled with the relevant exposure period. Nylon gauze was placed over the filter paper tops and secured with rubber bands to afford extra protection and prevent damage during transfers.

During the fumigation test period, gas samples were taken at regular intervals by means of a fine bore nylon sampling line run from each fumigation chamber to the gas chromatograph. Gas concentrations within the chamber were calculated based on 'standard' gas samples from calibrated cylinders. These were used to calculate CTPs as before.

All fumigated insect material remained at the test temperature until the longest exposure cultures had been removed from the chamber. After a period of 2 days airing, all the cultures were returned to 25°C, 60% rh (25°C, 70% rh for *A. advena*) in steps of 5°C. Immature insect cultures were sieved and examined on a weekly basis to assess survival rates by counting live adults which emerged. Counting commenced on the older cultures during the week following the fumigation test, while counts for the younger cultures started approximately three weeks later (this allowed time for the younger stages to develop into adults). Where possible, the data obtained were subjected to probit analysis. Sample numbers were estimated from the number of insects emerged from the test temperature control cultures.

Adults tested at 5 and 10°C were examined at intervals after exposures usually at 3, 6, 10 and 14 days after fumigation. Those tested at 7.5°C were usually counted at 14 days only. Where possible, results for each species were subjected to probit analysis.

RESULTS

A. advena

Immature stages of both strains of this species proved highly susceptible to phosphine (Tables 2 and 3) and many were severely affected by exposure to low temperature (Table 4). At 7.5°C and 10°C LD 99's were all less than 40 hours at 0.05 mg/l and no stage survived a 40-hour exposure. In the absence of phosphine at 5°C (Table 4) younger stages were all killed within 7 days and at 7.5°C only a few (10-15%) of the older stages survived as long as 9 days.

Adults were tested at 10 and 7.5°C for their rate of response to phosphine. For both strains deaths continued to be recorded after 6-10 days of observations and end points had not been fully reached by day 14 (Table 5). However, probit analysis illustrated (Table 6) that there was relatively little increase in mortality after the first 6-7 days.

T. castaneum

T.castaneum was another species severely affected by the cold, analysis of controls revealing that nearly all younger stages were killed by a 14-day exposure at 10°C, whereas older stages of the field strain were nearly all dead after 32 days at 7.5°C (Table 7).

The two strains of this species differed widely in their tolerance to phosphine and the field strain collected from Wells, Somerset, gave a positive result when tested for resistance to phosphine by the FAO resistance test method (Anon, 1975). A total of 176 out of 700 adults survived the discriminating concentration of 0.04 mg/l for a 20-hour exposure at 25°C. All stages of the laboratory strain succumbed after a 24 hour exposure to 0.11 mg/l of phosphine at 10°C (Table 8), but older, immature stages of the field strain required 7 days at 0.29 mg/l for control. At 5°C probit analysis revealed a further increase in tolerance for this strain (Table 9, Fig. 2).

Adults of both strains increased in tolerance to phosphine as temperatures were lowered but in the laboratory strain adults remained more susceptible (judged by the 14-day assessment) than the older immature stages even at 5°C (Tables 10 and 11). Those of the field strain, however, appeared equally tolerant to older immature stages at 5°C, the LD99 after 14 days being 41.19 (Table 11) compared with 41.21 mg h/l for the immatures (Table 9). Tolerance of adults showed little difference between 7.5 and 10°C (Table 11, Fig. 3).

O. surinamensis

Like *A. advena* and the laboratory stock of *T. castaneum*, the *O. surinamensis* strains showed a high susceptibility to phosphine at all the low temperatures tested, particularly as eggs or younger larvae. At 10°C all immature stages were killed within 3 days at concentrations near 0.1 mg/l and at 7.5°C there were only low levels of survival of older stages of the field strain after 4-5 day exposures at 0.05 mg/l with the laboratory stock being more susceptible (Table 12).

At 5°C all immature stages died after a 4-day exposure at 0.05 mg/l. Probit analysis of the experimental data revealed little change in susceptibility between 7.5 and 10°C in the older stages of the field stock while those of the laboratory stock were more susceptible at the lower temperature (Table 13, Figs. 4 and 5). A CTP of 7-8 mg h/l was sufficient for complete control of all stages at all low temperatures tested.

Adults were less affected than immature stages by exposure at low temperatures but were no more tolerant of exposure to phosphine (Table 14). None were alive 14 days after a 2-day exposure to phosphine concentrations around 0.1 mg/l at 10°C. Exposures at 7.5°C were not extended beyond 32h but at 0.15 mg/l phosphine, a 24-hour exposure was sufficient to kill all adults after 14 days. Probit analysis of results showed that there was in fact little difference between 7.5 and 10°C in the CTP's required for control (Table 15). A marked increase in the level of kill recorded continued up to the tenth day after exposure.

C. ferrugineus

A much higher level of tolerance to phosphine was apparent in all stages of this species at low temperatures. The tolerance of older immature stages to prolonged exposure at low temperature in the absence of phosphine was high, over a third being capable of producing normal adults at 25°C after a 37-day exposure of a fumigation control culture at 5°C, as judged by the emergence in the 25°C control. At 10°C immature stages of the field strain showed a higher level of tolerance to phosphine than the laboratory strain, older larvae and pupae surviving up to an exposure of 8 days at 0.1 mg/l and 4 days at 0.36mg/l (Table 16). Younger stages of both strains were all killed within three days at the higher concentration.

Tolerance of younger rather than older stages was increased at 7.5°C, though older stages remained the most tolerant at this temperature. At 0.11 mg/l a 12-day exposure was required for complete kill of the field strain, and 8 days for the laboratory strain. At 5°C, with a much increased concentration of 0.78 mg/l, the laboratory strain appeared the most tolerant strain, a result borne out by the probit analyses (Table 17).

Higher CTP's were required at 5°C than at the other temperatures tested. However, the slopes of regression lines for the 5°C data were less steep than those for the other temperatures (Figs. 6 and 7). The 5°C lines crossed the others near the LD_{50} in both strains.

Results for adults reflected the findings with immature stages with tolerance tending to increase as temperatures were lowered (Tables 18 and 19). However, the increase in tolerance that was obtained may also have been due to increased concentration as the much higher level of 0.78 mg/l was tested at 5°C, compared with 0.1-0.3 mg/l at 7.5 and 10°C. Complete kill of adults was not achieved in the high-dose 5°C fumigation, even after the 14-day assessment. However, the longest exposures tested were only 3 or 4 days. Regression lines at 5°C have low slopes because of the bunching of data between 40 and 90% mortality (Figs. 8 and 9). Nevertheless, adults appear to be the most tolerant life stage at 5°C. Problems were encountered in assessing mortality 3 days after exposure, some adults initially regarded as dead subsequently recovering.

S. granarius

Older immature stages of this species survived the longest exposures tested at most temperatures. Younger immature stages were, however, susceptible to cold, over three quarters of control cultures dying after 2-3 week exposures at 10°C, and they were very easily killed by the lowest CTP's at the shortest exposures tested at 10°C. As a result, they were omitted from most tests at 5 or 7.5°C.

Only concentrations above 0.6 mg/l were tested against *S. granarius* older larvae and pupae. Survival of a small proportion of the insects in each sample persisted through most of the dose range. This gave rise, especially at 10°C, to 'flat' zones in mortality dose response data (Table 21). In spite of the wide dose ranges tested, kill at even the lowest exposures of this flat region ranged from 75-95% (Tables 21-23). At 10°C, mortality started to increase progressively only when exposure times were extended beyond 140 hours. This effect was apparent at both concentration levels in spite of the very different CTP's involved. A similar picture was evident at 7.5 and 5°C (Tables 22 and 23) with the exception of the field stock at 0.7-0.8 mg/l where 'flat' zones of mortality response were less obvious.

Probit analysis of the results was attempted (Table 24) and resulted in very low slopes of regression lines (Figs. 10 and 11). Nevertheless, in most cases, predicted LD99 levels agreed well with expectations from observing survivals at the highest CTP's probably because all the data points, especially in the field strain, were grouped together at the higher mortality levels (Tables 20 -23).

Adults of *S. granarius* were relatively susceptible to exposure at 5-10°C, and were less tolerant than those of *C. ferrugineus*. Time to end-point mortality was protracted, particularly in the laboratory strain, and the level of kill attained continued to increase between 10 and 14 days after exposure at low temperatures and return to rearing conditions (Table 25). As with tests on *C. ferrugineus*, difficulties were encountered in obtaining a realistic mortality assessment 3 days after treatment, some individuals subsequently recovering. Tolerance of adults of the field stock appeared to increase as temperatures were lowered from 10°C. Probit analysis of results indicated that any changes in susceptibility with temperature were small (Table 26). High levels of kill in all test exposures at the 14-day assessment prevented the computation of several lines and resulted in very low slopes for some of those that were computed. Regression lines for the 0.70-0.78 mg/l concentration tests at 5 and 7.5°C are shown in Figs. 12 and 13 for laboratory and field strains respectively.

DISCUSSION

There were wide differences in the tolerances to phosphine of the five species tested with *A. advena* being the least and *S. granarius* the most tolerant. In every case the younger immature stages, comprising eggs and younger larvae, proved highly susceptible to phosphine at the low temperatures tested. The older immature stages, comprising older larvae and pupae, were almost always the stages of highest tolerance with the exception of adults of both strains of C. *ferrugineus* at 5°C. This unusual finding warrants further investigation.

Many immature stages, particularly eggs, were killed by cold in the absence of phosphine. Fields (1992) in his comprehensive review of the effect of extreme temperatures on insects states that eggs are usually the most cold-susceptible stage. In the present study, eggs of *O. surinamensis* proved particularly susceptible. Mullen and Arbogast (1979) found that exposures of less than 3 days at 5°C killed 95% of young eggs exposed. Jacob and Fleming (1986) found that complete kill of all age groups of eggs of this species at 5°C was obtained after a 4-day exposure.

A. advena, O. surinamensis and the laboratory strain of *T. castaneum* were all controlled by the low dosage of 0.1 mg/l held for 4 days at all temperatures tested. It is thus possible that low doses of phosphine can be recommended for grain infested only with these species, but with caution. In practice the evolution of gas from metal phosphide formulations is slow at these temperatures and this should be borne in mind

when setting treatment times, together with making allowance for the time required for gas to distribute throughout the bulk.

Studies at CSL to determine the evolution of phosphine from Detia sachets, containing aluminium phosphide, have shown that they liberate 50% of their theoretical phosphine content after 40 hours and 4 days at 15°C and 10°C respectively. At 5°C it is likely that a much longer time would be required to liberate the same percentage. The rate of evolution appears little faster for Degesch plates containing magnesium phosphide. Therefore, it is likely that at the end of some treatments at 5-10°C some unreacted metal phosphide may remain. Careful attention will, therefore, have to be taken of the manufacturers' recommendations on the safe disposal of the partially-spent formulations either by burying, burning or by careful addition to water. The slow breakdown at these temperatures may offer some advantage to the maintenance of effective concentrations in situations where all leak sources cannot be eliminated.

Estimation of the time required for the unaided distribution of phosphine from the site of generation throughout the grain bulk can be difficult. Complex interactions between the depth of grain and temperature differences within the bulk leading to convection currents can occur. Frequently in floor-stored bulks, there is an up-flow caused by grain being warmer than the ambient air. Provided that the surface is well sealed, this can aid distribution when the phosphine is applied either from solid formulations or from cylinders at the base of the bulk in ventilation ducting (Bell *et al.*, 1991; Chakrabarti, 1994). However, if phosphine is applied at the surface the up-flow can result in a considerable delay before useful concentrations reach the bottom, if indeed they ever do so. In tall silos, a strong up-flow caused by the 'chimney effect' means that it is very difficult for surface-dosed phosphine to reach the bottom. In general, the geometry and size of the treated bulk will affect phosphine distribution. A uniform distribution of a metallic phosphide formulation throughout a bulk is the ideal though this can only be readily achieved in practice by its addition to the grain stream when rapid filling of a storage structure is possible. Friemel (1983) found that phosphine moves through grain at a rate of 3-5 metres per day. Experimental fumigations by CSL corroborate these findings.

A very wide difference in tolerance was apparent between the two strains of *T. castaneum,* and the field strain was, in fact, subsequently diagnosed as resistant. All stages, including adults, showed increased tolerance, necessitating a concentration of 0.3 mg/l to be held for 8 days to obtain complete control at the temperatures tested. Regression line slopes for this strain were very low, the result of testing a population containing both susceptible and resistant individuals.

Resistance to phosphine has often been detected in strains of stored product beetles overseas. There is thus a danger of importing resistant strains on imported commodities. A recent survey of storage pests in commercial grain stores conducted by CSL on behalf of the Authority (Prickett and Muggleton, 1991) showed that 8 out of 28 strains (28.6 %) of *O. surinamensis,* 2 out of 21 strains (9.5%) of *C. ferrugineus* and 1 out of 11 (9.1%) strains of *S. granarius* were identified as resistant. A CSL report in preparation (A. J. Prickett and J. Muggleton) on the situation in provender mills indicates that in these premises the incidence of resistance is higher in these species and that resistance commonly occurs in *T. castaneum* also. The implications of these findings are serious and they may require that both concentration level and exposure time are increased when phosphine is used.

As reported by other workers (Smith, 1970; Fields, 1992) *C. ferrugineus* showed high tolerance of cold, the adult stage being particularly tolerant. This is perhaps surprising as the species requires temperatures in excess of 22°C for population increase, a threshold higher than for the other species (Howe, 1965). When exposed to phosphine at 5°C and 0.78 mg/l, adults were the most tolerant life stage in both strains. The very high CTPs which appeared to be required for control may well have been the result of the inefficiency of such a high concentration of phosphine in short exposure periods. Bell (1979) showed that for diapausing larvae of *Ephestia elutella* (Hubner) at 20°C phosphine concentrations above 0.49 mg/l did not significantly affect the exposure time required for 99% mortality and that the level of kill attained at higher concentrations was largely determined by exposure period. In further experiments over a range of temperatures, phosphine concentrations between 0.05 and 0.10 mg/l gave the greatest efficiency of fumigant action (Bell, 1992).

At 7.5 and 10°C all stages of C. ferrugineus were killed by lower CTPs than at 5°C. However, this was achieved by using the lower concentrations of 0.10-0.36 mg/l, with exposure periods extending up to 12 days. At all temperatures tested an 8-day exposure at 0.3 mg/l would achieve complete control of this species in the absence of resistance.

S. granarius has for some time been recognised as the most tolerant stored product species to phosphine and is responsible for the higher dosages and exposures currently recommended for grain treatment (Hole *et al.*, 1976; Winks *et al.*, 1980; Anon, 1984). The present results for this species at 10°C agree closely with the earlier results of Hole *et al.* (1976) showing some survival even after 15-16 day exposures. This survival is due to a highly tolerant phase early in the pupal period

(Howe, 1973) which at 25°C may only last 4-5 days. The threshold temperature for development of the species is 13-15°C (Evans, 1977, 1983; Howe, 1965). At lower temperatures development of some stages may proceed slowly although transition to the next stage will not occur. Hence, phases of tolerance are enormously extended and this is reflected in the fumigation results obtained here. The tolerance of these stages and adults to cold is high and this species has no difficulty in overwintering in cooled grain (Armitage and Llewellin, 1987).

An examination of the raw data in Tables 21-23 shows persistent survival of a narrow age band of tolerant individuals in samples derived from 3-week oviposition periods and containing older larvae and pupae. In the majority of cases the lowest CTP tested killed 75% or more of the insects estimated to be present, as judged from the emergence in control replicates. In the initial probit analysis (Table 24) the fits for regression lines were poor because of the resulting clustered distribution of data points, and calculated slopes were small. The results for this species, taken at face value, indicate that at temperatures of 10°C or below the use of phosphine as a control measure may be inappropriate. The picture is, however, far from complete because of the lack of precision in estimating extreme mortality levels.

In an attempt to clarify this situation, another method of arriving at sample size was investigated. In many respects the samples tested contained stages which had no chance of surviving the dose range tested and hence these may be regarded as having been effectively absent from the treatment. To obtain a further estimate of the CTPs required for control the effect of these stages on the relevant sample size was removed by reducing the estimate for number tested to include tolerant stages only. This was done by regarding the population as being divided into 21 equal units based on the original oviposition period used to produce the cultures, and finding the number of these units required to account for the level of survival observed in the shortest exposure. It was recognised that this method was to some extent arbitrary, because individuals develop at different rates, but the distribution of insects at each stage of development at the time of fumigation can be assumed to reflect that at oviposition, although the span may be wider. Moreover, the choice of age categories based on days at 25°C enabled the samples to be related to the findings of Howe (1973) in his examination of the tolerance of pupae aged to within one day at 25°C.

Howe (1973) observed a peak in tolerance to phosphine at the start of the pupal stage with survival of a 32-hour exposure to 1 mg/l at 25°C starting in the 26-day age group and reaching a peak in the 32-day age group. No survival was recorded in the age groups up to 26 days. Percentage survivals for 31-34 day age groups were 6.9, 9.8,

8.1 and 5.4 respectively. In the current work, depending on the level of survival observed in the lowest dose, the sample sizes of the fumigated batches were re-cast to represent the number of insects produced in a limited oviposition period of between 1 and 5 days out of a total of 21 days duration for the culture as a whole. Therefore, 1 to 5 twenty-firsts of the control emergence was used to re-estimate the number treated and the probit analyses were repeated (Table 27). Although increased, slopes of regression lines were still low and there were grounds to suspect that in some tests the lower exposures were not contributing to the kill of the tolerant age group. The option of merging such exposures with the control was not followed as it was recognised that too few data would remain for analysis.

As suspected in examining the results for the 5°C test on *C. ferrugineus,* there was again evidence with *S. granarius* that higher concentrations were much less efficient than lower ones in achieving kill at the temperatures tested. Taken together, the results of Tables 24 and 27 show little consistency in the CTPs required for 99% mortality for the higher and lower concentrations tested at 7.5 and 10°C. Calculating the exposures necessary to achieve this level of kill showed that times were actually longer for the higher concentrations for the laboratory strain at 7.5°C (Tables 24 and 27) and, in the revised analysis, at 10°C also (Table 27). Hence, there is little point in relying on further increases in concentration to achieve kill in a shorter exposure time. From a practical viewpoint it would in any case be very difficult to maintain concentrations above 1 mg/l throughout a bulk for long treatment times. From the present data and that of Hole *et al.* (1976), a concentration of 1 mg/l could be regarded as an upper limit for any improvement of efficacy in phosphine treatments at low temperature.

For the laboratory strain tolerance was higher as temperature was lowered provided that a similar concentration level was compared at each temperature, regardless of the method of analysis. For the field strain changes in tolerance with temperature were inconsistent but tolerance was not significantly less than that of the laboratory strain at 5°C. At 10°C in the revised analysis based on very small sample sizes, LD_{99} values for the field strain were enormously increased, whereas complete kill was observed after 15 days at the higher concentration. For the laboratory strain an examination of the results of both sets of analyses, and the original data, indicate that control of all stages could only be expected by an exposure of at least 18 days at the concentrations tested. This finding is in agreement with the data of Hole *et al.* (1976), who observed a small survival after a 16-day exposure to a concentration of 0.76 mg/l at 10°C.

A dosage schedule can now be constructed from the data obtained here and in other work (Table 28). Fumigation at 10°C can only be recommended for all species if appropriate measures are used to provide the necessary concentration throughout the exposure. With conventional treatments based on metal phosphide formulations the maintenance of high concentration levels throughout a long exposure can only be achieved by the use of a recirculation system, and a means of periodic redosing, as necessary. Alternatively, a cylinder-based formulation of phosphine could be employed as the source of a continuous flow of gas, as proposed in our previous report (Bell *et al.*, 1991) and in the Australian SIROFLO system (Winks, 1993).

Fumigation can only be recommended at temperatures below 10°C in the absence of *S. granarius.* For the other species exposures of 8-12 days, depending on the concentration level within the range of 0.1 and 0.3 mg/l, should remain effective down to 5°C. It should be noted that other stored product species, such as *E. elutella*, *Ptinus tectus* Boield and *Trogoderma granarium* Everts, may have a tolerance to phosphine at low temperatures intermediate between *S. granarius* and the others tested here (Bell, 1976; Hole *et al.*, 1976). For these species at 10°C, exposures of 8-16 days at 1 mg/l are recommended (Anon., 1984). Additional time has to be allowed in all cases for the necessary concentration levels to be established throughout the bulk.

A separate entry is included in Table 28 for the resistant strain of *T. castaneum.* It can be seen that resistance in any species, but particularly in a naturally tolerant species, will have a profound effect on phosphine dosages. Often the effect of resistance is that susceptible stages reduce the gap with tolerant stages (Price and Mills, 1988; Mills *et al.*, 1990) so that all require high dosages for control and poor fumigations will result in even higher levels of survival.

In the majority of situations infestations detected in cooled grain comprise adults only. The dosages presented in Table 28 pre-suppose the presence of immature stages in the infestation detected. Nevertheless, in the case of phosphine-susceptible strains of *C. ferrugineus*, adults may be the most tolerant life stage to phosphine at low temperature, and thus there is limited scope for reduction in the dosages recommended in Table 28.

The timing of adult mortality assessments after exposure is likely to affect the evaluation of treatment efficacy in two ways. Firstly, as some insects recover after sub-lethal exposures between 3 and 6 days after incubation, an early assessment may be misleadingly optimistic. Secondly, many insects continue to die as a result of the

treatment during the second week of incubation after exposure. The present 14-day assessment recommended by CSL is therefore best retained.

CONCLUSIONS

1. Fumigation with phosphine at temperatures below 10°C can only be recommended if *S. granarius* is absent.

2. At 10°C, a concentration of 1 mg/l held for at least 18 days is required for control of *S. granarius.*

3. At 5-10°C, all stages of the other 4 species tested can be controlled by a 12-day exposure at phosphine concentrations above 0.1 mg/l.

4. Adult mortality can not be reliably assessed in any of the species tested within a week of returning to warm conditions, nor in some cases within two weeks. A 14-day assessment remains the best option.

5. The eggs and younger larvae of all 5 species were adversely affected by the low temperature exposures in the absence of phosphine.

6. The difference in tolerance to phosphine between adults and immature stages tends to reduce as temperatures are lowered.

7. The difference in tolerance between the stages of phosphine-resistant strains is less than that in susceptible strains and the presence of adult resistant insects is likely to affect dosage recommendations.

REFERENCES

Anon. (1975) Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides. Tentative method for some adults of some major pest species of stored cereals, with methyl bromide and phosphine. F.A.O. Method No. 16. *F.A.O. Pl. Prot. Bull.* **23,** 12-25.

Anon. (1984) Standard 18. Phosphine fumigation of stored products (UK method). *EPPO Bull.* **14,** 589-599.

Armitage, D. M. and Llewellin, B. (1987) The survival of *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) and *Sitophilus granarius* (L.) (Coleoptera Curculionidae)
in aerated bins of wheat during British winters. *Bull. ent. Res.* **77,** 457-466.

Bell, C. H. (1976) The tolerance of developmental stages of four stored product moths to phosphine. *J. stored Prod. Res.* **12,** 77-86.

Bell, C. H. (1979) The efficiency of phosphine against diapausing larvae of *Ephestia elutella* (Lepidoptera) over a wide range of concentrations and exposure times. *J. stored Prod. Res.* **15,** 53-58.

Bell, C. H. (1992) Time, concentration and temperature relationships for phosphine activity in tests on diapausing larvae of *Ephestia elutella* (Hubner) (Lepidoptera: Pyralidae). *Pestic. Sci.* **35,** 255-264.

Bell, C.H., Chakrabarti, B. and Mills, K.A. (1991) New application methods for the use of phosphine to disinfest bulk grain. Project Report No. 41, Home-Grown Cereals Authority, London. 44pp.

Chakrabarti, B. (1994) Methods of distributing phosphine in bulk grain. Research Review No. 27, Home-Grown Cereals Authority, London. 44pp.

Evans, D. E. (1977) Some aspects of acclimation to low temperatures in the grain weevils *Sitophilus oryzae* (L.) and *S. granarius* (L.). *Austral. J. Ecol.* **2,** 309-318.

Evans, D. E. (1983) The influence of relative humidity and thermal acclimation on the survival of adult grain beetles in cooled grain. *J. stored Prod. Res.* **19,** 173-180.

Fields, P. G. (1992) The control of stored-product insects and mites with extreme temperatures. *J. stored Prod. Res.* **28,** 89-118.

Friemal, W. (1983) The "Detia Bag Blanket", its use and application. Developments in Agricultural Engineering 5, Controlled Atmosphere and Fumigation in Grain Storages, Proceedings of an International Symposium 'Practical Aspects of Controlled Atmosphere and Fumigation in Grain Storages', 11-22 April 1983, Perth, Australia. Ed. B.E. Ripp *et al.*, Elsevier, Amsterdam, 1984, 359-367.

HGCA (1994) Weekly Bulletin, Vol. 29, No.12, 16pp.

Hole, B.D., Bell, C.H., Mills, K.A. and Goodship, G. (1976) The toxicity of phosphine to all developmental stages of thirteen species of stored product beetles. *J. stored Prod. Res.* **12,** 235-244.

Howe, R. H. (1965) A summary of estimates of optimal and minimal conditions for population increase of some stored product insects. *J. stored Prod. Res.* **1,** 177-184.

Howe, R. H. (1973) The susceptibility of the immature and adult stages of *Sitophilus granarius* to phosphine. *J. stored Prod. Res.* **8,** 241-262.

Jacob, T. A. and Fleming, D. A. (1986) The effect of temporary exposure at low temperature on the viability of eggs of *Oryzaephilus surinamensis* (L.) (Col., Silvanidae). *Ent. Month. Mag.* **122,** 117-120.

Mills, K.A., Clifton, A.L., Chakrabarti, B. and Savvidou, N. (1990) The impact of phosphine resistance on the control of insects in stored grain by phosphine fumigation. Proceedings of Brighton Crop Protection Conference, 1990, Pests and Diseases pp. 1181-1187.

Mullen, M. A. and Arbogast, R. T. (1979) Time-temperature-mortality relationships for various stored-product insect eggs and chilling times for selected commodities.
J. econ. Ent. **72,** 476-478.

Price, L. A. and Mills, K. A. (1988) The toxicity of phosphine to the immature stages of resistant and susceptible strains of some common stored product beetles, and implications for their control. *J. stored Prod. Res.* **24,** 51-59.

Prickett, A. J. and Muggleton, J. (1991) Commercial grain stores 1988/9, England and Wales. Pest incidence and storage practice. Project Report No. 29, part 2, Home-Grown Cereals Authority, London.

Reynolds, E. M., Robinson, J. M. and Howells, C. (1967) The effect on *Sitophilus granarius* (L.) (Coleoptera, Curculionidae) of exposure to low concentrations of phosphine. *J. stored Prod. Res.* **2,** 177-186.

Smith, L. B. (1970) Effects of cold-acclimation on supercooling and survival of the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Cucujidae) at sub-zero temperatures. *Can. J. Zool.* **48,** 853-858.

Winks, R. G. (1993) The development of SIROFLO in Australia. Proceedings of the International Conference on Controlled Atmosphere and Fumigation in Grain Storages, Winnipeg, Canada, June, 1992, ed. Navarro, S. and Donahaye, E., Caspit Press, Jerusalem, pp. 399-410.

Winks, R. G., Banks, H. J., Williams, P., Bengston, M. and Greening, H. G. (1980) Dosage recommendations for the fumigation of grain with phosphine. Standing Committee on Agriculture Technical Report Series No. 8, CSIRO, Canberra, 9pp.

Table 1 Origins of laboratory and field strains and their culturing details.

SPECIES	ORIGINS OF LABORATORY STRAINS	ORIGINS OF FIELD STRAINS	FOOD MIXTURES	FOOD WEIGHT IN TEST CULTURES
A.advena	West African Ship at Liverpool Docks 1956	Pebworth - Worcester 1989	5 parts wheatfeed 5 parts rolled oats 2 parts dried yeast	75g (164g)
C.ferrugineus	Insectaries 1977	Pluckley - Kent 1989	12 parts rolled oats 6 parts whole-wheat flour 1 part dried yeast	75g (155g)
O.surinamensis	Insectaries 1977	Cornwall 1989	Rolled oats	75g (150g)
S.granarius	Windsor - 1970	Plympton - Devon 1987	Whole wheat	160g (320g)
T.castaneum	Insectaries 1976	Wells - Somerset 1992	9 parts whole-wheat flour 1 part dried yeast	100g (200g)

( ) = Quantity of food used in 750 ml cultures in preliminary tests

Table 2 Highest exposures and CTP's tested which allowed survival, and the lowest which achieved complete kill of immature stages of *Ahasverus advena* exposed to phosphine at low temperatures.

Strain	Temp. (°C)	Stage*	Conc^{n}. (mg/l)	Last Exposure (h)	Survivor CTP (mgh/l)	100% Exposure (h)	Kill CTP (mgh/l)
Laboratory	10	O	0.05	34[a]	1.66[a]	>34	>1.66
		Y	**0.05	2	0.14	4	0.27
	7.5	O	0.05	32	1.70	40	2.60
	5	O	**0.05	16[b]	0.94[b]	>16	>0.94
		Y	**0.05	<2	<0.15	2	0.15
Field	10	O	0.05	34[c]	1.66[c]	>34	>1.66
		Y	**0.05	<4	<0.27	4	0.27
	7.5	O	0.05	24	1.30	32	1.70
	5	O	**0.05	16	0.94	32	1.54
		Y	**0.05	<4	<0.27	4	0.27

* O = Older larvae and pupae , Y = Eggs and younger larvae.
** = Results of preliminary tests.
a - c = complete kill not achieved at the highest dose tested:
a = 2 survivors out of 903 individuals expected from control emergence.
b = 6 survivors out of 58 individuals expected from control emergence.
c = 3 survivors out of 132 individuals expected from control emergence.

Table 3 Probit analysis parameters for the older developmental stages of *Ahasverus advena*.

Strain	Temp. (°C)	Conc^{n}. (mg/l)	Slope ± S.E.	LD 50	LD 99	Degrees of Freedom	HF
Laboratory	10	0.05	4.29 ± 0.44	0.35	1.22	12	18.705
	7.5	0.05	2.88 ± 0.48	0.24	1.53	16	10.381
Field	10	0.05	7.09 ± 1.12	0.60	1.29	6	8.382
	7.5	0.05	7.38 ± 1.13	0.51	1.05	8	9.988

HF = Heterogeneity factor

Table 4 Cold susceptibility of immature stages of *Ahasverus advena.*

Strain	Stage*	Temp. (°C)	Number emerged in 25°C control	Exposure (Days)	Percentage (of control) emerging
Laboratory	O	10	1992	7	43.5
		7.5	3179	9	15.8
	Y	10	1791	4	37.0
			1791	7	25.2
			1791	10	8.8
		5	1791	3	4.6
			1791	5	0.5
			1791	7	0.0
Field	O	10	376	7	49.7
		7.5	1143	9	13.8
	Y	10	323	4	15.8
			323	7	17.2
			323	10	9.3
		5	215	3	0.93
			215	5	0.0

* O = Older larvae and pupae, Y = Eggs and younger larvae

Table 5 Highest exposures and CTP's tested which allowed survival, and the lowest which achieved complete kill of adult *Ahasverus advena* exposed to phosphine at low temperatures.

Strain	Temp. (°C)	Concn. (mg/l)	Days after Treatment	Last Exposure (h)	Survivor CTP (mgh/l)	100% Exposure (h)	Kill CTP (mgh/l)
Laboratory	10	0.05	6	15	0.77	23	1.19
			14	10	0.50	15	0.77
	7.5	0.05	4	18	0.95	>18	>0.95
			7	18	0.95	>18	>0.95
			10	18	0.95	>18	>0.95
			14	18[a]	0.95[a]	>18	>0.95
		0.15	14	18	2.61	24	3.93
	5	**0.05	14	16	0.94	>16	>0.94
Field	10	0.05	6	23	1.19	34	1.66
			14	15	0.77	23	1.19
	7.5	0.05	4	24	1.30	>24	>1.30
			7	24	1.30	>24	>1.30
			10	24	1.30	>24	>1.30
			14	18	0.95	24	1.30
		0.15	14	24[b]	3.93[b]	>24	>3.93

** = Results of preliminary tests.
a & b = Complete kill not achieved at the highest dose tested:
a = 32 survivors out of 149 individuals tested.
b = 1 survivor out of 150 individuals tested.

Table 6 Probit analysis parameters for the adults of *Ahasverus advena* from mortality counts 3 - 14 days after treatment.

Strain	Temp. (°C)	Conc^{n}. (mg/l)	Days after Treatment	Slope ± S.E.	LD 50	LD 99	Degrees of Freedom	HF
Laboratory	10	0.05	3	-	-	-	-	-
			6	7.08 ± 0.92	0.41	0.87	9	2.625
			10	-	-	-	-	-
			14	6.74 ± 0.94	0.37	0.82	8	2.671
	7.5	0.05	4	2.60 ± 0.54	1.74	13.66	13	1
			7	3.78 ± 0.37	0.81	3.40	16	1
			10	3.70 ± 0.47	0.74	3.17	16	1
			13	3.84 ± 0.34	0.70	2.82	16	1
		0.15	14	5.11 ± 0.38	0.98	2.78	5	1
Field	10	0.05	3	-	-	-	-	-
			6	5.77 ± 0.84	0.56	1.42	9	4.881
			10	-	-	-	-	-
			14	5.97 ± 0.95	0.44	1.07	7	3.593
	7.5	0.05	4	3.55 ± 0.35	0.61	2.78	18	1.686
			7	4.46 ± 0.32	0.47	1.56	18	1
			10	4.56 ± 0.33	0.47	1.51	17	1
			13	4.64 ± 0.33	0.46	1.46	17	1
		0.15	14	4.38 ± 0.36	0.73	2.47	7	1

HF = Heterogeneity factor

Table 7 Cold susceptibility of immature stages of *Tribolium castaneum.*

Strain	Stage*	Temp. (°C)	Number emerged in 25°C control	Exposure (Days)	Percentage (of control) emerging
Laboratory	O	10	5530	6	88.0
			4525	14	43.2
	Y	10	5669	6	6.8
			5176	14	0.6
Field	O	10	5977	6	51.4
			3144	11	59.2
			3806	14	60.8
		7.5	2785	32	0.6
		5	2672	11	46.7
	Y	10	3803	6	12.0
			4692	14	0.1

* O = Older larvae and pupae, Y = Eggs and younger larvae

Table 8 Highest exposures and CTP's tested which allowed survival, and the lowest which achieved complete kill of immature stages of *Tribolium castaneum* exposed to phosphine at low temperatures.

Strain	Temp. (°C)	Stage*	Conc^{n}. (mg/l)	Last Survivor Exposure (h)	CTP (mgh/l)	100% Kill Exposure (h)	CTP (mgh/l)
Laboratory	10	O	**0.05	16	0.72	>16	>0.72
			0.11	16	1.71	24	2.57
			0.16	7	1.14	10	1.74
		Y	**0.05	8	0.38	16	0.72
			0.11	<6	<0.59	6	0.59
			0.16	<5	<0.80	5	0.80
	5	O	**0.05	16[a]	0.72[a]	>16	>0.72
		Y	**0.05	4	0.18	8	0.38
Field	10	O	**0.05	32	1.44	>32	>1.44
			0.11	64	6.19	>64	>6.19
			0.16	168	23.76	>168	>23.76
			0.29	144	42.53	168	47.71
		Y	**0.05	32	1.44	>32	>1.44
			0.11	48	5.95	>48	>5.95
			0.16	<48	<8.34	48	8.34
	7.5	O	0.30	<122	<36.60	122	36.60
	5	O	**0.05	32	1.40	>32	>1.40
			0.34	170[b]	55.13[b]	>170	>55.13
		Y	**0.05	32[c]	1.40[c]	>32	>1.40

* O = Older larvae and pupae , Y = Eggs and younger larvae.
** = Results of preliminary tests.
a - c = complete kill not achieved at the highest dose tested:
a = 118 survivors out of 281 individuals expected from control emergence.
b = 7 survivors out of 1248 individuals expected from control emergence.
c = 1 survivors out of 14 individuals expected from control emergence.

Table 9 Probit analysis parameters for the immature developmental stages of *Tribolium castaneum.*

Strain	Temp. (°C)	Stage*	Conc^{n}. (mg/l)	Slope ± S.E.	LD 50	LD 99	Degrees of Freedom	HF
Laboratory	10	O	0.11	5.69 ± 0.23	0.65	1.67	8	5.413
		O	0.16	12.11 ± 2.15	0.69	1.08	4	7.979
Field	10	O	0.11	2.42 ± 0.16	2.27	20.89	17	28.795
		O	0.16	3.07 ± 0.52	2.24	12.89	16	4.652
		O	0.29	2.58 ± 0.42	2.82	22.46	14	2.890
		Y	0.11	1.88 ± 0.181	0.72	12.48	19	4.270
	5	O	0.34	2.24 ± 0.26	3.79	41.21	19	2.900

* O = Older larvae and pupae, Y = Eggs and younger larvae.
HF = Heterogeneity factor

Table 10 Highest exposures and CTP's tested which allowed survival, and the lowest which achieved complete kill of adult *Tribolium castaneum* exposed to phosphine at low temperatures.

Strain	Temp. (°C)	Concⁿ. (mg/l)	Days after Treatment	Last Exposure (h)	Survivor CTP (mgh/l)	100% Exposure (h)	Kill CTP (mgh/l)
Laboratory	10	**0.05	14	16	0.72	>16	>0.72
		0.11	3	16	1.71	24	2.57
			6	16	1.71	24	2.57
			10	16	1.71	24	2.57
			14	10	0.98	16	1.71
		0.16	3	10	1.74	14	2.37
			6	7	1.14	10	1.74
			10	5	0.80	7	1.14
			14	5	0.80	7	1.14
	7.5	0.15	14	9	1.35	14	2.15
	5	**0.05	14	16[a]	0.72[a]	>16	>0.72
Field	10	**0.05	14	32	1.44	>32	>1.44
		0.11	3	48	5.95	>48	>5.95
			6	48	5.98	>48	>5.95
			10	48	5.95	>48	>5.95
			14	48[b]	5.95[b]	>48	>5.95
		0.16	6	48	8.34	>48	>8.34
			10	48	8.34	>48	>8.34
			14	48[c]	8.34[c]	>48	>8.34
	7.5	0.15	14	80[d]	12.28[d]	>80	>12.28
	5	0.34	7	96	31.35	>96	>31.35
			11	96	31.35	>96	>31.35
			14	96[e]	31.35[e]	>96	>31.35

** = Results of preliminary tests.
a - e Complete kill not achieved at the highest dose tested:
a = 5 survivors out of 50 individuals tested.
b = 19 survivors out of 150 individuals tested.
c = 6 survivors out of 152 individuals tested.
d = 2 survivors out of 148 individuals tested.
e = 1 survivor out of 99 individuals tested.

Table 11 Probit analysis parameters for the adults of *Tribolium castaneum* from mortality counts 3 - 14 days after treatment.

Strain	Temp. (°C)	Conc^{n}. (mg/l)	Days After Treatment	Slope ± S.E.	LD 50	LD 99	Degrees of Freedom	HF
Laboratory	10	0.11	3	8.40 ± 1.18	0.85	1.60	7	3.135
			6	8.29 ± 0.67	0.80	1.52	7	1
			10	11.46 ± 0.82	0.72	1.14	7	1
			14	14.87 ± 1.10	0.68	0.98	5	1
		0.16	3	9.00 ± 0.62	0.72	1.31	10	1
			6	10.76 ± 0.80	0.67	1.10	7	1
			10	12.60 ± 0.98	0.59	0.90	7	1
			14	14.75 ± 1.20	0.56	0.81	4	1
	7.5	0.15	14	3.68 ± 1.29	0.35	1.51	5	1
Field	10	0.11	3	2.63 ± 0.18	3.10	23.73	16	1
			6	2.78 ± 0.19	2.96	20.30	16	1
			10	2.52 ± 0.17	2.28	19.17	16	1
			14	2.68 ± 0.16	2.02	14.89	19	1
		0.16	3	-	-	-	-	-
			6	1.72 ± 0.16	1.80	40.51	19	1
			10	2.14 ± 0.18	1.58	19.32	19	1
			14	2.29 ± 0.19	1.49	15.38	19	1
	7.5	0.15	14	2.57 ± 0.23	1.96	15.72	13	1
	5	0.34	7	2.10 ± 0.56	3.57	45.85	7	1
			11	2.45 ± 0.60	4.19	37.36	7	1
			14	1.76 ± 0.65	1.98	41.19	7	1

HF = Heterogeneity factor

Table 12 Highest exposures and CTP's tested which allowed survival, and the lowest which achieved complete kill of immature stages of *Oryzaephilus surinamensis* exposed to phosphine at low temperatures.

Strain	Temp. (°C)	Stage*	Conc^{n}. (mg/l)	Last Survivor Exposure (h)	Last Survivor CTP (mgh/l)	100% Kill Exposure (h)	100% Kill CTP (mgh/l)
Laboratory	10	O	**0.05	16	0.83	32	1.45
		O	0.06	24	1.59	32.5	2.10
			0.12	24	3.00	32	3.96
		Y	0.06	10	0.67	16	0.98
			0.12	10	1.19	15	1.87
	7.5	O	0.05	32[a]	1.70[a]	>32	>1.70
	5	O	**0.05	32	1.44	64	2.88
		Y	**0.05	<16	<0.60	16	0.60
Field	10	O	**0.05	64	2.89	96	4.14
		O	0.06	96	4.61	>96	>4.61
			0.12	32	3.96	49	7.14
		Y	0.06	16	0.98	24	1.59
			0.12	15	1.87	20	2.54
	7.5	O	0.05	104[b]	5.14[b]	>104	>5.14
	5	O	**0.05	64	2.88	96	4.34
		Y	**0.05	16	0.60	24	1.02

* O = Older larvae and pupae , Y = Eggs and younger larvae.
** = Results of preliminary tests.
a & b = complete kill not achieved at the highest dose tested:
a = 5 survivors out of 834 individuals expected from control emergence.
b = 3 survivors out of 949 individuals expected from control emergence.

Table 13 Probit analysis parameters for the immature developmental stages of *Oryzaephilus surinamensis.*

Strain	Temp. (°C)	Stage*	Conc^{n}. (mg/l)	Slope ± S.E.	LD 50	LD 99	Degrees of Freedom	HF
Laboratory	10	O	0.06	3.16 ± 0.39	0.37	2.04	11	6.930
		O	0.12	3.55 ± 0.21	0.58	2.61	15	6.476
		Y	0.06	5.58 ± 0.28	0.33	0.86	8	1.000
		Y	0.12	5.04 ± 0.72	0.47	1.36	10	18.269
	7.5	O	0.05	3.42 ± 0.21	0.27	1.28	16	4.547
Field	10	O	0.06	2.20 ± 0.19	0.49	5.62	17	6.360
		O	0.12	3.60 ± 0.34	1.24	5.48	14	21.988
		Y	0.06	4.16 ± 0.64	0.36	1.32	11	12.492
		Y	0.12	4.34 ± 0.30	0.46	1.57	8	4.685
	7.5	O	0.05	2.20 + 0.20	0.53	5.99	19	13.79

* O = Older larvae and pupae, Y = Eggs and younger larvae.
HF = Heterogeneity factor

Table 14 Highest exposures and CTP's tested which allowed survival, and the lowest which achieved complete kill of adult *Oryzaephilus surinamensis* exposed to phosphine at low temperatures.

Strain	Temp. (°C)	Conc^{n}. (mg/l)	Days after Treatment	Last Exposure (h)	Survivor CTP (mgh/l)	100% Exposure (h)	Kill CTP (mgh/l)
Laboratory	10	**0.05	14	16	0.50	24	1.19
		0.06	3	32.5	2.10	>32.5	>2.10
			6	32.5	2.10	>32.5	>2.10
			10	16	0.98	24	1.59
			14	16	0.98	24	1.59
		0.12	3	20	2.54	24	3.01
			6	20	2.54	24	3.01
			10	15	1.87	20	2.54
			14/16	15	1.87	20	2.54
	7.5	0.05	3	32	1.70	>32	>1.70
			6	32	1.70	>32	>1.70
			10	32	1.70	>32	>1.70
			14	32	1.70	>32	>1.70
		0.15	14	8	1.10	14	2.15
	5	**0.04	14	24	1.02	32	1.44
Field	10	**0.05	14	24	1.19	32	1.45
		0.06	3	32.5	2.10	48	3.12
			6	24	1.59	32.5	2.10
			10	24	1.59	32.5	2.10
			14	24	1.59	32.5	2.10
		0.12	3	24	3.01	32	3.96
			6	24	3.01	32	3.96
			10	15	1.87	20	2.54
			14/16	15	1.87	20	2.54
	7.5	0.05	3	24	1.30	>24	>1.30
			6	24	1.30	>24	>1.30
			10	24	1.30	>24	>1.30
			14	24	1.30	>24	>1.30
		0.15	14	14	2.15	24	3.93
	5	**0.04	14	32	1.44	64	2.88

** = Results of preliminary tests.

Table 15 Probit analysis parameters for the adults of *Oryzaephilus surinamensis* from mortality counts 3 - 16 days after treatment.

Strain	Temp. (^{o}C)	Conc^{n}. (mg/l)	Days After Treatment	Slope ± S.E.	LD 50	LD 99	Degrees of Freedom	HF
Laboratory	10	0.06	3	8.89 ± 0.66	1.01	1.85	5	1
			6	6.21 ± 0.34	0.78	1.84	10	1
			10	7.12 ± 0.72	0.63	1.34	10	2.238
			14	6.98 ± 0.79	0.63	1.36	10	2.709
		0.12	3	8.11 ± 0.52	1.26	2.44	8	1
			6	6.09 ± 0.36	0.93	2.23	11	1
			10	7.33 ± 0.69	0.72	1.51	10	1.871
			16	7.57 ± 0.57	0.72	1.45	10	1
	7.5	0.05	3	10.97 ± 0.73	1.02	1.66	8	1
			6	7.02 ± 0.38	0.65	1.39	10	1
			10	7.97 ± 0.50	0.64	1.25	11	1
			14	8.01 ± 0.53	0.64	1.25	10	1
		0.15	14	4.06 ± 1.85	0.37	1.37	4	1
Field	10	0.06	3	12.99 ± 0.92	1.26	1.90	4	1
			6	11.02 ± 0.85	1.16	1.89	4	1
			10	9.60 ± 0.78	1.01	1.76	8	1
			14	10.18 ± 0.93	1.01	1.70	7	1
		0.12	3	8.01 ± 0.87	1.59	3.11	6	3.207
			6	6.78 ± 0.42	1.38	3.05	9	1
			10	7.06 ± 0.49	1.24	2.65	8	1
			14	6.90 ± 0.48	1.22	2.66	8	1
	7.5	0.05	3	5.44 ± 1.17	1.74	4.67	7	1
			6	4.89 ± 0.57	1.26	3.77	10	1
			10	5.26 ± 0.58	1.21	3.34	10	1
			14	5.87± 0.60	1.11	2.77	10	1
		0.15	14	6.52 ± 0.61	1.09	2.49	7	1

HF = Heterogeneity factor

Table 16 Highest exposures and CTP's tested which allowed survival, and the lowest which achieved complete kill of immature stages of *Cryptolestes ferrugineus* exposed to phosphine at low temperatures.

Strain	Temp. (°C)	Stage*	Conc^{n}. (mg/l)	Last Exposure (h)	Survivor CTP (mgh/l)	100% Exposure (h)	Kill CTP (mgh/l)
Laboratory	10	O	0.10	136	12.67	193	18.38
			0.36	45	15.84	69	24.80
		Y	0.10	48	4.81	67	6.79
			0.36	21	7.84	30	11.21
	7.5	O	0.11	162	18.09	193	20.79
			0.30	96	27.53	>96	>27.53
		Y	0.11	96	12.90	>96	>12.90
	5	O	**0.09	192	15.72	>192	15.72
			0.78	96	80.44	>96	>80.44
		Y	**0.09	48	3.18	96	8.16
Field	10	O	0.10	193	18.38	>193	>18.38
			0.36	96	33.09	144	50.47
		Y	0.10	96	9.52	>96	>9.52
			0.36	45	15.84	69	24.80
	7.5	O	0.11	193	20.79	286	27.06
			0.30	96	27.53	144	41.53
		Y	0.11	162	18.09	>162	>18.09
	5	O	**0.09	96	8.16	192	15.72
			0.78	72.5	58.73	96	80.44
		Y	**0.05	48		96	

* O = Older larvae and pupae , Y = Eggs and younger larvae.
** = Results of preliminary tests. Concentration quoted is that from the second day of the test.

Table 17 Probit parameters for the immature developmental stages of *Cryptolestes ferrugineus.*

Strain	Temp. (°C)	Stage*	Conc^{n}. (mg/l)	Slope ± S.E.	LD 50	LD 99	Degrees of Freedom	HF
Laboratory	10	O	0.10	7.44 ± 0.48	3.30	6.77	10	8.428
		O	0.36	3.75 ± 0.30	3.16	13.16	10	2.017
		Y	0.10	8.27 ± 1.31	1.61	3.07	8	19.375
	7.5	O	0.11	7.36 ± 1.13	6.53	13.53	8	30.098
		O	0.30	6.23 ± 0.93	5.65	13.34	10	13.144
		Y	0.11	3.41 ± 0.35	1.55	7.45	10	2.981
	5	O	0.78	1.96 ± 0.23	4.72	72.37	18	3.112
Field	10	O	0.10	3.98 ± 0.27	1.98	7.60	12	4.749
		O	0.36	3.79 ± 0.21	4.99	20.52	13	4.118
		Y	0.10	11.50 ± 2.23	1.99	3.17	5	56.746
		Y	0.36	4.26 ± 0.51	1.69	5.94	10	12.398
	7.5	O	0.11	4.06 ± 0.35	3.64	13.64	13	5.913
		O	0.30	3.06 ± 0.20	3.35	19.27	16	2.425
		Y	0.11	2.88 ± 0.45	2.54	16.33	19	17.957
	5	O	0.78	2.31 ± 0.37	4.27	43.39	12	2.394

* O = Older larvae and pupae, Y = Eggs and younger larvae.
HF = Heterogeneity factor

Table 18 Highest exposures and CTP's tested which allowed survival, and the lowest which achieved complete kill of adult *Cryptolestes ferrugineus* exposed to phosphine at low temperatures.

Strain	Temp. (°C)	Conc^{n}. (mg/l)	Days after Treatment	Last Exposure (h)	Survivor CTP (mgh/l)	100% Exposure (h)	Kill CTP (mgh/l)
Laboratory	10	0.10	6/7	96	9.52	>96	>9.52
			14	96	9.52	>96	>9.52
		0.29	7	72	19.89	96	28.01
			11/12	72	19.89	96	28.01
			14	72	19.89	96	28.01
	7.5	0.11	21	96	12.90	162	18.09
		0.30	14	53	15.50	72	21.29
	5	**0.07	14	24	1.11	48	3.18
		0.78	3/4	72	58.73	>72	>58.73
			6-9	72	58.73	>72	>58.73
			10-12	72	58.73	>72	>58.73
			14-16	72[a]	58.73[a]	>72	>58.73
Field	10	0.1	6/7	96	9.52	>96	>9.52
			14	67	6.79	96	9.52
		0.29	7	72	19.89	96	28.01
			11/12	72	19.89	96	28.01
			14	72	19.89	96	28.01
	7.5	0.30	14	72[b]	21.29[b]	>72	>21.29
	5	**0.09	14	48	3.18	96	8.16
		0.78	3/4	96	80.44	>96	>80.44
			6-9	96	80.44	>96	>80.44
			10-12	96	80.44	>96	>80.44
			14-16	96[c]	80.44[c]	>96	>80.44

** = Results of preliminary tests. Concentration quoted is that from the second day of the test.
a - c = Complete kill not achieved at the highest dose tested:
a = 15 survivors out of 151 individuals tested.
b = 1 survivor out of 158 individuals tested.
c = 4 survivors out of 151 individuals tested.

Table 19 Probit analysis parameters for the adults of *Cryptolestes ferrugineus* from mortality counts 3 - 14 days after treatment.

Strain	Temp. (^{o}C)	Conc^{n}. (mg/l)	Days After Treatment	Slope ± S.E.	LD 50	LD 99	Degrees of Freedom	HF
Laboratory	10	0.10	6/7	3.32 ± 0.36	3.77	18.92	16	2.749
			14	3.45 ± 0.24	3.03	14.31	16	1
		0.29	7	3.51 ± 0.30	6.21	28.58	17	1
			11	2.55 ± 0.41	2.59	21.15	8	1
			14	2.38 ± 0.44	2.14	20.40	7	1
	7.5	0.11	21	5.04 ± 0.77	4.61	13.33	11	2.549
		0.30	14	2.85 ± 0.26	3.74	24.52	17	1
Field	10	0.10	6/7	3.91 ± 0.29	2.07	8.15	14	1.745
			14	4.30 ± 0.56	1.67	5.80	13	3.985
		0.29	7	2.67 ± 0.40	5.07	37.82	15	1.944
			12	2.04 ± 0.47	1.41	19.49	9	1
			14	3.08 ± 0.90	1.95	11.10	5	1
	7.5	0.30	14	1.99 ± 0.37	1.70	25.25	17	3.514

HF = Heterogeneity factor

Table 20 Highest exposures and CTP's tested which allowed survival, and the lowest which achieved complete kill of immature stages of *Sitophilus granarius* exposed to phosphine at low temperatures.

Strain	Temp. (°C)	Stage*	Conc^{n}. (mg/l)	Last Survivor Exposure (h)	CTP (mgh/l)	100% Kill Exposure (h)	CTP (mgh/l)
Laboratory	10	O	0.64	359	224.09	>359	>224.09
			1.35	360[a]	467.64[a]	>360	>467.64
		Y	0.64	<28.5	<20.20	28.5	20.20
			1.35	<24	<36.20	24	36.20
	7.5	O	0.70	555	366.00	674	434.31
			1.62	460[b]	707.79[b]	>460	>707.79
	5	O	0.78	673[c]	459.08[c]	>673	>459.08
			**1.90	96	181.34	192	367.30
Field	10	O	0.64	359	224.09	>359	>224.09
			1.35	264	353.44	360	467.64
		Y	0.64	<28.5	<20.20	28.5	20.20
			1.35	<24	<36.20	24	36.20
	7.5	O	0.70	555	366.00	>555	>366.00
			1.62	307	491.25	460	707.79
	5	O	0.78	504	356.73	673	459.10

* O = Older larvae and pupae , Y = Eggs and younger larvae.
** = Results of preliminary tests.
a - c = complete kill not achieved at the highest dose tested:
a = 1 survivor out of 264 individuals expected from control emergence.
b = 3 survivors out of 2836 individuals expected from control emergence.
c = 2 survivors out of 1254 individuals expected from control emergence.

Table 21 Mean emergence from three replicates of older cultures of *S. granarius* tested at 10°C.

Concⁿ. (mg/l)	Exposure (h)	CTP (mgh/l)	Mean no. emerged ± S.E.	
			Laboratory	Field
0.64	Control	0	311.83 ± 42.59	333.33 ± 25.88
	54	38.27	76.33 ± 25.30	11.33 ± 3.54
	74	51.98	72.00 ± 15.94	11.67 ± 2.60
	99	68.24	65.33 ± 11.08	15.67 ± 3.84
	140	88.25	40.33 ± 18.62	15.67 ± 5.19
	190	125.85	1.33 ± 0.54	3.33 ± 1.09
	262.5	169.10	10.33 ± 7.62	4.00 ± 0.47
	358.5	224.09	0.67 ± 0.54	0.67 ± 0.27
1.35	Control	0	88.00 ± 13.27	361.00 ± 62.59
	55	82.42	9.67 ± 4.84	15.33 ± 3.31
	75	110.90	2.67 ± 1.19	13.00 ± 3.56
	100	145.23	8.00 ± 2.05	12.67 ± 3.54
	140	182.12	4.67 ± 1.09	11.67 ± 4.48
	191	263.07	0.33 ± 0.27	7.33 ± 1.36
	264	353.44	2.00 ± 0.47	3.33 ± 1.52
	360	467.64	0.33 ± 0.27	0 -

Table 22 Mean emergence from three replicates of older cultures of *S. granarius* tested at 7.5°C.

Concn. (mg/l)	Exposure (h)	CTP (mgh/l)	Mean no. emerged ± S.E.	
			Laboratory	Field
0.70	Control	0	503.00 ± 35.78	374.67 ± 38.67
	48	37.36	-	89.00 ± 4.64
	72	52.74	102.00 ± 15.37	-
	96.5	71.00	100.00 ± 8.73	58.33 ± 1.19
	170	125.43	56.50 ± 1.77	40.00 ± 7.13
	264	175.48	35.00 ± 2.12	14.33 ± 1.44
	385	265.40	9.00 ± 3.68	9.33 ± 9.11
	555	366.00	1.33 ± 0.27	0.33 ± 0.27
	673.5	434.31	0 -	not tested
1.62	Control	0	945.33 ± 95.24	514.67 ± 114.12
	48	84.87	139.00 ± 7.41	47.33 ± 9.66
	70	113.54	118.33 ± 7.31	33.33 ± 8.06
	100	162.52	132.33 ± 3.81	41.33 ± 6.13
	146	232.82	95.00 ± 8.96	22.00 ± 5.19
	210	346.41	55.67 ± 7.31	7.67 ± 1.66
	307	491.24	14.33 ± 2.60	3.00 ± 1.41
	459.5	707.79	1.00 ± 0.47	0 -

Table 23 Mean emergence from three replicates of older cultures of *S. granarius* tested at 5°C.

Conc^{n}. (mg/l)	Exposure (h)	CTP (mgh/l)	Mean no. emerged ± S.E.	
			Laboratory	Field
0.78	Control	0	418.00 ± 60.86	424.67 ± 18.23
	72	58.73	85.00 ± 0.94	67.33 ± 7.89
	96	80.44	76.67 ± 11.59	42.33 ± 3.84
	168	132.05	34.00 ± 4.64	24.00 ± 3.30
	264	203.27	25.67 ± 4.23	22.33 ± 5.82
	335.5	247.65	16.33 ± 5.89	6.67 ± 0.27
	504	356.73	7.00 ± 2.16	4.00 ± 0.94
	673	459.08	0.67 ± 0.27	0 -

Table 24 Probit analysis parameters for the older developmental stages of *Sitophilus granarius*.

Strain	Temp. (°C)	Conc^{n}. (mg/l)	Slope ± S.E.	LD 50	LD 99	Degrees of Freedom	HF
Laboratory	10	0.64	2.27 ± 0.52	23.32	246.25	17	27.046
		1.35	1.47 ± 0.49	11.42	438.87	15	4.300
	7.5	0.70	1.93 ± 0.20	23.28	374.38	15	7.746
		1.62	1.30 ± 0.17	16.95	1058.22	19	12.086
	5	0.78	1.72 ± 0.16	21.52	485.41	19	4.760
Field	10	0.64	0.92 ± 0.31	0.65	217.24	19	5.424
		1.35	1.08 ± 0.29	2.60	366.14	17	4.370
	7.5	0.70	1.54 ± 0.16	14.43	465.66	14	4.921
		1.62	1.43 ± 0.24	11.60	490.56	17	7.257
	5	0.78	1.66 ± 0.19	14.69	369.68	17	4.784

HF = Heterogeneity factor.

Table 25 Highest exposures and CTP's tested which allowed survival, and the lowest which achieved complete kill of adult *Sitophilus granarius* exposed to phosphine at low temperatures.

Strain	Temp. (°C)	Conc$^{n.}$ (mg/l)	Days after Treatment	Last Survivor Exposure (h)	CTP (mgh/l)	100% Kill Exposure (h)	CTP (mgh/l)
Laboratory	10	0.64	3	74	51.98	>74	>51.98
			6	40	28.21	74	51.98
			10	40	28.21	74	51.98
			14	40	28.21	74	51.98
		1.35	6	75	110.90	>75	>110.90
			10	75	110.90	>75	>110.90
			14	44	64.21	55	82.42
	7.5	0.70	14	48	37.36	>48	>37.36
		1.62	14	19	33.22	25	43.81
	5	0.78	3/4	72	58.73	>72	>58.73
			6-8	72	58.73	>72	>58.73
			10/11	40	34.86	54.5	43.73
			14	30	27.40	40	34.86
Field	10	0.64	3	74	51.98	>74	>51.98
			6	74	51.98	>74	>51.98
			10	74	51.98	>74	>51.98
			14	40	28.21	74	51.98
		1.35	6	44	64.21	75	110.90
			10	44	64.21	75	110.90
			14	24	36.20	31	46.69
	7.5	0.70	14	48	37.36	>48	>37.36
		1.62	14	25a	43.81a	>25	>43.81
	5	0.78	6-8	40	34.86	54.5	43.73
			10/11	40	34.86	54.5	43.73
			14	40	34.86	54.5	43.73

** = Results of preliminary tests.
a = Complete kill not achieved at the highest dose tested, 32 survivors out of 149 individuals tested.

Table 26 Probit analysis parameters for the adults of *Sitophilus granarius* from mortality counts 3 - 14 days after treatment.

Strain	Temp. (°C)	Conc^{n}. (mg/l)	Days After Treatment	Slope ± S.E.	LD 50	LD 99	Degrees of Freedom	HF
Laboratory	10	0.64	3	1.79 ± 0.35	4.23	84.16	8	1
			6	3.10 ± 0.55	6.17	34.71	4	1
			10	2.40 ± 0.63	3.29	30.51	2	1
		1.35	6	1.76 ± 0.39	5.00	104.94	12	1
			10	2.41 ± 0.62	6.02	55.52	5	1
	7.5	0.70	14	2.91 ± 0.16	6.56	41.30	16	1
		1.62	14	1.19 ± 0.17	0.70	63.39	13	1.991
	5.0	0.78	3/4	5.36 ± 0.62	35.06	95.18	16	2.118
			6-8	3.93 ± 0.54	21.26	83.15	17	3.515
			10/11	2.06 ± 0.42	5.73	77.56	14	3.051
			14	1.61 ± 0.46	1.00	28.17	4	1
Field	10	0.64	3	2.48 ± 0.29	8.63	75.09	11	1
			6	2.44 ± 0.36	5.76	51.55	11	1
			10	2.67 ± 0.43	5.32	39.43	8	1
		1.35	6	2.75 ± 0.52	10.51	73.86	8	1
			10	3.21 ± 0.78	9.47	50.29	5	1
	7.5	0.70	14	3.00 ± 0.16	5.88	35.14	17	1
		1.62	14	1.53 ± 0.14	1.51	49.88	15	1
	5.0	0.78	6-8	3.35 ± 0.33	14.69	72.83	13	2.377
			10/11	2.24 ± 0.23	5.32	57.95	13	1
			14	1.59 ± 0.26	2.04	59.03	13	1

HF = Heterogeneity factor

Table 27 Alternative probit analysis using projected smaller sample sizes for the older developmental stages of *Sitophilus granarius*. Sample sizes reduced to a fraction of the control emergence based on the survival at the lowest CT products.

Strain	Temp. (°C)	Conc^{n}. (mg/l)	Fraction of original population#	Slope ± S.E.	LD 50	LD 99	Degrees of Freedom	HF
Laboratory	10	0.64	$^{5}/_{21}$	4.40 ± 0.94	75.25	254.22	13	30.240
		1.35	$^{3}/_{21}$	2.82 ± 0.82	118.24	788.30	13	4.400
	7.5	0.70	$^{5}/_{21}$	3.91 ± 0.32	114.67	450.08	13	6.150
		1.62	$^{4}/_{21}$	2.79 ± 0.30	197.07	1346.74	19	12.820
	5	0.78	$^{5}/_{21}$	3.41 ± 0.34	120.56	581.44	19	7.270
Field	10	0.64	$^{1}/_{21}$	3.11 ± 0.94	104.74	586.18	11	8.180
		1.35	$^{1}/_{21}$	3.68 ± 0.77	202.92	869.59	11	5.129
	7.5	0.70	$^{5}/_{21}$	3.88 ± 0.39	106.20	422.44	12	5.960
		1.62	$^{2}/_{21}$	4.04 ± 0.59	206.10	777.15	11	8.309
	5	0.78	4/21	3.15 ± 0.39	99.84	547.36	17	7.463

HF = Heterogeneity factor

= Fraction, based on the number of days out of the 21-day egg-laying period, of insects arriving at a stage of tolerance by the time of fumigation.

Table 28 Estimated dosages required for control of five species of stored product beetles at 5 - 10°C.

Species	Temperature (°C)	Dose (mg/l)	Exposure (days)*
A. advena	10 7.5 5	0.10 0.05 0.05	2 2 2
T. castaneum Laboratory strain	10	0.10	2
O. surinamensis	10 7.5 5	0.10 0.10 0.05	4 4 4
T. castaneum Phosphine resistant strain	10 5	0.30 0.30	8 8
C. ferrugineus	10 7.5 5	0.10 0.30 0.10 0.30 0.10 0.80	12 8 12 8 12 6
S. granarius	10 7.5 5	1.00 1.00 1.00	18 >23 >28

* Extra time has to be allowed for the decomposition of a solid formulation and the distribution of the gas through the grain bulk.

Figure 1 The apparatus used to generate phosphine for dosing desiccators

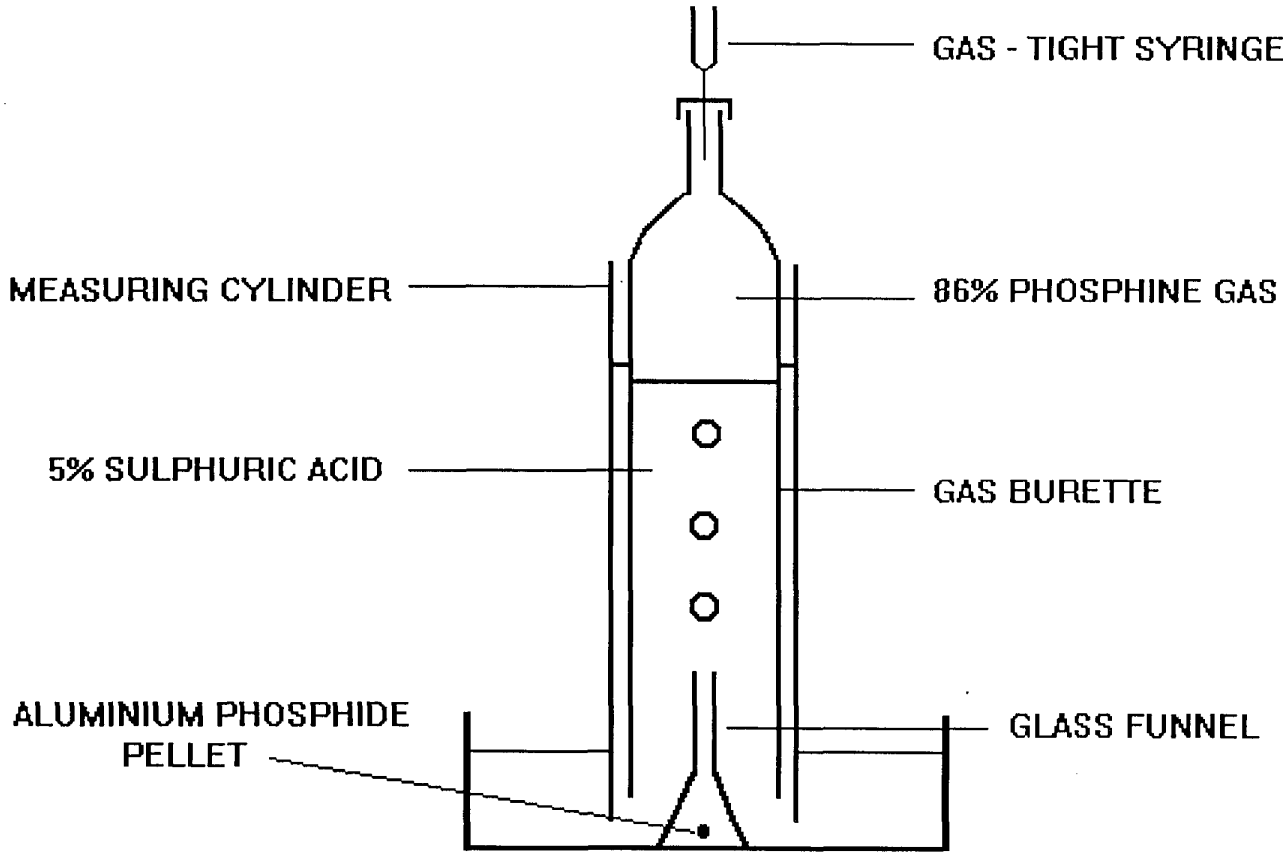

Figure 2 Probit response lines for older stages of the field strain of *Tribolium castaneum* at 5 and 10°C.

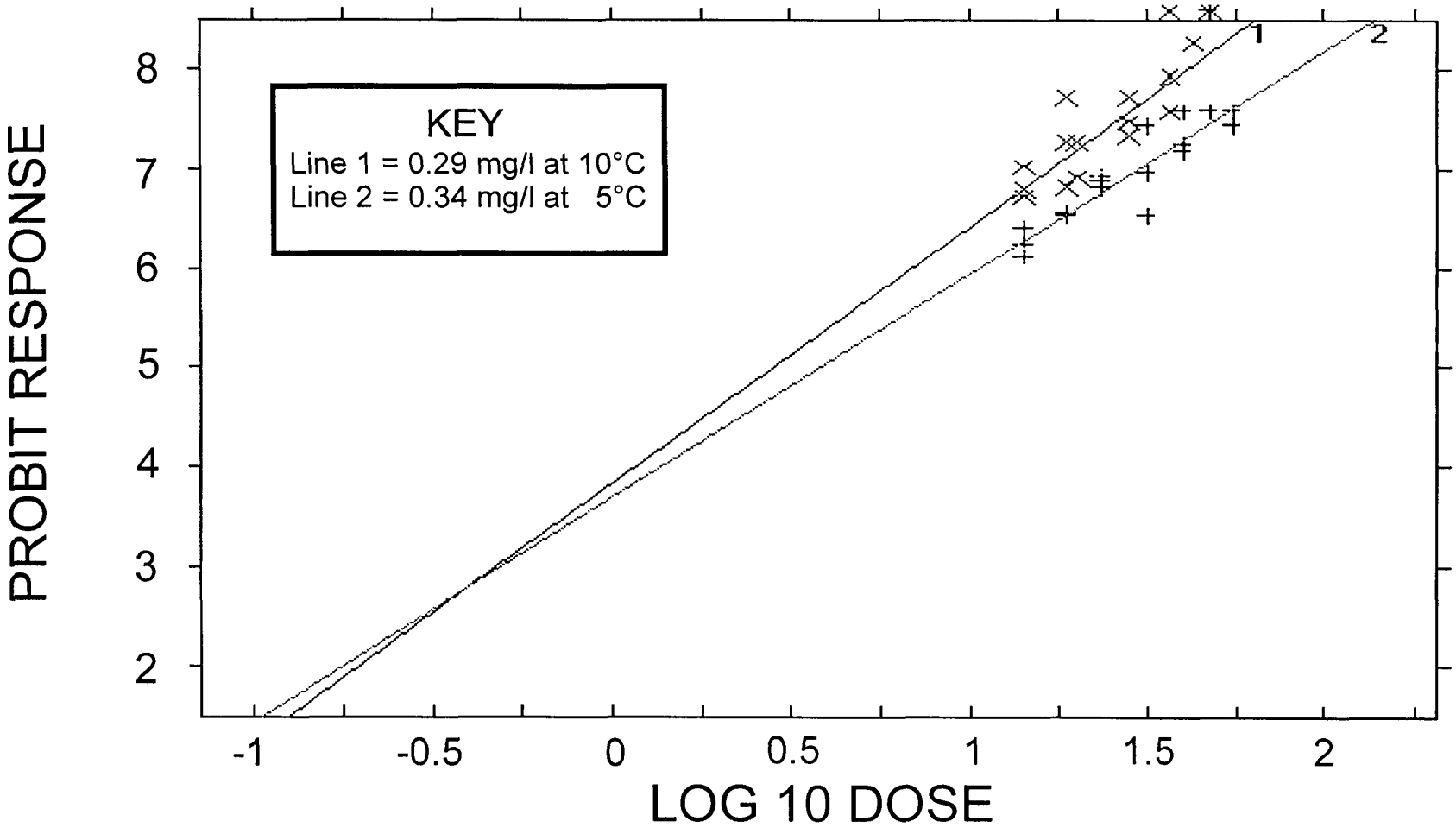

Figure 3 Probit response lines for adults of the field strain of *Tribolium castaneum* at 5, 7.5 and 10°C.

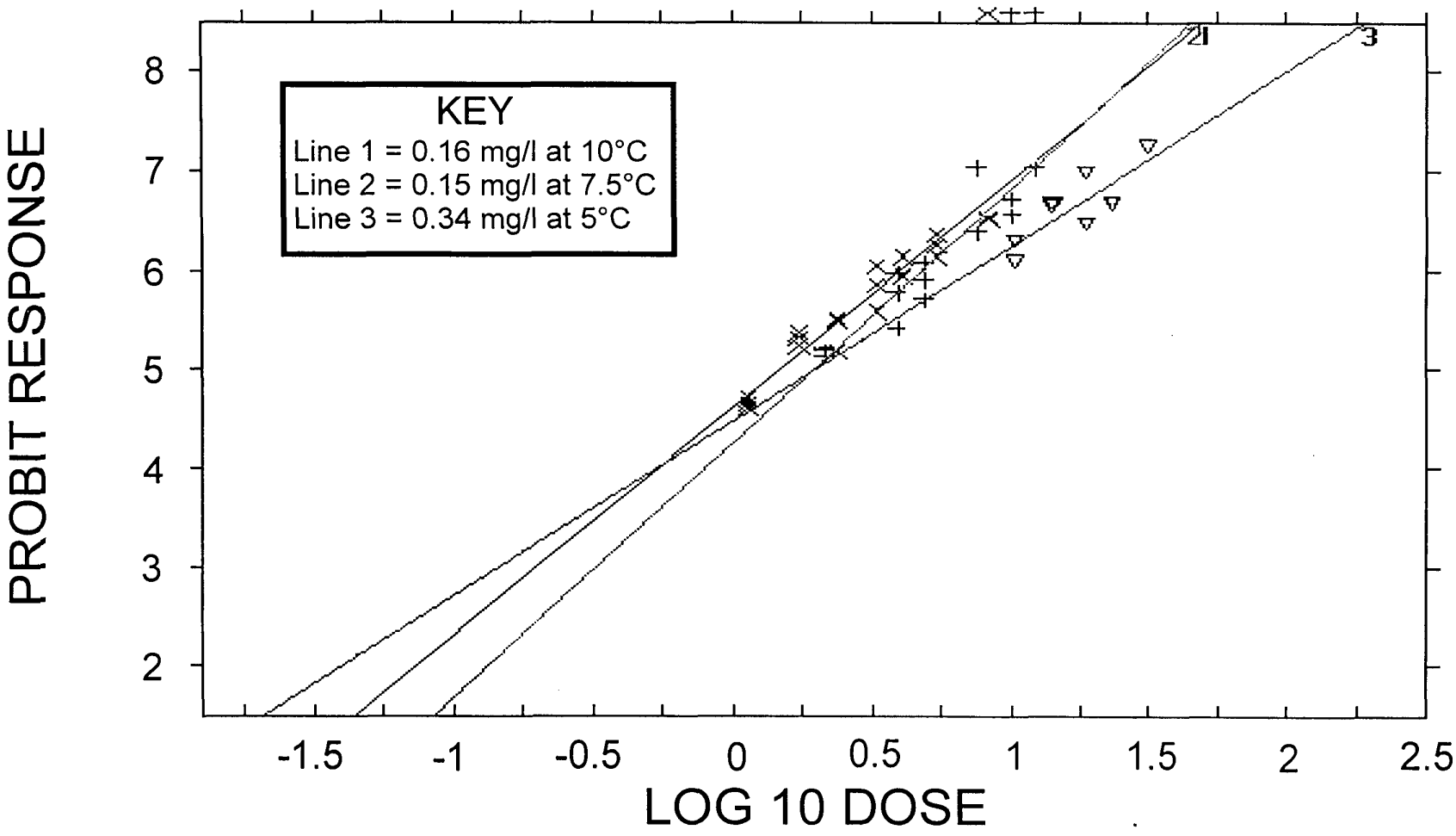

Figure 4 Probit response lines for older stages of the laboratory strain of *Oryzaephilus surinamensis* at 7.5 and 10°C.

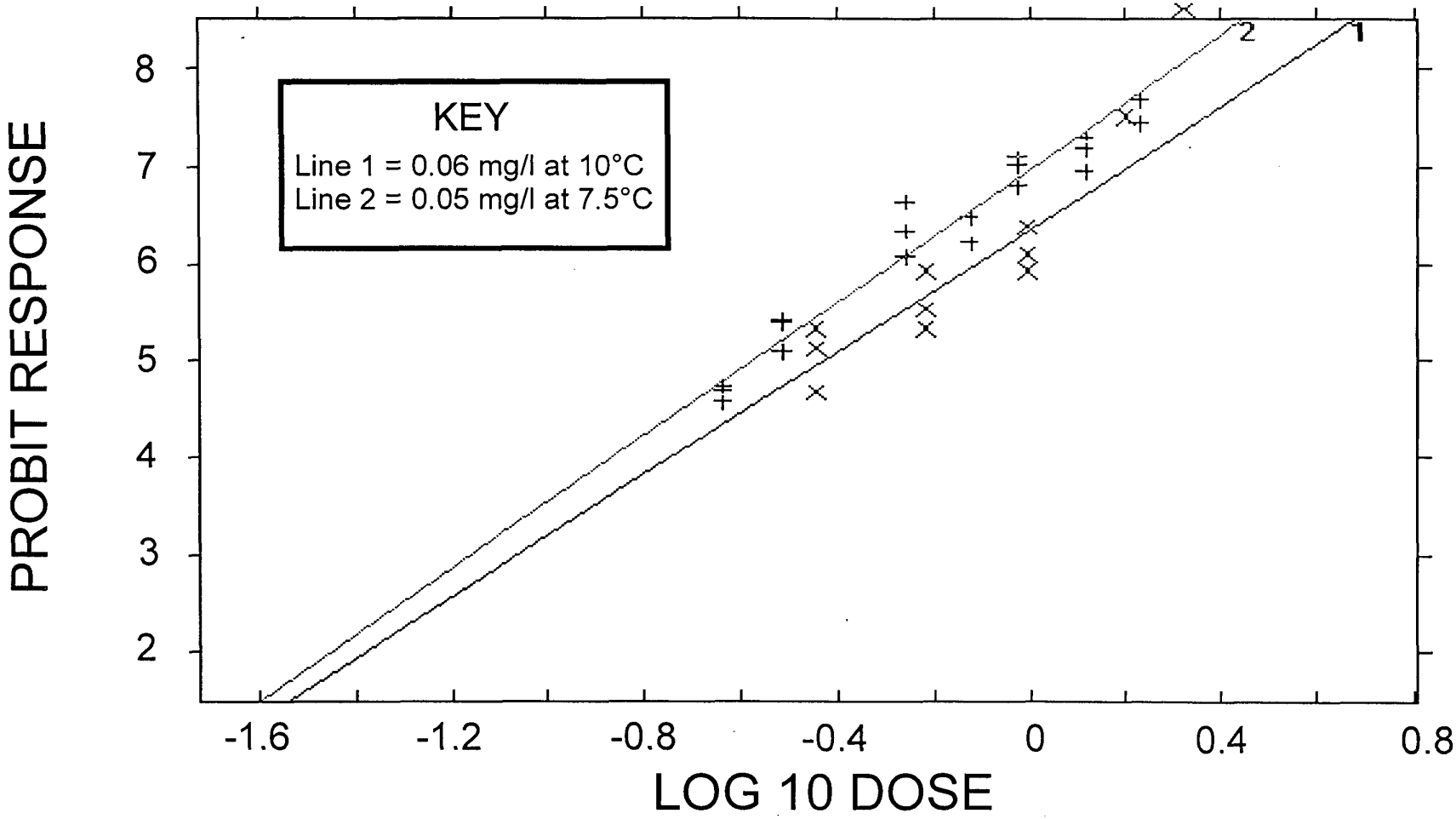

Figure 5 Probit response lines for older stages of the field strain of *Oryzaephilus surinamensis* at 7.5 and 10°C.

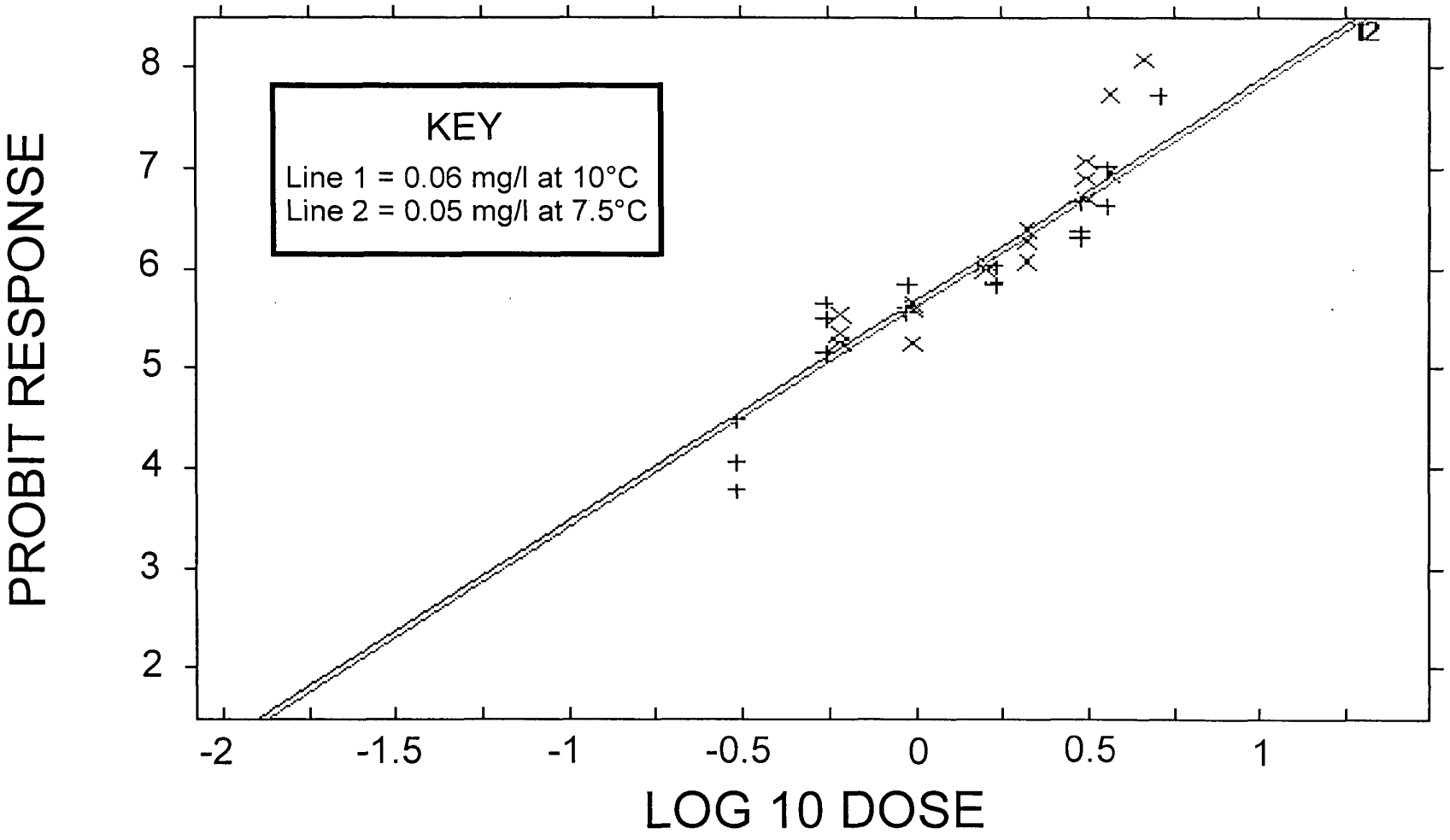

Figure 6 Probit response lines for older stages of the laboratory strain of *Cryptolestes ferrugineus* at 5, 7.5 and 10°C.

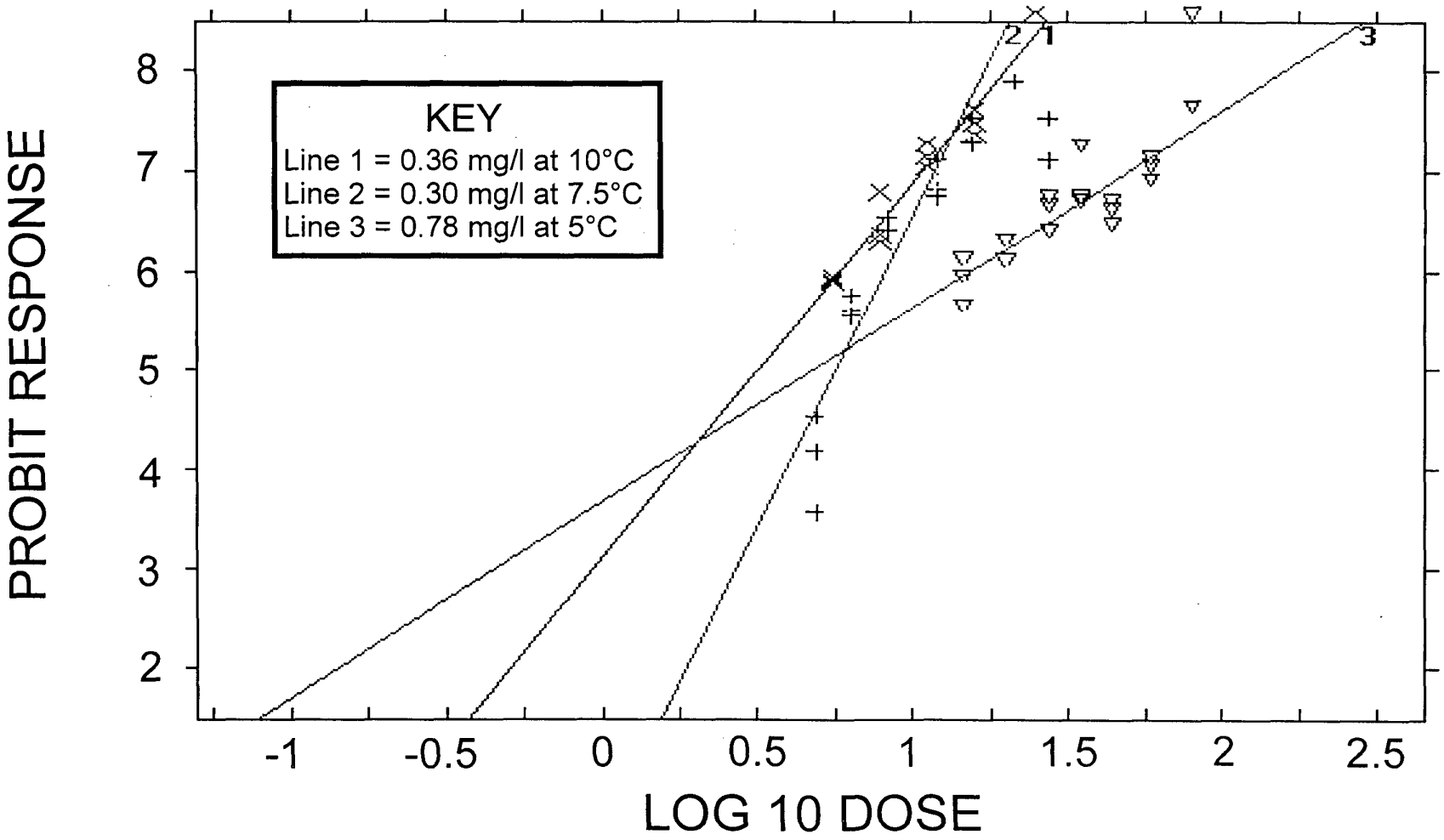

Figure 7 Probit response lines for older stages of the field strain of *Cryptolestes ferrugineus* at 5, 7.5 and 10°C.

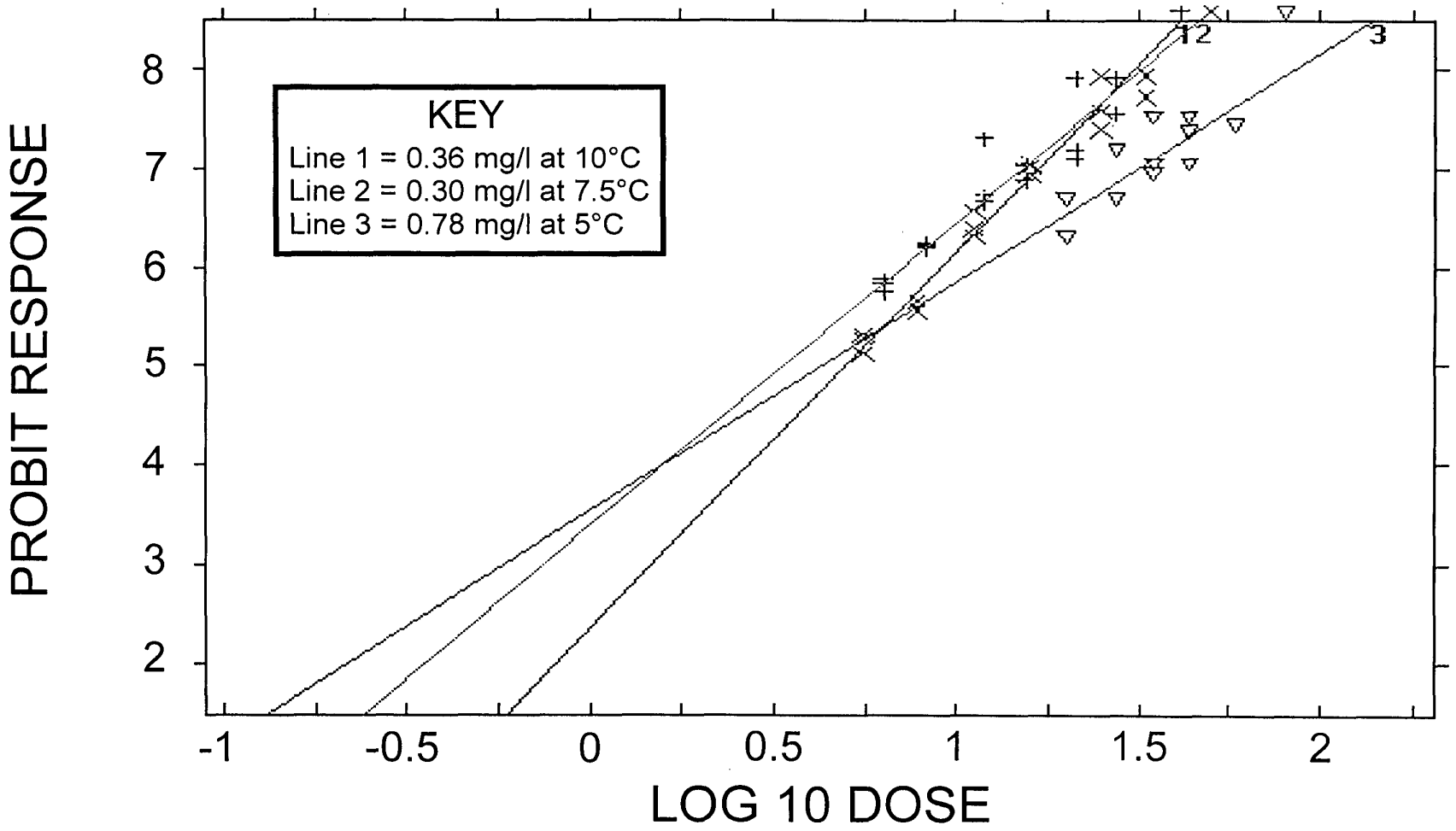

Figure 8 Probit response lines for adults of the laboratory strain of *Cryptolestes ferrugineus* at 5, 7.5 and 10°C.

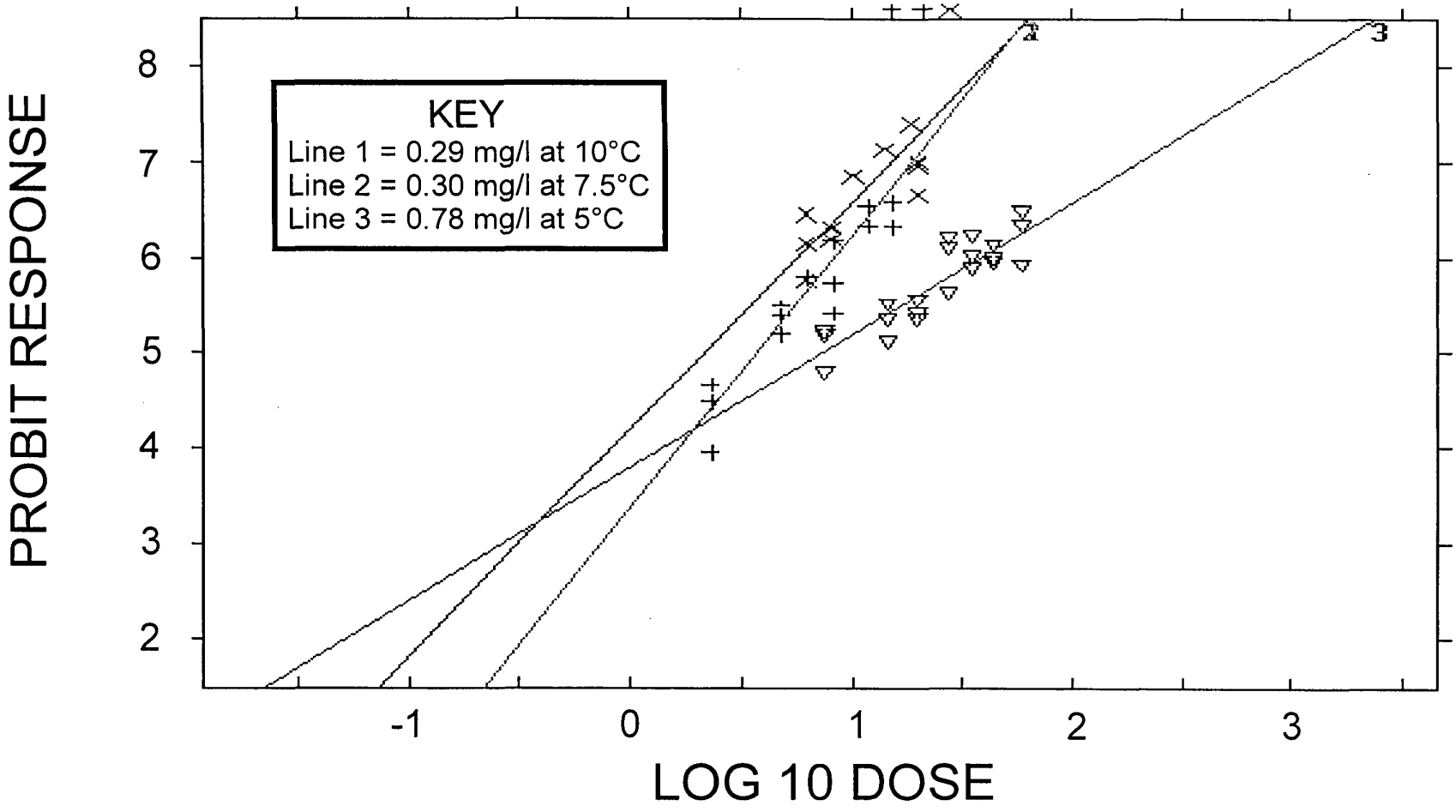

Figure 9 Probit response lines for adults of the field strain of *Cryptolestes ferrugineus* at 5, 7.5 and 10°C.

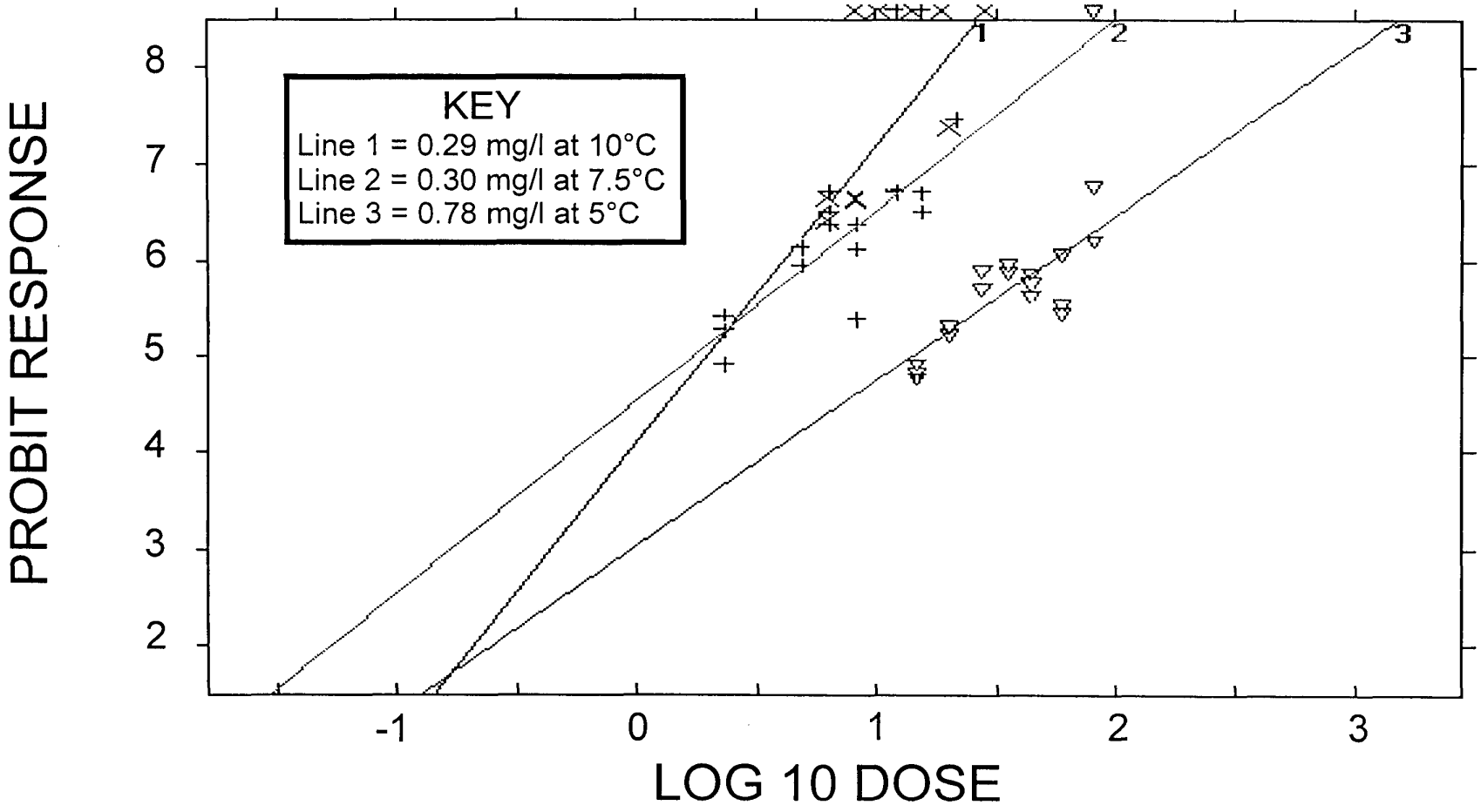

Figure 10 Probit response lines for older stages of the laboratory strain of *Sitophilus granarius* at 5, 7.5 and 10°C.

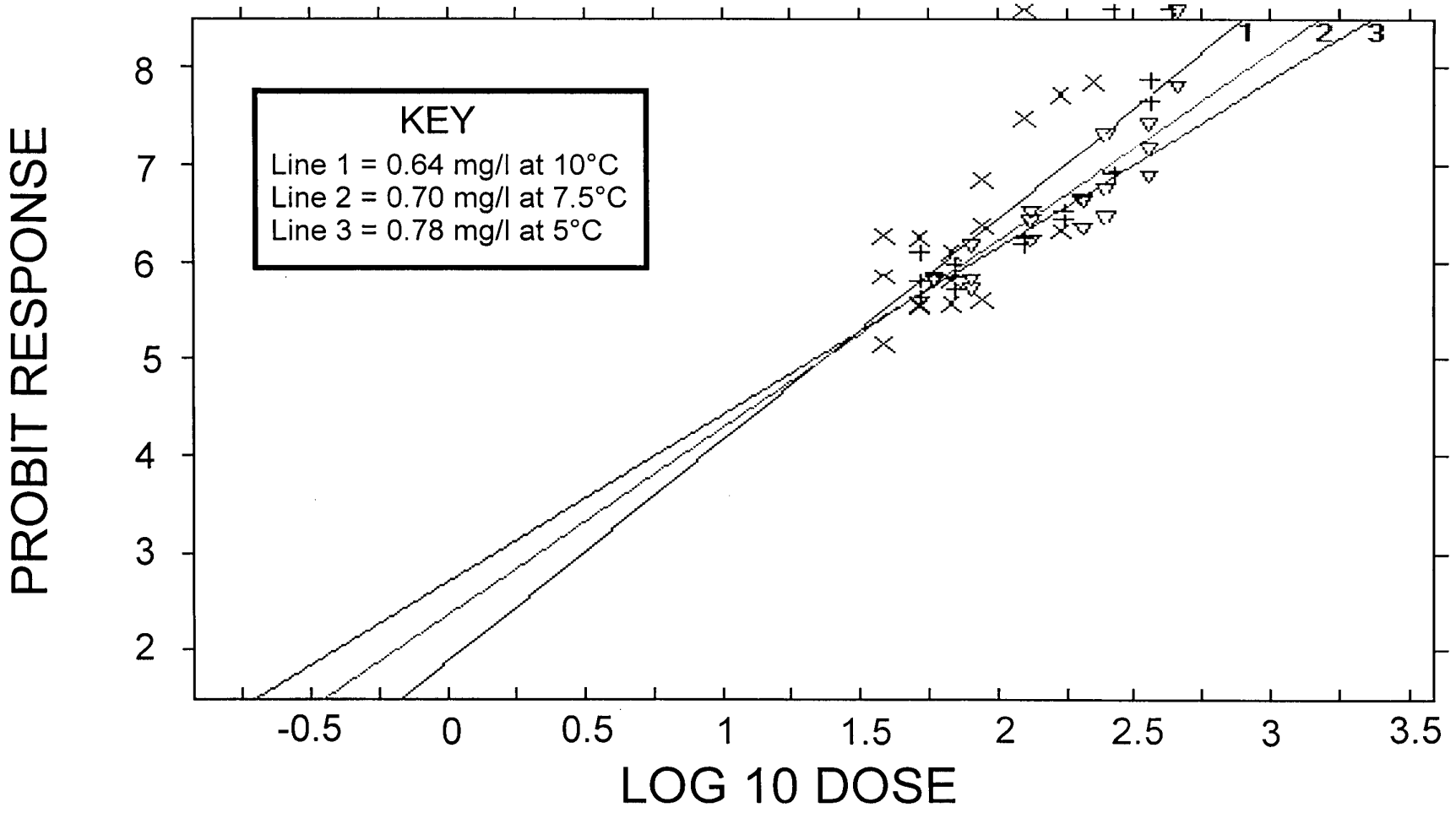

Figure 11 Probit response lines for older stages of the field strain of *Sitophilus granarius* at 5, 7.5 and 10°C.

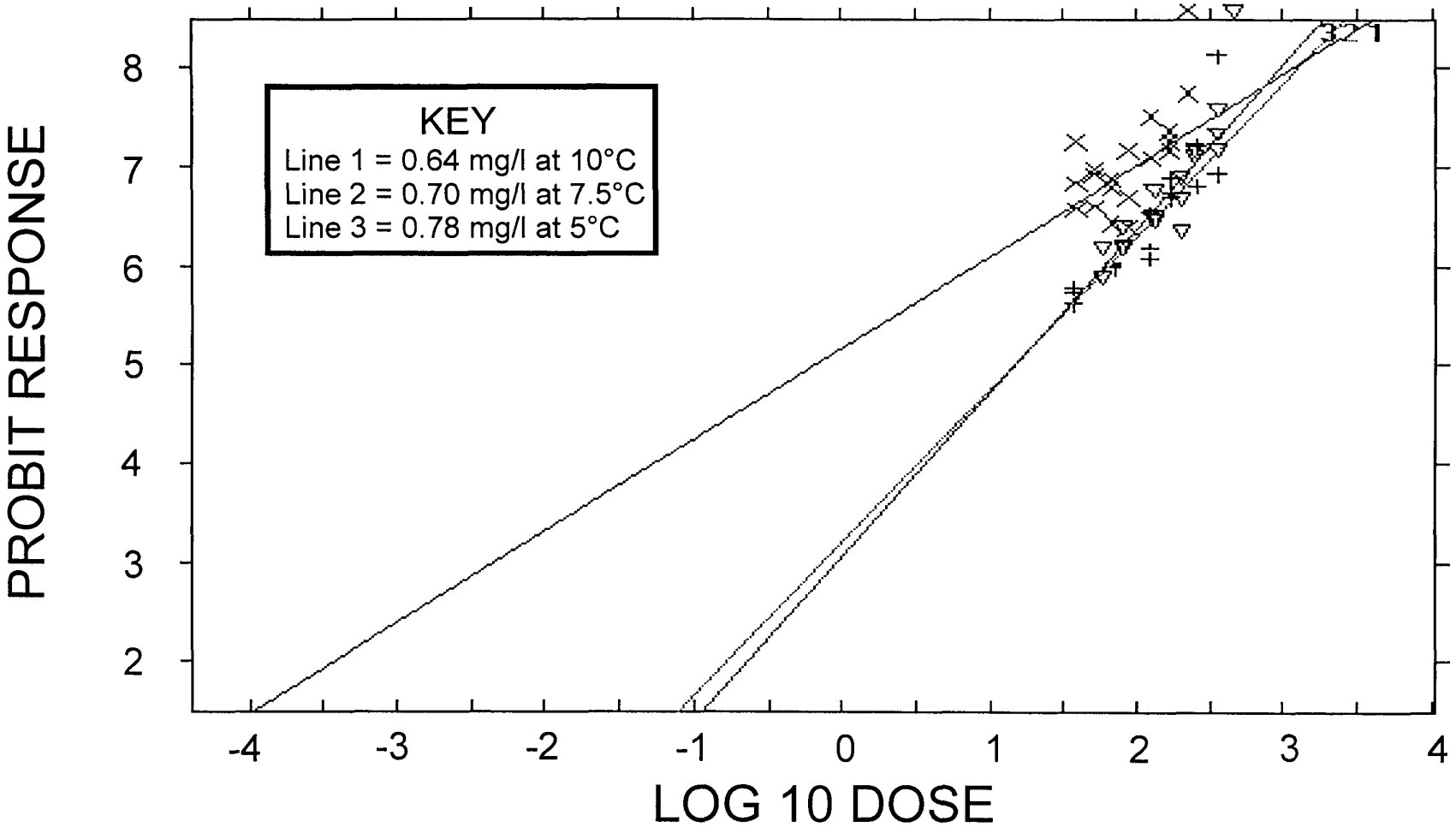

Figure 12 Probit response lines for adults of the laboratory strain of *Sitophilus granarius* at 5 and 7.5°C.

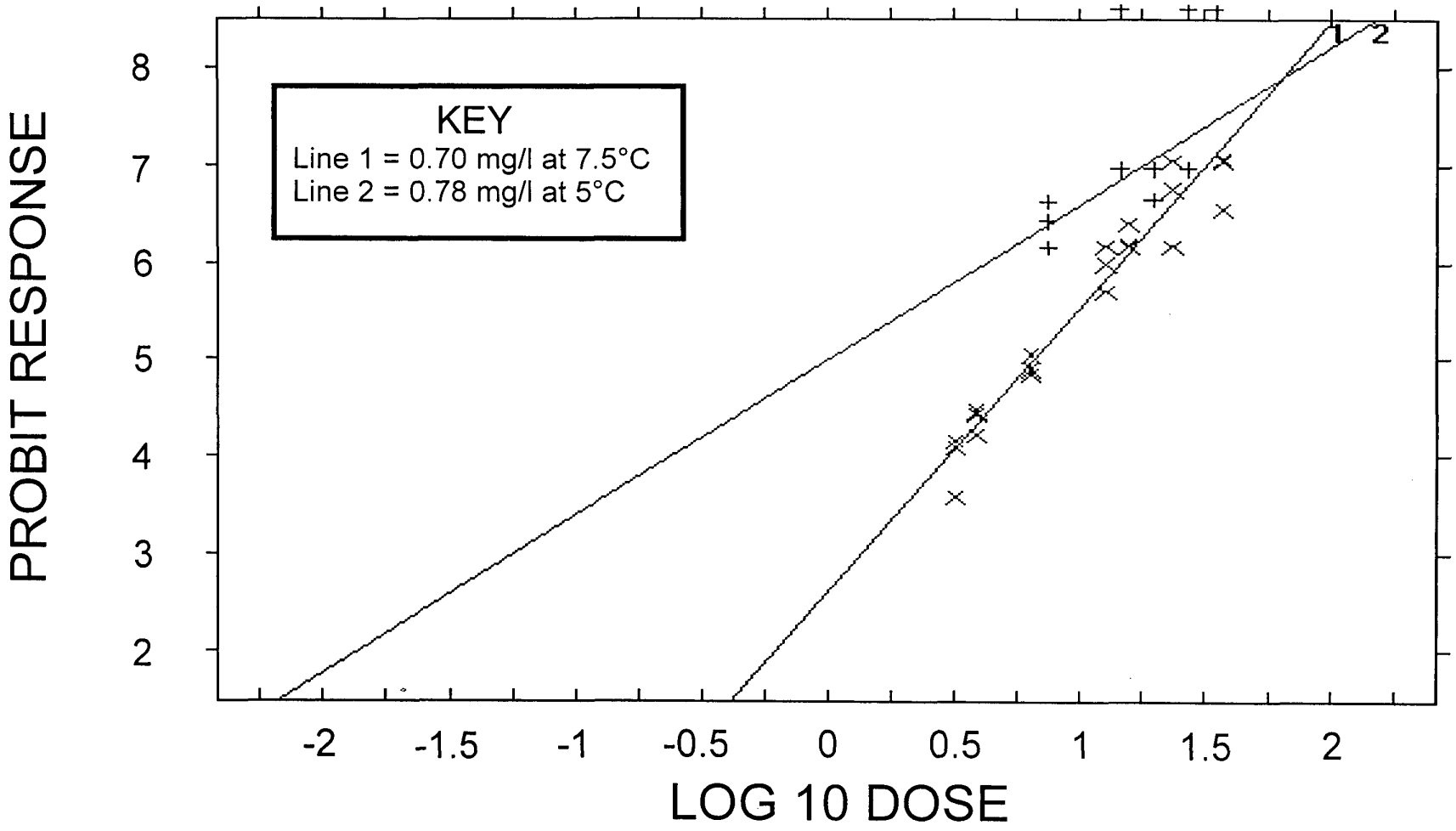

Figure 13 Probit response lines for adults of the field strain of *Sitophilus granarius* at 5 and 7.5°C.