

# Measurement of resistance to grain fumigants with particular reference to phosphine

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## Abstract

The dosage of a fumigant is a function of the concentration to which an insect is exposed and the duration of exposure to that concentration. Since any measure of resistance is usually a comparison of dosages that elicit a similar response, it follows that fumigant resistance may be influenced by the choice of variables used to measure or characterise resistance. When the ratio of the coefficients of the dosage variables (the toxicity index) of the strains being compared is equal, the magnitude of resistance will be constant over a range of dosages. The converse is true when the ratios are not equal. In addition, when the ratios are greater than, or less than one, the measure of resistance will depend on the choice of the independent variable on which the comparison is based. With phosphine it has been shown that the toxicity index varies between strains and is usually less than one. This can have a significant effect on the interpretation of resistance data and the implications of such resistance in practical fumigations.

This paper describes the underlying principles that influence the detection and measurement of fumigant resistance and describes results of experiments that examine the differences associated with the choice of the independent variable on which dosage comparisons are made. Resistance factors obtained for two strains of *Rhyzopertha dominica* varied  $\times 5$  and  $\times 13$  in one strain and between  $\times 10$  and  $\times 31$  in another strain depending on whether the comparisons were based on estimates of LC<sub>99</sub> from a 20-hour test or on estimates of LT<sub>99</sub> from a test at 0.05 mg/L. By contrast, resistance factors, derived from times to population extinction of mixed age cultures of the same two strains were  $\times 1.4$  and  $\times 2.4$  at 0.05 mg/L and  $\times 1.4$  and  $\times 1.4$  at 0.1 mg/L.

The results of this study question whether any of the reported resistances to phosphine pose a threat to the continued usefulness of this fumigant, except in fumigation enclosures that are inherently leaky and as such, unsuitable for fumigation. They also raise questions about the methods used for detecting and measuring resistance.

## Introduction

Resistance to phosphine was first observed in stored-product insects over 30 years ago as a cross resistance following selections of *Sitophilus granarius* with methyl bromide (Monro et al. 1961). Moreover, the FAO survey of resistance in stored-product pests (Champ and Dyte 1976), in which widespread resistance to phosphine was detected, is now almost 20 years old. Clearly, phosphine resistance has been present in insect populations around the world for a long time and over the last decade some quite high levels of resistance have been

reported. Nevertheless, phosphine is still an effective fumigant and is likely to remain so for some years to come.

At the time of the FAO survey, in 1973, it was decided to include testing for resistance to the fumigants methyl bromide and phosphine. For that purpose a method that one of us (Winks) had developed was employed. This method was later adopted as the FAO test method for fumigant resistance (Anon. 1975). The method uses modified laboratory desiccators as fumigation chambers and is based on the exposure of adult insects to selected concentrations for a fixed exposure period. The exposure period nominated was 20 hours, which was chosen to accommodate a 24-hour testing routine by a single person when many samples had to be tested, i.e. allowing time before testing commenced to terminate tests started on the previous day and to prepare insects for that day's dosing. A 24-hour test period was considered unsuitable for such a requirement. A fixed exposure period was chosen as the basis of the method, partly for convenience and partly because it was the basis of apparatus and techniques that were simpler and cheaper than those required for tests based on fixed concentrations. Moreover, many earlier studies on fumigants, particularly methyl bromide, had been based on fixed exposure periods.

The FAO test method has been employed in a number of laboratories over the years and has proved to be an effective method for detecting fumigant resistance. A number of laboratories have pursued studies of resistance to phosphine using methods derived from the FAO method. Largely, the methods employed have simply extended the exposure times to periods more suited to some of the strains being tested (e.g. Mills 1983). While the FAO test method has proved to be a valuable tool for detecting resistance to fumigants, its limitations, particularly in the case of phosphine, need to be recognised. Perhaps its greatest limitation in this context lies in its inability to provide a meaningful estimate of the level of resistance. This is partly because of the choice of fixed exposure periods as the basis of the method and partly because of the intrinsic characteristics of the toxicity of phosphine, i.e. the unequal contribution of concentration and time in the toxic action of phosphine. However, at the time the method was developed, the relationship between concentration and time in the toxicity of phosphine to insects was not fully understood.

In this paper we report results of experiments that highlight the intrinsic characteristics of fumigant resistance using strains of *Rhyzopertha dominica* that are resistant to phosphine and, in so doing, demonstrate one of the limitations of the FAO method.

## Theoretical considerations

The dosage of a fumigant is the product of the concentration ( $C$ ) and time ( $t$ ) of exposure. If these dosage variables acted equally we would have a simple relationship of the form  $C \times t = k$ , where  $k$  is a constant describing the dosage required for a specified level of response, e.g. mortality. This equation is sometimes referred to as Haber's Rule. Although this relationship might be a convenient description for calculating dosages

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in some field situations, it does not provide an acceptable description of the toxicity of a fumigant, or any other dose/response relationship in which the dosage is comprised of more than one variable. Indeed, in most cases, Haber's Rule is the exception to the rule. However, it would seem that Haber's Rule (Haber 1924) has been largely misquoted for a good many years. In comparing the toxicity of a number of gases with potential use in chemical warfare, he proposed that 'the smaller the product of concentration and time producing a fatal response in the laboratory animal, the more poisonous the chemical weapon'. This, however, does not imply that the product of concentration and time for a specific level of response for a given gas is constant.

The more general form of the relationship between concentration and time is  $C^n t = k$  (Winks 1984) in which the value of  $n$ , the 'toxicity index', provides a measure of the relative importance of concentration and time to the dosage. The toxicity index is a measure of the slope of the regression of time-to-a-given-response-level (e.g. LT99) over a range of fixed concentrations and described by the equation:

$$\log t = \log k + n \log C$$

When  $n = 1$  concentration and time contribute equally to the effectiveness of the dosage. When  $n$  is less than one, time is the more significant component of dosage and when  $n$  is greater than 1 the concentration has greater effect.

Apart from being significant in terms of the influence of the dosage variables on a given dosage, the value of  $n$  also influences the significance of resistance measurements. When  $n$  is greater than or less than 1, the choice of the fixed dosage variable, concentration or time, will influence the magnitude of a resistance factor determined for the same strain. If  $n$  is less than 1, experiments in which exposure time is the fixed component of dosage will produce a higher resistance factor than when concentration is the fixed component of dosage. The converse is true when  $n$  is greater than 1. This may be demonstrated by

considering a range of say LT99 values that could be expected from a range of fixed concentrations for a resistant strain and a susceptible strain both of which have a value of  $n$  of 0.5. Hypothetical data for such strains are plotted in Figure 1.

From the equations to these lines it may be deduced that the hypothetical resistant strain is 10 times resistant when LT99s are compared, i.e. when concentration is the fixed component of dosage, but is 100 times resistant when LC99s are compared, i.e. when exposure time is the fixed component of dosage, e.g., a comparison of the LC99s (or LD99) from an experiment based on a range of concentrations at a fixed exposure period of 20 hours (or any other fixed time).

When the values of  $n$  are different between two strains, a simple comparison (i.e. one in which the resistance level remains constant over the range chosen) no longer applies. In this case the level of resistance will not only depend on the choice of the fixed component of dosage, but will also depend on the particular value of the fixed component. This is illustrated in the data of Figure 2 in which the value of  $n$  of the resistant strain is 0.4 and that in the susceptible strain is 0.95.

From the data of Figure 2 it may be deduced that, if a fixed exposure period was used, the resistance factor would vary depending on the length of the exposure period chosen (Table 1).

It may also be deduced that if a fixed concentration was used as the basis of a test the resistance factor would vary depending on the concentration chosen (Table 2).

Clearly, when strains being compared have different values of  $n$ , large differences in the measured resistance factor can be obtained depending on the choice of the fixed component of dosage. The principal significance of these differences lies in their interpretation. Frequently they are interpreted to indicate the likelihood of failure of the fumigant in question to control the resistant strain, or the extent by which the dosage needs to be increased to control the resistant strain. With only a single

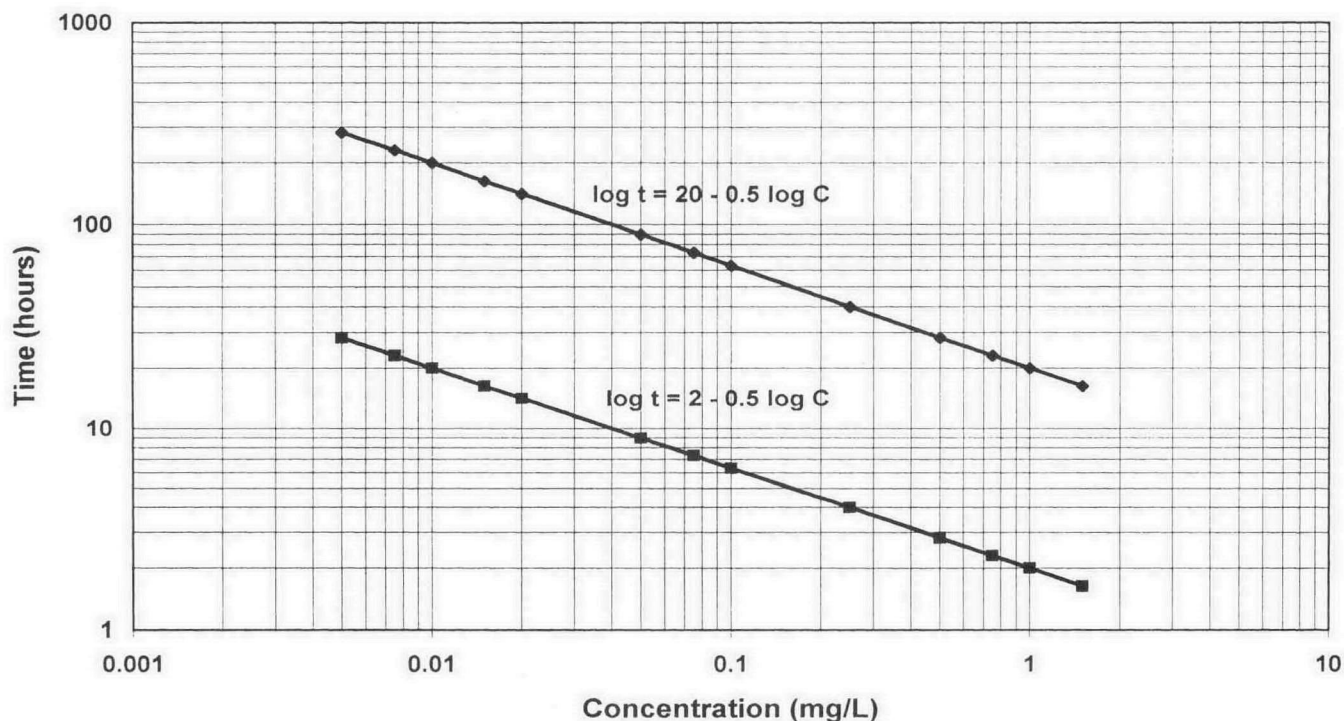


Fig. 1. Comparison of two strains with the same slopes of the regressions of LT99 over a range of fixed concentrations.

**Table 1.** Resistance factors derived for fixed exposure periods from the data of Figure 2.

Fixed exposure time (hours)	Strain 1 (mg/L)	Strain 2 (mg/L)	R factor
6	20.29	0.073	277
20	1.00	0.021	49
48	0.11	0.008	14
96	0.02	0.004	5

**Table 2.** Resistance factors derived from different fixed concentrations from the data of Figure 2.

Concentration (mg/L)	Strain 1 (hours)	Strain 2 (hours)	R factor
0.01	126.2	39.7	3
0.05	66.3	8.6	8
0.1	50.2	4.5	11
0.5	26.3	1.0	27
1	20.0	0.5	40

fixed concentration or single fixed exposure time being used as the basis of comparison between two such strains, gross errors of judgement could be made.

While the data of Figures 1 and 2 represent specific response levels such as the LD99, a more general description, embracing all response levels, is provided by the equation:

$$Y = a + b_1X_1 + b_2X_2$$

where  $Y$  is the probit response,  $X_1$  is log concentration ( $C$ ),  $X_2$  is log time ( $t$ ) and  $b_1$  and  $b_2$  are the coefficients of log  $C$  and log  $t$ , respectively. This equation describes a plane representing the probit response to a range of dosages obtained by

various combinations of the dosage variables, concentration and time (Finney 1971). For such a plane, in which an interaction term is not significant, a simple relationship between concentration and time may be derived in the form  $C^n t = k$  as follows:

$$(Y - a)/b_2 = (b_1/b_2)\log C + \log t$$

taking antilogs:

$$k = C^n t$$

where  $n = b_1/b_2$ .

For  $n$  to equal 1,  $b_1$  must equal  $b_2$ . In addition, for the values of  $n$  from two strains to be the same, the ratio  $b_1/b_2$ , for each strain, must be the same. Clearly, both propositions are unlikely to be true except in a few cases as convenient approximations. Therefore, resistance factors, derived in accordance with the FAO test method, and those that have been reported in the literature, (e.g. Mills 1983; Zettler 1990) are unlikely to provide any real guide to their practical significance. Their only value lies in determining some measure of relative resistance between strains.

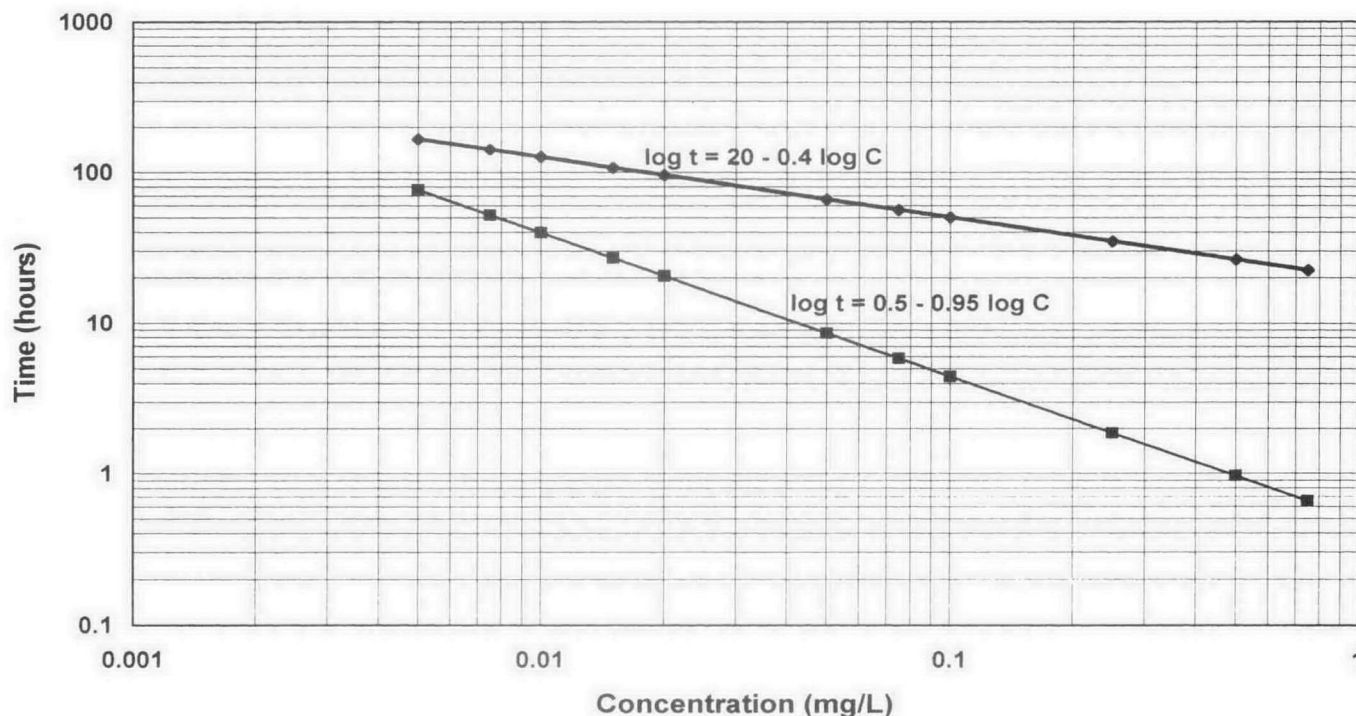
To examine the foregoing theory, experiments were conducted with resistant and susceptible strains of *R. dominica* using various concentrations and times and then comparing these results with comparisons based on times to population extinction of mixed-age cultures.

### Materials and Methods

Three experiments were conducted, two with adults and the third with mixed-age cultures.

*Experiment 1:* Adult insects were exposed for a fixed time (20 hours) to various concentrations of phosphine.

*Experiment 2:* Adults were exposed to a fixed concentration of phosphine (50 µg/L) for various exposure periods.



**Fig. 2.** Comparison of two strains with different slopes of the regressions of LT99 over a range of fixed concentrations.

**Experiment 3:** Mixed-age cultures were exposed to a fixed concentration of phosphine (50 µg/L) for a range of exposure times.

### Origin and maintenance of insect material

Three strains of *R. dominica* were used in all experiments:

1. A phosphine-susceptible strain, CRD2, cultured in the laboratory since 1967 from stock obtained from the Pest Infestation Control Laboratory (U.K.).
2. A phosphine-resistant strain, CRD235P10, derived from laboratory selection with phosphine of 10 generations of a strain from Borivli, India 1972 that was detected as resistant to phosphine during the FAO survey of resistance in stored-product insects (Champ and Dyte 1976).
3. CRD316, a phosphine-resistant strain collected in 1989 from a grain store at Trangie, New South Wales.

The insects were reared on whole wheat containing some whole wheat flour. Before use, the wheat was conditioned to 12% m.c. and sterilised by heating to 60°C. Cultures were established by placing 200 adults on 30 g of flour in glass jars sealed with filter paper tops. After 7 days the adults were removed and 400 g of whole wheat was added to the jar. This method produced an F<sub>1</sub> generation without any F<sub>2</sub> adults at 6 weeks. The age of the adult insects ranged from 2 to 4 weeks at the time of the experiments. Mixed-age cultures were established by placing 300 adults on 1000 g of wheat and 200 g of whole wheat flour in 2 L jars. The wheat and flour were arranged in layers in the jar. The adults were not removed from these cultures. This method produces a culture in which every stage of the life cycle is present. The mixed-age cultures were 6 weeks old at the time of the experiment. Samples from these cultures were examined using X-rays to determine the relative abundance of the various developmental stages and to ensure that there were sufficient pupae and late instar larvae present to give a reasonable expectation that pupae of all ages were present at the start of experiments. In most species, the pupa is the most tolerant stage to phosphine and young pupae, the most tolerant of this stage. All insect cultures were reared at 30°C, 70% r.h. and insects used in experiments were conditioned at 25°C for at least 1 week before use.

### Fumigation chambers

#### Experiment 1

The fumigation chambers used were modified 2.5 L desiccators described in Anon. (1975). Insects were confined in the desiccators within glass rings 2.5 cm high and 2.4 cm inner diameter. The glass rings had stainless steel mesh glued to one end and a stainless steel mesh lid was attached to the other end with a rubber band to prevent insects from escaping. The glass cages were assigned to each desiccator randomly. For all insect strains, two replicates of 50 insects were used for each dose and four replicates used for the control.

#### Experiment 2

A multi-chamber apparatus, from which insects can be removed at selected times following exposure to a constant phosphine concentration, was used (2nd apparatus, Winks and Waterford 1983). There was no recirculation and gas was supplied by mass flow controllers as outlined below. Insects were confined to glass rings and assigned to chambers in the same manner as in Experiment 1.

#### Experiment 3

Each culture was divided into two 3.1 L perspex flow-through fumigation chambers (Fig. 3). Each chamber was fitted with perspex screw top lids (1) and made gastight with

11.5 cm diameter neoprene O-rings. The lids were lined with stainless steel mesh to prevent insects and dust escaping through the inlet (2) and outlet (3). Six chambers were connected in parallel and supplied with a constant, flow-through concentration of phosphine.

### Fumigation procedures

All insects were placed into the fumigation chambers 24 hours before the start of the experiments.

#### Experiment 1

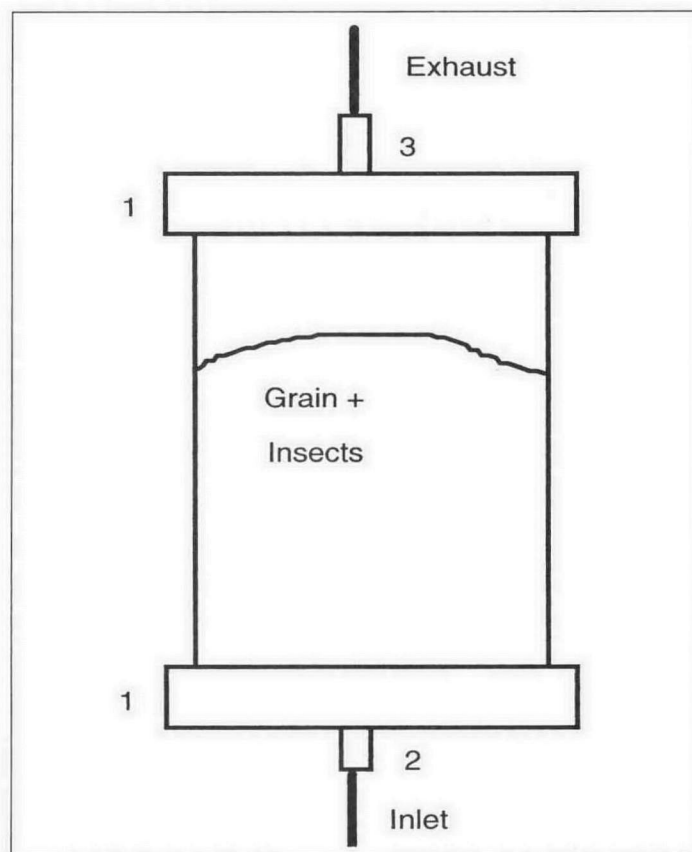
A source of phosphine was prepared according to Anon. (1975). The required dose of phosphine for each desiccator was achieved by injecting calculated volumes of the phosphine source using gas tight syringes.

#### Experiments 2 and 3

The multi-chamber apparatus and the flow-through fumigation chambers were supplied with a constant concentration of phosphine. This was achieved by blending compressed air and phosphine from cylinders of phosphine in nitrogen using two Brooks mass flow controllers (5850E series). The diluted phosphine was humidified to 57%, by passing it through distilled water maintained at 15°C before being warmed to the laboratory temperature of 25°C.

The flow of the phosphine/air mixture to the multi-chamber apparatus was at a rate of 2 L/minute. In Experiment 3 the total flow of the phosphine-air mixture from the mass flow controllers was 200 mL/minute. The flow into each of the six fumigation chambers was regulated using Brooks flowmeters.

Throughout each experiment phosphine concentrations were monitored using a gas chromatograph fitted with a flame



**Fig. 3.** Flow-through fumigation chamber of apparatus used to expose mixed-age cultures of insects to a constant concentration of phosphine.

photometric detector. The flame photometric detector was calibrated using samples of a gas mixture, the concentration of which was determined using a gas density balance. The response of a gas density balance is predictable from the molecular weights of the components of the sample.

### Post-fumigation procedures

#### Experiments 1 and 2

At the end of the exposure periods adult insects were removed from the chambers and transferred to 9 cm polystyrene Petri dishes containing a layer of wholemeal flour. During the post treatment holding period, the insects were held at 25°C, 67% r.h. Mortality assessments were made at 7, 14 and 21 days following the end of exposure. These periods were chosen to ensure that a mortality end-point was reached in each of the treatments (Winks 1982).

#### Experiment 3

At intervals of 24 hours for the experiment at 50 mg/L and of 2–3 days at 100 mg/L, samples of approximately 200 g were taken from the whole cultures and placed in glass jars the tops of which were black filter paper sealed around the edges with paraffin wax. These samples were incubated at 25°C, 60% r.h. and assessed for the presence of live adults by sieving. Assessments were made 24 hours after exposure and then at 8 weeks and 16 weeks later. From these observations, the time to population extinction (TPE) was determined as the first exposure time that produced no survival of insects at 8 weeks or 16 weeks, providing subsequent exposure periods also produced no survival.

## Results

Using the methods described in Anon. (1975), and in accordance with Experiment 1 above, discriminating dosage tests were conducted on the three strains. The mortality response of each strain at the dosages chosen is given in Table 3. It is clear from these results that both strains CRD316 and CRD235P10 would be diagnosed as resistant according to this test and the mortalities obtained were either at or below the lowest mortality recorded during the FAO survey of phosphine resistance in 1973. Moreover, from the data of Mills (1983) the strain CRD235P10 would appear to be similar to the Bangladesh strain at the discriminating dosage, judged from its response at 0.8 mg.hours/L.

In accordance with experiments 1 and 2 the response of these strains was determined to a graded series of concentrations at a fixed exposure period of 20 hours and to a graded series of exposure times at a fixed concentration of 0.05 mg/L. Probit lines were fitted to the data and resistance factors determined from comparisons of both the LD<sub>50</sub> and LD<sub>99</sub> (Table 4). There was a marked difference in the measured level of resistance depending on the method chosen.

Probit planes fitted to data from a number of experiments in accordance with the protocol of Experiment 2 for CRD2 and CRD235P10 and over a range of fixed concentrations of 0.0025 to 0.05 mg/L for CRD2, and from 0.05 to 0.35 mg/L for CRD235P10, were also examined. The probit planes satisfied the simple model in which the interaction between concentration and time was not significant. The parameters of these planes in which *Y* is the probit response, are given in the following equations:

$$\text{CRD2 } Y = 5.96 + 6.86 \log C + 10.88 \log t$$

$$\text{CRD235P10 } Y = -4.89 + 9.40 \log C + 13.14 \log t$$

From these planes, estimates of the LC<sub>99</sub> and the LT<sub>99</sub> were derived and resistance factors calculated at 0.05 mg/L and at 20 hours. At 0.05 mg/L the resistance factor was ×8 while at 20 hours the resistance factor was ×22 (Fig. 4).

**Table 3.** The response of strains of *Rhyzopertha dominica* to dosages of phosphine at or above the discriminating dosage for this species (Anon. 1975).

Concentration (mg/L)	Exposure time (hours)	Mortality response (%)		
		CRD2	CRD316	CRD235P10
0.03 <sup>a</sup>	20	100	3	NT <sup>b</sup>
0.04	20	100	NT	1
0.052	20	100	47	NT
0.075	20	100	NT	3
0.093	20	100	86	NT

<sup>a</sup> FAO test discriminating dosage

<sup>b</sup> NT = not tested

**Table 4.** Resistance factors derived from the response of adults of strains of *Rhyzopertha dominica* exposed to phosphine over a graded series of concentrations for 20 hours and over a graded series of times at 0.05 mg/L

	CRD2 (mg. hours/L)	CRD316 (mg. hours/L)	Resistance factor	CRD235P10 (mg.hours/L)	Resistance factor
Fixed exposure time of 20 hours					
LD <sub>50</sub>	0.11	1.71	16	2.93	28
LD <sub>99</sub>	0.19	2.50	13	5.77	31
slope	9.29	14.15		7.91	
Fixed concentration of 0.05 mg/L					
LD <sub>50</sub>	0.31	1.1	4	2.46	8
LD <sub>99</sub>	0.52	2.33	5	5.13	10
slope	10.54	7.13		7.26	

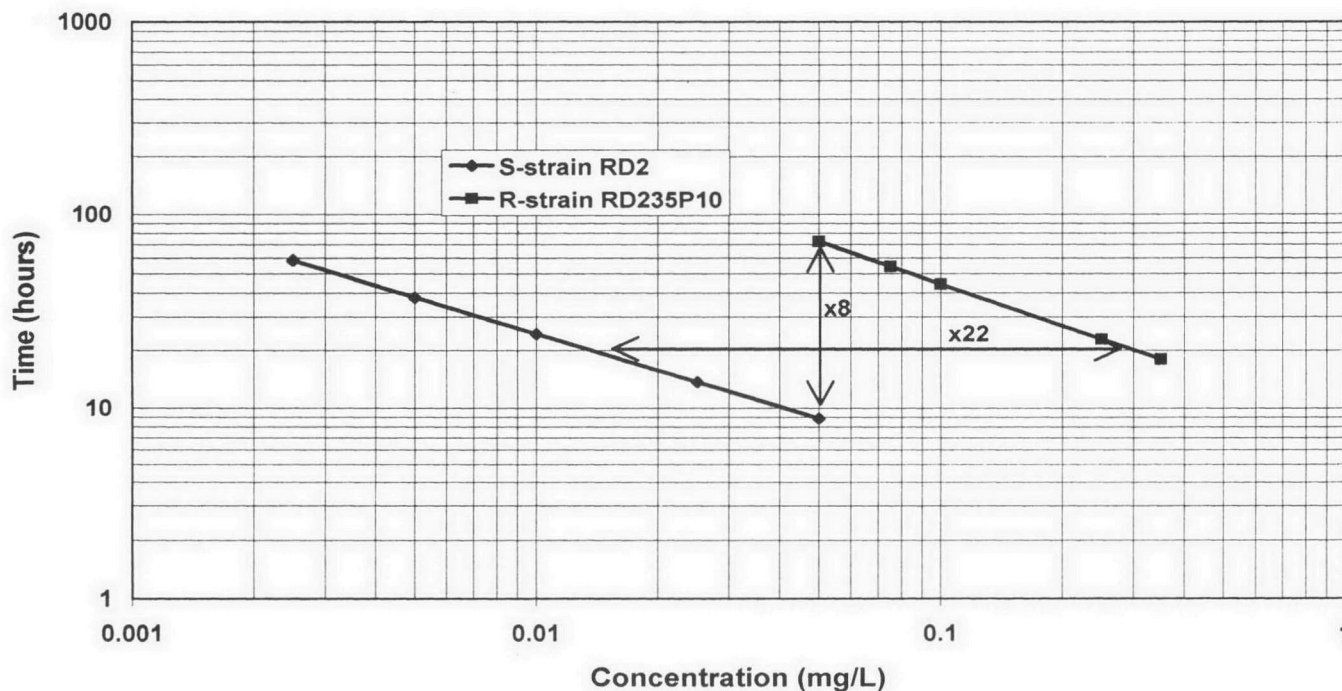


Fig. 4. The relationship between concentration and time in the LD99 values derived from probit planes fitted to mortality response data for adults of a susceptible strain (CRD2) and a resistant strain (CRD235P10) of *Rhyzopertha dominica* exposed to a range of fixed concentrations of phosphine for various exposure times at 25°C, 60% r.h.

The values of the toxicity index for these two strains were 0.63 for CRD2 and 0.72 for CRD235P10. Thus the resistance factor does not change greatly over a wide range of concentrations and decreases with increasing concentration. With a resistant strain of *Tribolium castaneum* the opposite change was found and the resistant factor decreased with decreasing concentration (Winks and Waterford 1986).

Resistance factors derived from mixed-age cultures exposed to 0.05 mg/L are given in Table 5 together with results from a similar experiment at 0.1 mg/L. The times to population extinction (TPE) are based on emergence in samples up to 16 weeks from the end of the exposure periods. TPEs are recorded as the earliest exposure period from which there was no emergence in samples providing that this was also true in samples from longer exposure periods.

At 0.05 mg/L TPEs ranged from 5 to 7 days for the three strains and the resistance factors for the two strains based on TPE was  $\times 1.4$  for CRD316 and  $\times 2.4$  for CRD235P10. A comparison with similar data obtained at 0.1 mg/L showed that a doubling of the concentration made no discernible difference in strains CRD2 and CRD316 but did reduce the TPE and the resistance factor in CRD235P10.

### Discussion

The results of these experiments with different strains of *R. dominica* demonstrate that estimates of the level of resistance can vary widely depending on the method chosen. This is particularly so in terms of the choice of the independent variable of dosage. In the experiments of this study with adults, there was an approximate 3-fold difference in the estimated levels of resistance at the LD99 and about a 4-fold difference at the LD50 level. If inferences had been drawn from the results with adults about the practical difficulties of controlling such strains, such inferences would have been seriously at odds with the reality of the situation. For example, a resistance factor of  $\times 31$  obtained from the 20-hour test with CRD235P10 might have suggested that this strain would not have been controlled with normal dosages of phosphine and that an alternative method of control may have been needed. In reality, a dosage of 1.5 g/m<sup>3</sup>, which is a common recommendation, would have achieved control.

The various quick resistance tests based on the correlation between the narcotic response and resistance (Reichmuth 1991; Bell et al., these proceedings; Waterford and Winks, these proceedings) also have the potential to exaggerate the level of resistance if they are used for anything more than

Table 5. Times to population extinction (TPE) and derived resistance factors following exposure of mixed-age cultures of three strains of *Rhyzopertha dominica* to constant concentrations of phosphine at 25°C and 60% r.h.

Strain	0.05 mg/L		0.1 mg/L	
	TPE (days)	Resistance factor	TPE (days)	Resistance factor
CRD2	5		5	
CRD316	7	1.4	7	1.4
CRD235P10	12	2.4	7	1.4

detecting the presence or absence of resistance in a strain. These tests can be quite misleading if they are used to estimate resistance levels. Moreover, they are based on an, as yet, unproven correlation between resistance and the narcotic response and in addition, have the potential to fail to detect resistance arising from a mechanism that is not related to the narcotic response.

Tests like the FAO method and any derivatives based on simple increases of the exposure periods should only be used as means to detect resistance. When comparisons with the reference (susceptible) strain are made, inferences about the practical significance of such comparisons should be resisted.

### Conclusion

The results of this study demonstrate that, with phosphine, where the value of the toxicity index  $n$  is likely to be less than one:

- any resistance factor derived from experiments in which fixed exposure periods are used with a range of concentrations, *will exaggerate the level of resistance.*
- any resistance factor based on comparisons at, for example, only one fixed concentration, *will be inadequate and could be quite misleading.*
- any resistance factor derived from adults only, *should not be used as the basis for adjusting dosage rates or exposure times in the field.*
- any resistance level estimated from a quick test method, *should not be used as the basis for adjusting application rates or exposure periods in practice.*

While times to population extinction provide estimates of levels of resistance that give reasonable guides to the practical significance of such resistance, experiments can take up to 6 months to complete.

### References

Anon. 1975. Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides. 16 Tentative method for adults of some major species of stored cereals, with methyl bromide and phosphine. FAO Plant Protection Bulletin, 23, 12–25.

- Champ, B.R. and Dyte, C.E. 1976. Report of the FAO global survey of pesticide susceptibility of stored grain pests. Rome, Food and Agriculture Organization of the United Nations, FAO Plant Production and Protection Series, No. 5, 297 p.
- Finney, D.J. 1971, Probit analysis, 3rd ed. Cambridge, Cambridge University Press.
- Haber, F. 1924. Fünf vorträge aus den jahren 1920–1923 über die darstellung des ammoniaks aus stickstoff und wasserstoff die chemie im kriege das zeitalter der chemie neue arbeitsweisen zur geschichte des gaskrieges. Berlin, Julius Springer, 23–92.
- Mills, K.A. 1983. Resistance to the fumigant hydrogen phosphide in some stored-product species associated with repeated inadequate treatments. Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie, 4, 98–101.
- Monro, H.A.U., Musgrave, A.J. and Uptis E. 1961. Induced tolerance of stored-product beetles to methyl bromide. Annals of Applied Biology 49, 373–377.
- Reichmuth, C. 1991. A quick test to determine phosphine resistance in stored product insects. GASGA Newsletter, 15, 14–15.
- Winks, R.G. 1982. The toxicity of phosphine to adults of *Tribolium castaneum* (Herbst): time as a response factor. Journal of Stored Products Research, 18, 159–169.
- Winks, R.G. 1984. The toxicity of phosphine to adults of *Tribolium castaneum* (Herbst): time as a dosage factor. Journal of Stored Products Research, 20, 45–46.
- Winks, R.G. and Waterford, C.J. 1983. Multi-chamber apparatus for laboratory studies with gases. Laboratory Practice, 32, 62–65.
- Winks, R.G. and Waterford, C.J. 1986. The relationship between concentration and time in the toxicity of phosphine to adults of a resistant strain of *Tribolium castaneum* (Herbst). Journal of Stored Products Research, 22, 85–92.
- Zettler, J.L. 1990. Phosphine resistance in stored product insects in the United States. Proceedings of the Fifth International Working Conference on Stored-product Protection, Bordeaux, France, September 1990, 1075–1081.