Mitteilungen aus der Biologischen Bundesanstalt
für Land- und Forstwirtschaft
Berlin-Dahlem

Heft 220 Juli 1984

Effect of carbon dioxide on the efficacy of phosphine against different stored product insects

by

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Berlin 1984

Herausgegeben
von der Biologischen Bundesanstalt für Land- und Forstwirtschaft
Berlin-Dahlem

Kommissionsverlag Paul Parey, Berlin und Hamburg
Lindenstraße 44-47, D-1000 Berlin 61

ISSN 0067-5849 ISBN 3-489-22000-5

CIP-Kurztitelaufnahme der Deutschen Bibliothek
Desmarchelier, James Michael:
Effect of carbon dioxide on the efficacy of phosphine against different stored product insects / by James Michael Desmarchelier. Hrsg. von d. Biol. Bundesanst. für Land- u. Forstwirtschaft Berlin-Dahlem. –
Berlin; Hamburg: Parey [in Komm.] 1984.
(Mitteilungen aus der Biologischen Bundesanstalt für Land- u. Forstwirtschaft Berlin-Dahlem; H. 220)
ISBN 3-489-22000-5
NE: Biologische Bundesanstalt für Land- und Forstwirtschaft <Berlin, West; Braunschweig>: Mitteilungen aus der . . .

1984 Kommissionsverlag Paul Parey, Berlin und Hamburg, Lindenstraße 44–47, D-1000 Berlin 61.
Printed in Germany by Arno Brynda GmbH, 1000 Berlin 62.

Introduction

Fumigants, that is, chemicals with high vapour pressures at normal temperature and pressures, have been, and are, a preferred method of controlling insects in stored products because of their ability to penetrate grain bulks, their speed of action and the low levels of the fumigant in the fumigated commodity. However, many of the previonsly-accepted fumigants, such as ethylene oxide or acrylonitrile, are no longer registered for admixture with grain in the Federal Republic of Germany as a result of strict safeguard regulations controlling the presence of metabolic products of fumigants in food. In this respect phosphine is a favoured fumigant because its major metabolic product, inorganic phosphate, is universally present in living systems (BANKS, 1979). Controlled atmospheres, that is, atmospheres containing low oxygen concentrations (approximately 1 %) in nitrogen or high carbon dioxide (approximately 3o - 8o %) concentrations in air are of increasing interest especially because of the absence of chemical residues (BAILEY and BANKS, 1978, (198o), BANKS, 1979).

Although modified atmospheres and phosphine have the desirable property of, at most, low residues they have the undesirable property of slowness of action, especially at "low" temperatures, that is, at less than 2o°C. (BANKS, 1975; BAILEY and BANKS 1977, 198o). This statement must be slightly qualified for mixtures of carbon dioxide and air, as the data at low temperatures are inadequate (BAILEY and BANKS, 198o; BANKS 1979). Although both carbon dioxide in air and phosphine are relatively slow acting against certain stages of insects, it is possible that a mixture of these fumigants could act more quickly than either component alone, as a result of either synergistic joint-action, or of additive effects where, for example, one component is effective against one stage and the other against another stage.

The joint action of low levels of carbon dioxide and several fumigants, but not phosphine, was summarized by Peters (1945).

According to this author, carbon dioxide potentiates the effect of fumigants against adults and larvae, but not against eggs and pupae, of the species

investigated. Carbon dioxide, in the range o - 5o % in air, has been shown to potentiate the action of phosphine at 26°C against adults of Sitophilus granarius (L.) and Tribolium confusum (du Val) (KASHI and BOND, 1975).

It was accordingly decided to investigate the effects of mixtures of carbon dioxide and air, in the range o - 75 % carbon dioxide, and low levels of phosphine, 5o and 2oo vpm, against adults, pupae, larvae and eggs of six species of stored product pests. Those species were S. granarius, S. oryzae (L.), T. confusum, T. castaneum (Herbst), Trogoderma granarium (Everts) and Rhizopertha dominica (F.) These species were chosen partly because of their importance in stored products and partly because certain stages of the species investigated, e. g. Sitophilus pupae and larvae of T. granarium, are especially tolerant of both phosphine and inert atmospheres (LINDGREN and VINCENT, 1965; HOWE, 1973; HOLE et. al. 1976; BANKS, 1975). This comment must be qualified by the observation of BAILEY and BANKS, 198o, that there are "several inconsistencies and gaps in our knowledge of the biological actions of various controlled atmospheres". These authors point out in particular that the relative order of susceptibilities of S. oryzae and S. granarius is disputed, and that there is a need for a study of the comparative susceptibility of T. granarium and S. granarius to inert atmospheres.

In the work discussed in this report attention was given to mixtures of phosphine in carbon dioxide and air, rather than to mixtures of phosphine in low concentrations of oxygen. These latter atmospheres were not investigated, except for comparative purposes, because of studies which show the necessity of oxygen for the lethal action of phosphine (BOND and MUNRO, 1967; BOND, MUNRO and BUCKLAND, 1967; BOND, ROBINSON and BUCKLAND, 1969; KASHI, 1981a, 1981b).

In the work outlined in this report, studies were conducted at 19 ± 1°C, partly because of the need for toxicological data at "lower" temperatures (BAILEY and BANKS, 198o) and partly because studies at 25 or 3o°C are not immediately relevant to the conditions in central Europe. Even in the hotter

Australian conditions, the summer temperature of grain at base of silos is approximately 2o°C (WILSON and DESMARCHELIER, personal communication); this cooler grain could provide a refuge for insects under conditions where phosphine or inert atmospheres were lethal in the warmer centre of the silo.

The aim of this present study was, therefore, to provide data on the efficacy of phosphine and of mixtures of carbon dioxide and air and of mixtures of phosphine, carbon dioxide and air against all stages, namely eggs, larvae, pupae and adults, of six species of stored product insects. Mixtures of carbon dioxide, air and phosphine can be considered under two aspects, namely as the addition of phosphine to an essentially carbon dioxide fumigation, with the aim of reducing the time of exposure, or as the addition of carbon dioxide to an essentially phosphine fumigation, with the aim of reducing the amount of phosphine required. These studies should be seen in the background of the relatively high amounts of phosphine (5 - 1o g/tonne) used in practice (BOGGS, 1975) in phosphine fumigations and the slightly less than 1oo % control achieved in the practice of fumigation with inert atmospheres (BANKS, 1979), which is attributed to insufficent periods of exposure.

1. Materials and Methods

1.1 Culture of Insects

All insects were cultured at 25 ± 1°C and 7o ± 5 % relative humidity. S. oryzae, S. granarius, R. dominica and T. granarium were cultured on wheat. T. castaneum and T. confusum were cultured an wholemeal flour, enriched with glycerine. Each stain of test insect was a standard laboratory strain, susceptible to phosphine.

1.2 Establishment of Atmospheres

1.2.1 Experiments with Phosphine in 5oo l Fumigant chambers

Two fumigation chambers, originally designed for vacuum fumigation, each of 5oo l and equipped with a manometer, were sealed for work at atmospheric pressure so that at least 9o % of an initial measured phosphine concentration of approximately 5o vpm PH_3 was present after 24 h. The chambers, illustrated in Figure 1, were equipped with valves (a_1, a_2) and insect sampling ports (b) and attaches to a pump for evacuation (c). The valves a_1 and a_2 were connected to each other by plastic tubing over a system comprising a Dreschel tube containing a saturated solution of oxalic acid (d), a 'T' piece with a rubber septum for gas sampling (e) and a recirculatory pump (f).

Before use, chambers were purified by evacuation to o,o5 atmospheres and by admission of air after disconnection of the join between d, and e and by opening of the valve a_1 until the pressure in the chamber was, for the third time, evacuated to o,o5 atmospheres. Air was introduced over the Dreschel tube (d) by opening of the valve a_1 until the pressure in the chamber had attained the required partial pressure of air in the desired mixture of air and carbon dioxide. The valve a_1 was then closed, the opening d_2 in the Dreschel tube was connected to a flash of carbon dioxide and

after opening of the valve a_1 the chamber was filled with carbon dioxide until the total pressure was atmospheric. After the valve a_1 had been closed, it was connected to valve a_2 as shown in Fig. 1 (system a_1 - d - e - f - a_2).

After the valves a_1 and a_2 had been opened, the gas in the chamber was recirculated until the required r. h. had been atteined. This was measured after substituting the connection f-g, g-a_2 for the connection f-a_2 (cf. Fig. 1).

After reestablishment of the connection f-a_2, phosphine was introduced by a syringe into the 'T' joint e. Approximately 95 % of the theoretically required amount of phosphine was introduced at this stage. The gas was then recirculated for at least 3 h, using the system a_1 d, e, f and a_2 as shown in Fig. 1. After the phosphine concentration had been measured, the amount of phosphine required to achieve the desired concentration was injected into the 'T' piece, e. After recirculation for one hour, the pump was switched off and insects were introduced into the chamber.

The phosphine concentration was measured daily. In experiments with phosphine in air, phosphine levels were daily adjusted, via the 'T' piece e, provided that phosphine levels had not dropped below 85 % of the required level. In the case of a phosphine loss in excess of 15 %, the experiment was discarded. In the case of experiments with phosphine in mixtures of carbon dioxide and air, similar procedures were used as in the case of phosphine in air, with one exception. This exception occured after the total accumulated loss of phosphine exceeded 15 %. In this case the initial desired atmosphere was reconstituted in the second chamber, and insects were transferred into this second chamber.

1.2.2 Establishment of Atmospheres in Glass Flasks

The equipment used (Figure 2) comprised Dreschel flasks, with ground-glass joints. The flasks, which were of capacities o,25, o,75 and 1o L, were connected, as required, to each other with plastic tubing, and the joints were greased. Each flask contained a glass vial containing a saturated solution of oxalic acid for the control of the r.h. The total assembly was kept in a constant environment room at 19 ± 1°C and 7o ± 5 % r.h.

The atmosphere was created on a modular basis which was so designed that the required final atmosphere was achieved by mixing of the (modular) parts. There were flasks containing pure carbon dioxide, flasks containing air and where phosphine was used, a flask containing phosphine. The details of the system were:

Flasks with a total volume of x L (e.g. $a_1 - a_x$, Figure 2) were purged with carbon dioxide and other flasks with a total volume of y L (e.g. $b_1 - b_x$ or $b_1 - b_x$ plus b_p, Figure 2) were purged with air so that the ratio x/x+y equalled the desired partial pressure of carbon dioxide. The flask b_p contained the amount of phosphine, z ml, so that z/x+y equalled the desired phosphine concentration in vpm (ppm, V/V). Insects were introduced into the flasks $b_1 - b_x$, which were open to room air. Phosphine was first recirculated with all flasks that did not contain insects, after the appropriate flasks had been connected to each other (e.g., $a_x - c_1$, $c_2 - b_{p2}$, $b_{p1} - a_1$, Figure 2). The insect-containing flasks were then connected with this system (e.g., by substituting the connections $a_x - b_1$ and $b_x - c_1$ for that of $a_x - c_1$) and the system was recirculated.

In the system outlined in Figure 2, the insect density was kept low, less than 5/L, to prevent build-up of carbon dioxide. The volume of the insect-containing flasks was also held at less than 2o % of the total volume to enable a daily change of at least 8o % of the atmosphere. This was achieved by, firstly, seperation of the insect-containing flasks by joining b_1 to b_x, secondly by reconstitution of the required atmosphere, via the system $a_x - c_1$, $c_2 - b_{p2}$, $b_{p1} - a_1$ and by, thirdly, recirculation in the system $a_x - b_1$, $b_x - c_1$, $c_2 - b_{p2}$, $b_{p1} - a_1$.

The procedure described above was also used for experiments in the absence of phosphine. In such cases, of course, the concentration of phosphine in the flask b_p was zero.

1.3 Measurement of Gas Concentrations

Phosphine was determined with the aid of gas-chromatography, on a Carlo Erba 4100 chromatograph. A thermionic detector, in the phosphorus mode, was used. Operating conditions were: column, 10 % OV101 on Chromosorb W, H-P, 80 - 100 mesh; column temperature, 160°C and flow rate (nitrogen) 60 ml/min.

Carbon dioxide and nitrogen were determined by gas-density chromatography, on an Intersmat gas chromatograph (IGC 112 M). The support was Spherocarb in an stainless steel column.

Relative humidity was determined by dew-point on a MBW dew-point and dry-temperature indicator, model DP 6.

1.4 Bioassay Conditions

Assay conditions were 19 ± 1°C and 70 ± 5 % r.h. Insects were mainly exposed to fixed concentrations for varying periods although, in some cases, also to varying concentrations for fixed periods. Insects were exposed with food in guaze cylinders and, after completion of the exposure period, were transferred to Petrie dishes which were stored at 25°C, 70 % r.h. for a holding period before assessment of mortality.

All assays were duplicated and most triplicated. Assays for external stages such as adults and Tribolium larvae were corrected for control mortality by Abbot's formula and results were discarded when control mortality exceeded 10 %. Mortalities of internal stages, i.e. all juvenile stages of Sitophilus species and larvae and pupae of R. dominica, were calculated as the reduction in progeny as compared with the mean of three controls.

Most data were subjected to probit analysis using the program of NOACK and REICHMUTH, 1978. Data for probit mortality are only given when the correlation between mortality in probits and the logarithm of the dose was statistically highly significant.

Adults used in the assay were, unless otherwise specified, 1 - 4 weeks old. Approximately 3o adults were exposed, together with 2 g of wheat and 1 g of wholemeal flour, in guaze cylinders. Similar procedures were used for larvae of T. granarium, T. confusum and T. castaneum. These larvae were selected from cultures of mixed age and only the largest 25 % of the population was used. Young pupae of these three species were also exposed, together with 3 g of wheat, in guaze cylinders. Failure to emerge to adults over a period of 4 weeks at 25°C was the criterion of mortality for these stages.

Those stages that developed inside wheat kernels were exposed in guaze cylinders, each of which contained 16 ml of wheat including approximately 3o insects. These stages (with the time from ovideposition in brackets) were eggs of S. oryzae or S. granarius (o - 36 h), larvae of S. oryzae or S. granarius (15 - 18 days), larve of R. dominica (16 - 2o days), pupae of S. oryzae of S. granarius (27 - 31 days) and pupae of R. dominica (29 - 33 days). Each sample was kept at 25°C until 4 weeks after the first emergence of adults in that sample. Complete absence of emergence in a sample 8 weeks after the first emergence in the control sample was assessed as 1oo % mortality.

Eggs of R. dominica and of T. granarium, o - 3 days old, (approximately 3o eggs in 1 g flour) were exposed in guaze cylinders. After exposure, the eggs and flour were transferred to a glass petrie dish, which was then covered with Parafilm which was perforated with a fine needle. Live larvae were removed twice weekly over a period of three weeks: eggs failing to emerge over this period were assessed as dead. Eggs of T. castaneum and T. confusum were exposed using the procedure of TUNÇ (in press), i.e. eggs were placed in an indented plastic plate, which was covered with perforated Parafilm. The holding period for these species was also three weeks.

2. Results

2.1 Presentation of Results

Results are presented in the text in Section 2 and analyzed, in terms of additive, synergistic or antagonistic effects, in Section 3. Detailed results, i.e., mean and fiducial limits of the LT_{50} and LT_{99} values, are given in the Appendix (Tables A 1 - A 34). Values for the LT_{99} are also presented graphically in the Appendix (Figures A 1 - A 7).

2.2 Mortalities in phosphine-free mixtures of carbon dioxide and air

Calculated LT_{99} values for four stages of six insect species after exposure to 75 % carbon dioxide, 25 % air are given in Figure A 1 and further data are tabulated (Table A 1). The stages most tolerant to this mixture were larvae of T. granarium and pupae of S. granarius. The long exposure periods, in excess of six weeks, required for 99 % mortality of these stages confirm the results of BANKS (1979) that very long exposure periods are required for successful use of carbon dioxide-air mixtures.

Among the stages especially susceptible to 75 % carbon dioxide, 25 % air were adult species, especially adults of S. oryzae; and eggs of Tribolium species (Figure A 1, Table A 1).

Each stage of the tested species was also exposed for 7 days at 19°C, 7o % r.h., to a mixture of 25 % carbon dioxide, 75 % air. Although mortality was often very low (Table A 2), it was high for eggs of Tribolium species and for adults of S. oryzae and R. dominica. These susceptibilities were confirmed with the aide of probit analysis (Table A 3).

BANKS and BAILEY (198o) have drawn attention to conflicting results on the relative susceptibilities of S. oryzae and S. granarius to inert atmospheres. In the results reported in Tables A 1 and A 2 the more susceptible species was S. oryzae.

2.3 Mortalities of Adults in Phosphine-Containing Atmospheres

2.3.1 Mortalities of adults in mixtures of phosphine, air and carbon dioxide

Time to 99 % mortality for adults of T. confusum, T. granarium and S. granarius, after exposure to 5o vpm of phosphine, is plotted against the concentration of carbon dioxide in air in Figure A 2 (cf. Tables A 4 - A 6). Time to mortality was generally reduced by the presence of carbon dioxide. For example time to 5o % mortality for adults of T. confusum fell from 11,o h in air to 2,o h in 75 % carbon dioxide and 25 % air (Table A 4). The case of adults of S. granarius in 75 % carbon dioxide, 25 % air is discussed in Section 2.3.3.. In Figure A 3 (cf. Tables A 7 - A 9) are plotted LT_{99} values for adults of S. granarius, S. oryzae, R. dominica, T. castaneum and T. granarium, after exposure to 2oo vpm of phosphine against concentration of carbon dioxide in air. In all cases examined, insects died more quickly as the concentration of carbon dioxide in air was increased from o - 12,5 % and from 12,5 % to 25 %. For four species, T. castaneum, S. oryzae, S. granarius and R. dominica, the reduction in LT_{99} values with increasing carbon dioxide concentration was significant only over the range o - 25 % and not over the range 25 - 75 %. On the other hand, LT_{99} values, at the 95 % level of significance, were inversely proportional to the concentration of carbon dioxide in the range o - 5o %, in the case of T. granarium (Table A 5), and o - 75 %, in the case of T. confusum (Table A 4).

The effect of 12,5 % carbon dioxide on the time to mortality was greater in the case of S. granarius, 1 - 4 weeks old, exposed to 2oo vpm of phosphine than for any other insect (Figure A 3). The effect of carbon dioxide on the toxicity of phosphine at 2oo vpm against adults of S. granarius was accordingly further investigated by studying adults of another age group, namely o - 1 weeks. Both adults of age 1 - 4 weeks (Table A 6) and o - 1 weeks (Table A 1o) showed similar responses to carbon dioxide: e.g., time to 5o % mortality in 25 % carbon dioxide, 75 % air was, in each case, 9 ± 1 % of the value in air. It is also to be noted that the young adults (Table A 1o) died some 5o % more slowly than the older adults (Table A 6) in all mixtures tested at 2oo vpm of phosphine.

2.3.2 Mortalities of adults in mixtures of phosphine, nitrogen, oxygen and carbon dioxide

Adults of T. castaneum (Table A 11), R. dominica (Table A 12) and S. oryzae (Table A 13), all aged 1 - 4 weeks, and adults of S. granarius, both o - 1 and 3 - 4 weeks (Tables A 14 - A 15), were exposed to 2oo vpm of phosphine in atmospheres containing reduced amounts of oxygen relative to air and low amounts of carbon dioxide (o - 15 %). These atmospheres were: 1o % carbon dioxide, 8o % nitrogen and 1o % oxygen; 15 % carbon dioxide, 8o % nitrogen and 5 % oxygen; 9o % nitrogen and 1o % oxygen; and 95 % nitrogen, 5 % oxygen.

Time to mortality in 95 % nitrogen and 5 % oxygen or 9o % nitrogen and 1o % oxygen were not significantly different from those in air in the case of R. dominica (Table A 12). Time to 5o % but not to 99 % mortality in 95 % nitrogen, 5 % oxygen was significantly different to that in air in the case of S. oryzae (Table A 13). In the case of T. castaneum (Table A 11) time to mortality in air was significantly greater at the LT_{5o} but not at the LT_{99} level than time to mortality in 9o % nitrogen, 1o % oxygen or in 95 % nitrogen, 5 % oxygen. On the other hand, reduction of the oxygen level to 1o or 5 % by increasing the content of nitrogen resulted in a significantly quicker kill, at both the LT_{5o} and the LT_{99} levels, in the case of adults of S. granarius, aged o - 1 weeks (Table A 14), or 3 - 4 weeks (Table A 15).

In contadistinction to the comparatively small and irregular effect of reduced oxygen levels associated with increasing levels of nitrogen, reduced oxygen levels associated with increasing levels of carbon dioxide of 1o or 15 % resulted in a significantly reduced time to both 5o and 99 % mortality, relative to air, in all cases tested (Tables A 11 - A 15). Results in 1o % carbon dioxide, 8o % nitrogen and 1o % oxygen, at least at the LT_{5o} levels, in the case of adults of S. oryzae (Table A 13), R. dominica (Table A 12), T. castaneum (Tables A 11) and S. granarius (Table A 14 - 15), time to 5o % mortality was significantly lower in 15 % carbon dioxide, 8o % nitrogen and 5 % oxygen than in 95 % nitrogen, 5 % oxygen.

From these results it can be concluded that the effect of increasing the carbon dioxide content in mixtures of carbon dioxide and air is more important than the concomitant decrease in the oxygen concentration. The results also have a practical significance in that phosphine fumigations are sometimes carried out under hermetic conditions where, as a result of grain metabolism, atmospheres such as 1o - 15 % carbon dioxide, 8o % nitrogen and 5 - 1o % oxygen are typical. It is accordingly pleasing to note the increased efficacy of carbon dioxide under such conditions.

2.3.3 Response of S. granarius adults to phosphine in atmospheres with low oxygen concentrations

Despite repeated replication, it was not possible to obtain a linear plot between mortality in probits and the logarithm of the time of exposure in the case of adults of S. granarius, either o - 1, 1 - 4 or 3 - 4 weeks, exposed to 5o vpm phosphine in an atmosphere of 75 % carbon dioxide, 25 % air. Although this mixture killed approximately 8o % of the insects some eight times more quickly than did phosphine in air (Figure A 4), the remaining adults survived exposure periods in excess of the LT_{99} value for phosphine in air. Typical of these data is the plot for 1 - 4 week old adults that is shown in Figure A 4. That the ability of some 2o % of the insects to survive was not the result of resistance was shown by exposing progeny of the survivors to phosphine and to a mixture of carbon dioxide and phosphine.

Results for these progeny were not different from the original results (Figure A 4). It is also to be noted that this apparent antagonism of carbon dioxide to phosphine was not observed at the higher phosphine level of 2oo vpm (Table A 6, A 1o, A 14 - 15). The observed deviation from linearity in the plot of mortality in probits against the logarithm of the time of exposure was not observed in the case of exposure to phosphine in air (Figure A 4). In this plot special attention was placed on mortalities in excess of 9o % by

exposing 12 replicates, each of 33 insects, to phosphine in air. The observed mortalities of 92 %, 97 % and 99 % were consistent with those expected from extrapolation of the data obtained in the range 5 - 9o % mortality.

The data pertaining to the apparent immunity in atmospheres containing both low levels of phosphine and low levels of oxygen can be explained by the hypothesis that insects require time in which to utilize low levels of oxygen as a protective mechanism against phosphine, and that this time is too short for the more susceptible insects, exposed to 5o vpm phosphine, or for insects exposed to 2oo vpm phosphine.

2.3.4 Mortality of Pupae in Mixtures of Phosphine, Carbon Dioxide and Air

External pupae that develop outside of grain kernels, i.e. pupae of Tribolium species and of T. granarium, were exposed to 5o vpm of phosphine and internal pupae that develop inside grain kernels, i.e., pupae of Sitophilus species and of R. dominica, were exposed to 2oo vpm of phosphine. Data are summarized in Tables A 16 - A 21 and in Figure A 5. The LT_{99} values, after exposure to 2oo vpm of phosphine, were significantly greater for S. granarius (Table A 16) than for S. oryzae (Table A 17) and significantly greater for S. oryzae than for R. dominica (Table A 18). In addition, times to mortality of S. granarius, after exposure to 2oo vpm of phosphine, were significantly greater (Tables A 16) than times to mortality of the external pupae, T. granarium (Table A 19), T. castaneum (Table A 2o) and T. confusum (Table A 21) after exposure to only 5o vpm of phosphine.

The effect of the two tested carbon dioxide concentrations on the LT_{99} values for Sitophilus species was not significant (Table A 16 - A 17). A preciser estimimate of the effect of carbon dioxide on the efficacy of phosphine can be obtained from the LT_{5o} values. The upper and lower limit of the LT_{5o} value in the case of S. oryzae (Table A 17) were covered by

the range 11,4 ± 2,2 h, irrespective of concentration of carbon dioxide, and the best estimates of the LT_{50} values were covered by the range 11,o ± o,6 h. In the case of S. granarius, the best estimate of the LT_{50} values fell within the range 34,2 ± 2,3 h. It is concluded that the effect of carbon dioxide on the toxicity of phosphine to pupae of Sitophilus species is, at most, small and not established at the 95 % level of confidence. There was also no significant effect of carbon dioxide concentration, at either the LT_{50} or the LT_{99} levels, on the toxicity of phosphine to pupae of T. granarium (Table A 19), T. castaneum (Table A 2o) and T. confusum (Table A 21).

In the case of pupae of R. dominica (Table A 18), both LT_{50} and LT_{99} values in air were significantly greater than in 25 % carbon dioxide, 75 % air, although there were no significant differences between values in 25 % air, 75 % carbon dioxide and those in 75 % carbon dioxide, 25 % air. These observations, which were obtained from insects from the same culture exposed to different atmospheres at the same time, were unexpected.

It is pointed out at this stage that an analysis of the results in Section 2.3.4 is given in Section 3.

2.3.5 Mortalities of Larvae in Mixtures of Phosphine, Carbon Dioxide in Air

Larvae of the six tested species died more quickly in mixtures of 5o vpm of phosphine, 75 % carbon dioxide and 25 % air than in mixtures of 5o vpm of phosphine in air, and these differences were significant at the LT_{99} level (Figure A 6, Tables A 22 - A 27). The relative order of tolerance to phosphine, viz. T. granarium, S. oryzae, R. dominica, S. granarius, T. confusum and T. castaneum, was maintained in all tested mixtures of carbon dioxide and air (Figure A 6).

Of considerable practical importance was the reduction in the time required to control the larval stage of T. granarium which, in addition

to being tolerant to phosphine (Figure A 6), is the insect stage most tolerant to 75 % carbon dioxide and 25 % air (Section 2.2). The larvae of T. granarium were accordingly examined in more detail than were other larvae.

Firstly, larvae from mixed aged cultures were collected from two sources, namely the culture medium and from a roll of paper which had been placed on top of the culture medium. There was little difference in the results from the different groups of larvae (Table A 22). Larvae of T. granarium, taken from the paper above the medium, were also exposed to 5o vpm of phosphine in various atmospheres containing low levels of carbon dioxide (o %, 1o %, 15 %) and/or low levels of oxygen (5 %, 1o %) (Table A 28). Mortality values in mixtures of 5 % oxygen, 95 % nitrogen or 1o % oxygen, 9o % nitrogen were not significantly different from values in air (Table A 28), but were significantly different from values in 75 % carbon dioxide, 25 % air (Table A 22). It is concluded that the effect of high levels of carbon dioxide is not due to the resulting depletion in oxygen levels.

2.3.6 Mortalities of Eggs in Mixtures of Phosphine, Carbon Dioxide in Air

Eggs of Sitophilus species, which are deposited within grain kernels, were exposed to 2oo vpm of phosphine and eggs of Tribolium species, R. dominica and T. granarium, which were collected from flour, were exposed to 5o vpm of phosphine. Data from probit analysis are summarized in Tables A 29 - A 34, and values for the LT_{99} are given in Figure A 7. It is clear from these data that the interactions between carbon dioxide and phosphine show different effects for the Tribolium species, on the one hand, and the Sitophilus species, R. dominica and T. granarium, on the other hand. For these latter species, viz. S. granarius (Table A 29), S. oryzae (Table A 3o), R. dominica (Table A 31) and T. granarium (Table A 32), times to both 5o and 99 % mortality in air were not significantly different from those in either 25 or 75 % carbon dioxide in air. In the cases of T. castaneum

(Table A 33) and T. confusum (Table A 34), in contrast, time to mortality was inversely proportional to the concentration of carbon dioxide in air over the range o - 75 %.

Although the susceptibilities of eggs of Tribolium species to mixtures of phosphine, carbon dioxide and air was not unexpected, in view of the susceptibilities of the eggs to mixtures of carbon dioxide and air in the absence of phosphine (Table A 1), it was decided to study in more detail the efficacy of both carbon dioxide and phosphine on eggs of these species. Accordingly, eggs of T. castaneum and of T. confusum, of age o - 1, 1 - 2, 2 - 3, 3 - 4 and 4 - 5 days, were exposed to either 1oo vpm of phosphine in air for 24 h or to 75 % carbon dioxide in air for 2o h. For each species (Table A 35) young eggs, o - 1 days old, were not only especially tolerant to phosphine but also especially susceptible to carbon dioxide in air. The eggs of each species became, with increasing age, more susceptible to phosphine and less susceptible to carbon dioxide (Table A 35).

The joint action of carbon dioxide and phosphine on 1 - 3 day old eggs of Tribolium species (Tables A 33 - A 34) can be therefore explained, at least partly, in terms of the efficacy of phosphine against older eggs, and the efficacy of carbon dioxide against younger eggs.

3. Analysis of Results

3.1 Outline of the Procedures Used

There are many possible types of joint action of two components, such as phosphine and mixtures of carbon dioxide and air, on mortality. In this section an attempt is made to determine which of three categories of joint action best describes the results. These categories are firstly that of independent or additive effects of the two components, secondly that of more-than-additive effects and thirdly that of less-than-additive effects. The words synergism and

antagonism are used in Section 3.1 to define, respectively, more-than additive and less-than-additive effects.

Independent, additive, mortality occurs when the observed mortality is the sum of the partial mortalities of each component. The well-known Abbott's formula (Equation 1) is an example of independent effects and is based on the assumption that the corrected mortality M_c, i.e., the mortality due to the toxic component under investigation, and the control mortality, a, act independently to result in the observed mortality, M.

$$M_c / 100 = (M - a)/(100 - a) \qquad \text{Equation 1}$$

The formula for independent action, Equation 1, was used to obtain a criterion of joint action that was amenable to statistical analysis. The first step in this analysis (Survey Table) was the measurement and probit analysis, by means of standard procedures, of mortalities in mixtures of carbon dioxide and air (Code No. 1, Survey Table), in mixtures of phosphine and air (Code No. 2, Survey Table) and in mixtures of phosphine, carbon dioxide and air (Code No. 3, Survey Table). The next step in the analysis was the use of Equation 1 to correct the mortalities observed in a mixture of phosphine, carbon dioxide and air for the mortality due to carbon dioxide and air alone (Code No. 4, 5a, Survey Table) and to obtain a probit analysis of the subsequent corrected data (Code No. 5 b, Survey Table). The resulting values for the LT_{50} or the LT_{99} are the values due to phosphine alone in a mixture of phosphine, carbon dioxide and air, on the assumption that the action of phosphine is independent of that of carbon dioxide. For the case of such calculated or corrected values being not significantly different from those obtained in phosphine in air (Code No. 2 b, Survey Table), the assumption of independent, additive, joint action was taken as validated. For the case of such

Survey Table

Steps taken to obtain a criterion of the type of joint action

Code No.	Steps taken
1a	Measurement of mortalities after exposure in carbon dioxide and air.
1b	Probit analysis of the data in 1a.
2a	Measurement of mortalities after exposure to phosphine in air.
2b	Probit analysis of the data in 2a.
3a	Measurement of mortalities after exposure in a mixture of phosphine (concentration as in 2a) and carbon dioxide in air (concentration as in 1a)
3b	Probit analysis of the data in 3a.
4	Calculation, from the equation obtained in 1b, of the mortalities (a) due to exposure to a mixture of carbon dioxide and air, for each exposure period used in 3a.
5	Use of these mortalities (a) to obtain, with the aide of Equation 1, the mortalities M_c. These are the mortalities due to phosphine in a mixture of phosphine, carbon dioxide and air, on the assumption of independent joint action.
5b	Probit analysis of the "corrected"mortalities (M_c) that were calculated in 5a.
6	Comparison of values such as the LT_{50} or LT_{99} values obtained in 2a and 5a. Additive joint action occurs in the case of no significant difference between the values in 2a and in 5a.

calculated times to mortalities being significantly less than the measured values, the interactions between carbon dioxide and air, on the one hand, and phosphine, on the other, were more than additive, that is, synergistic. Antagonism, by a similar analysis, occurred when the calculated values, assuming independent action, were greater than those observed.

3.2 Analysis of Results for the Case of Insignificant Mortality in Mixtures of Carbon Dioxide and Air

In Table A 36 are outlined the mortalities expected from an exposure to 75 % carbon dioxide, 25 % air, in the absence of phosphine after exposure periods equal to the LT_{50} and LT_{99} values in a mixture of 75 % carbon dioxide, 25 % air and either 5o or 2oo vpm of phosphine. In many cases, e.g., for adults and larvae of T. confusum (Table A 36), the expected mortality from carbon dioxide was negligible, i.e. 'a' in Equation 1 was less than o,5 %. In such (mathematically trivial) cases the values for the mortalities calculated to be due to phosphine in a mixture of carbon dioxide, phosphine and air (Code No. 5b, Survey Table) equal those observed in the mixture (Code No. 3a, Survey Table), and the question of the type of joint action is easily solved. Observed and corrected times to mortalities are summarized in Tables A 37 and A 38, even for the mathematically trivial cases, to enable a rapid appraisal of the type of joint action observed for each stage of each insect species tested.

In several of the cases outlined in Table A 36, e.g. in the cases of pupae of T. confusum or eggs of S. granarius, expected mortalities from carbon dioxide were low at times equal to the LT_{50} values in a mixture of carbon dioxide, air and phosphine but high at times equal to the LT_{99} values. In such situations the type of joint action cannot be determined by a simple perusal of values for times to mortalities in different atmospheres but can only be determined from the type of analysis outlined in Section 3.1. In two cases (Table A 36), namely for eggs of T. confusum and of T. castaneum, mortalities

expected from carbon dioxide (Table A 1) were high at times of exposures equal to both the LT_{50} and LT_{99} values observed in mixtures of phosphine, carbon dioxide and air. In these cases (Table A 38), the observed mortalities in the mixture were corrected not for carbon dioxide, but for phosphine mortality.

3.2.1 Analysis of Results for Adults and Larvae

For adults of all six tested species (Table A 37), the calculated or corrected values for mortality due to phosphine in mixtures of 75 % carbon dioxide, 25 % air and phosphine were significantly less, at both the LT_{50} and the LT_{99} levels, than those observed in phosphine alone. That is to say, phosphine synergized the toxicity of carbon dioxide against adults. The reduction in the LT_{99} value of adults of T. granarium, for example, in a mixture of carbon dioxide, air and phosphine, relative to that in air, was therefore due to a combined effect between phosphine and carbon dioxide that was more than the sum of the effects of carbon dioxide and phosphine.

Synergism at the LT_{50} level was also demonstrated for larvae of the six tested species. At the LT_{99} level, synergism was demonstrated for larvae of T. confusum, T. castaneum, T. granarium, S. granarius and R. dominica but not for larvae of S. oryzae. (Table A 37).

3.2.2 Analysis of Results for Pupae and Eggs

The type of joint action beween phosphine and carbon dioxide was more complex for eggs and pupae than for adults or larvae. The action of carbon dioxide and phosphine was additive for the three external pupae, namely pupae of T. castaneum, T. confusum and T. granarium (Table A 37). This was also the case for S. granarius, although the fiducial limits were large (Table A 37). In the case of pupae of S. oryzae antagonism between carbon dioxide and phosphine was observed at the LT_{99} level. This result is plausible, in that it is known that carbon dioxide retards the development of

pupae to the adult stages (BAILEY and BANKS, 1979) which are more tolerant to phosphine. In contradistinction to results for pupae of other species, synergism between carbon dioxide and phosphine was observed in the case of R. dominica (Table A 37). It should be recalled, however, that immature stages of defined age were used in this work as pupae in the cases where pupae are internal, namely Sitophilus species and R. dominica. The possibility of the presence of some insects in either the larval or pre-adult stages cannot be excluded.

In the case of eggs, additive effects were observed at the LT_{99} level for the four species summarized in Table A 37, namely S. oryzae, S. granarius, T. granarium and R. dominica, although antagonism at the LT_{50} level was observed in the case of S. oryzae. This antagonism could also be due to reduction from carbon dioxide of the rate of development of eggs.

In Table A 38 are outlined mortalities of eggs of Tribolium species after exposure to carbon dioxide, corrected for phosphine mortality. Additive effects were observed at the LT_{99} levels. The data on the dependence on age of the susceptibilities to phosphine of eggs of Tribolium species (Table A 35) offer an alternative and, in the authors view, a more plausible explanation of the results than synergism in the strict sense of potentiation by phosphine of the lethal action of carbon dioxide.

3.2.3 A Note on the Significance of Additive Effects

In Figure A 8 are outlined mortality values for o - 1 day old eggs of S. granarius after 24 h exposures to different concentrations of phosphine in air and in 75 % carbon dioxide and 25 % air. The 24 h mortality of these eggs to 75 % carbon dioxide and 25 % air, in the absence of phosphine, was also measured as 19 % in this experiment. The mortalities observed in a mixture of carbon dioxide, air and phosphine were corrected for this 19 % "control" mortality and the resulting

corrected mortalities for mortality due to phosphine were not different from those observed after exposure to phosphine in air (Figure A 8).

In this example of independent, additive effects (Figure A 8), the difference between mortalities in carbon dioxide, air and phosphine, on the one hand, and those in phosphine in air, on the other, are greater for low overall mortalities than for high overall mortalities. This type of effect is a necessary consequence of additive effects as can be seen by rearrangement of Equation 1 to Equation 2: as the ratio $M_c/100$ tends to its maximum value of 1 (that is, as mortality approaches 100 %), the observed mortality M tends towards the corrected value, M_c. In other words, the additive effects of two components have little influence on high mortality values, such as the LT_{99} or LC_{99} values, which have similar results in a mixture as those obtained in the absence of the least toxic component of the mixture. Where high mortalities in one stage are required, little is to be gained by the use of a mixture of components with additive effects.

$$M_c + a(1 - M_c/100) = M \qquad \text{(Equation 2)}$$

4. Discussion

The use of a mixture of 50 or 200 vpm of phosphine and 75 %, V/V, carbon dioxide, 25 % air has several advantages relative to carbon dioxide plus air alone or to higher concentrations of phosphine in air. Firstly, the mixture results in a more rapid kill of the most numerical stages, the larvae and the adults. A second advantage of the mixtures is control of the larvae of T. granarium which is particually resistant to carbon dioxide in air. The third advantage of the mixtures is the relative speed of kill against Tribolium species. On the other hand, pupae of Sitophilus species, which are tolerant to both carbon dioxide and to phosphine, are tolerant to mixtures containing each component.

Mixtures of carbon dioxide and phosphine could find practical applications in stored products that are not generally affected by Sitophilus species, such as milled (e.g., flour) or processed (e.g., chocolates) products. Such products are precisely those where high dosages of phosphine are undesirable. Attention is drawn in this context to recent work (Dr. Friemel, of the firm Dr. W. Freyberg) which indicates that low levels of phosphine are not corrosive to machinery or electrical fittings.

In the factory certain commodities, such as chocolates, are only infested in the brief interval between processing and packaging. In such products, where pupae are absent, mixtures of phosphine and carbon dioxide could be especially useful. Subject to further data on the age-dependence of the sensitivity of eggs, mixtures of carbon dioxide and air, in the absence of phosphine, could also be used where the only stages present were eggs or adults.

It can be said in general that the amounts of insecticides in stored products can be reduced by a more selective use of chemicals (DESMARCHELIER 1979), namely by a program aimed not at a universal remedy but at remedies appropriate to the given physical and biological circumstances. The work outlined in this report could form the basis of some of these remedies.

Acknowledgments

The Alexander von Humboldt Foundation is thanked for its financial assistance, and C.S.I.R.O. is thanked for granting leave of absence. The staff of the BBA are thanked for their friendly assistance.

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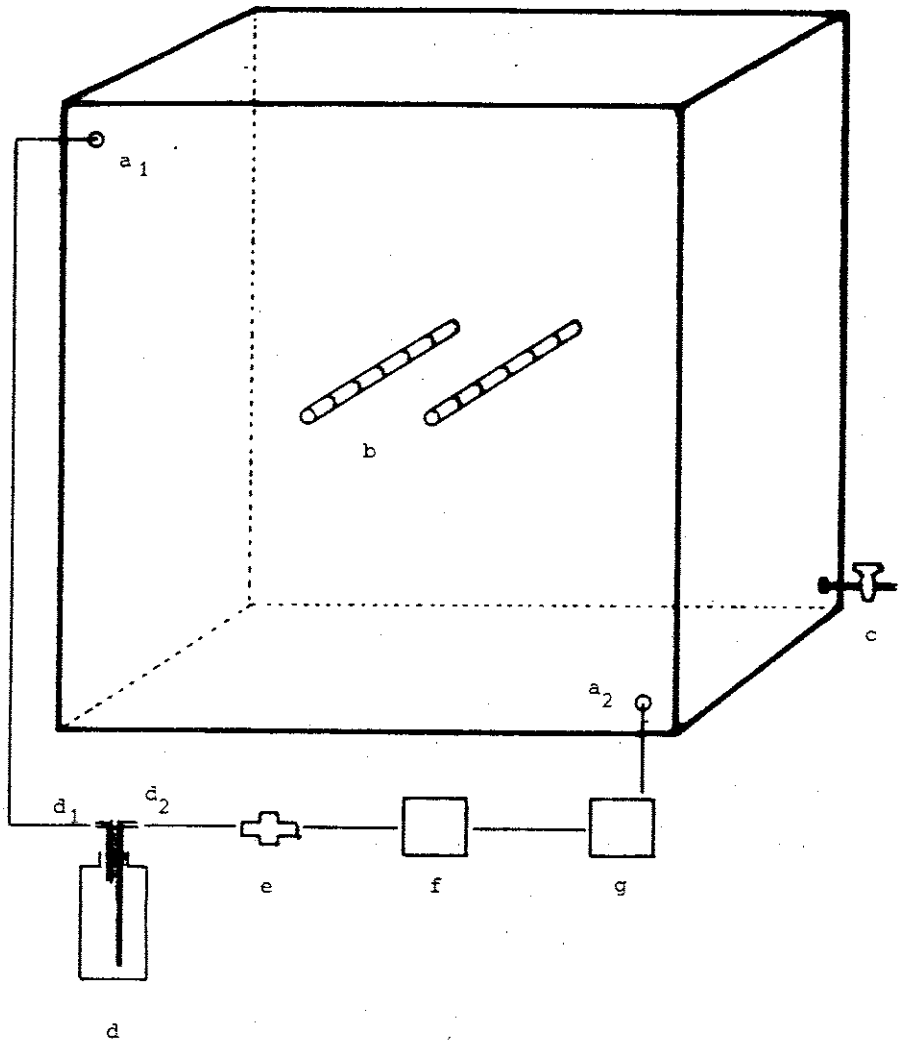

Figure 1: 500 litre fumigation chamber.

a_1 a_2 : valves
b: insect sampling ports
c: evacuating pump with valve
d: Dreschel flask with connections d_1 d_2
e: "T" piece, with septum
f: recirculatory pump
g: apparatus for measurement of dew-point

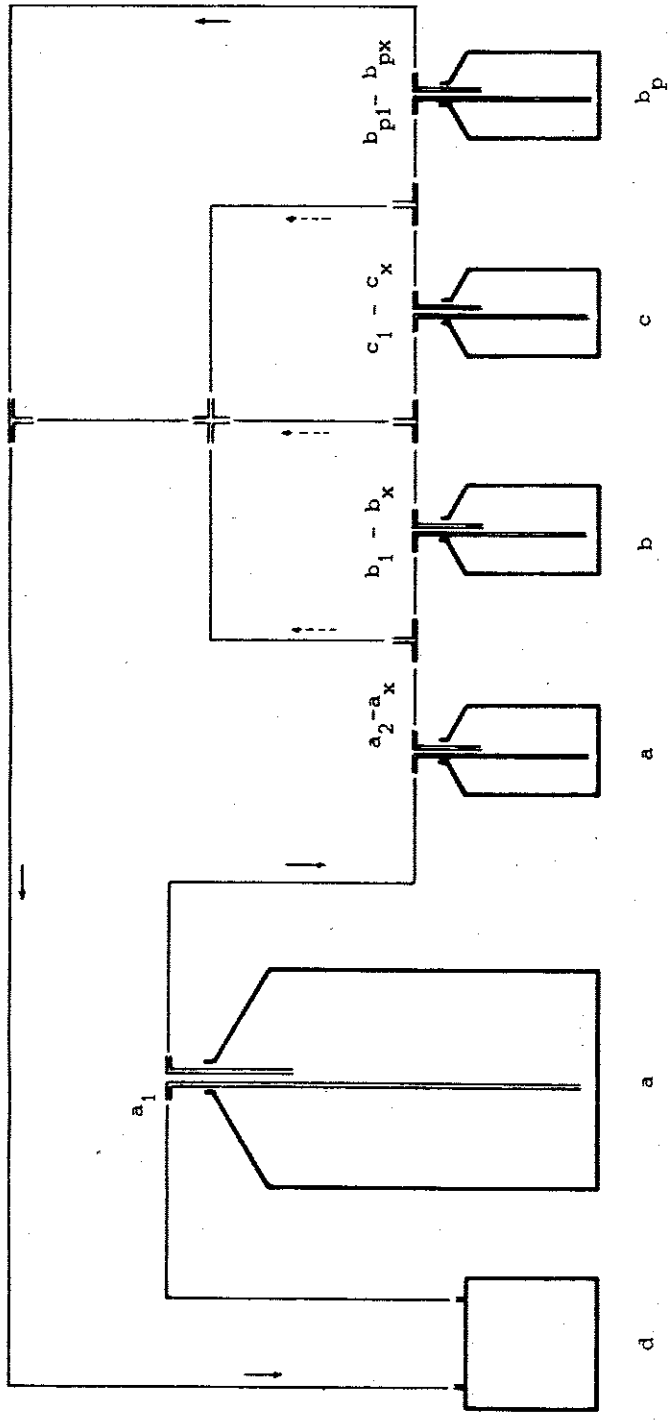

Figure 2. Equipment used for the constitution of different atmospheres.
a. flask(s) containing CO_2.
b. flask(s) containing insects in air.
c. flask(s) containing air.
d. recirculatory pump.
b_p. flask containing phosphine in air.

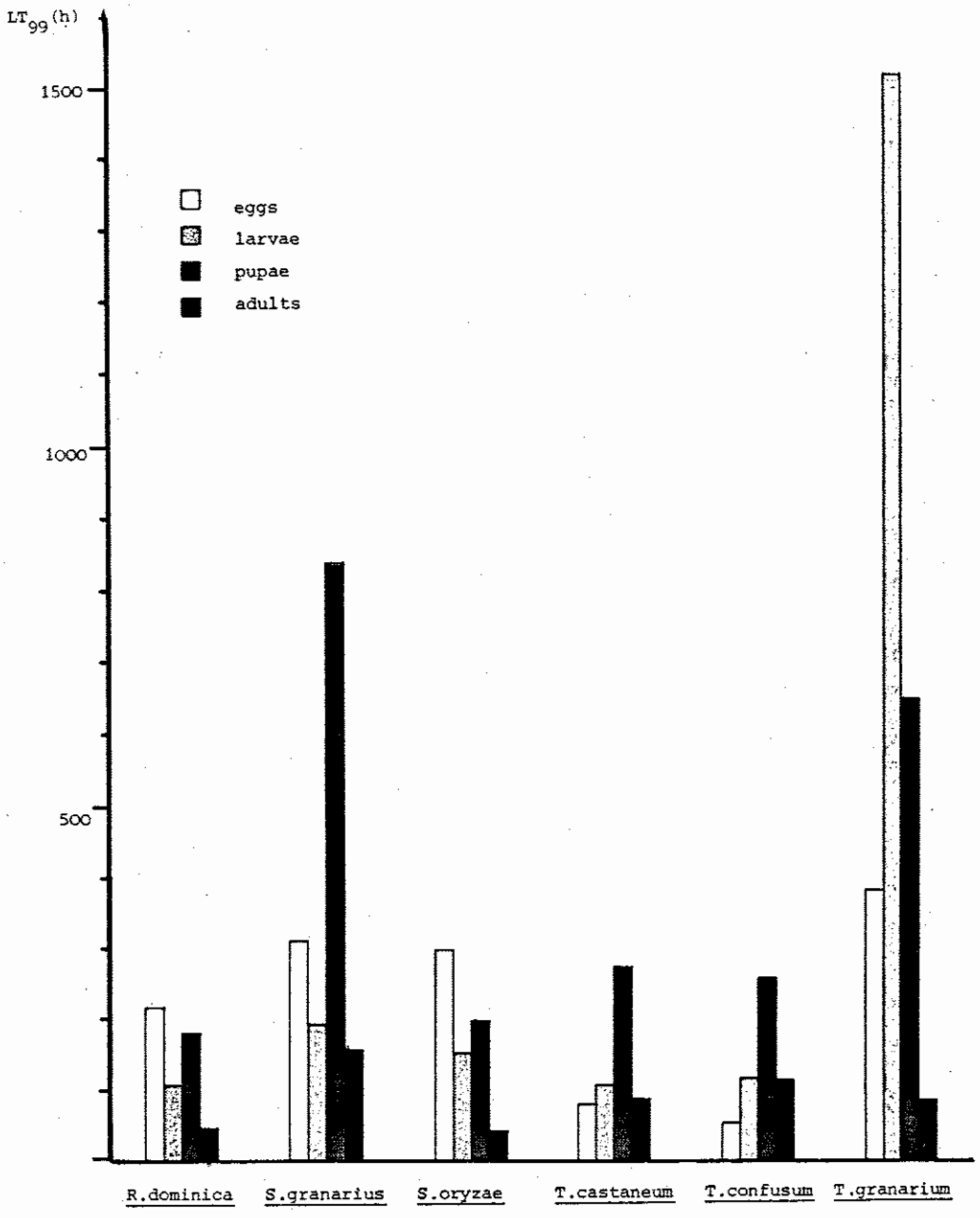

Figure A1. Time to mortality of eggs, larvae, pupae and adults of S.granarius, S.oryzae, R.dominica, T.castaneum, T.confusum and T.granarium, after exposure to 75% CO_2 and 25 % air at 19 C. and 70 % r.h.

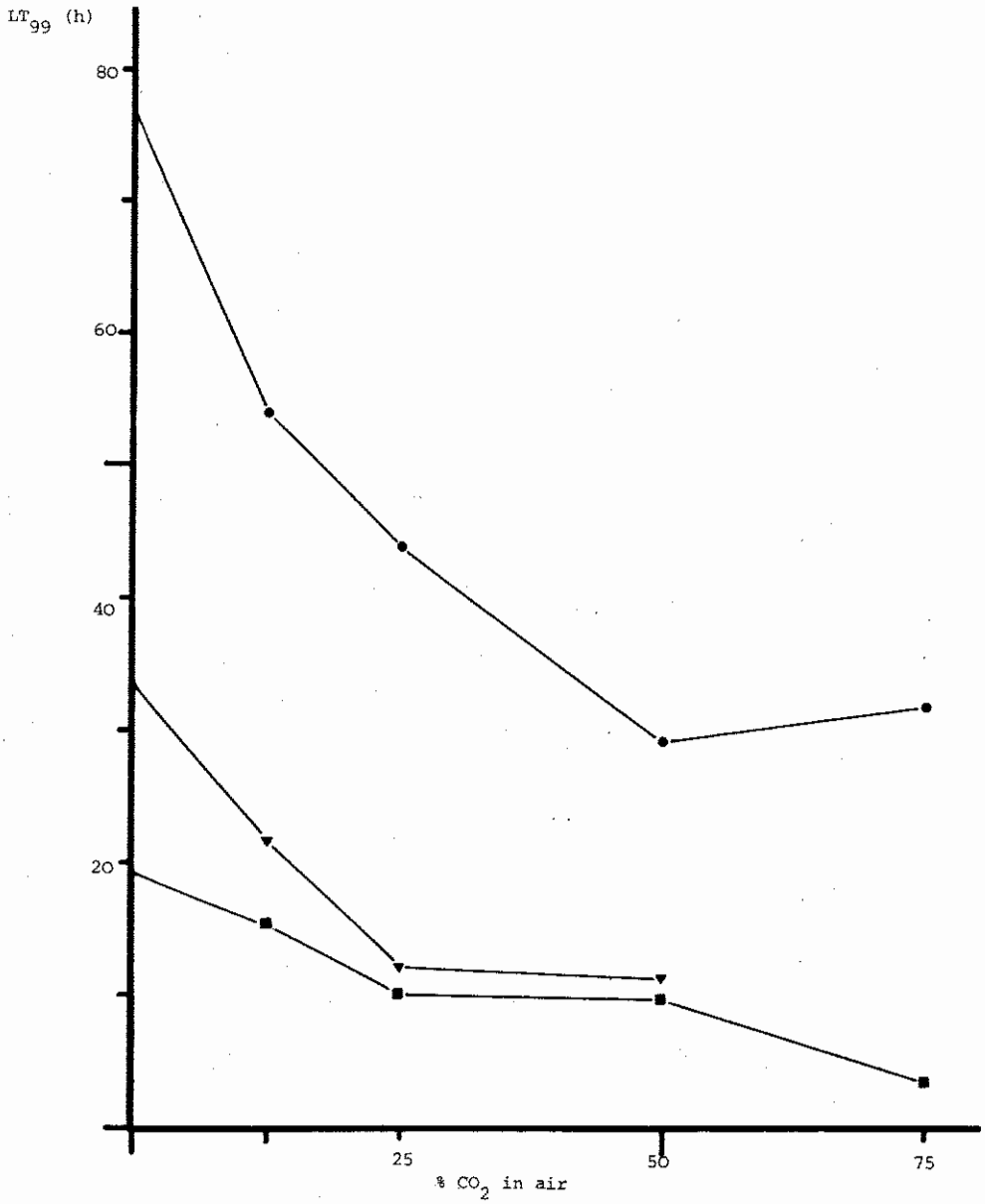

Figure A2. Plot of time to mortality of adults of T.confusum (■), T.granarium (●) and S.granarius(▼)after exposure to 50 vpm phosphine, against concentration of CO_2 in air.

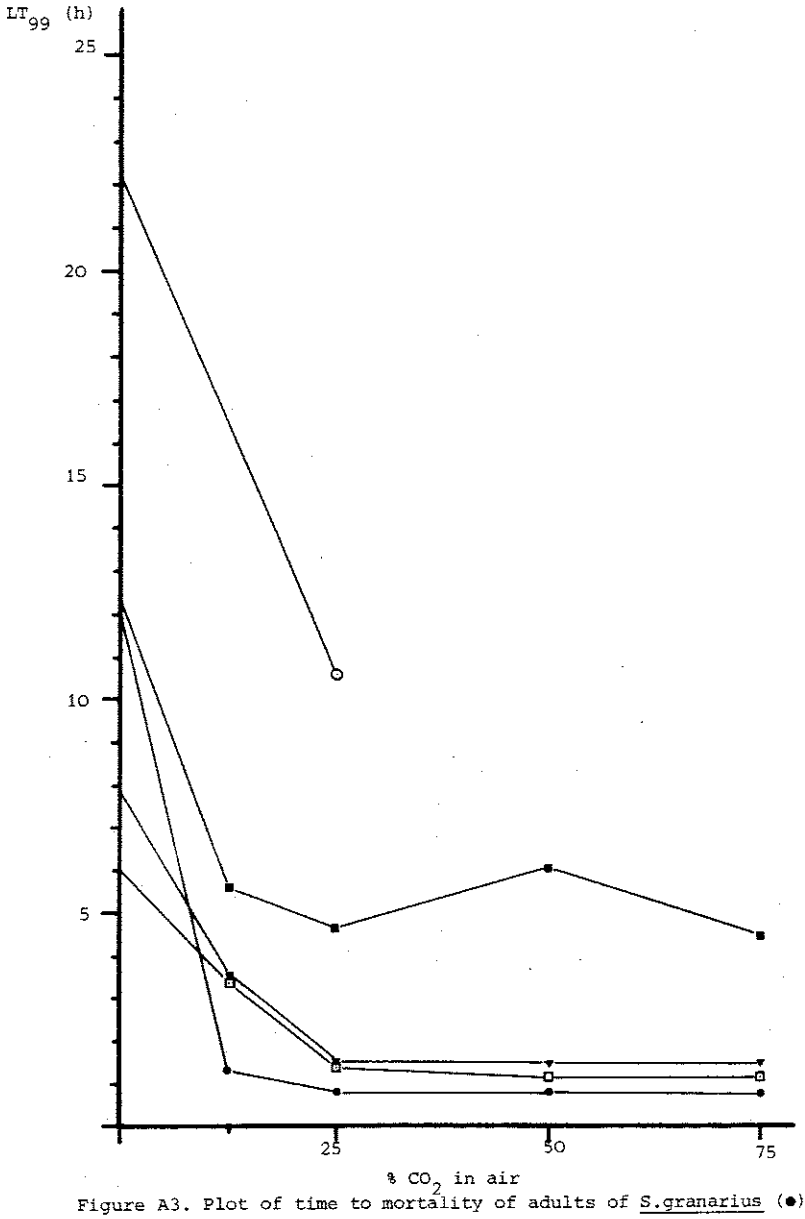

Figure A3. Plot of time to mortality of adults of S.granarius (●), S.oryzae (▼), R.dominica (■), T.castaneum (□) and T.granarium (○), after exposure to 200 vpm of phosphine, against concentration of CO_2 in air.

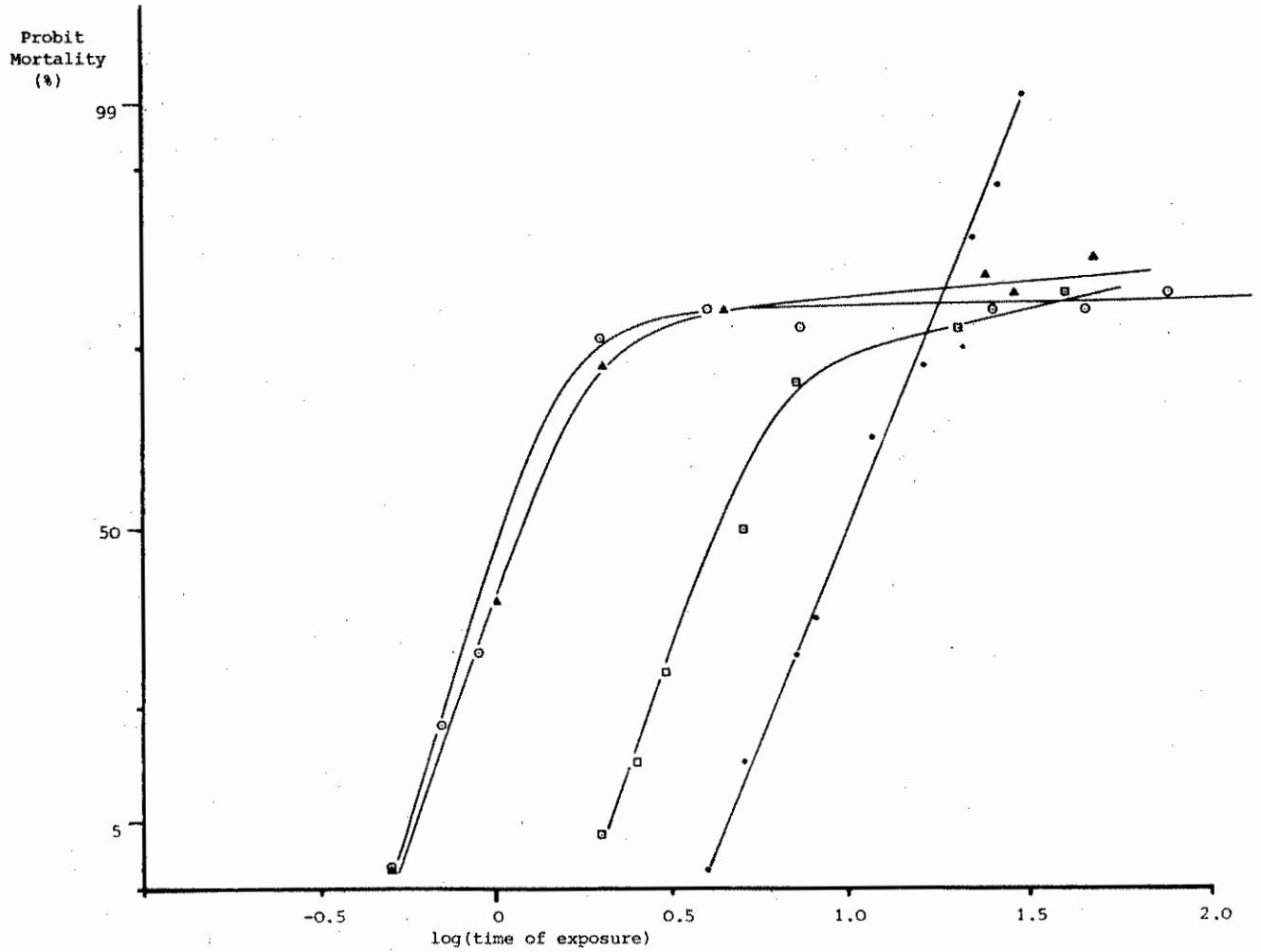

Figure A4. Plot of mortality (probit scale) of adults of S.granarius, after exposure to 50 vpm of phosphine in three atmospheres, against the logarithm of the time of exposure (h).

air (●). 95% N_2, 5% O_2 (□). 75% CO_2, 25% air: parents (○); progeny (▲).

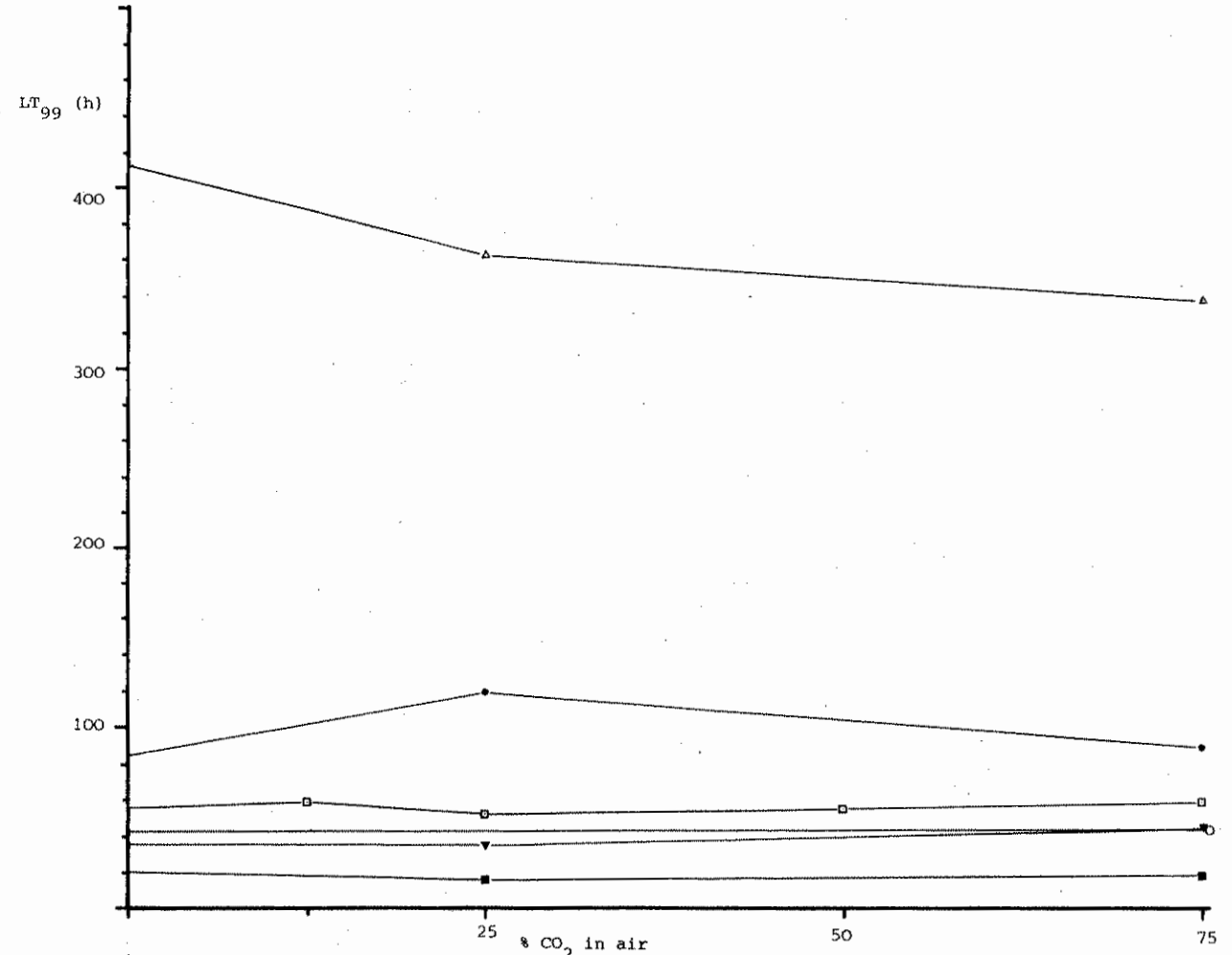

Figure A 5. Plot of time to mortality of pupae of S.oryzae (●), S.granarius (△) and R.dominica (■), after exposure to 200 vpm of phosphine, and of pupae of T.confusum (○), T.castaneum (▼) and T.granarium (□), after exposure to 50 vpm of phosphine, against concentration of carbon dioxide in air.

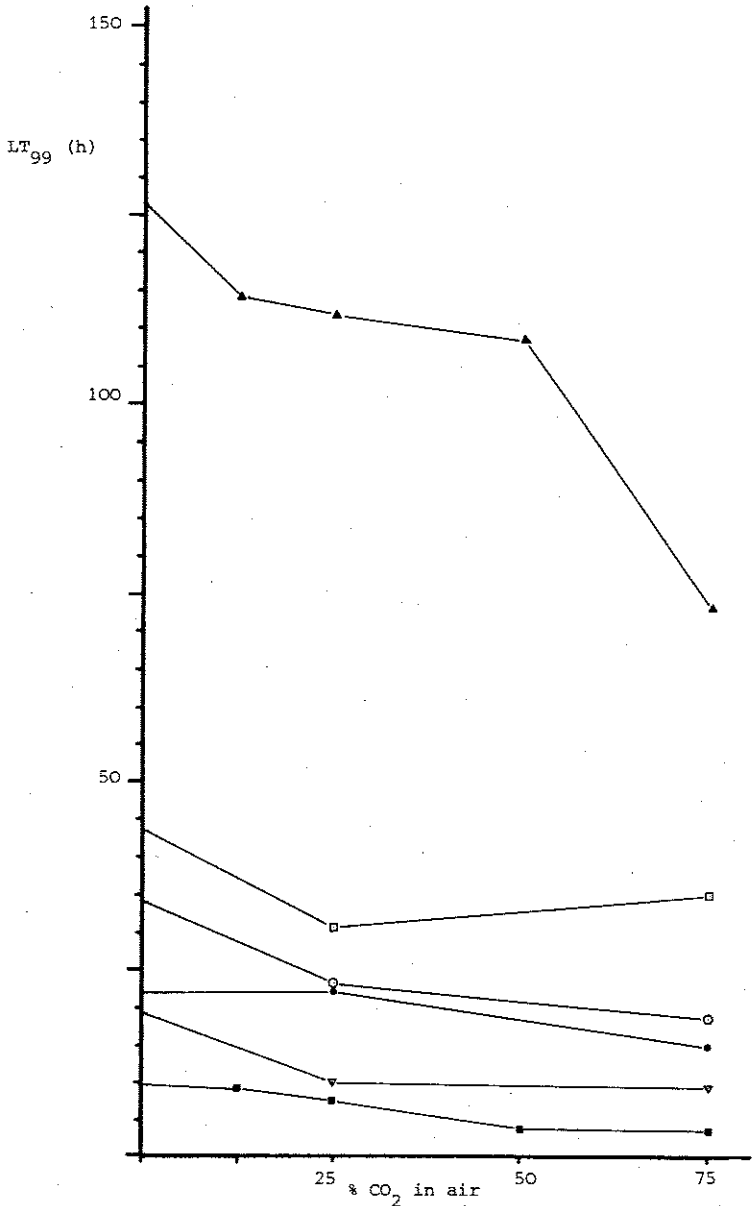

Figure A6. Plot of time to mortality of larvae of S.oryzae (□), R.dominica (○), S.granarius (●), T.confusum (▽), T.castaneum (■) and T.granarium (▲), after exposure to 50 vpm of phosphine, against concentration of CO_2 in air.

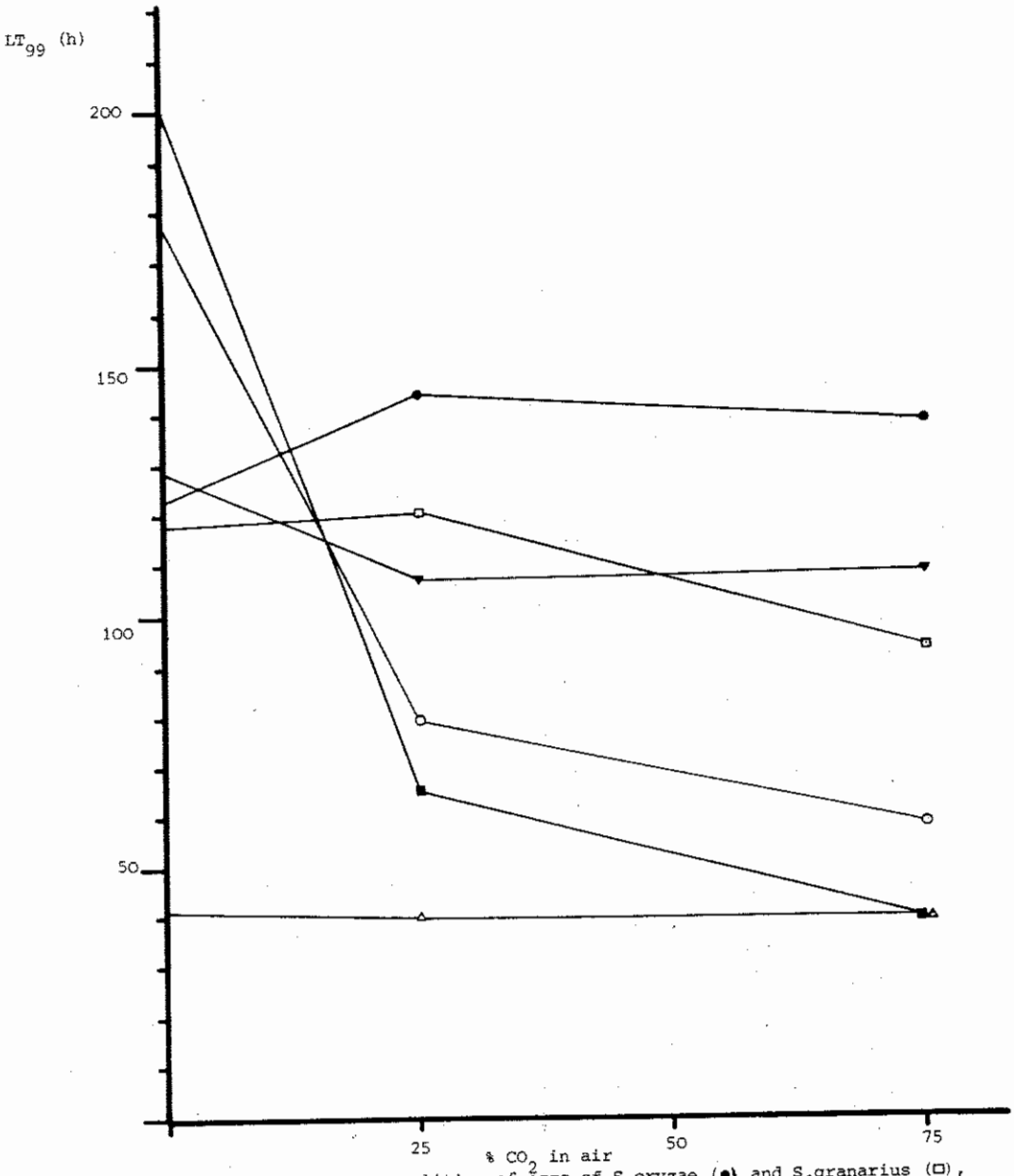

Figure A7. Plot of times to mortalities of eggs of S.oryzae (●) and S.granarius (□), after exposure to 200 vpm phosphine, and of eggs of T.confusum (○), R.dominica (△), T.castaneum (■) and T.granarium (▼), after exposure to 50 vpm phosphine, against concentration of CO_2 in air.

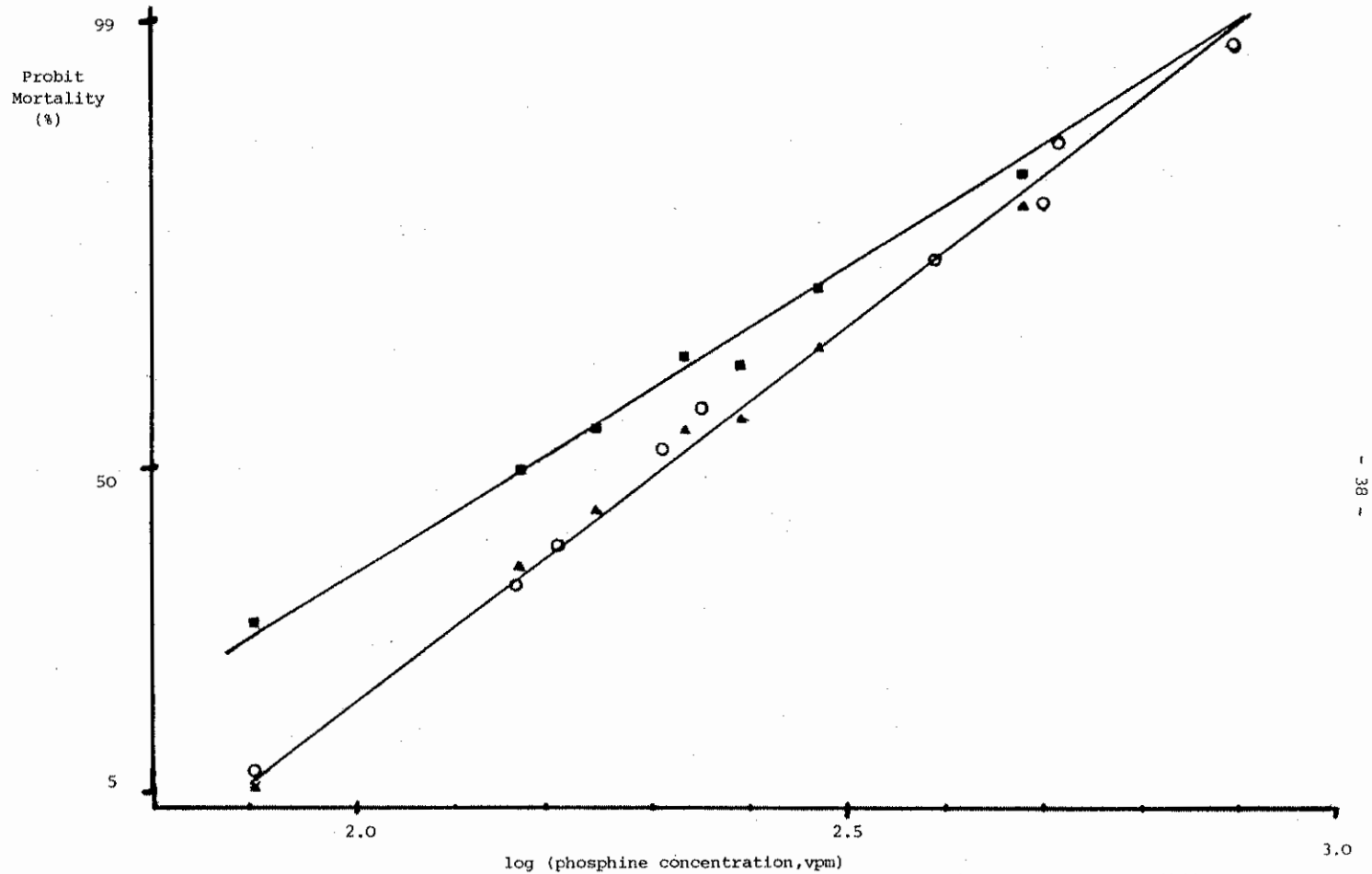

Figure A8. Plot of probit mortality of eggs of S.granarius, after exposure for 24h to phosphine in different atmospheres, against the logarithm of the phosphine concentration.
(O) in air. (■) in 75% CO_2,25% air. (▲) in 75% CO_2,25% air, corrected for mortality due to CO_2.

Table A1

Times to mortality for four stages of six insect species resulting from exposure to 75 % carbon dioxide, 25 % air at 19°C and 7o % r.h.

Species	Stage	Data Number	LT_{5o} (h)	LT_{99} (h)	Limits LT_{5o} lower (h)	Limits LT_{5o} upper (h)	Limits LT_{99} lower (h)	Limits LT_{99} upper (h)
S. oryzae	adults	8	14,5	3o,8	13,1	15,9	25,o	38,o
	pupae	11	63,2	196	59,8	66,7	167	237
	larvae	9	48,4	151	45,6	51,5	125	181
	eggs	29	73,o	297	7o,7	75,4	277	32o
S. granarius	adults	1o	63,o	157	57,9	68,5	12o	2o5
	pupae	16	2o7	829	195	22o	654	1o51
	larvae	13	68,3	19o	63,5	73,7	165	221
	eggs	8	56,o	3o6	47,7	65,8	193	485
R. dominica	adults	11	27,o	39,9	26,2	27,9	36,1	44,2
	pupae	17	49,4	184	47,8	51,o	169	2o1
	larvae	14	31,4	1o4	3o,4	32,3	96,1	112
	eggs	11	94,5	21o	88,3	1o1	166	264
T. granarium	adults	8	4o,1	85,7	36,6	43,9	67,3	1o8
	pupae	11	238	65o	186	283	475	876
	larvae	22	312	1522	288	345	1o4o	1926
	eggs	12	115	383	1o7	124	294	5o1
T. castaneum	adults	15	39,o	89,8	36,3	41,8	74,5	1o8
	pupae	2o	98,8	278	93,6	1o4	229	337
	larvae	14	51,2	1o8	43,8	58,9	87,6	138
	eggs	13	28,3	81,o	25,3	31,7	67,3	1o7
T. confusum	adults	9	45,2	11o	4o,3	5o,6	73,8	164
	pupae	1o	74,5	266	66,o	84,o	179	396
	larvae	11	47,5	114	43,8	51,6	89,o	145
	eggs	12	15,3	51,1	13,5	17,4	38,4	68,o

Table A2
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Mortality of four stages of six insect species after a 7-day exposure to 25 % carbon dioxide, 75 % air at 19°C, 7o % r. h.

Species	Mortality, M, and Standard Error, SE, for:							
	adults		pupae		larvae		eggs	
	M	SE	M	SE	M	SE	M	SE
S. oryzae	1oo	o,o	4o,8	4,7	27,3	7,3	47,5	2,4
S. granarius	49,8	4,6	6,8	4,9	21,7	4,5	3,o	8,8
R. dominica	9o,4	6,9	1o,4	4,2	45,o	o,8	64,2	16,1
T. granarium	1oo	o,o	o,o	o,o	o,o	o,o	o,o	o,o
T. castaneum	o,o	o,o	1o,3	7,8	1,6	4,o	1oo	o,o
T. confusum	o,o	o,o	o,8	4,9	o,o	o,o	1oo	o,o

Table A3
========

Times to mortalities of adults of R. dominica and of S. oryzae and of eggs of T. castaneum and of T. confusum after exposure to 25 % carbon dioxide, 75 % air, at 19°C and 7o % r.h.

Species	Stage	Data No.	LT_{5o} (h)	LT_{99} (h)	95 % Fiducial Limits LT_{5o} (h)	95 % Fiducial Limits LT_{99} (h)
R. dominica	adults	2o	91,6	287	85,8-98,2	228 -359
S. oryzae	adults	1o	34,7	56,4	33,1-36,4	51,1- 62,1
T. castaneum	eggs	11	3o,1	118	25,1-35,2	99 -137
T. confusum	eggs	14	22,1	1o7	19,2-25,5	74,8-153

Table A4

Time to mortality for adults of T. confusum, 1 - 4 weeks, exposed to 5o vpm of phosphine in mixtures of carbon dioxide and air at 19°C and 7o % r.h.

Atmosphere CO_2 (%, V/V)	air	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
o	1oo	16	11,o (1o,4 - 11,8)	29,o (24,1 - 34,8)
12,5	87,5	8	5,7 (5,o - 6,5)	15,2 (11,o - 21,o)
25	75	16	5,o (4,8 - 5,2)	1o,3 (9,3 - 11,4)
5o	5o	1o	2,9 (2,6 - 3,1)	9,3 (6,7 - 14,o)
75	25	8	2,o (1,8 - 2,1)	3,2 (2,7 - 3,8)

Table A5

Time to mortality for adults of T. granarium, o - 2 days, exposed to fixed concentrations of phosphine in mixtures of carbon dioxide and air at 19°C, 7o % r.h.

Atmosphere CO_2 (%, V/V)	air	PH_3 Concentration vpm	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
o	1oo	5o	23	14,4 (13,3 - 15,6)	76,9 (61,7 - 95,8)
		2oo	8	4,6 (3,8 - 5,4)	22,2 (11,9 - 41,6)
12,5	87,5	5o	23	9,2 (8,6 - 9,9)	53,8 (42,8 - 67,6)
25	75	5o	2o	8,8 (8,1 - 9,5)	43,5 (35,4 - 53,5)
		2oo	8	3,3 (1,9 - 3,8)	1o,6 (6,9 - 16,2)
5o	5o	5o	13	8,6 (7,9 - 9,4)	28,1 (24,o - 32,9)
75	25	5o	19	11,3 (1o,7 - 11,9)	31,9 (28,o - 36,6)

Table A6

Time to mortality for adults of S. granarius, 1 - 4 weeks, exposed to fixed concentrations of phosphine in mixtures of carbon dioxide and air at 19°C and 7o % r.h.

Atmosphere CO_2 (%, V/V)	air	PH_3 Concentration vpm	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
o	1oo	5o	13	9,8 (9,2 - 1o,5)	33,3 (29,4 - 37,8)
		2oo	8	2,8 (2,5 - 3,2)	11,9 (9,4 - 15,2)
12,5	87,5	5o	2o	5,2 (4,7 - 5,7)	22,3 (18,6 - 26,7)
		2oo	11	o,52 (o,47 - o,56)	1,3 (1,1 - 1,5)
25	75	5o	21	4,o (3,7 - 4,2)	11,7 (1o,1 - 13,5)
		2oo	8	o,24 (o,22 - o,27)	o,83 (o,61 - 1,1)
5o	5o	5o	16	4,2 (4,o - 4,5)	1o,7 (9,4 - 12,2)
		2oo	15	o,22 (o,2o - o,25)	o,84 (o,65 - 1,1)
75	25	2oo	12	o,75 (o,13 - o,17)	o,78 (o,51 - 1,2)

Table A7

Time to mortality for adults of T. castaneum, 1 - 4 weeks, exposed to 2oo vpm of phosphine in mixtures of carbon dioxide and air at 19°C and 7o % r.h.

Atmosphere CO_2 (%, V/V)	air	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
o	1oo	11	2,5 (2,3 - 2,7)	6,o (4,7 - 7,6)
12,5	87,5	13	1,3 (1,2 - 1,4)	3,3 (2,6 - 4,3)
25	75	15	o,65 (o,63 - o,68)	1,3 (1,1 - 1,5)
5o	5o	9	o,5o (o,43 - o,56)	1,1 (o,94 - 1,4)
75	25	8	o,57 (o,51 - o,63)	1,1 (o,91 - 1,4)

Table A8
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Time to mortality for adults of R. dominica, 1 - 4 weeks, exposed to 2oo vpm of phosphine in mixtures of carbon dioxide and air at 19°C and 7o % r.h.

Atmosphere CO_2 (%, V/V)	air	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
o	1oo	1o	3,7 (3,3 - 4,3)	12,2 (9,1 - 16,4)
12,5	87,5	16	2,4 (2,2 - 2,5)	5,6 (4,6 - 6,8)
25	75	12	1,7 (1,5 - 1,8)	4,6 (3,5 - 6,1)
5o	5o	15	1,1 (1,6 - 1,8)	6,o (4,2 - 8,5)
75	25	8	1,5 (1,4 - 1,7)	4,4 (3,4 - 5,5)

Table A9
========

Time to mortality for adults of S. oryzae, 1 - 4 weeks, exposed to 2oo vpm of phosphine in mixtures of carbon dioxide and air at 19°C and 7o % r.h.

Atmosphere CO_2 (%, V/V)	air	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
o	1oo	1o	3,5 (3,2 - 3,7)	7,8 (6,2 - 9,8)
12,5	87,5	11	1,2 (1,1 - 1,4)	3,5 (2,5 - 4,8)
25	75	9	o,61 (o,51 - o,7o)	1,4 (1,1 - 1,7)
5o	5o	11	o,51 (o,46 - o,57)	1,5 (1,o - 2,2)
75	25	12	o,47 (o,43 - o,51)	1,5 (1,1 - 2,o)

Table A1o

Time to mortality for adults of S. granarius, o - 1 weeks, exposed to 2oo vpm of phosphine in mixtures of carbon dioxide and air at 19°C and 7o % r.h.

Atmosphere CO_2 (%, V/V)	air	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
o	1oo	8	4,8 (4,1 - 5,6)	28,4 (16,3 - 54,o)
12,5	87,5	1o	o,96 (o,86 - 1,1)	3,4 (2,4 - 4,7)
25	75	8	o,47 (o,42 - o,53)	1,3 (o,97 - 1,8)
5o	5o	9	o,43 (o,38 - o,49)	1,2 (o,87 - 1,9)
75	25	1o	o,44 (o,38 - o,5o)	1,2 (o,81 - 1,9)

Table A11

Time to mortality for adults of T. castaneum, 1 - 4 weeks, exposed to 2oo vpm of phosphine in mixtures of CO_2, N_2 and O_2 at 19°C and 7o % r.h.

Atmosphere CO_2	N_2	O_2 (%, V/V)	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
1o	8o	1o	15	1,2 (1,1 - 1,2)	2,8 (2,3 - 3,2)
15	8o	5	11	1,o (o,9o - 1,1)	2,7 (2,1 - 3,4)
o	9o	1o	14	1,4 (1,3 - 1,5)	4,2 (3,1 - 5,8)
o	95	5	13	1,3 (1,2 - 1,5)	4,5 (3,6 - 5,9)
o	8o	2o	11	2,5 (2,3 - 2,7)	6,o (4,4 - 7,6)

Table A12

Time to mortality for adults of R. dominica, 1 - 4 weeks, exposed to 2oo vpm of phosphine in mixtures of CO_2, N_2 and O_2 at 19°C and 7o % r.h.

Atmosphere CO_2 N_2 O_2 (%, V/V)	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
1o 8o 1o	21	2,o (1,8 - 2,1)	7,1 (5,6 - 7,9
15 8o 5	14	2,o (1,8 - 2,1)	6,8 (5,2 - 8,8)
o 9o 1o	11	2,6 (2,o - 3,1)	7,4 (6,o - 9,o)
o 95 5	14	3,2 (3,o - 3,5)	1o,5 (7,9 - 12,7)
o 8o 2o	1o	3,8 (3,3 - 4,3)	12,2 (9,o - 16,4)

Table A13

Time to mortality for adults of S. oryzae, 1 - 4 weeks, exposed to 2oo vpm of phosphine in mixtures of CO_2, N_2 and O_2 at 19°C, 7o % r.h.

Atmosphere CO_2 N_2 O_2 (%, V/V)	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
1o 8o 1o	8	1,2 (1,1 - 1,3)	4,1 (3,1 - 5,5)
15 8o 5	12	1,1 (1,o - 1,2)	4,o (3,o - 5,4)
o 9o 1o	13	3,o (2,7 - 3,2)	7,4 (6,o - 9,3)
o 95 5	1o	2,8 (2,6 - 3,o)	6,4 (5,1 - 8,o)
o 8o 2o	1o	3,5 (3,2 - 3,8)	7,4 (6,1 - 9,8)

Table A14

Time to mortality for adults of S. granarius, o - 1 weeks, exposed to 2oo vpm of phosphine in mixtures of CO_2, N_2 and O_2 at 19°C, 7o % r.h.

Atmosphere CO_2 N_2 O_2 (%, V/V)	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
1o 8o 1o	1o	1,4 (1,2 - 1,5)	5,o (3,o - 8,4)
15 8o 5	12	1,o (o,79 - 1,1)	5,o (3,3 - 7,1)
o 9o 1o	12	1,7 (1,4 - 1,9)	1o,4 (6,9 - 15,8)
o 95 5	13	2,1 (1,9 - 2,4)	12,6 (8,1 - 19,5)
o 8o 2o	8	4,8 (4,1 - 5,5)	28,4 (16,3 - 54,o)

Table A15

Time to mortality for adults of S. granarius, 3 - 4 weeks, exposed to 2oo vpm of phosphine in mixtures of CO_2, N_2 and O_2 at 19°C, 7o % r.h.

Atmosphere CO_2 N_2 O_2 (%, V/V)	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
1o 8o 1o	9	o,63 (o,56 - o,7o)	1,8 (1,3 - 2,5)
15 8o 5	1o	o,61 (o,54 - o,7o)	2,3 (1,6 - 3,4)
o 9o 1o	11	1,6 (1,4 - 2,9)	6,o (4,1 - 7,9)
o 95 5	1o	1,o (o,85 - 1,2)	3,6 (2,7 - 4,7)
o 8o 2o	8	2,8 (2,6 - 3,2)	11,6 (9,4 - 15,2)

Table A16
=========
Time to mortality for pupae of S. granarius exposed to 2oo vpm of phosphine in mixtures of carbon dioxide and air at 19°C, 7o % r.h.

Atmosphere CO_2 (%, V/V)	air	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
o	1oo	12	32,1 (28,8 - 35,8)	41,2 (282 - 6o4)
25	75	1o	36,5 (32,5 - 41,1)	36o (23o - 564)
75	25	12	31,9 (28,7 - 35,3)	338 (24o - 476)

Table A17
=========
Time to mortality for pupae of S. oryzae exposed to 2oo vpm of phosphine in mixtures of carbon dioxide and air at 19°C, 7o % r.h.

Atmosphere CO_2 (%, V/V)	air	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
o	1oo	11	11,5 (1o,6 - 12,5)	84,6 (7o,6 - 1o1)
25	75	11	11,6 (9,8 - 13,6)	119 (85,3 - 163)
75	25	8	1o,4 (9,2 - 11,7)	87,7 (68,4 - 112)

Table A18
=========
Time to mortality for pupae of R. dominica exposed to 2oo vpm of phosphine in mixtures of carbon dioxide and air at 19°C, 7o % r.h.

Atmosphere CO_2 (%, V/V)	air	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
o	1oo	22	7,6 (7,4 - 7,8)	23,2 (21,8 - 24,7)
25	75	1o	3,3 (3,1 - 3,5)	14,5 (12,3 - 16,1)
75	25	17	4,2 (4,o - 4,4)	16,3 (14,7 - 18,1)

Table A19
=========

Time to mortality for pupae of T. granarium exposed to 5o vpm of phosphine in mixtures of carbon dioxide and air at 19°C, 7o % r.h.

Atmosphere CO_2 (%, V/V)	air	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
o	1oo	1o	26,8 (25,o - 28,7)	56,o (46,9 - 66,9)
12,5	87,5	8	28,1 (25,7 - 3o,8)	56,9 (47,7 - 68,2)
25	75	13	27,o (25,6 - 28,5)	52,1 (45,o - 6o,3)
5o	5o	14	27,2 (25,9 - 28,7)	52,8 (45,8 - 6o,9)
75	25	12	25,o (22,1 - 28,1)	58,5 (49,5 - 76,4)

Table A2o
=========

Time to mortality for pupae of T. castaneum exposed to 5o vpm of phosphine in mixtures of carbon dioxide and air at 19°C, 7o % r.h.

Atmosphere CO_2 (%, V/V)	air	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
o	1oo	14	6,3 (5,5 - 7,2)	35,7 (2o,9 - 61,2)
25	75	1o	6,5 (5,6 - 7,6)	35,1 (17,6 - 7o,3)
75	25	9	6,7 (5,5 - 8,1)	42,6 (21,6 - 64,o)

Table A21
=========

Time to mortality for pupae of T. confusum exposed to 5o vpm of phosphine in mixtures of carbon dioxide and air at 19°C, 7o % r.h.

Atmosphere CO_2 (%, V/V)	air	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
o	1oo	1o	1o,2 (8,7 - 12,o)	42,5 (28,3 - 63,9)
75	25	1o	1o,2 (8,8 - 11,9)	45,4 (29,3 - 7o,3)

Table A22
=========

Time to mortality for larvae of T. granarium exposed to 5o vpm of phosphine in mixtures of carbon dioxide and air at 19°C, 7o % r.h.

Atmosphere CO_2 (%, V/V)	air	Source of larvae	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
o	1oo	Paper	17	42,3 (4o,o - 44,7)	127,o (11o,4 - 146,1)
		medium	14	41,6 (38,6 - 45,o)	125,9 (1o7,1 - 148,o)
12,5	87,5	Paper	13	42,8 (39,9 - 46,o)	116,9 (99,o - 137,9)
		medium	23	42,1 (4o,3 - 44,o)	11o,o (99,4 - 121,8)
25	75	Paper	14	43,4 (4o,5 - 45,8)	1o8,3 (94,3 - 119,5
		medium	16	39,8 (37,4 - 42,4)	117,3 (1o1,4 - 135,6)
5o	5o	Paper	12	39,8 (37,7 - 42,o)	1o5,5 (91,9 - 121,2)
		medium	17	39,3 (37,4 - 41,3)	112,4 (99,2 - 127,6)
75	25	Paper	2o	32,6 (31,4 - 33,7)	68,9 (62,7 - 75,7)
		medium	14	33,7 (31,9 - 35,5)	78,5 (68,3 - 9o,2)

Table A23

Time to mortality for larvae of S. oryzae exposed to 5o vpm of phosphine in mixtures of carbon dioxide and air at 19°C, 7o % r.h.

Atmosphere CO_2 (%, V/V)	air	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
o	1oo	16	1o,8 (1o,5 - 11,3)	43,o (38,5 - 67,9)
25	75	1o	7,9 (7,3 - 8,5)	3o,5 (25,1 - 37,o)
75	25	19	7,4 (7,1 - 7,7)	34,3 (3o,8 - 37,o)

Table A24

Time to mortality for larvae of R. dominica exposed to 5o vpm of phospine in mixtures of carbon dioxide and air at 19°C, 7o % r.h.

Atmosphere CO_2 (%, V/V)	air	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
o	1oo	11	8,6 (8,2 - 8,9)	34,o (29,o - 39,9)
25	75	13	5,6 (5,4 - 5,8)	22,7 (2o,3 - 25,3)
75	25	11	4,7 (4,6 - 4,9)	17,6 (15,8 - 19,5)

Table A25

Time to mortality for larvae of S. granarius exposed to 5o vpm of phosphine in mixtures of carbon dioxide and air at 19°C, 7o % r.h.

Atmosphere CO_2 (%, V/V)	air	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
o	1oo	14	7,1 (6,8 - 7,5)	22,5 (19,6 - 25,1)
25	75	1o	4,8 (4,1 - 5,6)	22,o (16,o - 3o,o)
75	25	12	3,7 (3,5 - 3,9)	14,4 (12,2 - 16,7)

Table A26

Time to mortality for larvae of T. confusum exposed to 5o vpm of phosphine in mixtures of carbon dioxide and air at 19°C, 7o % r.h.

Atmosphere CO_2 (%, V/V)	air	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
o	1oo	11	6,6 (6,o - 7,3)	18,7 (13,9 - 25,1)
25	75	1o	3,5 (3,2 - 3,9)	9,8 (7,4 - 13,2)
75	25	1o	3,1 (2,7 - 3,5)	9,7 (6,8 - 13,8)

Table A27

Time to mortality for larvae of T. castaneum exposed to 5o vpm of phosphine in mixtures of carbon dioxide and air at 19°C, 7o % r.h.

Atmosphere CO_2 (%, V/V)	air	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
o	1oo	6	4,1 (3,6 - 4,6)	9,6 (7,3 - 12,8)
12,5	87,5	16	2,9 (2,7 - 3,1)	8,8 (6,7 - 11,4)
25	75	12	2,6 (2,3 - 2,8)	7,3 (5,5 - 9,8)
5o	5o	12	1,7 (1,6 - 1,8)	3,8 (3,1 - 4,5)
75	25	9	1,4 (1,3 - 1,5)	3,o (2,1 - 4,o)

Table A28

Time to mortality for larvae of T. granarium exposed to 5o vpm of phosphine in mixtures of CO_2, N_2 and O_2 at 19°C, 7o % r.h.

Atmosphere CO_2	N_2	O_2 (%, V/V)	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
1o	8o	1o	7	45,2 (39,4 - 51,9)	117 (83,o - 164)
15	8o	5	7	48,8 (42,7 - 55,9)	121 (83,4 - 175)
o	9o	1o	7	49,1 (41,9 - 54,8)	125 (85,6 - 171)
o	95	5	5	48,o (38,8 - 59,4)	13o (55,6 - 3o2)
o	8o	2o	14	42,3 (4o,o - 44,7)	127 (11o - 146)
75	2o	5	2o	32,6 (31,4 - 33,7)	68,9 (62,7 - 75,7)

Table A29

Time to mortality for eggs of S. granarius, o - 36 h, exposed to 2oo vpm of phosphine in mitxtures of carbon dioxide and air at 19°C, 7o % r.h.

Atmosphere CO_2	air (%, V/V)	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
o	1oo	2o	21,o (19,5 - 22,6)	117 (1o1 - 135)
25	75	11	18,8 (16,8 - 21,o)	12o (9o,7 - 157)
75	25	11	22,1 (2o,7 - 24,3)	1o3 (77,7 - 137)

Table A3o

Time to mortality for eggs of S. oryzae, o - 36 h, exposed to 2oo vpm of phosphine in mixtures of carbon dioxide and air at 19°C, 7o % r.h.

Atmosphere CO_2 (%, V/V)	air	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
o	1oo	16	44,6 (42,8 - 46,2)	123 (111 - 137)
25	75	11	44,4 (41,7 - 47,2)	144 (119 - 174)
75	25	14	43,7 (41,o - 45,4)	138 (12o - 158)

Table A31

Time to mortality for eggs of R. dominica, 1 - 3 days, exposed to 5o vpm of phosphine in mixtures of carbon dioxide and air at 19°C, 7o % r.h.

Atmosphere CO_2 (%, V/V)	air	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
o	1oo	1o	8,3 (7,2 - 9,6)	4o,7 (27,4 - 6o,7)
25	75	11	7,9 (6,8 - 9,1)	4o,3 (27,3 - 59,3)
75	25	11	8,1 (7,1 - 9,3)	39,o (26,9 - 56,5

Table A32

Time to mortality for eggs of T. granarium, 1 - 3 days, exposed to 5o vmp of phosphine in mixtures of carbon dioxide and air at 19°C, 7o % r.h.

Atmosphere CO_2 (%, V/V)	air	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
o	1oo	11	33,3 (29,3 - 37,8)	128 (91,7 - 164)
25	75	9	32,6 (28,9 - 36,8)	1o7 (81,9 - 14o)
75	25	1o	32,8 (29,3 - 36,8)	1o7 (81,4 - 139)

Table A33

Time to mortality for eggs of T. castaneum, 1 - 3 days, exposed to 5o vpm of phosphine in mixtures of carbon dioxide and air at 19°C, 7o % r.h.

Atmosphere CO_2 air (%, V/V)	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
o 1oo	12	44,2 (39,2 - 49,8)	199,7 (149,3 - 265,6)
25 75	11	24,6 (22,6 - 26,7)	65,o (51,9 - 81,5)
75 25	15	14,o (12,8 - 15,2)	39,o (31,o - 48,9)

Table A34

Time to mortality for eggs of T. confusum, 1 - 3 days, exposed to 5o vpm of phosphine in mixtures of carbon dioxide and air at 19°C, 7o % r.h.

Atmosphere CO_2 air (%, V/V)	Data No.	LT 5o (h) (lower - upper limits)	LT 99 (h) (lower - upper limits)
o 1oo	12	5o,o (45,5 - 56,2)	177 (12o - 26o)
25 75	7	24,8 (21,3 - 28,9)	79,2 (5o,o - 125)
75 25	11	11,4 (9,5 - 13,7)	47,6 (34,o - 68,3)

Table A35

Mortalities of eggs of T. castaneum and of T. confusum, as a function of age, after exposure to either 75 % carbon dioxide, 25 % air for 2o h or to 1oo vpm of phosphine in air for 24 h.

Species	Gas Mixture	Mean and range of % mortalities for eggs of age in days				
		o - 1	1 - 2	2 - 3	3 - 4	4 - 5
T.castaneum	75 % CO_2	84,o ±3,6	18,8±4,2	7,9±5,5	1o,o±o,o	15,o± 6,8
T. confusum	75 % CO_2	1oo ±o,o	8o,o±o,o	58,4±6,8	47,6±7,8	39,2±1o,2
T.castaneum	1oo vpm PH_3	27,6 ±3,4	6o,o±o,o	67,6±9,6	61,6±1,6	53,6± 3,6
T. confusum	1oo vpm PH_3	4o,o ±5,o	41,7±4,5	63,8±7,9	94,3±1,3	93,o± o,o

Table A36

Mortalities due to 75 % carbon dioxide, 25 % air at the times equal to the LT_{50} and LT_{99} values in a mixture of phosphine, 75 % carbon dioxide and 25 % air, on the assumption of independent, additive, effects

Species	Mortalities (%) due to 75 % CO_2, 25 % air, at the LT_{50} and LT_{99} values in phosphine, CO_2 and air for:							
	Adults		Pupae		Larvae		Eggs	
	LT_{50}	LT_{99}	LT_{50}	LT_{99}	LT_{50}	LT_{99}	LT_{50}	LT_{99}
T. confusum	< 0,5	< 0,5	< 0,5	55	< 0,5	< 0,5	31	98
T. castaneum	< 0,5	< 0,5	< 0,5	2,8	< 0,5	< 0,5	4o	78
R. dominica	< 0,5	< 0,5	< 0,5	1,1	< 0,5	4,5	< 0,5	0,9
T. granarium	< 0,5	23	< 0,5	< 0,5	< 0,5	1,2	0,6	43
S. granarius	< 0,5	< 0,5	< 0,5	78	< 0,5	< 0,5	1o	76
S. oryzae	< 0,5	< 0,5	< 0,5	79	< 0,5	22	18	8o

Table A37

Measured times to mortality after exposure to phosphine in air compared with those calculated as due to phosphine in mixtures of phospine, 75 % CO_2 and air, on the assumption of independent joint action

Species	Stage	PH_3 conc. (vpm)	Mean and range of LT_{50} (h) measured	calculated	Mean and range of LT_{99} (h) measured	calculated	
T. confusum	5o	adults	11,1 ± o,7	2,o ± o,2	s	29,5 ± 5,5	3,2 ± o,6 s
T. castaneum	2oo	"	2,5 ± o,2	o,84 ± o,27	s	6,2 ± 1,5	1,2 ± o,3 s
R. dominica	2oo	"	3,8 ± o,5	1,6 ± o,2	s	12,7 ± 3,7	4,4 ± 1,1 s
S. granarium	5o	"	14,4 ± 1,2	11,4 ± o,4	s	79 ± 17	34 ± 3,2 s
S. oryzae	2oo	"	3,4 ± o,3	o,47 ± o,o4	s	8,o ± 1,8	1,5 ± o,5 s
S. granarius	2oo	"	2,8 ± o,4	o,12 ± o,o1	s	33,6 ± 4,2	o,85 ± o,35 s
T. confusum	5o	larvae	6,7 ± o,7	3,1 ± o,4	s	19,5 ± 5,6	1o,3 ± 3,5 s
T. castaneum	5o	"	4,1 ± o,5	1,4 ± o,1	s	1o,1 ± 2,7	3,1 ± 1,o s
R. dominica	5o	"	8,5 ± o,5	4,8 ± o,3	s	34,5 ± 5,5	18,2 ± 1,7 s
T. granarium	5o	"	43,2 ± 2,7	32,6 ± 1,4	s	127 ± 17	77 ± 7,7 s
S. oryzae	5o	"	1o,9 ± o,4	9,o ± o,7	s	43,2 ± 4,7	39,2 ± 9,3 i
S. granarius	5o	"	7,2 ± o,4	3,7 ± o,2	s	22,3 ± 2,8	14,4 ± 2,3 s
T. confusum	5o	pupae	1o,3 ± 1,7	1o,2 ± o,8	i	46 ± 18	46 ± 9 i
T. castaneum	5o	"	6,3 ± o,9	6,7 ± o,4	i	41 ± 2o	43 ± 21 i
R. dominica	2oo	"	7,6 ± o,2	4,2 ± o,2	s	23,2 ± 1,5	16,4 ± 1,8 s
S. granarium	5o	"	26,8 ± 1,8	25,1 ± 3,o	i	57 ± 1o	63 ± 14 i
S. oryzae	2oo	"	11,5 ± 1,1	11,2 ± 2,2	i	86 ± 15	134 ± 17 a
S. granarius	2oo	"	32,1 ± 3,7	38,5 ± 5,4	i	44o ± 162	692 ± 25o i
R. dominica	5o	eggs	8,4 ± 1,2	8,1 ± 1,2	i	44 ± 17	39 ± 17 i
T. granarium	5o	"	33,5 ± 4,3	33,3 ± 1,9	i	128 ± 56	115 ± 17 i
S. oryzae	2oo	"	44,5 ± 1,7	56,8 ± 3,4	a	124 ± 13	126 ± 17 i
S. granarius	2oo	"	21,o ± 1,6	25,3 ± 2,8	i	118 ± 18	156 ± 44 i

a: antagonism between carbon dioxide and phosphine at the 95 % probability level.

i: independent joint action: neither synergism or antagonism proved.

s: synergism between carbon dioxide and phosphine at the 95 % probability level.

Table A38

Measured times to mortality after exposure to 75 % carbon dioxide, 25 % air, compared with those calculated as due to carbon dioxide in mixtures of 5o vpm of phosphine, 75 % carbon dioxide and 25 % air, on the assumption of independent joint action

Species	Stage	Mean and range of LT_{50} (h)		Mean and range of LT_{99} (h)	
		measured	calculated	measured	calculated
T. confusum	eggs	15,4 ± 2,o	11,7 ± o,9 s	43,2 ± 14,8	57,6 ± 11,5 i
T. castaneum	eggs	28,5 ± 3,2	12,7 ± o,8 s	87,1 ± 19,9	6o,6 ± 2o,1 i

i: independent joint action: neither synergism nor antagonism proved.

s: synergism between carbon dioxide and phosphine at the 95 % probability level.