

# Uptake of phosphine by stored-product pest insects during fumigation

C. Reichmuth\*

## Abstract

Phosphine (PH<sub>3</sub>) is still the most important fumigant in grain storage world wide. Pest control of insects in large bulks of harvested agricultural products is often carried out with phosphine released from special formulations or even from cylinders.

A better knowledge of the mode of action enables the development of appropriate application of control procedures. In this process, the comparison of the efficacy of exposure to slowly increasing and decreasing phosphine concentrations with constant concentrations led consequently to the development of SIROFLO® by CSIRO Australia.

This study was carried out at the Stored Grain Research Laboratory of the Division of Entomology of CSIRO and describes the uptake of phosphine by *Sitophilus granarius*, *Tribolium castaneum*, *Rhyzopertha dominica*, and *Trogoderma variabile* including susceptible as well as phosphine-resistant or diapausing larvae. Two special fumigation chambers with pairs of Geiger tubes were constructed and used to establish uptake simultaneously during exposure to radio labelled PH<sub>3</sub>. In addition, undecomposed PH<sub>3</sub> was determined as residue in fumigated insects with a special micro method.

The results show that the linear uptake rate decreases from living but already immobilised insects to deadly poisoned insects. The occurrence of change in slope can be linked to the time of a lethal effect of phosphine. Remarkably, resistant insects take up phosphine at a much lower rate. The total amount of incorporated phosphine leading to death is smaller or equal in resistant insects compared to susceptible strains. But even the resistant insects show a pronounced change in uptake rate after the lethal exposure period. Resistant insects remain active much longer when fumigated (concentration > 0.3 mg PH<sub>3</sub>/L) and can clearly be distinguished from susceptible strains which are immobilised after a few minutes.

## Introduction

In the last 60 years one chemical stands out for stored-product pest control: hydrogen phosphide or phosphine (PH<sub>3</sub>). Experiments have been carried out to understand the mode of action of this fumigant (for example Banks 1975; Price and Mills 1988; Bolter and Chefurka 1990). One part of such experiments in toxicology consists in research in the speed of incorporation and investigations of the metabolic rate at which the poison is metabolised (Bond et al. 1969; Robinson 1969; Price 1984; Price and Mills 1988). This kind of research has been supported by surprising findings (Reichmuth 1986),

that changing concentrations of phosphine, as occurring in practice, are much less effective than constant concentrations at a low level when comparing the CT-(concentration × time) products. Winks (1992) transferred this knowledge into the practice of fumigation as the SIROFLO technique, where a constant low amount of phosphine is continuously purged through a bulk of grain in silo bins. How can this property of phosphine of increased efficacy at low concentrations be explained? This study tries to highlight the uptake behaviour of phosphine in treated insects of different species and strains during fumigation at constant and varying concentrations in comparison with chemical reaction of the incorporated gas into other non-gaseous compounds.

## Materials and Methods

### Preparation of radioactively labelled phosphine

Magnesium powder, 'cold' phosphoric acid and labelled ortho-phosphoric acid, H<sub>3</sub><sup>32</sup>PO<sub>4</sub>, were mixed together with water and some dilute hydrochloric acid in a clear quartz vessel. The mixture is dried gently under a stream of dry CO<sub>2</sub> over night. The CO<sub>2</sub> also removes residual oxygen. On heating the dry mixture, the exothermic fusion leads to radiolabelled magnesium phosphide. After cooling down, the phosphide is treated with 5% sulphuric acid giving an immediate production of phosphine. The phosphine is collected in a gas burette, with the CO<sub>2</sub> present later being removed by absorption with KOH solution.

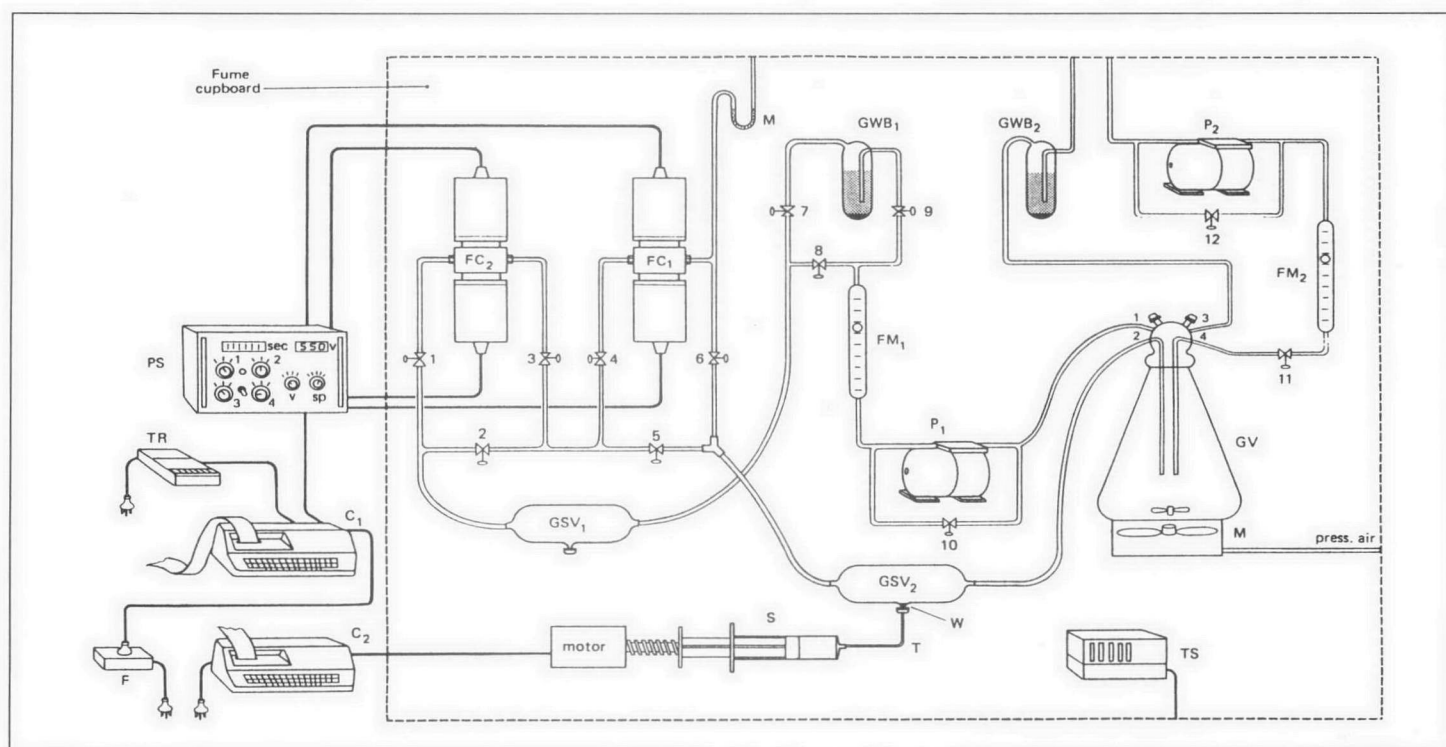
### Determination of phosphine uptake with radiolabelled gas

Phosphine uptake at 25°C by fumigated insects was determined on line by Geiger counting <sup>32</sup>PH<sub>3</sub> in a specially constructed fumigation cell. The apparatus used for fumigation and radioactivity counting is shown in Figure 1. Insects, in batches of 10, were weighed and narcotised in the refrigerator prior to exposure into the fumigation cell FC<sub>1</sub>. The phosphine concentration in the system and the required radioactivity was adjusted before the treatment of the insects started by opening clamp 5 and closing clamps 4 and 6. The fumigation of the insects commenced by opening clamps 4 and 6 and closing clamp 5. The counting device was set to zero. The cpm (counts per minute) values were recorded on paper together with the elapsed time. After correcting these values for the half life of <sup>32</sup>P (14.31 days), the uptake in ng PH<sub>3</sub> per 10 insects or per mg of insect could be calculated with the knowledge of the background counts, the phosphine concentration and the volume of the cell.

### Determination of uptake by use of a new micro method

The tracer method described above does not distinguish between incorporated <sup>32</sup>PH<sub>3</sub> and metabolised <sup>32</sup>P in any other form. Robinson and Bond (1970) have determined the eventual fate of incorporated <sup>32</sup>PH<sub>3</sub> as in excreted hypophosphite

\* Federal Biological Research Centre for Agriculture and Forestry, Institute for Stored Product Protection Königin-Luise-Straße 19, 14195 Berlin, Germany.



**Fig. 1.** Apparatus and installation for monitoring phosphine uptake by insects at constant or varying concentrations: Fumigation chambers  $FC_1$  and  $FC_2$ ; during most of the experiments,  $FC_1$  contains the insects; power supply (PS) and counting device (computer  $C_1$ ) for the Geiger tubes; the voltage of the Geiger tube (550 V) can be adjusted with switch V for the 4 tubes 1, 2, 3, and 4; the elapsed time of the experiment is indicated in seconds or minutes depending on the switch between potentiometer 3 and 4; time reset button between potentiometer 1 and 2; sp for loudspeakers linked to the Geiger tubes; Filter F saves the computer from spikes derived from the main voltage supply; pump  $p_1$  circulates the gas; flow adjustment at tube clamp 10, flow indication at flow meter  $FM_1$ , humidifying of the gas in gas washing bottle  $GWB_1$  which contains a saturated NaCl solution and some crystalline NaCl at the bottom; regulation with clamps 7, 8, and 9; monitoring of the gas concentration at gas sampling vessel  $GSV_1$  with syringe through the septum of this vessel; clamps 1 to 6 determine the actual path of the gas; manometer M shows the pressure in the system relative to ambient; the septum port in gas sampling bottle  $GSV_2$  can be used to introduce gas slowly from a syringe S and tubing T; the plunger of the syringe is driven by a motor according to gas release characteristics which can be programmed into computer  $C_2$ ; the tip of the syringe inside  $GSV_2$  is covered with water W to avoid free diffusion of phosphine from the tip into the system; the gas in the gas mixing vessel GV with two septum ports for injection or withdrawing of gas is stirred by a magnet which is driven by another magnet outside on top of a rotor being moved by pressurised air to avoid electric heat transfer; for simulation of leakage port 3 and 4 of GV can be used to withdraw gas with pump  $p_2$ , regulated with clamp 12; flow indication at flow meter  $FM_2$ ; the withdrawn gas is continuously replaced by fresh humidified (gas washing bottle  $GWB_2$ ) air; the temperature of the installation is regulated constantly to  $25 \pm 0.3^\circ\text{C}$  using a thermosensor TS which is placed close to the apparatus inside a fume cupboard which is continuously sucking air outside the laboratory to install a negative pressure difference because of safety reasons; the leads of the electrical equipment are lead through holes in the walls of the cupboard.

and polyphosphate. To determine residual phosphine as such in fumigated insects, a micro headspace technique was developed, based on suggestions of Nowicki (1978) for the residue determination in treated foodstuffs. Figure 2 shows the apparatus for determination of residual phosphine released from fumigated insects. The insects are heated with concentrated KOH solution, which destroys the chitin membranes without significantly reacting with phosphine. This heating process transported most of the sorbed phosphine into the free air space of the vessel. After cooling, the released phosphine was determined with gas chromatography. Prior to phosphine determination fumigated insects were stored in liquid nitrogen to avoid losses of phosphine.

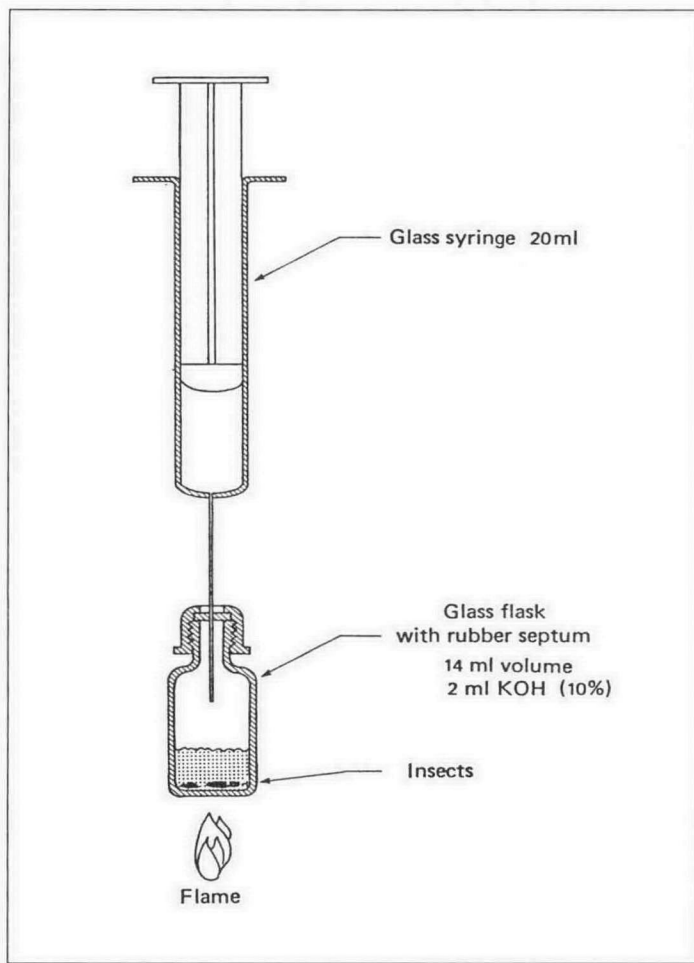
#### Test insects

All insects were taken from cultures of the Stored Grain Research Laboratory (SGRL), CSIRO Division of Entomology, Canberra.

## Results

### Experiments with radiolabelled phosphine

Figure 3 contains transformed cpm values transformed into phosphine content in  $\text{ng PH}_3/\text{mg insect}$ . *Sitophilus granarius* incorporated phosphine with  $^{32}\text{P}$  tracer differently at the four investigated concentrations of between 0.1 mg and 1 mg  $\text{PH}_3/\text{L}$ . With ascending concentration, the speed of uptake or the slope of the uptake rate increased correspondingly. The upper curve in Figure 3 starts bending after about 300 minutes of exposure, the second from top after about 400 minutes, the other second change slope within between 500 minutes and 1000 minutes with less pronounced change in rate. These times can be linked to lethal exposure at the given concentration. After about 30 minutes, all 10 weevils were immobilised at 0.95 mg  $\text{PH}_3/\text{L}$ . The place where the slope changed was not correlated with this immobilisation. The final slopes of the uptake curve for 0.53 mg  $\text{PH}_3/\text{L}$  and 0.26 mg  $\text{PH}_3/\text{L}$  do not correspond to the tendency of proportional reduction with

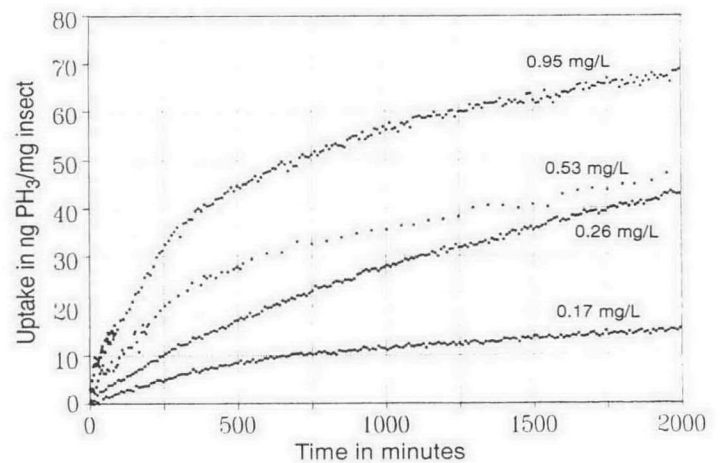


**Fig. 2.** Gas flask with variable volume: instrumentation to release phosphine from fumigated insects; the movable plunger of the syringe enables the expansion of the air-gas mixture in the glass flask when heated and contraction when cooling down again.

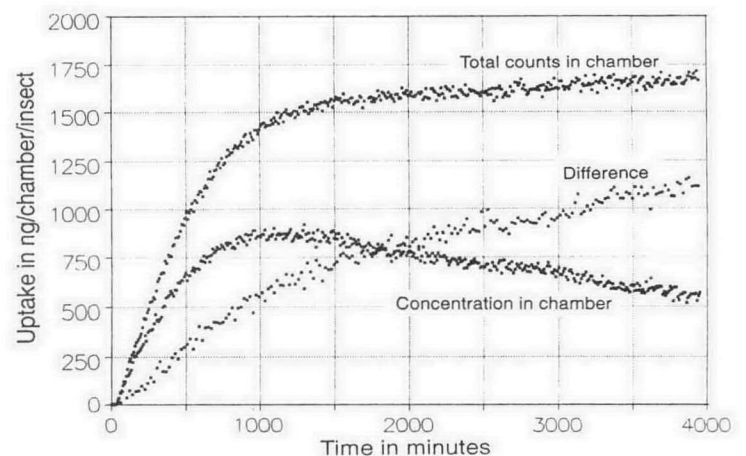
reduced concentration during exposure. These slopes of lines which result from uptake of mortally poisoned granary weevils are not parallel but fairly constant as are those of the curves during the beginning of exposure.

The response to varying concentrations of phosphine, as occurring in practice, are shown in Figure 4. The curve with the maximum value at 1000 minutes shows the gas concentration as measured in reference cell FC<sub>2</sub> (see Figure 1). The concentration is given in ng PH<sub>3</sub>/567 μl, in order to have results comparable directly with uptake, which is given in ng PH<sub>3</sub>/10 insects. The maximum concentration reached is 1.6 mg PH<sub>3</sub>/L (Figure 5, top graph). The upper curve in Figure 4 contains combined data of concentration counts and uptake counts as measured in fumigation chamber FC<sub>1</sub> (see Figure 1). The subtraction of the concentration counts led to the typical uptake curve for 10 weevils of 20 mg weight in Figure 4 with the change in slope at about 750 minutes of exposure.

The difference in uptake of susceptible and resistant insects is shown for *Rhyzopertha dominica* (Figure 6) and *Tribolium castaneum* (Figure 7) at 1 mg PH<sub>3</sub>/L. Even from the raw data in Figure 6, the difference is obvious. As in Figure 7, the resistant insects (TC<sub>4</sub>P<sub>10</sub> from the SGRL) take up only minute amounts of phosphine during exposure compared with susceptible strains (TC<sub>4</sub>). Similar results were obtained with susceptible (RD<sub>2</sub>) and resistant (RD<sub>235</sub>P<sub>10</sub>) *Rhyzopertha dominica* (Figure 6).



**Fig. 3.** Uptake in ng phosphine per mg insect of phosphine during 2000 minutes by *Sitophilus granarius* at constant concentrations of 0.17 mg/L (bottom line), 0.26 mg/L (second line from the bottom), 0.53 mg/L (second line from the top), and 0.95 mg/L (top line) at 25°C.

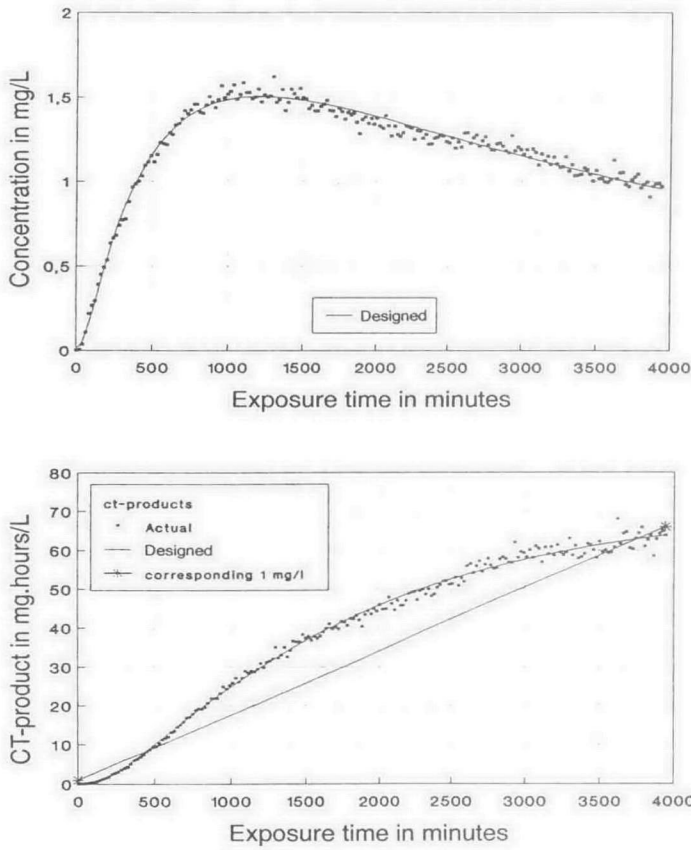


**Fig. 4.** Concentration of phosphine in ng per chamber (567 μl) (line with maximum after 1000 minutes), or as total counts in chamber ng per 10 granary weevils (20.08 mg) (uppermost line). Line starting as the lowest line represents the difference between the two lines as ng per 10 granary weevils.

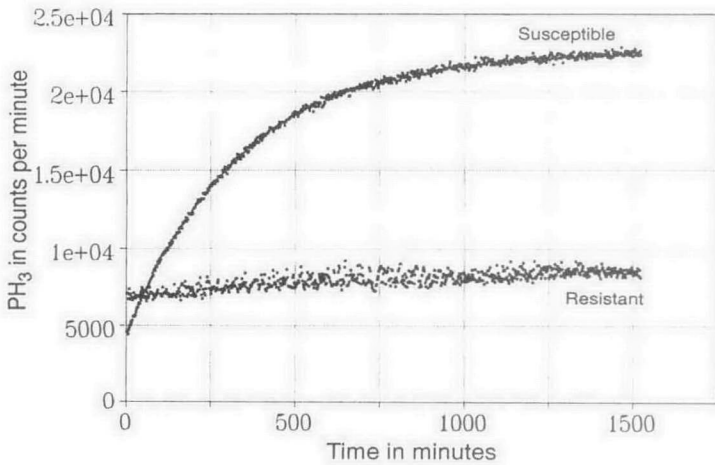
Whereas the susceptible insects lost weight from 16.89 mg to 14.49 mg (14 %) within 1525 minutes of exposure to phosphine, the weight loss in resistant beetles was determined only from 11.36 mg to 10.56 mg (8 %). This tendency was supported by other experiments.

The investigation of *Trogoderma variabile* (Tv) (strain CTV5 of SGRL) showed that diapausing larvae behave similarly to resistant insects in taking up less phosphine during exposure than nondiapausing Tv (Figure 8). According to Banks (pers. comm. 1984), diapausing larvae are defined here as larvae which have been separated from each other and not turned into pupa or adult during 5-7 weeks of insulation at 25°C. The results were obtained with batches of 5 larvae each and show the pronounced uptake of normal larvae compared to significantly reduced uptake by diapausing individuals.

The irregularities in Figure 8 may have been caused by mechanical disturbances during the experiment.



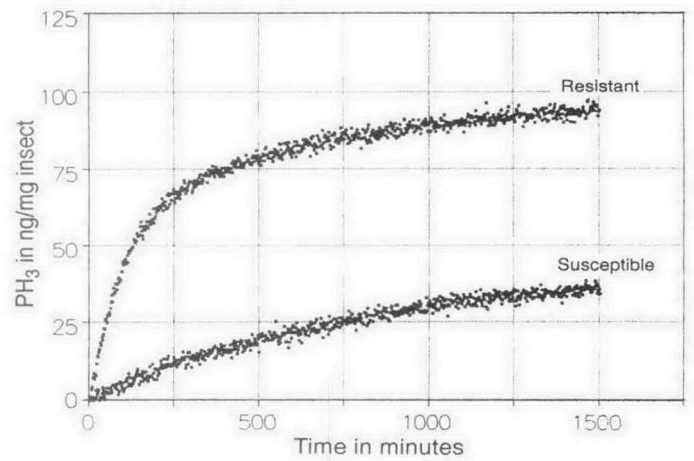
**Fig. 5.** Concentration profile corresponding to data in Figure 4, (upper graph) CT-product obtained under this profile and CT-product expected for constant phosphine concentration 1 mg/L as comparison (straight line, lower graph).



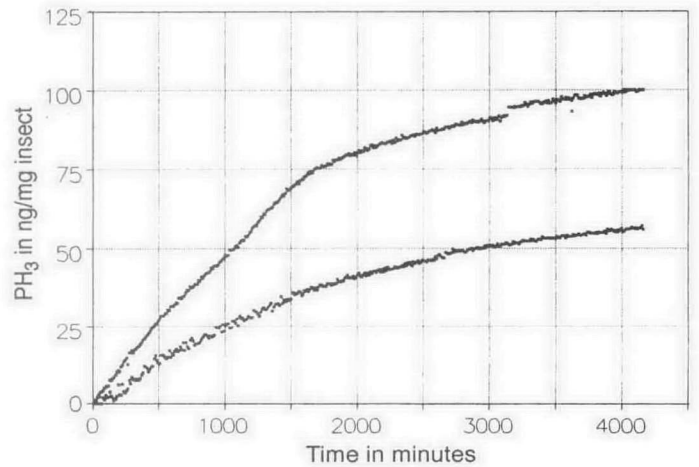
**Fig. 6.** Relative uptake in counts per minute versus time of susceptible and resistant adult *Rhyzopertha dominica* at 1 mg phosphine/L.

**Experiments to determine phosphine micro chemically**

After exposure of batches with 200 2–3 weeks old *Sitophilus granarius* to 1 mg PH<sub>3</sub>/L, the content of undecomposed phosphine and the increase of difference in weight between untreated and fumigated insects was determined is a function of length of exposure (Figure 9). The two lines show fitted trends. Figure 10 and Figure 11 include results at gradually increasing phosphine concentrations. The concentration



**Fig. 7.** Incorporation of phosphine in ng/mg insect by susceptible (bottom line) and resistant (top line) adult *Tribolium castaneum* during treatment with 1 mg/L, the amount of incorporated phosphine in susceptible insects after a lethal exposure time of 250 minutes corresponds roughly to the amount of incorporated phosphine in resistant insects after 750 minutes leading to death in this strain, the slope of both lines after 750 minutes is 0.0119 ng PH<sub>3</sub>/mg insect/minute (upperline), and 0.0136 ng PH<sub>3</sub>/mg insect/minute (lower line), respectively.

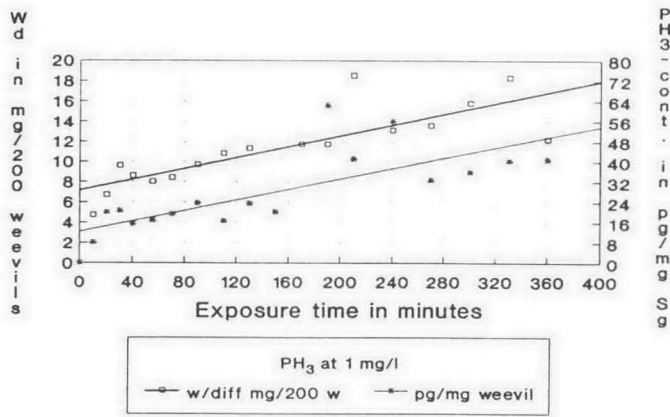


**Fig. 8.** Phosphine uptake in ng/mg insect of diapausing (lower line) and nondiapausing (upper line) larvae of *Trogoderma variable* during exposure to 1 mg phosphine.

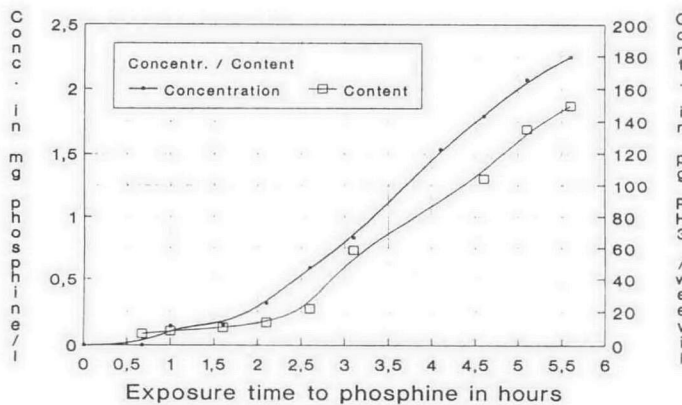
changes, observed time and percentage of immobilisation, content of phosphine, and mortality are indicated. Phosphine is still being incorporated after the insects have been immobilised and mortally poisoned. With the weight of 1 weevil of about 2 mg, the insects picked up about 70 pg phosphine within 5 hours at the given changing concentrations. There was a steep change in phosphine content in the insects after 2 hours of exposure, when immobilisation and mortal poisoning reached the 50% region (Fig. 11). Immobilisation did not necessarily lead to death at the same percentage as can be seen in Figure 11, where percentage of immobilisation reached about 90% after 3 hours of exposure with the same percentage of mortality requiring 4–5 hours.

**Discussion**

During the experiments with radiolabelled phosphine in the perspex fumigation chamber, I observed that resistant insects



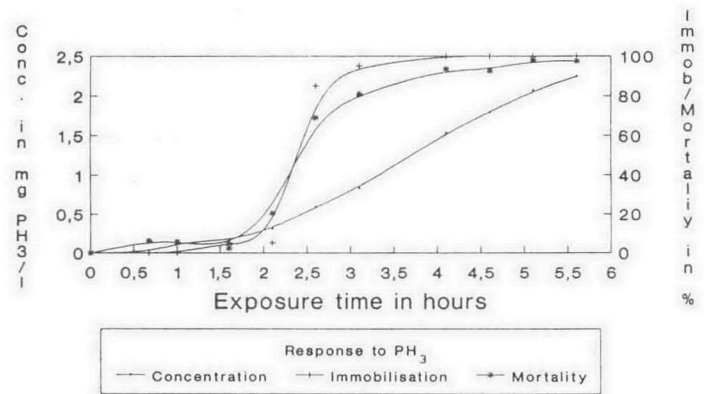
**Fig. 9.** Content of undecomposed phosphine (lower line) and difference in weight (upper line) following the exposure of *Sitophilus granarius* (14–21-day-old adults) to 1 mg/L at 26°C and 65% r.h.



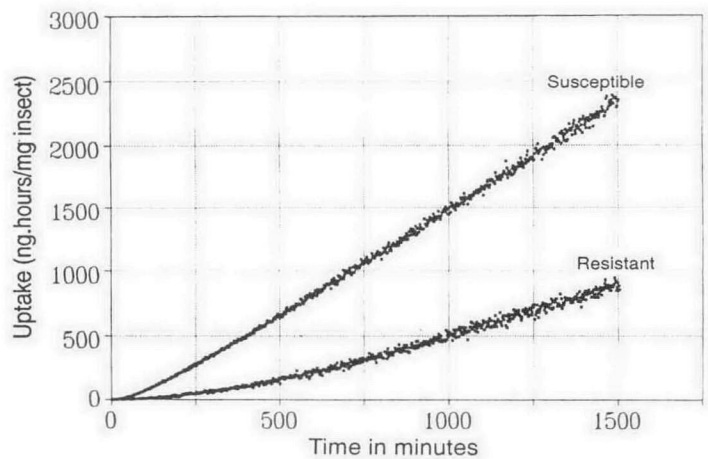
**Fig. 10.** Content of phosphine following the exposure of *Sitophilus granarius* to slowly rising concentrations at 25°C and 65% r.h.

have the ability to move around in the fumigated cell whereas the susceptible strains are immobilised after less than 1 hour at concentration above 0.5 mg/L. This finding has been developed into a rapid resistance test method (Reichmuth 1991 and 1992). At a given concentration, resistant insects pick up the gas at a rate equivalent to that for susceptible insects at lower phosphine concentrations (Figure 3, between 0.17 mg PH<sub>3</sub>/L and 0.26 mg PH<sub>3</sub>/L). The comparison of the CT-products for these strains of *Tribolium castaneum* (Winks 1984 and 1986) and the incorporated amounts of phosphine for control of susceptible TC<sub>4</sub> and the resistant TC<sub>4</sub>P<sub>10</sub> leads to the conclusion that resistant insects have at least the sensitivity to incorporated phosphine as susceptible ones. In Figure 7, the change in slope of the phosphine uptake occurs between 200 minutes and 300 minutes. Presumably, the incorporated amount of phosphine at this point is deadly. This can also be seen in Figure 12, which shows the integral of uptake over time for both lines from Figure 7. After 250 minutes, this integral is 250 ng h/mg insect in susceptible *Tribolium castaneum* (Tc). The latter value is reached in resistant Tc after about 750 minutes in Figure 12, again within the range of changing uptake rate in resistant Tc as shown in Figure 7 for the bottom line. The actual amount of incorporated phosphine seems to lead to death in all strains, with resistant or diapausing insects taking it up at a lower rate only.

The new micro method revealed, that, as in the other experiments with radiolabelled phosphine, the time of dramatic changes within the dying insects can be derived from uptake



**Fig. 11.** Mortality and immobilisation in percent following the exposure of *Sitophilus granarius* to slowly rising concentrations at 25°C and 65% r.h.



**Fig. 12.** Integral uptake in ng phosphine \* hour per mg insect of resistant (lower line) and non-resistant (upper line) *Tribolium castaneum* at 1 mg/L phosphine.

data. Most of the incorporated phosphine seems to have been transformed into non gaseous compounds as in Robinson (1972), since only pg amounts of actual phosphine per mg insect could be detected (Figures 9 and 10), compared with amounts of some ng/mg insect which were incorporated according to the radiotracer experiments. The magnitude of the metabolic rate and its kinetics can be calculated from these figures.

The results in Figure 7 suggest that the final uptake rate of mortally poisoned insects is similar in resistant and non-resistant insects: 0.0119 ng PH<sub>3</sub>/mg insect/minute for susceptible and 0.0135 ng PH<sub>3</sub>/mg insect/minute for resistant *Tribolium castaneum* over the last 750 minutes of observation.

Results in Figures 6 and 8 appear to support this finding of constant uptake in dead insects at a given concentration. Figure 3 contains information that this rate may be concentration-dependant. On the other hand, the amount of incorporable phosphine should be limited by the chemistry of the insect body. This is saturated after some time and unable to react with more phosphine, provided that the body does not work like a catalyst for the oxidation of phosphine.

There is now a better understanding possible of the mode of action of phosphine in insect pest control. Phosphine uptake and lethal poisoning, especially in resistant insects, can be linked together. The question arises if, in the future, continuous observation necessary of insects during exposure to insecticides can be used to reduce the number of experiments and the time to determine the lethal conditions for particular

fumigants. In the light of reduced funds for research, the technique described here could serve to save time and money in the course of development of a new compound like carbonyl sulphide (COS), for pest control. This material is in discussion as a replacement for the ozone depleting methyl bromide.

### Acknowledgments

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Finally, I thank my assistant, Mrs A. Paul, for patient work with thousands of data on activity counts. This work is not yet over.

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