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THE POTENTIAL OF METHYLPHOSPHINE AS A FUMIGANT FOR THE CONTROL OF PHOSPHINE-RESISTANT STRAINS OF FOUR SPECIES OF STORED-PRODUCT INSECTS

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ABSTRACT

The use of phosphine (PH_3) as the major fumigant of stored foodstuffs is threatened by the emergence of widespread resistance in stored-product insects in many countries. Studies have indicated that the mechanism of PH_3-resistance probably involves respiratory exclusion as well as detoxification of PH_3 in resistant insects. The search for a suitable alternative for control of PH_3-resistant insects led us to evaluate methylphosphine, a close analogue of PH_3, as a potential fumigant of stored products. Results indicated that exposure to methylphosphine produced much greater mortality in PH_3-resistant insects of four species of stored-product beetles compared to the corresponding susceptible conspecifics. It is possible that the presence of the methyl group in methylphosphine prevents exclusion by resistance mechanisms, and metabolism by the PH_3-detoxification process produces reactive products which lead to a higher mortality in resistant insects. The possibilities of using methylphosphine as a fumigant to control PH_3-resistant insects are discussed.

INTRODUCTION

Phosphine gas (PH_3) has been extensively used over the past four decades to control insect pests in grains and other stored commodities throughout the world (Bond, 1984). Many features, such as the lack of toxic residues after fumigation (Bruce *et al.*, 1962; Scudamore and Goodship, 1986) and the availability of solid phosphide formulations that generate the gas *in situ*, have made PH_3 the fumigant of choice. With recent restrictions on the production of the alternative, methyl bromide, due to alleged effects on the ozone layer (Taylor, 1994), PH_3 might soon become the only safe fumigant available for disinfesting stored commodities. The use of PH_3 as a fumigant is especially important for developing countries where most grain storage is in bags and the technology for the application of non-gaseous grain-protectants is not available. The future efficacy of this indispensable fumigant is, however, threatened by the emergence of widespread resistance in several species of stored-product insects in many countries (Champ and Dyte, 1976; Mills, 1983;

Tyler *et al.*, 1983; Champ, 1985; Taylor and Halliday, 1986; Taylor, 1989; Zettler *et al.*, 1989; Pacheco *et al.*, 1990; Udeaan, 1990; Chaudhry, 1991; Taylor, 1991; Zettler, 1991; Irshad *et al.*, 1992; Rajendran and Narasimhan, 1994; Zettler and Keever, 1994; Bell and Wilson, 1995). PH_3-resistance has been linked to poor fumigation practices that could lead to sub-lethal exposure and consequent selection of resistant insect populations (Halliday *et al.*, 1983; Mills, 1983; Tyler *et al.*, 1983). At least 11 species of stored-product insects, 10 species of beetles and 1 species of moth (Mills, unpublished) are now known to have developed resistance to PH_3.

Chemically, PH_3 is a strong reducing agent. Biological redox systems, especially the components of the mitochondrial electron transport chain, are its likely target sites in insects. However, the action of PH_3 differs from other known inhibitors of the respiratory chain, such as anoxia and hydrogen cyanide (Price, 1980; Price and Bell, 1981; Price and Dance, 1983; Price and Walter, 1987). The toxicity of PH_3 appears to arise from oxidation *in vivo* which could produce phosphorylating electrophilic species (Lam *et al.*, 1991), and it has been shown to cause generation of reactive oxyradicals in insects (Bolter and Chefurka, 1990; Chaudhry, 1991; Chaudhry and Price, 1992).

Compared to their susceptible counterparts, PH_3-resistant strains of several species of stored-product insects have been shown to absorb very small amounts of PH_3 (Price, 1981; Price and Dance, 1983; Price, 1984; Nakakita and Kuroda, 1986; Chaudhry and Price, 1989, 1990; Chaudhry, 1991; Reichmuth, 1994). The fact that some of the strains tested originated in different countries indicates a common mechanism of PH_3 resistance in insects. The amount of PH_3 absorbed by live resistant insects was even lower than that passively absorbed by dead insects, and Price (1984) suggests that there is active exclusion of PH_3 in the lesser grain borer *Rhyzopertha dominica*. This was supported by the findings that resistant strains of *R. dominica* excluded much greater amounts of gaseous [^{32}P] after treatment with [^{32}P]-PH_3 compared to a similarly treated susceptible strain (Chaudhry and Price, 1992). Other studies indicated that reduced uptake of PH_3 was not the only underlying mechanism of resistance in insects; a detoxification process was also involved (Chaudhry and Price, 1990). This was supported by conventional genetic studies on different species of stored-product insects which indicate that two or more genes are involved in PH_3 resistance (Ansell *et al.*, 1990; Li and Li, 1994).

Due to the importance of PH_3 in post-harvest protection of grains and other commodities, many improvements in fumigation practices have been suggested to increase efficacy against insects and control resistant pest populations. This includes application of PH_3 in multiple doses (Friendship *et al.*, 1986), use of formulations that release PH_3 at a slower rate (Halliday, 1986), fumigation under gas-proof sheets to minimise leakage (Taylor and Harris, 1994) and maintaining a low level of PH_3 by continuous supply from cylinders (Anderson, 1989). Increasing the concentration of CO_2 in the air to 14% has been reported to increase respiratory activity and enhance the uptake and efficacy of PH_3 in insects (Kashi and Bond, 1975). A mixture of PH_3 in CO_2 has therefore been used to increase the efficacy of PH_3 against insects (Desmarchelier, 1984; Desmarchelier *et al.*, 1984; El-Lakwah *et al.*, 1991). The usefulness of most of these methods in completely controlling

PH_3-resistant insects in a field situation is, however, doubtful. Resistance, at least in some instances, has reached levels where it can lead to control failures unless the best fumigation practices are applied in conditions of acceptable gastightness.

There is therefore a strong need to develop new fumigants as alternatives to PH_3, but the choice of suitable chemicals that exist as gases at normal temperature and pressure is very limited. Recently, the fumigant properties of carbonyl sulphide (COS) gas have been reported (Banks *et al.*, 1993). The insecticidal action of COS is thought to be due to hydrolysis by carbonic anhydrase to produce H_2S and CO_2 inside the insects. However, high aqueous solubility of COS and breakdown of the solubilised gas to H_2S might lead to sulphurous residues in the fumigated commodities. We previously carried out studies on arsine (AsH_3) and stibine (SbH_3), the group Vb analogues of PH_3. We found a negative correlation between resistance to PH_3 and tolerance to both of these gases which indicated that the PH_3-exclusion mechanism probably failed to exclude AsH_3 and SbH_3, and the oxidative breakdown by the PH_3-detoxification process produced toxic products (Chaudhry and Price, 1991). These findings suggested the possibility that the PH_3-resistance mechanism could be manipulated to selectively kill resistant insects using substances which are chemically similar to PH_3 but, unlike PH_3, produce reactive metabolites in oxidative breakdown. Unfortunately, AsH_3 and SbH_3 could not be used as fumigants because of the probability of toxic residues. We also tested two silicone hydride gases, silane (SiH_4) and methylsilane (CH_3SiH_3), but their insecticidal action was not comparable to the hydride gases of group Vb elements, i.e. PH_3, AsH_3 and SbH_3 (Chaudhry, unpublished). These studies led us to evaluate methylphosphine (CH_3PH_2), a close analogue of PH_3, as a potential fumigant against PH_3-resistant and PH_3-susceptible insects. The results of these tests are presented in this paper.

MATERIALS AND METHODS

The four species of stored-product insects used in the toxicity tests were the lesser grain borer, *R. dominica*; the rust-red flour beetle, *Tribolium castaneum*; the rice weevil, *Sitophilus oryzae*; and the flat grain beetle, *Cryptolestes ferrugineus*. All insects were cultured at 25°C and 70% r.h. except *C. ferrugineus* which was cultured at 30°C and 60% r.h. The strains of *R. dominica* and *S. oryzae* were cultured on whole wheat, *T. castaneum* on wheat flour containing 5% yeast and *C. ferrugineus* on food containing oats, flour and yeast.

Three strains of *R. dominica* used in the tests comprised a susceptible (reference), a laboratory selected PH_3-resistant (306sel) and a highly PH_3-resistant field strain (BR2) which originated from a population sampled in Bangladesh. The *T. castaneum* strains tested comprised a susceptible (lab) and a PH_3-resistant strain collected from Bangladesh (BT1vs). The strains of *S. oryzae* included a susceptible (reference) and a PH_3-resistant (476s) strain, and *C. ferrugineus* strains comprised a susceptible (reference) and a PH_3-resistant (BC12s) strain.

Methylphosphine was prepared by the reaction of dimethyl-methylphosphonate and lithium-aluminium-hydride ($LiAlH_4$) in ethylene-glycol-dimethyl-ether (monoglyme),

using a slight modification of the method described by Crosbie and Sheldrick (1969). In a typical synthesis, a pellet of $LiAlH_4$ weighing about 2.5 g (Aldrich Chemical Co., UK) was added to 15 ml of monoglyme, in a three-neck flask fitted with a rubber septum, and maintained under a flow of nitrogen (N_2) gas. The hydrogen gas produced by the reaction of $LiAlH_4$ with any trace of moisture was discarded through a bubbler containing 2% mercuric chloride ($HgCl_2$) solution while the pellet was stirred in monoglyme to form a slurry. About 1.60 g of dimethyl methylphosphonate (Aldrich Chemical Co., UK) were very slowly stirred into the slurry. Methylphosphine generated by the reaction was collected in a stream of N_2 gas over the surface of water in a gas burette fitted with a rubber septum. The identity of methylphosphine was confirmed by mass spectrometric analysis which was consistent with its previously reported mass spectrum (Wada and Kiser, 1964).

The concentration of methylphosphine was measured by Gas Chromatography (GC) using a Shimadzu-GC-9A which was fitted with a glass-lined packed column (1 m long, 1/8" O.D., Poropak-QS 80–100 mesh packing), flame photometric detector and automatic gas sampling loop. The column temperature was maintained at 200°C with injector and detector temperatures at 150°C. Methylphosphine was eluted from the column immediately after PH_3; retention times were 0.255 and 0.397 min, respectively, for PH_3 and methylphosphine, and the amounts were estimated by comparison to standard concentrations of PH_3 in N_2 gas. A correction factor was used to account for the difference in molecular weights of PH_3 and methylphosphine. In all syntheses, a trace of PH_3 (1–2% of methylphosphine) was present, presumably arising from phosphate contamination of the glassware and/or from breakdown of methylphosphine. In some cases, concentrations of methylphosphine measured by GC were verified by chemical analysis. This was carried out by reacting a known volume of methylphosphine with standard $HgCl_2$ solution and titrating the amount of HCl, produced in the resulting reaction, with NaOH solution: $CH_3PH_2 + 2HgCl_2 \rightarrow CH_3P(HgCl)_2 + 2HCl$.

We also carried out investigations into the nature of the oxidation products of methylphosphine by bubbling the gas through 2% $HgCl_2$ and oxidising the resulting precipitate of $CH_3P(HgCl)_2$ by boiling in bromine-water. The supernatant was isolated and used for paper chromatography together with ortho-phosphate, phosphite, hypophosphite and methylphosphonic acid standards. The paper chromatogram was developed in 1-butanol saturated with 2N HNO_3 (Robinson and Bond, 1970), dried in air, sprayed with Harrap's reagent (Harrap, 1960) and visualised under UV light to record *Rf* values for each compound.

Treatments of insects with methylphosphine gas were carried out in gastight 6.25-L desiccators, each fitted with a rubber septum. In all the tests, 50 or more adult insects aged 4 to 8 weeks were used. Each treatment with methylphosphine was replicated at least twice. Appropriate control insects were also sealed in similar desiccators to estimate mortality in untreated insects during the exposure period. After exposing insects to the fumigant for 24 h, the desiccators were opened and aired for 15–20 min. Both the untreated control insects and the treated insects were transferred to labelled glass jars containing appropriate food and kept there for 1 week before estimating mortalities.

RESULTS AND DISCUSSION

Initial tests to evaluate the efficacy of methylphosphine against insects were carried out before a suitable analytical method for methylphosphine was available. In these tests, a calculated amount of methylphosphine (based on theoretical yield) was applied to produce a nominal concentration of 0.2 mg/L in the desiccators. Subsequent information indicated that the actual concentration was, however, much lower than 0.2 mg/L. The results of these tests (shown in Fig. 1 and Fig. 2) should, therefore, be seen only as a preliminary assessment of the efficacy of methylphosphine against PH_3-resistant and PH_3-susceptible insects. The tests nevertheless indicated that methylphosphine had a much greater toxic effect on PH_3-resistant insects than it did on PH_3-susceptible insects of all four species of beetles tested over treatment periods of 24 h and 48 h.

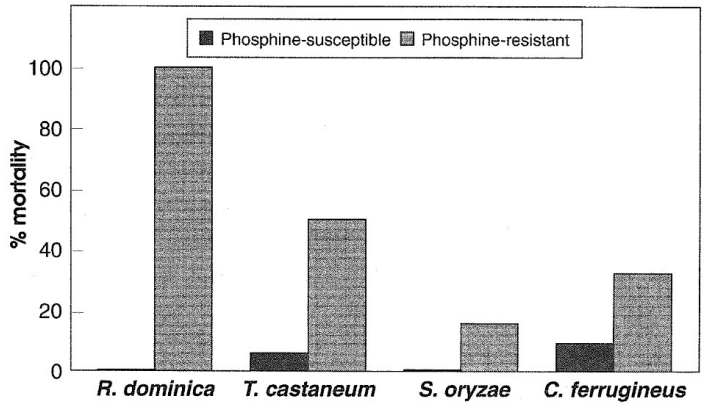

Fig. 1. Percentage of insect mortality after a 24-h exposure to a nominal concentration of 0.2 mg/L of methylphosphine (Chaudhry *et al.*, 1995).

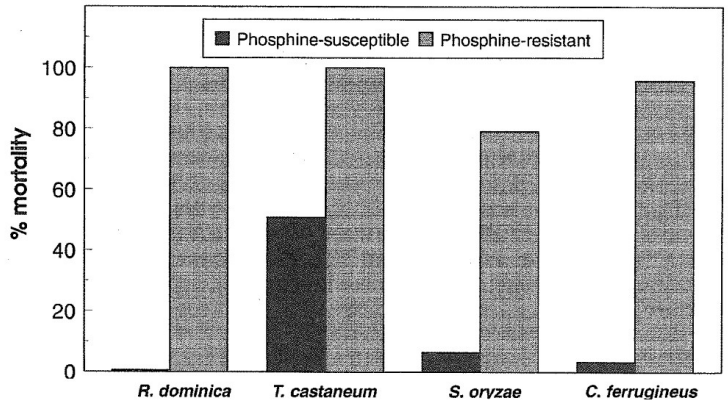

Fig. 2. Percentage of insect mortality after a 48-h exposure to a nominal concentration of 0.2 mg/L of methylphosphine (Chaudhry *et al.*, 1995).

This led us to carry out further toxicity tests, using different doses, to characterise dose-response relationships using methylphosphine on several species of stored-product insects. The results shown in Table 1 are, in some cases, preliminary, and further results are needed to obtain more reliable data. However, these results confirmed that methylphosphine is more toxic to resistant strains than to the corresponding susceptible insects in all the beetle species tested.

In the case of *R. dominica*, a comparison of LC_{50} values indicates that almost 4–8 times more methylphosphine was needed for PH_3-susceptible insects than for the two PH_3-resistant strains. It is also interesting to note that, in terms of molarity, the concentration of methylphosphine that produced 99.9% mortality of PH_3-resistant insects is comparable to the discriminating dose of PH_3 required to kill all PH_3-susceptible insects (Table 1).

Similar effects of methylphosphine on PH_3-resistant and PH_3-susceptible insects of *S. oryzae* were observed. Whereas a 24-h exposure to 0.0162 mg/L of methylphosphine would have killed 50% of the PH_3-resistant (476s) strain, a concentration more than seven times higher (0.1196 mg/L) was required to kill 50% of the PH_3-susceptible insects. A similar difference in the toxicity of methylphosphine to PH_3-resistant and PH_3-susceptible insects was observed at the $LC_{99.9}$ level. The concentration of methylphosphine which produced 99.9% mortality in PH_3-resistant insects is comparable to the discriminating concentration of PH_3 which kills all susceptible insects of this species (Table 1).

TABLE 1
Toxicity of methylphosphine (mg/L) to adults of phosphine-resistant and phosphine-susceptible strains of four species of stored-product beetles over a 24-h exposure at 25°C

Species/strain	LC_{50}	95% FL	$LC_{99.9}$	95% FL	Slope	p-value
Rhyzopertha dominica						
Susceptible	0.1016				*	0.0105
306-sel	0.0129	0.0118–0.0140	0.0468	0.0389–0.0615	5.54	0.9705
BR2	0.0229	0.0218–0.0242	0.0473	0.0417–0.0561	9.83	0.4276
Sitophilus oryzae						
Susceptible	0.1196	0.1084–0.1287	0.4607	0.3734–0.6393	5.28	0.1048
476s	0.0162	0.0147–0.0174	0.0590	0.0469–0.0857	5.51	0.4805
Tribolium castaneum						
Susceptible	0.0113	0.0017–0.0167	0.0860	0.0538–0.8594	3.50	0.9020
BT1vs	0.0168	0.0063–0.0215	0.0497	0.0303–0.0440	6.55	0.0050
Cryptolestes ferrugineus						
Susceptible	0.0747				*	0.8931
BC12s	0.0348	0.0277–0.0405	0.1102	0.0795–0.2427	6.17	<0.001

FL = fiducial limits; * = insignificant slope.
Discriminating dose of phosphine (mg/L, 20-h exposure): *R. dominica* = 0.03 (Anon., 1975); *T. castaneum* = 0.04 (Anon., 1975); *S. oryzae* = 0.04 (Anon., 1975); *C. ferrugineus* = 0.06 (Mills, 1983).

The dose-response line for the PH_3-resistant (BT1vs) strain of *T. castaneum* was not statistically significant, and the LC_{50} values indicate that a similar concentration of methylphosphine is required to kill PH_3-resistant and PH_3-susceptible insects. At the $LC_{99.9}$ level, however, about twice the concentration of methylphosphine was required to kill PH_3-susceptible insects than that required for the PH_3-resistant ones. Also, as with *R. dominica* and *S. oryzae*, the $LC_{99.9}$ for the BT1vs strain was comparable to the discriminating dose of PH_3 which kills all PH_3-susceptible insects of this species.

The PH_3-resistant and PH_3-susceptible strains of *C. ferrugineus* also responded similarly to methylphosphine treatment. At the LC_{50} level, almost twice the concentration of methylphosphine was required to kill PH_3-susceptible insects than that for PH_3-resistant insects. Because of the insignificant slope of the probit line for susceptible insects, a comparison of $LC_{99.9}$ values was not possible. However, on a molar basis, the $LC_{99.9}$ of methylphosphine for the PH_3-resistant (BC12s) strain was very close to the discriminating dose of PH_3 for this species.

Some physical and chemical properties of methylphosphine are presented in Table 2. As with PH_3, environmental breakdown of methylphosphine is expected to produce non-toxic products. The *Rf* value of the only visible oxidation product of methylphosphine (*Rf* = 0.73) on paper chromatograms was similar to that for methylphosphonic acid. The *Rf* values recorded for ortho-phosphate, phosphite and hypophosphite were 0.58, 0.75 and 0.70, respectively. This indicates that the main oxidation product of methylphosphine appears to be methylphosphonate which is non-toxic. However, the oxidation of the methylphosphine-$HgCl_2$ complex was more difficult than that of PH_3, requiring extensive boiling in bromine-water. In conjunction with observations on stability in air, this indicates that oxidative breakdown of methylphosphine may be a slow process.

In these preliminary studies there were indications that the concentration of methylphosphine in the test desiccators fell during the exposure period. This drop in concentration was considerable at low levels, and the cause of this phenomenon, which might be sorption into the nylon material used to retain insects in glass-dishes, is currently being investigated.

Although more tests are needed to improve the reliability of LC_{50} and $LC_{99.9}$ values, the results presented here clearly indicate that this newly discovered fumigant has greater toxic effects on PH_3-resistant insects than on PH_3-susceptible ones. The results also indicate that control of PH_3-resistant insects could be achieved by application of doses of methylphosphine that are comparable to the discriminating doses of PH_3 that kill PH_3-susceptible insects. A similar trend in the strains of all four insect species tested is consistent with earlier findings which indicate that there is a common mechanism of PH_3-resistance in insects. It is possible that the presence of the methyl group in the CH_3PH_2 molecule prevents exclusion by the resistance mechanism. The much greater mortality of PH_3-resistant insects further indicates that oxidative breakdown of methylphosphine by the PH_3-detoxification mechanism produces reactive products in insect tissues. Our earlier work with two other analogues of PH_3, arsine (AsH_3) and stibine

TABLE 2
Some physical and chemical properties of methylphosphine

Chemical formula	CH_3PH_2
Molecular weight	48
Physical state at STP	Colourless gas with garlic-like odour
Boiling temperature	–14°C (Kosolapoff and Maier, 1972)
Vapour pressure	28.1 mm Hg at –78.5°C, 72.5 mm Hg at –63.5°C (Crosbie and Sheldrick, 1969)
Flammability	Likely to be flammable
Method of detection	GC/chemical analysis. Concentration in air can be monitored by PH_3/AsH_3 detector tubes
Toxicity	Classified as very toxic although no data is available in the literature (Van Wazer, 1958). Toxic to insects
Mode of toxic action	Not known. Likely to act on mitochondrial respiratory chain in insects
Solubility in water	Not known
Stability in air	Not known. Appears to be reasonably stable in initial tests
Stability when mixed with foodstuffs	Not known
Breakdown products	Main oxidation product may be methylphosphonic acid
Residues in treated grain	Not known. Possibly methylphosphonate and other oxidation products (phosphite, phosphate, etc.)
Toxicity of residues	Not known. Oxidation products are likely to be non-toxic
Method of synthesis	By reaction of $LiAlH_4$ with dimethylmethylphosphonate (Crosbie and Sheldrick, 1969)
Likely formulation	Either as compressed gas mixture with an inert gas in cylinders or in solid formulation as HCl salt to release the gas *in situ* on reaction with H_2O (Groenweghe, 1965)
Corrosion of metals	Not known. Oxidation products may be corrosive to metals

(SbH_3), also showed that they had more effect on PH_3-resistant insects than on PH_3-susceptible ones (Chaudhry and Price, 1991). This was also probably due to the conversion of arsine and stibine into toxic metabolites by the PH_3-detoxification process which converts PH_3 to non-toxic products in resistant insects.

The more effective insecticidal action of methylphosphine on PH_3-resistant insects, therefore, appears to be due to a chemical structure which, avoiding the mechanism of active exclusion in resistant insects, is instead activated by the PH_3-detoxification mechanism. Methylphosphine could therefore be used to selectively control PH_3-resistant

insects, although either higher doses of this gas or longer exposure periods, or a combination of both, can be utilised to kill susceptible as well as resistant insects. The gaseous nature of methylphosphine means that its use as a fumigant, alone or in combination with PH_3, would be possible. Our preliminary tests also showed that the addition of 0.05 mg/L of PH_3 to methylphosphine at a nominal concentration of 0.2 mg/L for a 24-h exposure period killed all susceptible and resistant insects of *R. dominica* and *T. castaneum* species (data not shown). More work is needed to establish whether the toxic action of these two gases is cumulative or synergistic.

The discovery of the fumigant potential of methylphosphine and its greater efficacy against PH_3-resistant insects presents an extraordinary opportunity to use this gas in the management of PH_3-resistance. The fumigant use of methylphosphine, alone or in combination with PH_3, is expected to exert a negative selection pressure on PH_3-resistance genes, thus preventing further selection of resistance. This will enhance the useful life of PH_3 as a fumigant. Its effectiveness would ensure that any possible development of methylphosphine resistance in insects could be managed by alternating the use of these two gases.

The alkylphosphines' property of forming volatile phosphonium-halide salts has been reported (Groenweghe, 1965), and this represents a very useful way of generating methylphosphine gas by exposing a solid formulation to moist air. Unlike the residues left after decomposition of aluminium phosphide to generate PH_3 gas, those left after the generation of methylphosphine from phosphonium-halide salt may be acidic. This problem could, however, be overcome by using specially designed containers for the solid preparation which can be collected after the fumigation operation. Alternatively, appropriate solid formulations could contain other ingredients (such as carbonate salts) which would simultaneously neutralise the acid produced and generate carbon-dioxide gas, minimising the potential hazard of flammability associated with pure methylphosphine.

The results presented here have shown that methylphosphine has considerable potential as a new fumigant for the protection of stored products, especially as a complement to the use of PH_3 (threatened by the development of resistance). However, further studies will be needed to investigate such various aspects of the use of methylphosphine as the extent and nature of resulting residues, stability at higher relative humidity and other properties which might influence its use as a fumigant.

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