THE TOXICITY OF PHOSPHINE TO INSECTS

H. J. BANKS
Stored Grain Research Laboratory
CSIRO
Canberra City, A.C.T.
AUSTRALIA

Phosphine has been in use as a stored product fumigant for about 40 years [1]. It is highly toxic to insects, relatively easy to apply, not appreciably phytotoxic, does not taint most commodities and leaves little residue. With these advantages and length of use it is remarkable that the properties of phosphine relevant to stored product entomology are so poorly understood and that much of the work is conflicting.

The main areas of doubt are:

(1) The oxidation properties of phosphine in air [2,3,7].

(2) The presence and nature of phosphine-derived residues on grain [2,4,5,7,8,9,10].

(3) The mammalian toxicology of phosphine [11,12].

(4) The effective dose rate against various insects and various developmental stages [cf. 13].

(5) The nature of the dose-exposure-mortality relationship for insects [e.g. 13,14,15].

(6) The mode of action of phosphine [16,17].

(7) The toxicity of phosphine in nitrogen [16,18].

It is important to resolve these in order that the most efficient and safe use can be made of this important fumigant.

This paper reviews the physical properties, chemistry and biological properties relevant to the toxicity and possible modes of action of phosphine on insects and presents original data on the uptake of phosphine by insects.

PHYSICAL PROPERTIES: Phosphine is a colourless gas of a density close to that of air (1.5307 kg/m^3 at s.t.p. [3] cf. air = 1.2929 kg/m^3). The diffusion coefficient in air at 0°C($D = 1.73 \times 10^{-5} m^2$/sec, calc. from O_2, $D = 1.78 \times 10^{-5} m^2$/sec [19]) since it is related to molecular weight (M.W. (PH_3) = 34.00), will be higher than all fumigants except hydrogen cyanide. It obeys Henry's law in aqueous solution [20]. Since it is only weakly sorbed on surfaces and has low solubility in liquids generally its actual rate of travel through biological materials will be high. It can penetrate intact insect cuticle [21].

The exact sorption properties of phosphine on commodities and the reversible or irreversible nature of this sorption is still in debate. There is no doubt that a significant quantity of phosphine may be removed from the gas phase by stored products under fumigation. Table 1 summarises the sorption of phosphine on commodities at about 25°C. The values of sorption were obtained either by a radioactive tracer method [2,4,5,6] or by estimation

of loss of fumigant from the gas phase [22,23]. Because of the many factors involved, particularly the load factor and commodity moisture content, it can only be said that similar results are given here. Three of these reports [4,5,6,22] are for residues after extensive airing and are attributable to chemical reaction within the commodity (see below). Chemisorption of phosphine on grain products has been discounted [9]. High recoveries of phosphine were obtained by duplication of some earlier experiments [22] suggesting that the sorption of phosphine was a physical process only*. Further work is needed to clarify this point.

TABLE 1. Sorption of phosphine on wheat observed by different authors at approximately comparable conditions.

Dosage mg/l	Exposure hr.	% uptake of dose	Commodity moisture content (%)	Temperature °C	Load-factor (est.) %	Reference
0.15-0.6	72	19	12.5	24	30	[22]
0.15-0.6	72	35	15	24	30	[22]
1.2	96	5	15	26.7	20	[23]
1.2	96	27	15	26.7	80	[23]
3.0	120	4.5	14.7	25	4	[4,5]
3.0	120	1.3	10.0	25	4	[4,5]
3.2	96-120	7	11	?	42	[6]
5.1	96-120	37	11	?	90	[6]

CHEMICAL PROPERTIES: Phosphine under physiological conditions is neither acidic nor basic [3]. Its reactivity can be described in terms of its weak nucleophilicity, its action as a sluggish but powerful reducing agent, its suitability as heavy metal ligand and that of radicals created by hydrogen abstraction.

(a) Oxidative behaviour - Pure phosphine/air mixtures at levels occuring in normal fumigation practice appear to be stable [3]. However, it cannot be said that phosphine absorped onto surfaces or in the prescence of biological materials may not be labile to atmospheric oxidation. The ease of aerial oxidation of phosphine by copper-salt impregnated charcoals [24,25] is an example of this. Robinson and Bond [2] postulate the formation of diphosphine (P_2H_4) during air oxidation, but a main pathway by the direct, possibly catalysed, addition of oxygen to phosphine with rearrangement to give hypophosphorous acid appears more plausible.

$$PH_3 + O_2 \rightarrow H_3PO_2$$

The existence of the possible oxidation intermediates, phosphine oxide, PH_3O, or the hydroxylamine analogue, PH_2OH, at room temperature is discounted by Fluck [3]. Phosphine oxide decomposes

*Rauscher et al. [9] did not distinguish between physical absorption and a reversible chemical reaction.

to the polymeric hydride ($(PH)_x$) even at -115°C.

The residues noted by several authors arising from the fumigation of cereal products [2,5,6], tobacco [26] and cellulose [7] with radioactive phosphine have been shown to be largely oxy-acids of phosphorus, particularly hypophosphite [2,5,6]. It has been suggested [2] that these are formed by aerial oxidation but they could also be formed catalytically or by direct combination with biological constituents. It is notable that the oxidation of phosphine apparently occurs readily in aqueous solution [20].

Phosphine can reduce a number of organic compounds under mild conditions, particularly aromatic nitro-compounds [3]. It is said to reduce cystine [15] but this has been doubted [6].

(b) Nucleophilic reactions - Phosphine reacts as a weak nucleophile with carbonyl groups and activated olefins. For instance, under acid catalysis phosphine reacts with primary aliphatic aldehydes giving tetrakis (hydroxyalkyl) phosphonium salts [3]. Aliphatic ketones react much less readily forming primary phosphine oxides [3,27].

$$PH_3 + 4RCHO + H^+ \rightarrow P(CH(OH)R)_4^+$$

$$PH_3 + (CH_3)_2CO \rightarrow (CH_3)_2CH\ P(O)H_2$$

Phosphine may be cyanoethylated under base catalysis [3] and presumably will react with other similarly activated diene systems. p-toluquinone does not react with phosphine in air in ethanolic solution [28].

The initial step in the addition of phosphine to ketones can be expected to be reversible by analogy with reactions of amines. This and similar reactions may explain why prolonged airing may be required to remove all sorbed phosphine from commodities. Disney and Fowler [29] observed much larger recovery (18x) of sorbed phosphine by heating grain dry rather than by the normally used procedure [30] of heating with dilute sulphuric acid. This could be explained by acid-catalysed fixation of phosphine in a manner similar to that given above. Biological systems contain many important substances with activated carbonyl groups e.g., Coenzyme A and effective base or acid catalysts e.g. those in chymotrypsin [31] which could react in this manner, possibly with significant biological effect.

(c) Radical reactions - Phosphine is converted to a reactive radical PH_2 by UV irradiation, atomic irradiation and organic radical generation [3,32,33]. This radical will attack olefins to form organophosphines [3] and is implicated in the explosive reaction of phosphine with air [33]. These reactions are chain reactions [33] and thus in the absence of effective terminations require only small initial quantities of PH_2. There are biological systems with free radical character such as the mixed function oxidase system [34] possibly capable of generating such a radical. The m.f.o. system does not appear to play a major role in phosphine detoxification [35].

(d) Reaction with heavy metals - Phosphine readily forms phosphides with many heavy metal salts in solutions [3] and

can act as a heavy metal ligand [3]. There are no data on the exact chemical nature of the complexes formed by enzyme prosthetic groups with phosphine but reaction can occur. Oxidised cytochrome c apparently catalyses phosphine oxidation to hypophosphite but can be recovered unchanged [2]. Cytochrome oxidase is reduced and not reoxidisable with air after reduction [21]. Haemoglobin does not react in the absence of oxygen but oxyhaemoglobin is converted through Fe +++ containing compounds to a verdichromogen-like material [36]. Tkachuk [6] showed significant conversion of phosphine to phosphorus oxyacids in the presence of haemoglobin. Catalase is also affected by phosphine [37]. Phosphine may be expected to act as a ligand with components of the respiratory chain. Attack at this site has been suggested as a mode of toxic action of phosphine to insects [2,38]. By reaction with other enzyme metal prosthetic groups it may poison other vital enzymes.

OTHER REACTIONS: The nature of the reaction products of phosphine on proteins has not been elucidated but is obviously relevant here. Phosphine denatures the globin fraction during reaction with oxyhaemoglobin [36] and decreases a variety of enzymic activities [37]. ^{32}P-labelling experiments showed part of the phosphorus taken up by proteins to be firmly bound but to be released on acid hydrolysis [6].

The nature of the phosphorus-containing residues from phosphine fumigations of commodities [2,5,6,26], which is not eluted with hot water, is unknown.

This review clearly demonstrates that the known reactivity of phosphine provides many possibilities for the toxic interference of biological processes. It is at the chemical and fundamental physiological level that the mode of action of this toxicant must be understood. The gross symptomology discussed below is a consequence of these actions.

BIOCHEMICAL EFFECTS: Biochemical studies on phosphine are notably lacking. Phosphine has been shown to inhibit uncoupled but not controlled respiration in isolated rat mitochondria [17].

PHYSIOLOGICAL EFFECTS OF PHOSPHINE: (a) On mammals - Klimmer [11] critically summarises the poisoning action of phosphine on mammals with a discussion of the symptomology. He rejects the possibility of a chronic effect from phosphine (but see [12]). The primary effects appear to be [11] sleepiness, apathy, respiratory distress and disturbed circulatory regulation and the secondary effects such as anoxemic convulsions result from these. The poisoning syndrome does not appear to be unequivocably interpretable for a single mode of action.

(b) On Insects - Insects exposed to phosphine undergo a series of stages of behaviour. At mediumt concentrations an

---

†Low concentrations of phosphine <0.1 mg/l, medium concentrations 0.1 - 3 mg/l, high concentrations > 3 mg/l.

initial period of hyperactivity is followed by knockdown or "paralysis" [39]. Cockroaches at high concentrations close their spiracles and then undergo knockdown, convulsions, loss of coordination and paralysis [2]. In other insects the spiracles become fixed and open [21]. Sealing of the spiracles apparently increased phosphine toxicity [21]. In contrast to mammals, insect species retain the effects of phosphine poisoning for considerable lengths of time sometimes permanently [12,13,21].

The relationship between time after exposure to reach end-point mortality is linearly related to log dosage for adult *T. castaneum*. At high dosages, up to 20 days is required to reach terminal mortality [13]. Recovery rate from knockdown varies between strain and species but may take a similar period at medium concentrations [21]. Some *Sitophilus granarius* removed from a sublethal phosphine exposure appear completely unharmed, others exhibit various stages of incapacitation from a slightly abnormal gait to merely occasional twitching of the appendages. The slightly incapacitated individuals recover [28].

Metamorphosis is sometimes deranged when immature stages are exposed to phosphine [21]. In *Plodia interpunctella* only part of the larva acquired pupal skin on occasions [40] while *Tenebrioides mauritanicus* larvae pupated successfully but emerged incompletely and were often deformed [2]. The rate of development appears unaffected [41] though longevity is reduced [13].

Reproductive capacity can be either enhanced [41] or inhibited [13,41]. In the most thorough study on this subject Winks [13] showed that at doses around 2 mg/l reproduction of surviving *T. castaneum* was significantly and permanently inhibited (up to 40%). At lower and higher concentrations there was less effect. The decreasing effect of the latter was ascribed to the protective effect of narcosis.

NARCOSIS: A number of workers have observed that on occasions non-linear probit mortality-log dosage relationships are obtained for phosphine [see 14]. Although there may be other explanations (e.g. inadequate determination of end-point mortality [42]) the curvature would normally be interpreted as evidence of inhomogeneity of the population leading to a response deviating from a normal distribution (or that obtained after log-transformation). This occurs particularly at higher levels of concentration with phosphine as occurs in field practice. This response has been interpreted in terms of narcosis [13,43] or protective stupefaction by phosphine where some individuals are able to regulate their uptake of the fumigant by reduction of metabolism and activity. Winks [13] observed a reduced response attributed to narcosis with *T. castaneum* for exposures exceeding 1 mg/l at 25°C. The phosphine-resistant strain of *S. granarius* [43] selected at high concentrations exhibited a high resistance factor when tested again at these levels but this was markedly reduced at lower exposure levels. The resistance, which was genetically inherited, was attributed to narcosis at high levels and consequent failure to take up a lethal dose.

UPTAKE OF PHOSPHINE BY INSECTS: The most important parameter in the analysis of fumigant action is the quantity of uptake of the fumigant from the atmosphere into the insect. Without a knowledge of how much fumigant is present in the insect, and at what rate it enters, hypotheses such as those based on narcosis can only be sustained indirectly. As with all toxicology the measure sought is the concentration of toxicant at the site of action but usually, as here, only the gross uptake is measured. Removal by non-target areas complicates any subsequent analysis.

Many attempts have been made to relate action of fumigants to metabolic rate. This is particularly attractive with phosphine with its possible action on the respiratory chain and the probable oxidative metabolic pathway. The direct correlation is not well borne out in practice for different stages of the same insect [39] and has yet to be substantiated. However, it has been noted that when oxidative respiration is inhibited by cyanide phosphine toxicity is reduced [16]. In nitrogen phosphine is non-toxic and uptake is very low [16]. However Sato et al., [18] showed that under non-respiring conditions (0°C or under nitrogen) the insects are protected from phosphine action but died after a subsequent period of days in air. This conflict requires further work. In general phosphine reduces the respiration rate of insects [37,39].

Hitherto, uptake estimates have been based on indirect methods such as rate of loss from the gas phase or rate of oxygen consumption. These methods give average values of uptake but cannot distinguish between the dose taken up by individual insects; some of which may die, the others survive.

It has been shown that *Tribolium confusum* [16] and *T. castaneum* [21] can take up astonishingly large quantities of phosphine (over 100 µg/g insect body weight) under high dosage rates. The uptake apparently ceased after about 6 hours and was dependent on concentration up to about 4 mg/l (5 hr exposure) [16]. *Sitophilus granarius* took up much less gas than *T. confusum* and continued to absorb over a 24 hr period even at very high phosphine concentrations [16].

In order to investigate the uptake of individual insects a non-destructive radiochemical method has been developed. Insects were exposed to humidified $^{32}PH_3$/air mixtures in small flasks (~330 ml, 200 mg insects with 2 g whole wheat at 14% m.c.). After exposure the gas was aired off at 50 ml/min for 1 hr with nitrogen and absorbed for subsequent chemical and radiochemical analysis in mercuric chloride solution (0.5% aq.). Subsequent further insect handling and airing gave a period <9 hr before counting. The insects were weighed and then individually placed in 2" x 1/8" test-tubes suspended in scintillation vials. The ^{32}P-content was estimated by Cherenkov counting at an efficiency of about 18%.

Preliminary experiments were conducted to confirm the earlier results [16,21] and to establish if dead insects take up phosphine. Table II gives the uptake of phosphine by three species. Table III gives the uptake of batches of *T. confusum* exposed for different times. The uptake was estimated by two methods which

gave similar results. The values obtained for the uptake of phosphine by *Tribolium* spp. are consistent with that reported [16,21] and the apparent saturation of uptake at these levels is also demonstrated again.

TABLE II. Uptake of phosphine by live and dead insects and under N_2. *T. castaneum* included for comparison with published work.

Species	Medium	PH_3 conc. mg/l	Exposure hr	Uptake µg/g	Temperature °C
T. confusum	air	6.3	4	470	21
T. confusum (dead)	air	6.3	4	48	21
T. confusum	N_2	9.4	4	3.9	21
T. castaneum	air	0.91	2	112	23
S. granarius	air	0.015	24	1.3	25
S. granarius (dead)	air	0.015	24	0.3	25

TABLE III. Rate of uptake of phosphine by adult *T. confusum* (3.08 mg/l at 21°C) by Method A, radiochemical estimation and Method B, estimation by gas phase loss.

Exposure time hr	Uptake (µg/g) Method A	Method B
1.1	63	40
1.9	98	103
3.8	137	175
6.0	182	226
22.6	207	214

Insects were killed by immersion in liquid nitrogen. The dead insects took up appreciable quantities of phosphine but living insects under nitrogen took up <1% of that in air. This demonstrates either that phosphine is taken up oxidatively at this level or it reacts with a component activated by oxygen.

It has been found that phosphate once taken up is not desorbed by airing [16]. With dead insects long airing did not significantly reduce the radioactivity and PH_3-equivalent in the insects (Table IV). However, with living insects on food, radioactivity was lost. Since this did not occur on insects confined to ventilated counting vials without food over a period of days the radioactivity was presumably lost by excretion and abrasion, not as phosphine gas.

Samples of insects were exposed to $^{32}PH_3$ and assayed individually in order to investigate the range of sorption of phosphine taken up within a population. Table V gives strains used. Figure 1 shows the range obtained for a 24 hr exposure to

TABLE IV. Apparent loss of phosphine from living and dead insects on airing.

State	After ~ 3 hr Uptake (ng/insect)	S.E.	n	After 25 hr Uptake (ng/insect)	S.E.	n	Significance
Living	3.4	0.23	20	2.4	0.39	20	**
Dead	10.5	0.64	20	9.7	0.58	19	N.S.

concentration of about 18 µg/l PH_3 at 25°C (approximately LD_{50}). Only selected individuals of the fumigated sample were assayed in order to give values for living, dead, slightly incapacitated and "twitching" insects (24 hr activity assessment). No rigorous end point mortality observations were made at this stage but in general the slightly incapacitated and living survived at least for some days while the others did not revive. It can be seen that surviving insects take up less toxicant than those which are dead or twitching at 24 hr, the level at which death occurs being about 4 µg PH_3 uptake/insect. The phosphine preselected laboratory strain had some individuals which were more tolerant and the heavier pyrethrin-resistant strain took up to 12 µg without apparent ill-effect on occasion. The strain is known to be phosphine resistant [35]. A 48 hr exposure considerably extended the range of uptakes observed.

TABLE V. Ages and strains of test insects used for uptake studies.

Species	Strain	Age (days)
S. granarius	PH_3-selected at pupal stage (2x)	12-18
S. granarius	Pyrethrin-resistant	12-15
S. granarius	Field strain ex Windsor U.K.	4-20
S. granarius	Field strain ex Horsham U.K.	4-20
T. castaneum	FSS. Fumigant susceptible strain	21-35
T. castaneum	CTC12. Malathion non-specific resistant	21-35
T. castaneum	MSG. Melanotic stink gland mutant.	mixed

Tribolium castaneum adults tended to take up more phosphine under the same conditions, as has been previously observed in bulk samples (see Fig. 2). The malathion-resistant strain, CTC 12, appeared to be able to survive uptakes of 7 µg/insect. The melanotic stink gland mutant was included here since it does not produce defensive quinones [44]. The lower uptakes observed by this strain are consistent with the hypothesis that the quinone-forming system may normally be responsible for the high uptake found for *Tribolium* spp.

Figure 3 gives the 24 hr activity assessment of the complete fumigated samples from which insects were selected for assay.

A sample of *Sitophilus granarius* (PH_3 preselected

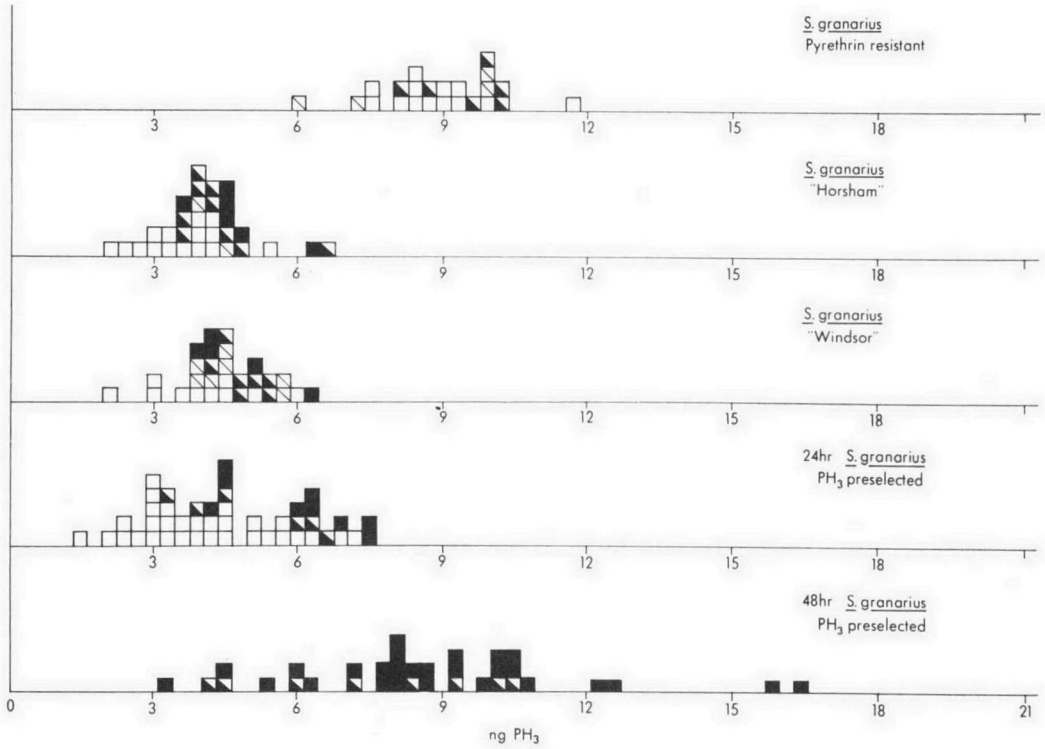

FIGURE 1. Uptake of phosphine by various strains of *S. granarius* at 15-18 µg PH_3/l, 25°C, 24 hr (one test, 48 hr as marked). □ = unaffected, ▨ = mildy incapacitated, ▩ = twitching, ■ = apparantly dead.

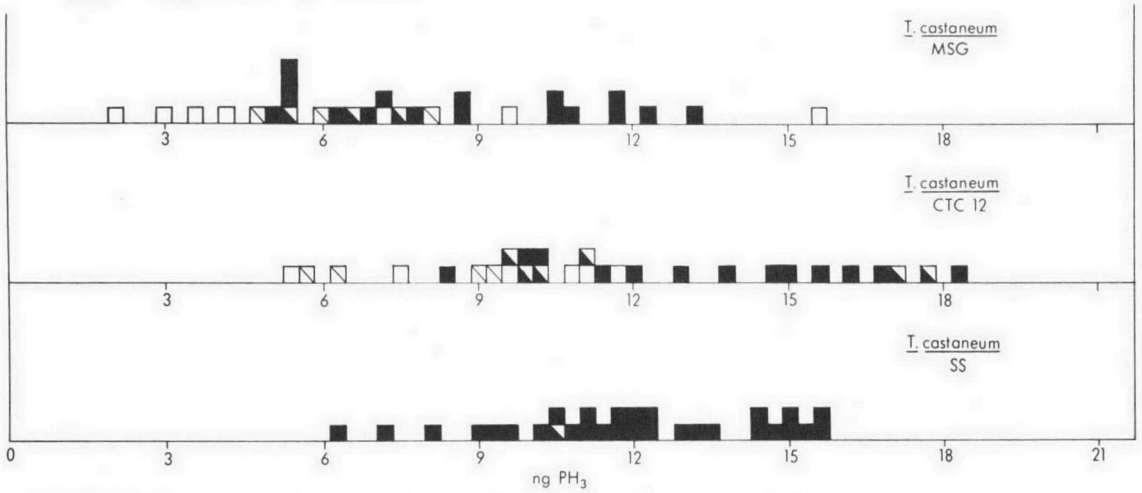

FIGURE 2. Uptake of phosphine by strains of *T. castaneum* at 18 µg PH_3/l, 25°C, 24 hr. Key as Fig. 1.

strain) was fumigated (18 µg/l, 24 hr, 25°C) and assessed for activity 24 hr after termination of treatment. The insects were placed in a 100 ml beaker and raced. On reaching the lip of the beaker they were placed in sequence for assay (alternate "active" insects were discarded). Those which could climb but not reach the rim and those which could not walk were graded on their abilities.

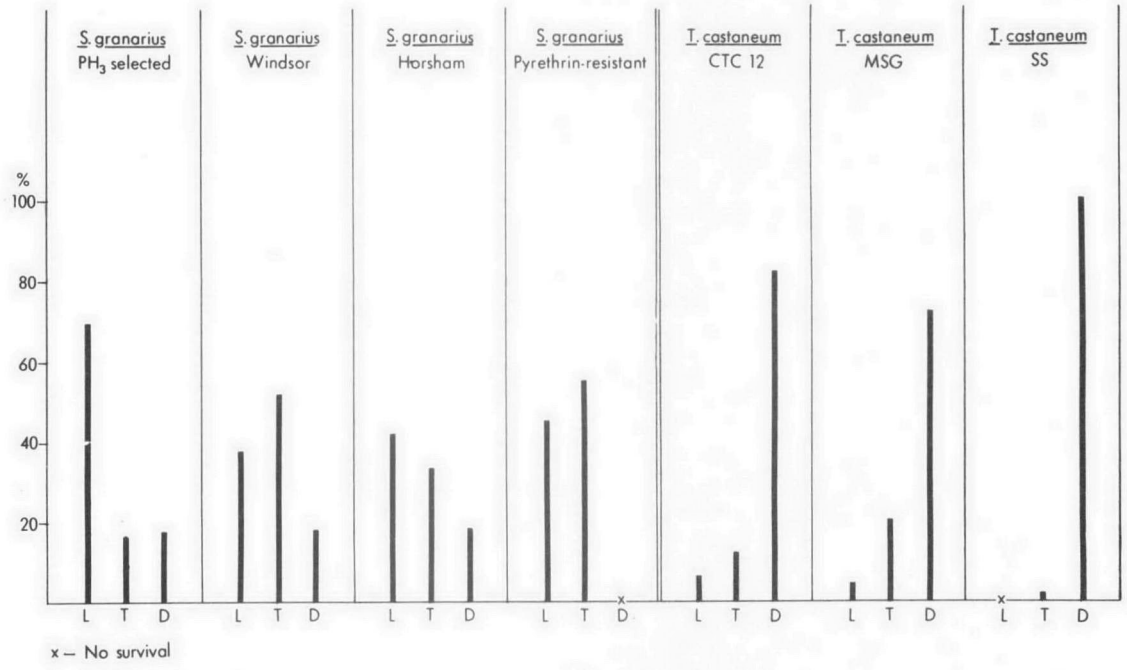

FIGURE 3. 24 hr activity assessment of complete samples of fumigated insects. 15-18 µg PH_3/l, 25°C, 24 hr.

A correlation (Fig. 4) is apparent between 24 hr activity and quantity of fumigant taken up. This demonstrates that uptake is a relevant measure of toxic action under these conditions.

Bulk measurement of uptake cannot distinguish between an insect which has taken up a just lethal dose and that which has taken up an "overkill". Nevertheless values obtained by other authors [16,18] for low dosages are consistent with those found here. The large uptakes at high doses corresponding to 50-200 µg/insect are presumably far in excess of that necessary for death. Bulk measurements must thus be used with caution.

The general uptake range is summarised in Table VI. It will be noted that all samples suffered severe weight loss during treatment despite adequate humidity control. This loss, which is found in the action of many insecticides on insects [e.g.45,46], may be a contributory factor in the death of the insect from phosphine.

This method of measurement of uptake is nondestructive, enabling survivors to be assessed either for lack of uptake or large uptake. It thus presents a method where narcosis-based resistance can be distinguished from metabolic detoxification and suggests criteria for selection for these resistance phenomena. Work is to be continued in this field.

ACKNOWLEDGEMENTS: The experimental work described herein was largely carried out while I was a visiting worker at the Pest Infestation Control Laboratory, London Road, Slough, U. K. I am most grateful to R. W. Disney, K. S. Fowler and R. W. Howe of that laboratory for their help and guidance and to R. G. Winks for

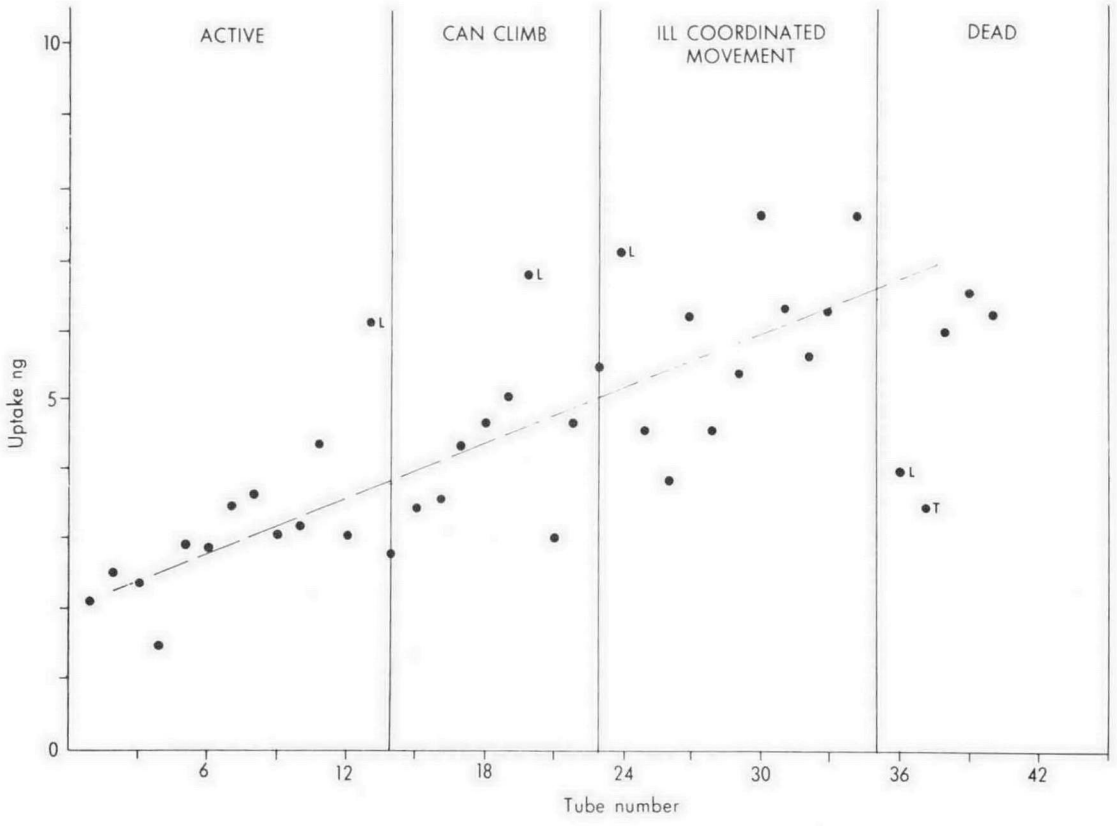

FIGURE 4. Correlation with activity 24 hr after exposure (18 µg PH_3/l, 25°C, 24 hr) with uptake of phosphine. Phosphine-preselected *S. granarius*.

TABLE VI. Range of uptake of phosphine and weight loss under treatment at 15-18 µg PH_3/l, 25°C for 24 hr.

Species	Strain	Range of Uptake ng/insect	Average Uptake µgPH_3/g insect	Average weight (mg) Before	After
S. granarius	preselected PH	1.5-7.5	1.7	2.76	2.57
	Horsham	2.1-6.6	1.7	2.39	-
	Windsor	2.1-6.3	1.7	2.60	-
	Pyrethin-resistant	6.0-11.7	2.1	4.30	4.18
T. castaneum	FSS	6.3-15.6	6.4	1.86	1.66
	CTC 12	5.4-25.4	6.1	1.98	1.82
	MSG	1.2-15.6	4.2	1.79	1.55

criticism of the manuscript.

REFERENCES:

[1] Heseltine, H. K., Thompson, R. H., The use of aluminium phosphide tablets for the fumigation of grain. Milling 129 (1957) 676-677, 730-732, 752, 774-775, 778, 783.

[2] Robinson, J. R., Bond, E. J. The toxic action of phosphine - studies with $^{32}PH_3$; terminal residues in biological materials. J. stored Prod. Res. 6 (1970) 133-146.

[3] Fluck, E., The chemistry of phosphine. Fortschr. Chim. Forsch. 35 (1973) 1-64.

[4] Disney, R. W., Fowler, K. S., Phosphorus-32-labelled phosphine in the determination of fumigation residues in grain. Proc. IUPAC 2nd Int. Conf. Pesticide Chem. Tel Aviv (1971) 423-425.

[5] Disney, R. W., Fowler, K. S., Residues in cereals exposed to hydrogen phosphide. In "Radiotracer Studies of Chemical Residues in Food and Agriculture". IAEA-PL-469/13 STI/PUB/332 Vienna (1972) 99-101.

[6] Tkachuk, R., Phosphorous residues in wheat due to phosphine fumigation. Cereal Chem. 49 (1972) 258-267.

[7] Robinson, J. R. Residues containing phosphorus following phosphine treatment: Measurement by neutron activation. J. stored Prod. Res. 8 (1972) 19-26.

[8] Dieterich, W. H., et al., Hydrogen phosphide as a fumigant for foods, feeds and processed food products. Residue Rev. 19 (1967) 135-149.

[9] Rauscher, H., Mayr, H. E., Sullivan, J. B., Sorption and recovery of phosphine. J. agric. Ed. Chem. 20 (1972) 331-333.

[10] Rauscher, H., Physical and chemical aspects of the use of phosphine as a fumigant against stored product Pests. Proc. 3rd Br. Pest Control Conf. Jersey (1971).

[11] Klimmer, O. R., Beitrag zur Wirkung des Phosphorwasserstoffes (PH_3). Arch. Toxicol. 24 (1969) 169-187.

[12] Bond, E. J., Upitis, E., Response of three insects to sublethal doses of phosphine. J. stored Prod. Res. 8 (1973) 307-313.

[13] Winks, R. G., "Some aspects of the response of *Tribolium castaneum* to Phosphine" Ph.D. Thesis, University of London (1973).

[14] Howe, R. W., Problems in the laboratory investigation of the toxicity of phosphine to stored product insects. J. stored Prod. Res. (In press).

[15] Bell, C. H., Glanville, V., The effect of concentration and exposure in tests with methyl bromide and with phosphine on diapausing larvae of *Ephestia elutella* (Hubner) (Lepidoptera, Pyralidae). J. stored Prod. Res. 9 (1973) 165-170.

[16] Bond, E. J., Robinson, J. R., Buckland, C. T. The toxic action of phosphine: absorption and symptoms of poisoning in insects. J. stored Prod. Res. 5 (1969) 289-298.

[17] Nakakita, H., Katsumata, Y., Ozawa, T., The effect of phosphine on the respiration of rat liver mitochondria. J. Biochem. 69 (1971) 589-593.

[18] Sato, K., Suwanai, M., Studies on the characteristics of action

of fumigants II. Entrance of hydrogen phosphide into weevil body under conditions of the failure to respire for the weevil. Botyu-Kagaku 38 (1973) 213-216.
[19] Weast, R. C., (ed.) "Handbook of Chemistry and Physics". The Chemical Rubber Co., Cleveland, Ohio. 45th ed. (1964) F-6, F-24.
[20] Van Wazer, J. W., "Phosphorus and its Compounds" Vol. I. Chemistry Interscience, New York (1958), 187.
[21] Rajak, R. L., "Studies Concerning the Toxic and Physiological Effects of Phosphine on Insects of some Pest Species". Ph.D. Thesis University of London (1971).
[22] Berck, B., Sorption of phosphine by cereal products. J. agric. Fd Chem. 16 (1968) 419-425.
[23] Vincent, L. E., Lindgren, D. L., Comparison of the sorption of hydrogen phosphine, methyl bromide, ethylene dibromide and hydrocyanic acid by wheat and corn of different moisture contents and load factors. J. econ. Ent. 64 (1971).
[24] Andreev, E. A., Kavtaradze, N. N., Catalytic oxidation of phosphine in absorbed layers. Doklady Akad. Nauk. S.S.S.R. 60 (1948) 1193-1195. Chem. Abstra. 43 (1949) 6898a. Also Chem. Abstra. 47 (1953) 5776-5778.
[25] Muthu, M., Krishnamurthy, T. S., Majumder, S. K., An evaluation of impregnated respirator canister carbons for phosphine removal from air. Pestic. Sci. 4 (1974) 245-249.
[26] Underwood, J. G., The radiochemical analysis of phosphine residues in fumigated tobaccos. Tob. Sci. 16 (1972) 123-126.
[27] Strizhevskii, I. I., Slizovskaya, L. V. Absorption of phosphine in an acetylene cylinder. Chem. Abstra. 70 (1969) 79530z.
[28] Banks, H. J., Unpublished observations.
[29] Fowler, K. S., Disney, R. W., Residues in cereals exposed to hydrogen phosphide. In "Isotope tracer studies of chemical residues in food and agricultural environment." IAEA-PL-S12/18. Vienna (1974) 21-23.
[30] Bruce, R. B., Robbins, A. J., Tuft, T. O., Phosphine residues from Phostoxin-treated grain. J. agric. Fd Chem. 10 (1962) 18-21.
[31] Blow, D. M., Birktoft, J. J., Hartley, B. S., Role of a buried acid group in the mechanism of action of chymotrypsin. Nature (Lond.) 221 (1969) 337-340.
[32] Kirby, A. J., Warren, S. G., "The Organic Chemistry of Phosphorus" Elsevier, Amsterdam (1967) 159.
[33] Halmann, M., "Photochemical and Radiation-induced Reactions of Phosphines". In Crawley, A. H., et al., "Topics in Phosphorus Chemistry" 4 (1967) 53-55.
[34] Staudinger, H., et al., A Study on the mechanism of microsomal hydroxylation. In King, T. E., Mason, H. A., Morrison, M., (eds.) "Oxidases and related Redox Systems". Vol. 2. Wiley New York (1965) 815-832 11.
[35] Rajak, R. L., Hewlett, P. S. Effects of some synergists on the insecticidal potency of phosphine. J. stored Prod. Res. 7 (1971) 15-19.

[36] Trimborn, H., Klimmer, O.R., Experimentelle Untersuchungen uber chemische Veranderungen des Blutfarbstoffs in vitro durch Phosphorwasserstoff. Arch. int. Pharmacodyn. 137 (1962) 331-347.
[37] Rohrlich, M., Meuser, F. Untersuchungen an mit Phosphorwasserstoff begastem Getreide. III. Getreide und Mehl. 20 (1970) 1-8, 13-16.
[38] Bond, E. J., Monro, H. A. U., Buckland, C. T. The influence of oxygen on the toxicity of fumigants to *Sitophilus granarius* (L.). J. stored. Prod. Res. 3 (1967) 289-294.
[39] Bang, Y. H., Telford, H. A., Effect of sublethal doses of fumigants on stored-grain insects. Washington Agr. expt. Stn. Tech. Bull. 50 (1966).
[40] Storey, C. L., Davidson, L. I., Relative toxicity of chloropicrin, phosphine, EDC-CCl_4, and CCl_4-OS_2 to various life stages of the Indian Meal moth. USDA. ARS-NC-6. June 1973.
[41] Howe, R. W., Susceptibility of the immature and adult stages of *Sitophilus granarius* to phosphine. J. stored Prod. Res. 8 (1973) 241-262.
[42] Hewlett, P. S., Time from dosage to death in beetles, *Tribolium castaneum*, treated with pyrethrins or DDT, and its bearing on dose-mortality relations. J. stored Prod. Res. 10 (1974) 27-41.
[43] Monro, H. A. U., Upitis, E., Bond, E. J., Resistance of a laboratory strain of *Sitophilus granarius* (L.) (Coleoptera, Curculionidae) to phosphine. J. stored Prod. Res. 8 (1972) 199-207.
[44] Engelhardt, M., Rapoport, H., Sokoloff, A., Odorous secretion of normal and mutant *Tribolium confusum*. Science 150 (1965) 632-633.
[45] Hewlett, P. S., Gostick, K. G., The loss of weight of pyrethrin-treated flour beetles, *Tribolium castaneum* (Herbst), and its application to bioassay. Ann. appl. Biol. 43 (1955) 213-236.
[46] Maddrell, S. H. P., Reynolds, S. E., Release of hormones in insects after poisoning with insecticides. Nature (Lond.) 236 (1972) 404-406.