Ren Y, Jansen M, Agarwal M, Emery R, Newman C, Cheng H (2012) Fate of ^{32}P labeled phosphine in grain and grain fractions. In: Navarro S, Banks HJ, Jayas DS, Bell CH, Noyes RT, Ferizli AG, Emekci M, Isikber AA, Alagusundaram K, [Eds.] Proc 9th. Int. Conf. on Controlled Atmosphere and Fumigation in Stored Products, Antalya, Turkey. 15 – 19 October 2012, ARBER Professional Congress Services, Turkey pp: 178-184

FATE OF ^{32}P LABELED PHOSPHINE IN GRAIN AND GRAIN FRACTIONS

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ABSTRACT

Murdoch University Stored Grain Research Laboratory investigated the fate of ^{32}P labelled phosphine in grain and grain fractions. Radioactively-labelled phosphine is a useful and sensitive tool to study residues of phosphine in grains and their fractions. This research will assist industry to improve phosphine fumigation practice. Five representative grains (wheat, barley, oats, canola and lupins) were fumigated with ^{32}P labelled phosphine at 700 ppm and 25±2°C for two weeks exposure. Two types of residues were formed in grains from phosphine fumigation – phosphine residue and phosphine converted non-volatile residues. After one week exposure to atmosphere, the levels of ^{32}P radioactive residue in the grains and their fractions were analysed. The results show that more than 85% of absorbed phosphine which was converted to non-volatile compounds cannot be removed by ventilation. Furthermore, 80-90% of non-volatile ^{32}P residues were present in water and acid soluble extractions. Radiation imagery shows that more than 80% of ^{32}P residues were located or distributed in the embryo, testa, pericarp and husk of wheat, barley, canola, lupins and oats. However, 90% of absorbed phosphine will be desorbed after one week exposure to the atmosphere.

Key words: fumigant, ^{32}P labelled phosphine, residue, grain.

INTRODUCTION

Phosphine (PH_3) has been used as a fumigant for many years. The world grain industry relies heavily on phosphine for control of insect pests during storage. However, we know very little of its physical and chemical behaviour or its residues in treated and re-treated grain (Desmarchelier and Ren 1999). Phosphine has been scheduled for a toxicological and residue Re-Evaluation Review in 2015 by the FAO Codex Alimentarius organisation that sets internationally agreed maximum residue limits (MRLs) for pesticides. Despite the international importance of phosphine there is limited information on the physical and chemical behaviour of phosphine in grain and its degradation. It is extremely important that the Australian grain industry has access to information on the fate of phosphine in grains so

that MRL breaches do not occur as these could jeopardise market access (Desmarchelier and Ren 1999; Ren and Desmarchelier 1998).

Therefore, it is necessary to understand phosphine residues in bulk grain to establish better procedures for fumigation and multi-fumigation (Ren et al. 2012). This will guide industry in the conduct of good phosphine fumigation practice, such as the time of exposure and aeration, as well as application methods. This could potentially support re-labelling phosphine in the future when higher dosages may be required to deal with increased resistance.

Murdoch Stored Grain Research Laboratory has investigated the fate of ^{32}P-labelled phosphine in grain and grain fractions. Radioactively-labelled phosphine is a useful and sensitive tool for studying residues of phosphine in grains and their fractions. This paper reports on the uptake of ^{32}P-labelled phosphine on grains of the three major food groups: cereals, legumes and oilseeds.

MATERIALS AND METHODS

Reagents and apparatus

Scintillation vials (20 mL) and scintillation fluid were obtained from Edwards Instruments Company. ^{32}P was purchased as high specific activity sodium orthophosphate in dilute (c. 0.015M) hydrochloric acid from the Australian Atomic Energy Commission (AAEC), Lucas Heights, NSW. The specific activity was 20 m*C*i (740 MBq) and concentration was 2-10 *C*i mg^{-1} (74-370 Bq mg^{-1}). All reagents were analytical grade, unless otherwise specified. Magnesium powder and orthophosphoric acid (>85%) were purchased from Ajax Chemicals, Australia.

The purity of synthetic ^{32}P labelled phosphine was determined on a GOW-MAC (Model 40-001) gas density detector (GOW-MAC Instrument Co., Madison, NJ), after separation on a 1 m × 5 mm (i.d.) Porapak Q 100/120 mesh (Alltech Associates, Sydney, Australia, Cat. No. 2702) at 105°C and carrier (N_2) flow of 150 mL min^{-1}. The reference gas was tetrafluoroethane (> 99.9 % pure).

A model LS 200 Beckman (Beckman Instrument Co., USA) liquid scintillation analyser was used for scintillation counting, operating at the appropriate wavelength for the radioisotope.

A model of FLA-5000 Fluorescent Image Analyzer (Fuji Photo Film Co. Ltd. Japan) was used for scanning radiation images of extracted and sectioned commodities.

Synthesis of ^{32}P- labelled phosphine

A modified method for laboratory phosphine production outlined in Reichmuth (1994) was used to produce the ^{32}P labelled phosphine used in this study.

Commodities conditioning and fumigant dosing

Five representative commodities (hard wheat, soft wheat, barley, oats, lupins and canola) were used. Grain samples (380-400 g) were placed into a sealed jar (500 mL) and allowed to equilibrate at 25°C and 65% relative humidity (r.h.). After a period of 6 weeks the commodities were removed and moisture content and equilibrium relative humidity checked.

The conditioned grains (10 g for each variety) were separately placed in beakers (20 mL), and all samples were placed in a desiccator (1.5 L), equipped with a septum. ^{32}P-labelled phosphine (3 mL and 35% purity balanced with CO_2) was injected into the desiccator by gastight syringe to give an initial concentration of 400 ppm, v/v. Grain samples were

fumigated for the typical industry maximum exposure period of 14 days at 25±2°C. Following initial dosing, the fumigant was circulated in the desiccator by a magnetic stir bar. After 14 days exposure, the desiccator was opened and the samples transferred to a fume hood where they were aired for 7 days at 25±2°C.

Measurement of total ^{32}P in whole grain kernels

This study involved the dynamic monitoring of ^{32}P degradation and reduction during aeration from day 0 to day 7. These results include ^{32}P labelled phosphine and non volatile residues. Fumigated grain samples (3 g) were collected at day 0, and then 1, 3 and 7 of aeration days after initial opening, and placed into a bottle (7 mL) containing 3 mL of $AgNO_3$ extracted for 7 days. The grains were then ground and 10 mL of distilled water was added after which 3 mL of the mixture was transferred to a 20 mL scintillation vial containing 5 mL scintillation fluid. Unfumigated samples of each commodity were used as controls and 4 replicates of each sample were prepared and stored at 25°C in the dark for 7 days prior to counting. These samples were counted three times to reduce error.

Determination of radioactivity in the extractions

The method of fractionating nutrients relies on the sequential solubilising of one fraction while leaving the residue as the substrate for the next extraction. The procedures were carried out to determine the fate of applied ^{32}P as described by Ren and Mahon (2007). Untreated grains were used as controls. All samples were prepared for duplicate testing.

(a) A weighed sample of the commodity (5 g) was removed and placed in a stainless steel mortar. The sample was crushed (not finely ground) and transferred to a Soxhlet extraction thimble.

(b) The extraction thimble containing the 5 g sample was placed into the Soxhlet apparatus and the sample extracted with 30 mL chloroform overnight. Following extraction, the solvent (containing lipid, fat-soluble vitamin, pigments etc) was transferred into a 25 mL volumetric flask for analysis of ^{32}P in total lipid. The thimble was placed in a fume hood to allow complete evaporation of residual chloroform and the sample was transferred to a 25 mL centrifuge tube.

(c) The sample (remaining from step b) was resuspended in 10 mL distilled water, vortexed and allowed to soak at 25°C for 3 hours. It was centrifuged for 10 minutes at 3500 r.p.m and the supernatant transferred to a 25 mL volumetric flask. The pellet was resuspended twice in 5 mL distilled water with a 15 min soak between each washing. Centrifuged washings were collected in the 25 mL volumetric flask for analysis of ^{32}P in this solution, which contained sugars, amino acids, inorganic acids and sulfide. The pellet was retained (for step d).

(d) The pellet (from step c) was resuspended in 10 mL 2N HCl, vortexed and allowed to soak at 25°C for 1 hour. It was centrifuged for 10 minutes at 3500 r.p.m and the supernatant transferred to a 25 mL volumetric flask. The pellet was resuspended twice in 5 mL distilled water with a 15 min soak between each washing. The centrifuged washings were collected in the 25 mL volumetric flask for analysis of ^{32}P in this solution which contained sugars, amino acids and sulfide. The pellet was retained (for step e).

(e) The remaining pellet was resuspended for three times with 20 mL 2N HCl in a 25 mL volumetric flask for subsequent analysis of residual ^{32}P.

Scintillation counting
Liquid samples (2 mL each, collected from steps b to e) were mixed with the scintillation fluid (5 mL) in a scintillation vial and placed in the dark. Precautions were taken to avoid complication due to photoluminescence. Four replicate samples were prepared and each was counted four times, and averaged. Quenching was determined on each fraction to allow correction for counting efficiency.

Radiation image
Commodity kernels treated with ^{32}P labelled phosphine were sectioned by cutting in half (crosswise and longitudinally). The sections were held by BLU TACK (Bostik Pty. Ltd. Australia) on the sample holder of the Fluorescent Image Analyzer for scanning of the radiation images.

Preparation of quenched standards
In scintillation counting, quench correction was carried out by calibrating a series of progressively quenched standards with reference to an external standard of ^{32}P labelled phosphine in $AgNO_3$ solution. Samples were replicated 4 times, and each was counted 4 times and averaged. All radioactive residue data were converted/calculated from scintillation counting data by calibrating with the quenching standard curve.

For calibration of ^{32}P decay during four weeks counting, the following method was used based on ^{32}P half-life decay (Eq. 1).

$$\mathbf{M_t = M_o\ (1/2)^{t/14.3}} \qquad \textbf{Eq. 1.}$$

Where: $\mathbf{M_t}$ is amount of ^{32}P at analysis time
$\mathbf{M_o}$ is starting amount of ^{32}P
$\boldsymbol{t}$ is time (days)
14.3 is ^{32}P half-life time (day)

RESULTS AND DISCUSSION

^{32}P labelled phosphine standard
The quenching standard curve (Fig. 1) was not linear over the tested region. However, it was very well fitted to equation: $Y=4E\text{-}11\chi^2+2E\text{-}08\ \chi\text{-}2E\text{-}05$ ($R^2=1$), where: Y is level of ^{32}P in mg and χ is counts of radioactivity. The counts increased with increasing dose of ^{32}P mixing with scintillation fluid. The standard error (SE) between replicates was < 10%. The levels of ^{32}P in grains and grain fractions were calculated on the basis of the counts, and calibrated periodically with Eq. 1.

Total uptake ^{32}P labelled phosphine and non-volatile ^{32}P by grains
The total uptake of radioactive ^{32}P on different grains at different time of aeration is shown in Fig. 2. The total radioactivity decreased with increasing periods of aeration due to desorption of ^{32}P labelled phosphine. The remaining radioactivity in the day seven aired grain samples was mainly due to non-volatile ^{32}P substances. After fumigation without aeration, the levels of radioactive residues were 5, 19-22 and 64 mg kg^{-1} in lupins, wheat/barley/canola and oats respectively. However, after 7 days aeration, radioactive residues remained at high levels 59.0, 65.2, 81.2 and 93.7% of non-volatile ^{32}P substances in canola, wheat/barley, lupins and

oats respectively. This portion of ^{32}P was permanent residue in treated grains. These results show that during exposure period, 59-93.7% absorbed phosphine is converted to non-volatile compounds which cannot be removed by ventilation.

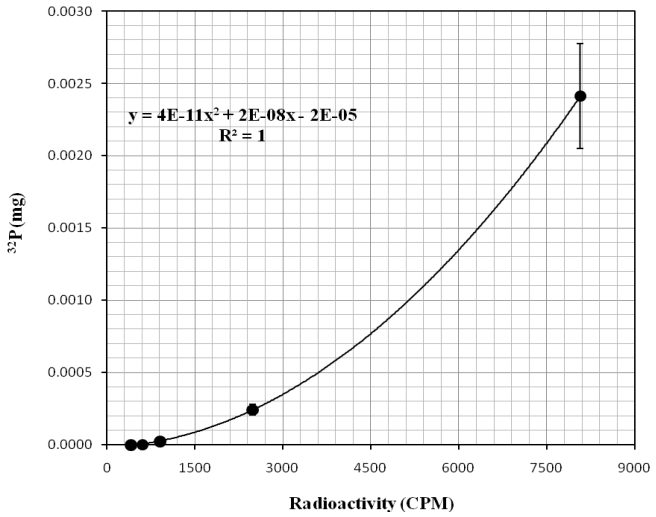
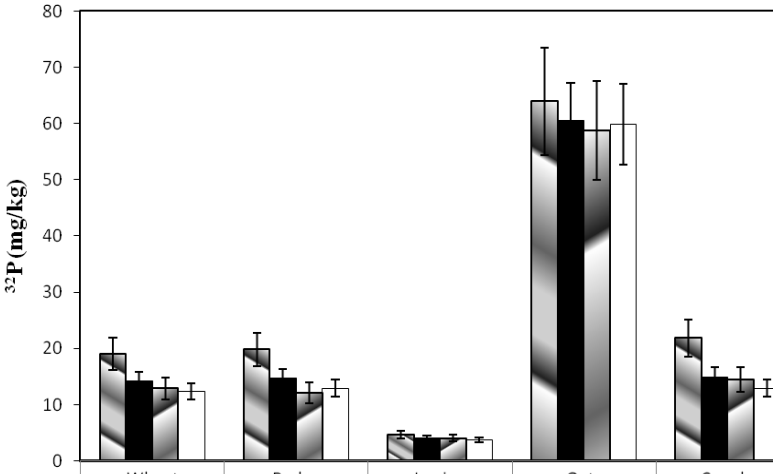


Fig. 1- Quenching standard curve plot from scintillation counting data (CPM) against series of progressively added ^{32}P labelled phosphine. Error bars indicate SD, n=9.

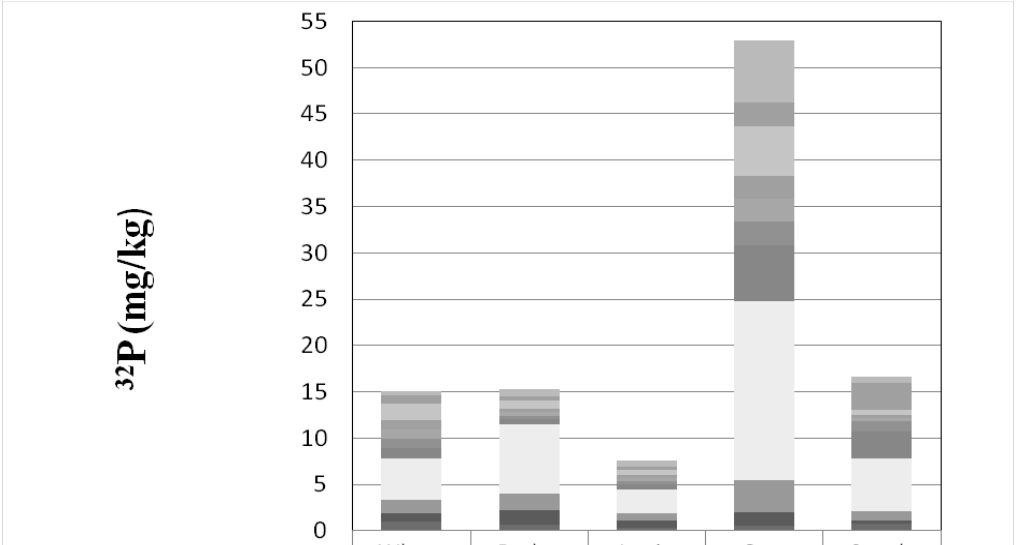


	Wheat	Barley	Lupine	Oats	Canola
Day 0	19.00	19.84	4.67	63.95	21.89
Day 1	14.26	14.73	4.10	60.50	14.95
Day 3	12.94	12.11	4.04	58.73	14.46
Day 7	12.37	12.94	3.79	59.93	12.91

Fig. 2- Total residue levels of ^{32}P (mg kg^{-1}) in grains at different times after aeration. Error bars indicate SD, n=9.

Uptake ^{32}P by grain fractions

The residues of ^{32}P in the extractions from treated grain samples are shown in Fig. 3. The amount of ^{32}P was calculated as mg ^{32}P equivalent to per kilogram of commodity (mg kg^{-1}). ^{32}P residues were found at high levels in organic acids, e.g., 29.2, 49.4, 35.1, 36.4 and 34.5% in wheat, barley, lupine, oats and canola. 66.3, 79.6, 66.9, 60.2 and 48.6% of total non-volatile ^{32}P residues in wheat, barley, lupins, oats and canola were present in water and acid soluble extractions (organic and amino acids, sugars, lignins and hemicelluloses). This could result in changes in grain and cereal nutrition and beer flavour. Further research will be conducted to determine how the phosphine affects grain qualities and beer flavour. In general, ^{32}P residues were at low levels in protopectins and non-cellulose polysaccharides, cellulose, lipids and hemicelluloses. The total amount of ^{32}P after seven days aeration period was 15.1, 15.3, 7.5, 52.9 and 16.6 mg kg^{-1} in fractions of wheat, barley, lupine, oats and canola. These results are consistent with results from whole grain extractions (Figures 2 and 3).



	Wheat	Barley	Lupin	Oats	Canola
Protopectins & non-cellulose Polysaccharides	0.47	0.79	0.61	6.71	0.71
Final Pellet	0.96	0.46	0.39	2.62	2.83
Lignins	1.75	0.91	0.55	5.25	0.60
Cellulose	0.95	0.35	0.33	2.55	0.35
Hemicellulose	1.03	0.40	0.33	2.37	0.34
Starch	1.00	0.40	0.37	2.67	1.13
Proteins	1.19	0.47	0.49	6.01	2.81
Organic Acids	4.42	7.58	2.65	19.28	5.73
Sugars	1.41	1.79	0.69	3.52	1.07
Amino Acids	0.95	1.52	0.82	1.46	0.32
Lipids	0.97	0.66	0.32	0.50	0.72

Fig. 3- The levels of ^{32}P residues (mg kg^{-1}) in different extractions from grains fumigated with ^{32}P labelled phosphine.

Distribution of ^{32}P in grains

Radiation imagery shows that more than 80% of ^{32}P residues were located or distributed in the embryo, testa, pericarp and husk of wheat, barley, canola, lupins and oats (Fig. 4). This work will contribute to the cereal and brewing industries by ensuring that residues that could affect nutrition and flavour will be minimized.

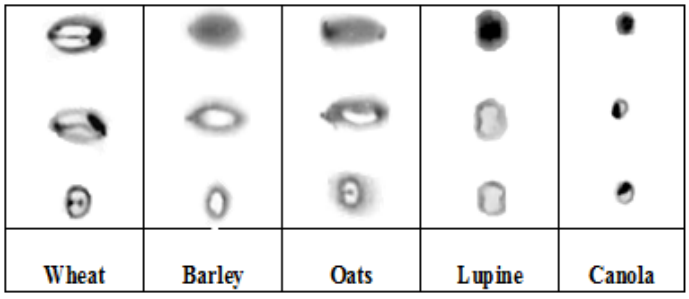


Fig. 4- Distribution and position of ^{32}P in the grains fumigated with ^{32}P labelled phosphine.

ACKNOWLEDGEMENTS

We would like to acknowledge the support of the Australian Government's Cooperative Research Centres Program. The support from the CRC for National Plant Biosecurity (CRCNPB) is gratefully acknowledged. We thank also Dr Mingbo Wang (CSIRO Plant Industry) for technical support.

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