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Project Leader Spencer S. Walse Location: USDA-ARS-SJVASC, Parlier, CA

Sorption and depuration of phosphine relative to methyl bromide following postharvest fumigation of grapes and citrus

Keywords: QPS fumigation, methyl bromide alternatives, food security, worker exposure

Abstract. Sorption, off-gassing (i.e., depuration), and residue data were obtained for both phosphine and methyl bromide following commercial procedures for chamber fumigation and cold-storage of table grapes, citrus, and bluberries. Results are presented in the context of quantifying fumigant inputs to ingestion exposure and worker inhalation exposure that are respectively derived from the consumption of fruit residues and off-gassing of palletized fruit in cold-storage. Relative to methyl bromide, ~10-fold less mass of phosphine is sorbed by palletized loads of fruit during fumigation, phosphine respectively off-gasses ca. >5-fold faster from loads of fruit in cold-storage, and respectively ca. >7-fold shorter amount of time is required for phosphine residues in fruit to meet USEPA food tolerances.

Problem and its Significance:

Further domestic and international regulation as outlined in the Montreal protocol is anticipated and may be directed at reducing the amount of methyl bromide that can be used to disinfest commodities involved for quarantine and preshipment (QPS) purposes. The fresh fruit industry relies heavily on methyl bromide to meet the phytosanitary treatment requirements of many QPS scenarios and will be greatly impacted by reduction and/or elimination of methyl bromide use, at least until an economically and technically feasible replacement is developed.

Fumigation with cylinderized phosphine (VAPORPH_3OS® or ECO_2FUME®) has marked potential as a methyl bromide replacement for fresh fruit treatments. Due to differences in physicochemical properties (e.g., Vapor pressure; phosphine @ 25°C = 2.93 E+04 mmHg, methyl bromide @ 25°C = 1.62 E+03 mmHg)(USEPA, 2011) and reactivity, phosphine offers several key advantages relative to methyl bromide with respect to nontarget exposure following treatment.

ARS conducted research that quantifies phosphine residues and off-gassing (depuration) rates in fresh fruit as related to ingestion exposure and worker exposure during post-fumigation handling/storage, respectively. Results are presented in direct comparison to analogous data obtained following postharvest methyl bromide fumigation and storage. The efficacy of phosphine and methyl bromide toward targeted pests, which is a critical component of establishing applied doses and durations for fumigations, was not evaluated nor discussed.

Long-term goal: Ensure pest-free security and food safety of fresh fruit in postharvest marketing channels via the development of efficient, economical, and environmentally benign chemical treatments. Retain and expand United States (US) imports and exports of fresh fruit.

Short-term goal: Obtain sorption and depuration data for both phosphine and methyl bromide following fumigation and storage of table grapes as well as citrus at treatment and storage temperatures based on industry-specific operational and logistical considerations.

Objectives:

1) Establish kinetic and equilibrium constants associated with phosphine sorption to fruit following fumigation of citrus at 37 °F as well as grapes and blueberries at 34°F. Establish kinetic and equilibrium constants associated with methyl bromide sorption to fruit following fumigation of citrus at 42 °F as well as grapes and bluberries at 40°F.

2) Conduct confirmatory-scale fumigations of packaged grapes (applied dose, duration; 64 mg/L methyl bromide, 3 h; 2500 ppmv (3.7 mg/L) phosphine, 24 h), citrus (applied dose, duration; 64 mg/L methyl bromide, 2 h; 2500 ppmv (3.6 mg/L) phosphine, 24 h), and blueberries (applied dose, duration; 72 mg/L methyl bromide, 2 h; 2500 ppmv (3.6 mg/L) phosphine, 24 h, using packaging materials and temperatures outlined in objectives 1 and 2.

3) Establish respective phosphine and methyl bromide depuration rates from palletized loads following confirmatory fumigations based on analyses conducted at post-fumigation sampling intervals of 0(just after aeration), 6 ,12, 18, 24, 30, 42, 48, 54, 60, 66, 72, 78, 84, 90, and 96 hours at storage temperatures of 37°F for citrus as well as 34°F for grapes and blueberries.

4) Quantify phosphine and methyl bromide residue levels inside/on fruit following confirmatory-scale fumigations (objective 2). Post-fumigation sampling intervals will be at 1, 4, 6, 11, 12, 14, 18, 24, 30, 42, 48, 54, 60, 66, 72, 78, 84, 90, and 96 h at storage temperatures of 37°F for citrus as well as 34°F for grapes and blueberries.

Sorption and Desorption Modeling.

The relative distribution of a fumigant between the solid and gas phases is described as the ratio:

$$K_d = [\text{fum.}]_s / [\text{fum.}]_g \qquad (1)$$

where K_d is termed the "solid to gas distribution coefficient", $[\text{fum.}]_s$ is the concentration of fumigant sorbed into the solid substrate, and $[\text{fum.}]_g$ is the concentration of fumigant in the air.

At equilibrium, the sorption and desorption of a gaseous fumigant to a solid are equal in magnitude. Upon the application of a fumigant to a solid substrate, there is a thermodynamic-based "drive" to achieve the solid-to-air distribution defined by the equilibrium where:

$$d[\text{fum.}]_s/dt = d[\text{fum.}]_g/dt = 0 \text{ and } K_d = K_{eq} \qquad (2)$$

K_d varies for each fumigant and changes as a function of many factors, including: total amount of fumigant applied, load of substrate, temperature, humidity, etc. However, over the range of conditions and concentrations that typify commercial fumigation scenarios, variation in K_d does not significantly affect modeling the kinetics of fumigant sorption and desorption.

Equilibrium distribution of a fumigant between headspace and substrate are never, or at least very rarely, reached during a conventional fumigation (nor during off-gassing in cold-storage). Under non-equilibrium conditions the sorption and/or desorption of MB is described as mass-transfer limited diffusion that is largely reversible (Darby 2008). Fruit and packaging sorb and desorb (i.e., off-gas) fumigants to varying extents due to differences in the rate of diffusion across the surface area of the load, as given by Fick's first and second laws describing gradient-flux (Banks 1985, Burg and Burg 1965, Walse 2012). Molecular diffusivity is generalized by the Stokes-Einstein equation

$$D = \frac{k_B T}{6\pi r \mu} \qquad (3)$$

where k_B is the Boltzmann constant (1.38×10^{-23} kg m^2 s^{-2} K^{-1}), and r is the hydrodynamic radius of "spherical" MB (~ 4.47 Å). Temperature (T) influences diffusivity via Brownian motion (numerator) and through changes to viscosity (μ) (denominator). However, the effect of temperature is expected to be negligible in this study where only 4 °F separated the temperature of the fruit types (grapes and oranges) undergoing phosphine fumigation and only 2 °F separated the temperature of the fruit types (grapes and oranges) undergoing methyl bromide fumigation (Walse et al., 2012b).

Fumigant sorption during fumigation, off-gassing (i.e., depuration) during storage (desorption), or loss as a gaseous "organic" residue from foodstuff (desorption) can be modeled as a non-steady state condition with variable surface concentration (Carslaw and Jaeger 1959, Crank 1975). If the case of sorption, the initial internal concentration is zero and the surface concentration at time, $\phi\,(t)$, varies linearly with a constant rate, k, such as:

$$\phi\,(t) = kt \qquad (4)$$

The converse occurs for desorption; the internal concentration at time, $\phi\,(t)$, varies

linearly with a constant rate, k, and the initial surface concentration is zero.

The change in fumigant concentration with time follows first order kinetics and is given by the equation:

$$\text{Sorption, } d[\text{fum.}]/dt = k_{OBS}\,[\text{fum.}] \quad (5)$$

$$\text{Desorption, } d[\text{fum.}]/dt = k_{\text{depuration}}\,[\text{fum.}] \quad (6)$$

where k_{OBS} (h^{-1}) is the observable rate constant of fumigant sorption and $k_{depuration}$ (d^{-1}) is the observable rate constant of depuration. Equations 5 and 6 can be further defined by,

$$k_{OBS} = a\ S\ k_{SPT} \quad (7)$$

where a (g L^{-1}) is the mass to volume ratio of the load, S (m^2 g^{-1}) is the surface area (SA) to mass ratio of the load, and k_{SPT} (Lm^{-2} h^{-1}) is the rate constant of fumigant sorption (or desorption) from the load.

The collective contribution of a fruit and its packaging toward the extent of fumigant sorption can be quantified indirectly via the measurement of fumigant loss from chamber headspace for each "packaged" load. Equation 7 was modified as in Walse (2012b) to conform with conventional measurements used in fumigation; mass is substituted with the volume of the packaged load, V_{PL}, such that

$$k_{OBS} = \left(\frac{V_{PL}}{V_{chamber}}\right)\left(\frac{SA}{V_{PL}}\right)\ k_{SPT\text{-combined}} \quad (8)$$

where the fractional load factor is $V_{PL}\ V_{chamber}^{-1}$ (unitless) and the surface area (SA) to volume (V_{PL}) ratio of the packaged load is $SA\ V_{PL}^{-1}$ (m^2 L^{-1}). Note that the $SA\ V_{PL}^{-1}$ term is nearly constant across commercial fumigations, at least in the US, because packaged loads are palletized into standard geometries (101.6 x 121.9 x 152.4 to 182.8 cm).

Solving equations 7 or 8 for k_{SPT} yields the rate of fumigant sorption (or desorption) that is intrinsic to a packaged load, independent of the load factor and the surface area to volume ratio. The kinetic description serves as a tool for quantifying the amount of fumigant that can be sorbed as well as the time it takes for this process to occur.

Methodology.

Objective 1. "Laboratory-scale sorption to fruit".

The kinetics and equilibrium of phosphine and methyl bromide sorption to fruit were evaluated in prelude to confirmatory testing. Briefly, the loss of each fumigant from chamber headspace was monitored in a series of exploratory fumigations performed in modified Labonco® 28.32-L vacuum chambers housed in a walk-in environmental incubator with programmable temperature and humidity (USDA, 2010). Source-gas cylinders, gas-tight syringes, non-fumigated control fruits, and test fruits of known volume and mass were acclimated to fumigation temperatures (i.e., tempered) for 12 h prior to treatment. The mass, volume, and surface area of fruits were measured or calculated from a representative population of fruit totaling 1 lb. These factors, which influence fumigant sorption (Banks 1985, Burg and Burg 1965, Walse 2012b), were used in the numerical models of sorption discussed below. Individual fruits were grouped into a fruit-specific 10-lbs load, bundled in cheesecloth, and transferred into a single chamber prior to fumigation.

Based on standard practices and recommendation of industry as related to importing Chilean-grown grapes as well as exporting US-grown fruits, methyl bromide fumigations were at temperatures of 42 °F for citrus and 40°F for grapes and blueberries. For VAPORPH$_3$OS® phosphine fumigations, exporters of US-grown fruit suggested treatment temperatures of 37 °F for citrus and 34°F for blueberries and grapes, the respective optimal storage temperatures. Commercial VAPORPH$_3$OS® phosphine fumigations of Chilean-grown exports are conducted with similar treatment temperatures for the respective fruit types.

Fruit pulp temperature was confirmed prior to fumigation by each of three probes (YSI scanning tele-thermometer) that record the respective pulp temperature in three uninfested berries distributed at different locations within the loaded chambers undergoing treatment. Temperature probes were then removed, circulation fans internal to the chamber turned on (circulations fans were not used for phosphine fumigations), and chamber lids clamp-sealed in preparation for treatment. A slight vacuum of approximately 76-127 mmHg was established in each chamber. Methyl bromide (>99.9%) was from a Meth-O–Gas 100 cylinder (Great Lakes, West Lafayette Indiana, USA) and phosphine was from a cylinder of 1.6 % (v/v) phosphine balanced with nitrogen (Cytec Canada, Inc., Niagara Falls, Ontario, Canada). Gas-tight super-syringes (Hamilton ® 500, 1000, or 1500 mL) were filled with a volume of fumigant to achieve the requisite dose. The syringes were fitted to a LuerLok ® sampling valve and subsequently opened so that fumigant was steadily drawn into the chamber. The syringe was then removed from the valve and normal atmospheric pressure (NAP) reestablished in each chamber before the valve was closed; this marked the beginning of the exposure period. Gas samples (40 mL) were taken from the chamber headspace through a LuerLok® valve using a B-D® 100 mL gas-tight syringe and quantitatively analyzed for methyl bromide with GC-FID or for phosphine with GC-PFPD at standard intervals corresponding to 5 (initial), 30, 60, 120, 180, 240, 1440 (one-day), and 2880 min (two-day).

Fumigant levels in headspace of fumigation chambers were measured using gas chromatography; retention time were used for chemical verification and the integral of peak area, referenced relative to liner least-squares analysis of a concentration – detector response curve, was used to determine concentration (Walse et al 2012a & b). Detector response and retention indices were determined each day in calibration studies by diluting known volumes of gaseous into volumetric gas vessels. Methyl bromide analyses were with a Varian 3800 and splitless injection (110 °C) using a gas sampling port with a 1-mL sample loop, a 2 mm id x 2 m Teflon® column packed with 10% OV-101 on Gas-Chrom Q® (100/120 mesh) held at 100 °C for 10 min. The FID detector was at 250 °C with respective flows of 30 mLmin^{-1} H_2 and 250 mLmin^{-1} air. Phosphine analyses were with a Varian 3800 and splitless injection (140 °C) using a gas sampling port with a 10 μL-sample loop, a Teflon column (L = 2 m, OD = 2 mm) packed with Porpak N (80/100 mesh)held at 130 °C for 10 min, and a PFPD detector (13 mL/min H_2, 20 mL/min air, and 10.0 mL/min N_2 make-up) at 250 °C that received only 10% of the 15 ml He/min column flow.

Objective 2. “Confirmatory-scale fumigation of palletized fruit”.

To simulate a commercial scenario, fumigations were conducted using a 15,000-L temperature-controlled Vacudyne steel chamber that accommodates a single pallet as the load (USDA, 2010). Chemcial analyses were as described above for the laboratory-scale fumigation studies. Packaging materials and treatment temperatures were consistent with the import and export scenarios described above and reported in Walse et al (2012c). Chamber load was estimated as a percentage ($V_{commodity}/V_{chamber}$ x 100) based on the method of (Monro, 1969) for table grapes (48.0%), blueberries (48.0%), and navel oranges (48.1%).

Procedures for methyl bromide fumigation were as reported in Walse et al. (2012a). Cylinderized phosphine fumigations were conducted using VAPORPH$_3$OS® that was dispensed with a Horn Diluphos System HDS-80. Application procedures were consistent with guidelines in the USDA-APHIS treatment manual (USDA, 2011). VAPORPH$_3$OS® phosphine

was applied using constant delivery of 3.7 mg/L phosphine. Delivery was terminated when three consecutive samples of chamber headspace, acquired through a sampling port that accessed the ventilation airstream, yielded ~3.7 mg/L via GC-analysis described above.

For methyl bromide fumigations, headspace samples were removed and analyzed at 5 (initial), 30, 60, 120 (orange end), and 180 min (grape end). Headspace samples of phosphine were removed and analyzed at 5 (initial), 30, 60, 240, 480, and 1440 min. Fumigant exposures were expressed as a concentration × time cross product, "CT", as calculated by the method of Monro (1969).

After the exposure period, chamber valves were opened to atmosphere and ventilation was initiated until headspace concentration of the fumigant was below mandated ventilation requirements for methyl bromide and phosphine, respectively 5 ppm (21μg/L) and 0.3 ppm (0.45μg/L). The chamber door was then opened and the treated pallet of fruit collected. Two boxes of navel oranges, 12 flats of blueberries, and four boxes of grapes were set aside from each fumigation to evaluate "off-gassing rates". Samples of fumigated fruit (75 g each) selected from 3 different locations of remaining pallet were placed into a cooler filled with dry ice within 5 minutes of the end of aeration and were used to estimate initial residue levels. The fruit on the treated pallets were transferred (along with non-fumigated control fruit) to cold-storage at 37 °F for citrus and 34 °F for blueberries and grapes. Fruit was temporally retrieved from storage and used for residue determination(s)(*vide infra*).

Objective 3. "Off-gassing rates".

Following confirmatory-scale fumigations of palletized fruit, representative fruit boxes were used to evaluate the rate of fumigant depuration from the treated load. Two 40-lb cardboard boxes of navel oranges(88 count, 30l × 46w × 32h cm)of the type intended for export to Korea, 12 cardboard trays (25.40 x 39.37 x 8.26 cm) with each tray consisting of two layers of six plastic clamshell containers (~ 170 g of fruit each), four 20-lb corrugated plastic boxes table grapes (60l × 12w × 40h cm) with packaging and materials (e.g., liners and sulfur dioxide pads) consistent with commercial import from Chile were transferred to respective 241.9-L steel chambers housed in a walk-in environmental incubator with programmable temperature and humidity (USDA, 2010) set to cold-storage at 37 °F for oranges and 34 °F for grapes and blueberries. The chamber accommodating the boxes of oranges was first loaded with four 0.5 ft^3 sand bags each wrapped in plastic packaging that displaced ~56.6 L total of chamber volume. Chamber loads of 48.0% 48.0, and 48.1%, respectively, for table grapes, blueberries, and navel oranges were consistent with the confirmatory fumigations.

At 6-h sampling intervals, aliquots of headspace were removed through the LuerLok ® sampling valve on each chamber with a 500-μL gas sampling syringe for each analysis of fumigant concentration. The concentration (mg/L) of fumigant measured in the headspace at a particular sampling interval is representative of duplicate analysis of headspace from the each of the five confirmatory fumigations for each fruit-fumigant pairing and are reported as $\bar{x} \pm s$ (n = 6). After completion of sampling at each time interval, chamber valves were opened to atmosphere and vacuum was pulled for 15 min to aerate the chamber (and ensure equilibrium was not achieved between headspace and load). Sampling was terminated when fumigant concentrations in headspace dropped below analytical limits of quantification.

Gas chromatography was used to analyze off-gassing (i.e., depuration) of methyl bromide as well as phosphine; chemical verification and quantification was as described above, however, instrumentation differed. For methyl bromide analyses, pulsed-splitless injections were with a Hewlett Packard 6890 gas chromatograph at port temperature of 125 °C and introduced via a gas-sampling valve with a 100μL-sample loop (100 °C) onto a GasPro analytical column (L = 30 m, ID = 0.32mm), Agilent Technologies, #113-4332) with an initial pressure pulse of 30 psi for 1.9 min that was reduced to 4.0 $mLmin^{-1}$ (59 $cmsec^{-1}$) He carrier flow. Initial oven temperature of

90°C was maintained for 10 min and ramped at 40 °Cmin^{-1} to a final temperature of 200 °C and held for an additional 3 min. Detection of methyl bromide was with a μECD at 275 °C with 60mL min^{-1} N_2 make-up flow. The method limit of quantification for methyl bromide was 0.00005 mg/L. For phosphine analyses, injections were with a Varian 3800 gas chromatograph and introduced via a gas-sampling valve with a 250μL-sample loop (100 °C) onto a PORAPLOT Q Column (L = 12.5 m, ID = 0.53mm, 20 μm flim thickness) with a column flow of 2.8 mLmin^{-1} He. Initial oven temperature of 80°C was maintained for 5 min. Detection of phosphine was with PFPD detector (13mL/min H_2, 17 mL/min air, and 10.0 mL/min N_2 make-up) at 250 °C. The method limit of quantification for phosphine was 0.00001 mg/L.

Objective 4. “Residue levels in fruit”.

Residues resulting from confirmatory-scale fumigations were quantified via a modified method of King et al. (1981) following each confirmatory trial. Samples for determining initial residues levels were gathered as described above within 5-10 min of the post-fumigation aeration/ventilation period. Subsequent residue sampling of fruit at storage temperatures (37°F for citrus and 34°F for grapes and blueberries) followed at 0 (initial), 1, 4, 6, 11, 12, 14, 18, 24, 30, 42, 48, 54, 60, 66, 72, 78, 84, 90, and 96 h. Fruit (~100 g) was randomly gathered from respective fumigation loads and pooled in a cloth bag to create a single sample. This process was repeated three times, and also at each sampling interval, to yield triplicate samples of fruit for each respective fumigation. Each bag was emptied, 75 g of fruit was gravimetrically measured, and then transferred to a 500-mL air-tight glass blending-vessel (Eberbach Corp., No. E8470.00) filled with 200 mL of freshly prepared and degassed 0.01M $NaHCO_3$ buffer at pH 7 (HCl-adjusted), 0.1μ NaCl, and ~15 °C. Polypropylene lids, equipped with rubber gaskets and a LuerLok ® sampling valve, were screwed into place, pressure tested for tightness-of-fit, and the fruit-buffer mixture was macerated for 1 min with a laboratory blender (Waring model no. 5BA60VL22) equipped with a General Electric 1/5 hp explosion proof motor. The motor speed was controlled with a Powerstat Variable Transformer, Type 116B, set at 80 % power. Vessels were stored at 15.0 ± 0.4 °C ($\bar{x} \pm s$) for 24 h and then an aliquot of headspace was withdrawn with a 250 μL-Pressure-Lok® glass syringe and analyzed using gas chromatography for methyl bromide or phosphine as described above. Two aliquots of headspace were removed from each vessel for each analysis.

Gas chromatography was used to analyze “organic” residues of gaseous methyl bromide as well as gaseous phosphine; chemical verification, quantification, and instrumentation were as described above for “off-gassing”. For calibration studies, volumetric gas-blending jars were filled with buffer solution and non-fumigated fruit. Measured volumes of gaseous fumigant are then injected through the septa covering the sampling port and then the samples are processed as described below. The Henry’s law liquid-to-gas distribution coefficient of each fumigant in the glass blending-vessels was determined for each fruit over the range of 1-100,000 ppb and were used to calculate the concentration of fumigant residues (ppm, ug/g - fruit) at a particular sampling interval. Residue levels are representative of duplicate analysis of headspace from the triplicate samples and is reported as $\bar{x} \pm s$ (n = 6). For illustrative purposes, a regression was fitted to the data to reflect change in residue levels over the sampling time course. Temporal sampling was terminated when residues levels fell below the limits of quantification.

Results and Discussion.

Objective 1. “Laboratory-scale sorption to fruit”.

In laboratory-scale fumigations of 10-lb fruit samples, experimental data support the kinetic model developed above, as plots of “ln ([fumigant]$_0$/[fumigant]$_t$)” versus time (t-t_o) measured as loss from headspace were linear over 2 h. The negative slope obtained from a least-squares analysis, k_{OBS} (h^{-1}), was determined; mean values and standard deviations ($\bar{x} \pm s$, n = 5) are shown in Figure 1 for methyl bromide and phosphine fumigations of grapes as well as oranges under the fumigation conditions

described above. Correlation coefficients (r^2) were based on composite regressions of replicate analyses and had values $r^2 > 0.9$. Figure 1 shows: phosphine is sorbed by oranges ~9-fold faster than methyl bromide; phosphine is sorbed by blueberries ~3-fold faster than methyl bromide, while table grapes sorb phosphine ~25-fold faster than methyl bromide.

As a fumigation progresses through time, there is an increase in the likelihood of equilibrium between the fumigant levels in chamber headspace and the sorbent (i.e., load). The equilibrium positioning respective to a particular dose/concentration is quantified by measuring the loss of gas from headspace over time and plotting headspace concentration per unit volume (x-axis) versus sorbent concentration per unit mass (y–axis).The change in the fruit to headspace distribution of fumigants, K_d, over a 48-h time course is shown in Figure 2 as composite regressions of triplicate analyses at respective treatment temperatures and initial applied headspace concentration (~applied dose). As change in the K_d decreases, equilibrium is approached. Note several salient features of Figure 2: 1) phosphine approaches equilibrium with the fruit faster than methyl bromide as shown by a more rapid "stabilization" of K_d, 2) phosphine has less of an affinity for fruit than does methyl bromide as shown by smaller K_d values, and 3) the "K_d trend-line" stabilizes (i.e., no Δ on y-axis) to a greater extent for phosphine than for methyl bromide. We have interpreted this final observation to be due to relatively less irreversible sorption and/or reactivity of phosphine with the substrate. Residue data presented below as well as numerous examples in literature (Flingelli et al., 2010; Klementz et al. 2005) support the interpretation that phosphine forms less bound-residues and reaction-byproduct residues in fresh fruit over 24 to 48-h fumigations than methyl bromide does over 2 to 4 h fumigations. Of course, one also must consider that the amount of methyl bromide typically employed in fruit fumigation is at least 30-fold greater in mass than is typically applied for a phosphine fumigation.

Objective 2. "Confirmatory-scale fumigation of palletized fruit".

Using procedures consistent with those of the fresh fruit industry, confirmatory-scale chamber fumigations were conducted to evaluate phosphine residues and off-gassing (depuration) during cold-storage as related to respective inputs for ingestion exposure and worker inhalation exposure. Analogous studies were conducted with methyl bromide for comparison. Differential sorption of phosphine between replicate trials of the same fruit type resulted in a range of toxic exposures, expressed as concentration x time products, for: grapes (84.8 to 88.8 mg h L^{-1}), bluberries (83.2 to 85.4 mg h L^{-1}), and oranges (81.7 to 82.8 mg h L^{-1}). Sorption following a 24-h exposure was estimated as a percentage ($[fumigant]_0$ -$[fumigant]_t$ x 100) and resulted in respective means of 15.1, 17.5, and 18.9% for table grapes, blueberries, and navel oranges (Table 1). Methyl bromide fumigation of table grapes for 3 h, blueberries for 2 h, and navel oranges for 2h also resulted in a range of CT exposures for grapes (175.9 to 181.8 mg h L^{-1}, blueberries (121.4 to 124.7 mg h L^{-1}) and oranges (120.6 to 123.1 mg h L^{-1}) with corresponding mean sorptions of 6.1, 34.0 and 19.9%(Table 2). It is interesting to note that ~4 mg/L of methyl bromide was sorbed by grapes, while only ~0.6 mg/L phosphine was sorbed. Even a larger disparity was observed for blueberry and orange fumigations. It is critical to note that the amount of fumigant sorbed by palletized fruit load serves as a conservative proxy for maximum inputs to ingestion exposure via fruit-residues and worker inhalation exposure to off-gassing palletized loads of fruit in cold-storage.

With respect to the aeration of chambers following fumigation, kinetic data on fumigant desorption presented below indicates that ~ 20-25% (*vide infra*) of the sorbed methyl bromide will off-gas (i.e., depurate) from the palletized loads of fruit during the (label-specified) 4-h ventilation time required for methyl bromide levels to drop below 5 ppm (21μg/L) in chamber headspace. It is difficult to estimate how much of the sorbed phosphine will off-gas during the aeration period that is required to last only until 0.3 ppm (0.45μg/L) is detected in chamber headspace. Under identical aeration times and conditions, however, a relatively greater percentage of the sorbed phosphine is expected to off-gas from palletized loads of fruit as compared to the percentage of sorbed methyl bromide expected to off-gas.

Objective 3. “Off-gassing rates”.

Experimental data support the first-order kinetic model of fumigant depuration from boxes of fruit; plots of “ln ($[fumigant]_0/[fumigant]_t$)” versus time ($t-t_o$) measured as loss from headspace were linear. The rate constant of depuration for each fumigant, $k_{depuration\text{-}fumigant}$ (h^{-1}), the negative slope obtained from a least-squares analysis, was reported as a mean ($\bar{x} \pm s$) and a composite regressions of replicate analyses is shown (Figure 3). Depuration half-life for grapes ($k_{depuration\text{-}methyl\ bromide} = 0.021 \pm 0.007\ h^{-1}$, $t^{1/2} \approx 33$ h; $k_{depuration\text{-}phosphine} = 0.44 \pm 0.03\ h^{-1}$, $t^{1/2} \approx 1.6$ h), oranges ($k_{depuration\text{-}methyl\ bromide} = 0.090 \pm 0.01\ h^{-1}$, $t^{1/2} \approx 7.8$ h; $k_{depuration\text{-}phosphine} = 0.46 \pm 0.03\ h^{-1}$, $t^{1/2} \approx 1.5$ h), and blueberries ($k_{depuration\text{-}methyl\ bromide} = 0.101 \pm 0.02\ h^{-1}$, $t^{1/2} \approx 6.8$ h; $k_{depuration\text{-}phosphine} = 0.47 \pm 0.03\ h^{-1}$, $t^{1/2} \approx 1.5$ h),were estimated for respective storage temperature of 37°F for citrus and 34°F for grapes and blueberries. It is interesting to note that the observable rate of fumigant sorption by the palletized load, k_{OBS} in equation 5, was nearly equivalent to the observable rate of off-gassing/depuration from the boxes of fruit, $k_{depuration}$ in equation 6. This result can be used to develop a protocol for determining aeration requirements to maintain headspace in cold-storages (of known volume) below thresholds that ensure worker safety, however, this application is outside the scope of the current investigation.

Objective 4. “Residue levels in fruit”.

Once the fruit were transferred to cold-storage at 37 °F for oranges and 34 °F for grapes and blueberries, residues were quantified through time (Figure 4). The rate of residue loss, via fumigant desorption/depuration from the fruit, also followed the first-order kinetic model (equation 6); plots of “ln ($[fumigant]_0/[fumigant]_t$)” versus time ($t-t_o$) were linear. The rate constant of residue loss for each fumigant, $k_{residue\text{-}fumigant}$ (h^{-1}), the negative slope obtained from a least-squares analysis, was reported as a mean ($\bar{x} \pm s$) and a composite regressions of replicate analyses is shown (Figure 5). Half-lifes for residue loss from grapes ($k_{residue\text{-}methyl\ bromide} = 0.019 \pm 0.005\ h^{-1}$, $t^{1/2} \approx 36$ h; $k_{residue\text{-}phosphine} = 0.371 \pm 0.02\ h^{-1}$, $t^{1/2} \approx 1.9$ h), blueberries ($k_{residue\text{-}methyl\ bromide} = 0.073 \pm 0.007\ h^{-1}$, $t^{1/2} \approx 9.5$h; $k_{residue\text{-}phosphine} = 0.461 \pm 0.02\ h^{-1}$, $t^{1/2} \approx 1.5$ h), and oranges ($k_{residue\text{-}methyl\ bromide} = 0.063 \pm 0.009\ h^{-1}$, $t^{1/2} \approx 11$ h; $k_{depuration\text{-}phosphine} = 0.45 \pm 0.03\ h^{-1}$, $t^{1/2} \approx 1.6$ h) were estimated for respective storage temperature of 37°F for citrus and 34°F for grapes and bluberries. Initial residue levels of methyl bromide in grapes, blueberries, and oranges were respectively 19.5 ± 1.3 ppm, 11.2 ± 1.1, and 12.4 ± 1.0 ppm. Considering these intial residue levels, cold-storage of grapes for ~9 d, blueberries for ~1.3 d, and oranges for ~1.4 d is required to reach USEPA TRED-listed residue tolerences of 0.5 ppm and 2 ppm for berries (grapes and blueberries) and citrus, respectively. Initial residue levels of phosphine in grapes, blueberries, and oranges were respectively 3.36 ± 0.07 ppm, 0.94 ± 0.06 ppm, and 1.0 ± 0.08 ppm, which would require cold-storage of all fruit types for < 13 h to reach residues levels of 0.01 ppm, the USEPA residue tolerence for phosphine in citrus.

References.

Banks, N. 1985. Estimating skin resistance to gas diffusion in apples and potatoes. J. Exper. Bot. 36: 1842-1850.

Banks, H.J. 1989. Needs for R & D in fumigation and controlled atmospheres for grain storage, pp. 237-246. *In* Champ, B.R., Highley, E. and Banks, H.J. (eds) Fumigation and Controlled Atmosphere Storage of Grain. Australia Centre for International Agricultural Research, National University of Singapore, Singapore.

Burg, S., and E. Burg. 1965. Gas Exchange in Fruits. Physiol. Plantarum. 18: 870-882.

Carslaw, H.S., and J.C. Jaeger. 1959. Conduction of Heat in Solids. 2nd ed., Oxford University Press, London

Crank, J. 1975. *The Mathematics of Diffusion*. London: Oxford University Press, London
King, Jimmie R., Benschoter, C. A., Burditt, Jr., A. K. 1981. Residues of methyl bromide in fumigated grapefruit determined by a rapid, headspace assay. J. Agric. Food Chem. 29: 1003-1005.

Flingelli, G.K., Klementz, D. W.,Brash, D.W.; Reichmuth, C. 2010 Residues of phosphine following fumigation of kiwifruit. Proceedings of Methyl Bromide Alternatives and Emissions Research Conference. November 2-5. Orlando FL.

Klementz, D. W., Heckemuller, H.; Reichmuth, C., Huyskens-Keil, S., Buttner, C., Horn, F., Horn, P. 2005. Disinfestation of table grapes with pure phosphine-residues and quality aspects. Proceedings of Methyl Bromide Alternatives and Emissions Research Conference. November 10-13. San Diego CA.

Monro, H.A.U., 1969. Manual of Fumigation for Insect Control. FAO Agricultural Studies 79, 381.

Obenland, D.M., Neipp, P.W. 2005. Forced hot air treatment of stone fruit to inhibit the development of mealiness. Acta Horticulturae 682:1171-1178.

Obenland, D.M., Collin, S., Mackey, B.E., Sievert, J., Arpaia, M. 2011. Storage temperature and time influences sensory quality of mandarin oranges by altering soluble solids, acidity and aroma volatile composition. Postharvest Biology and Technology. 59:187-193.

USDA. 2010. Fumigation and Chemistry Group of the Commodity Protection and Quality Research Unit, USDA, Agricultural Research Service, SJVASC, Parlier, CA 93648 http://www.ars.usda.gov//Main/site_main.htm?docid=18577 [Accessed on Mar 15, 2011].

USDA 2011. USDA-APHIS-PPQ Treatment manual. http://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/treatment.pdf. Date accessed, Dec. 20, 2011.

USEPA. 2011. MPVPWIN v4.10.

Walse, S.S; Bellamy, D.E., Krugner, R.; Tebbets, J.S.(2012a)Postharvest treatment of California USA strawberries with methyl bromide to eliminate the spotted wing drosophila, Drosophila suzukii, in exports to Australia. *J. Asian-Pac. Entomol.* 2012, 15, 451-456.

Walse, S.S; Liu, Y.B.; Myers, S.W.; Bellamy, D.E., Obenland, D; Simmons, G.S.; Tebbets, J.S. (2012b)The treatment of fresh fruit from California with methyl bromide for postharvest control of light brown apple moth*, Epiphyas postvittana* (Walker) *J. Econ. Entomol.* (in press) 2012.

Walse, S.S.; Krugner, R. Tebbets, S. (2012c) Residues and phytotoxicity associated with postharvest treatment of sweet cherries with methyl bromide to control spotted wing drosophila, *Drosophila suzukii* California Cherry Advisory Board and Northwest Horticulture Council report for USDA-APHIS for negotiation with Korea and Japan, June 17, 2012.

Winchester, R.V.; (1978) Determination of total bromide in cereals, nuts, and dried fruits by X-ray fluorescence spectroscopy*. New Zealand Journal of Science*, 21, 335.

Figure 1. Relative rate of methyl bromide and phosphine sorption into fruit under conditions used by industry for each fruit and fumigant. Phosphine sorbs faster to grapes and oranges over the initial 2-h period of fumigation.

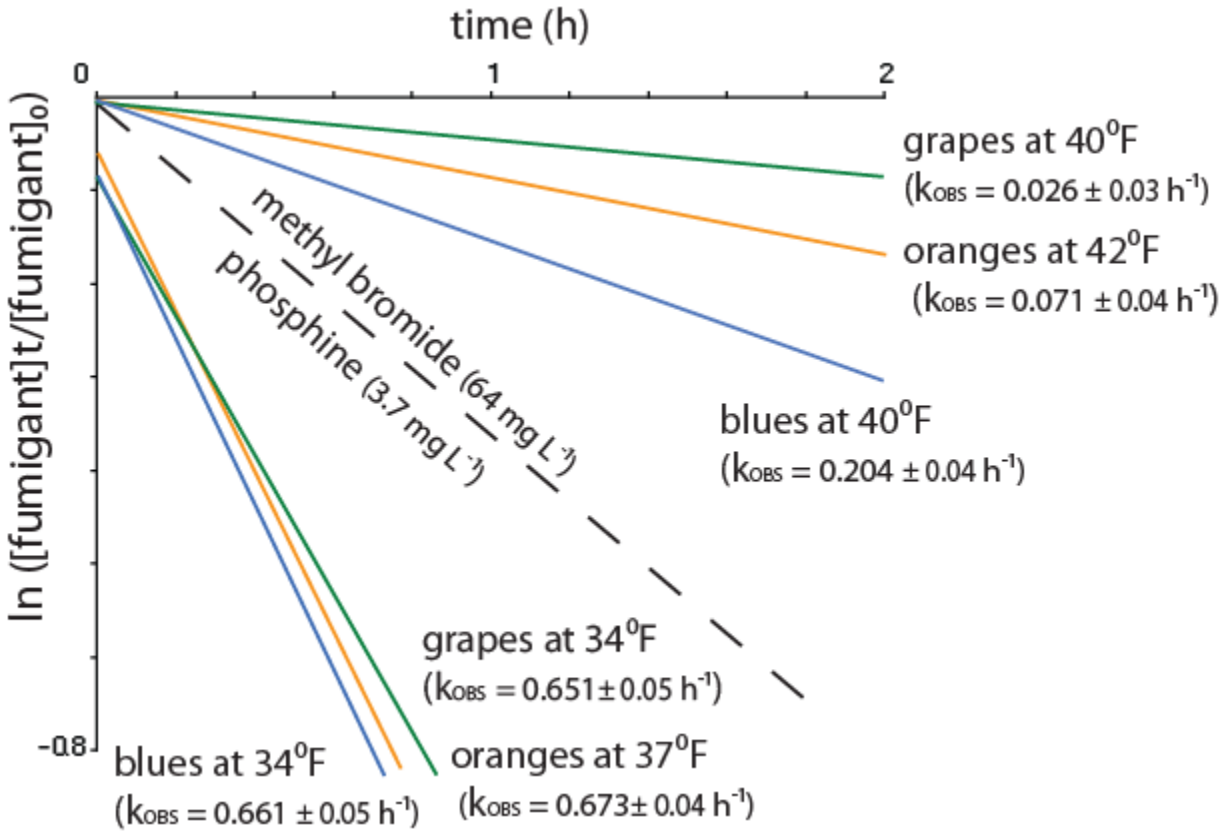

Figure 2. Change in the fruit to headspace distribution of fumigants, K_d, over a 48-h time course. As change in the K_d decreases, equilibrium is approached, but never reached due to the formation of irreversible-bound residues and reaction of the fumigant with substrate.

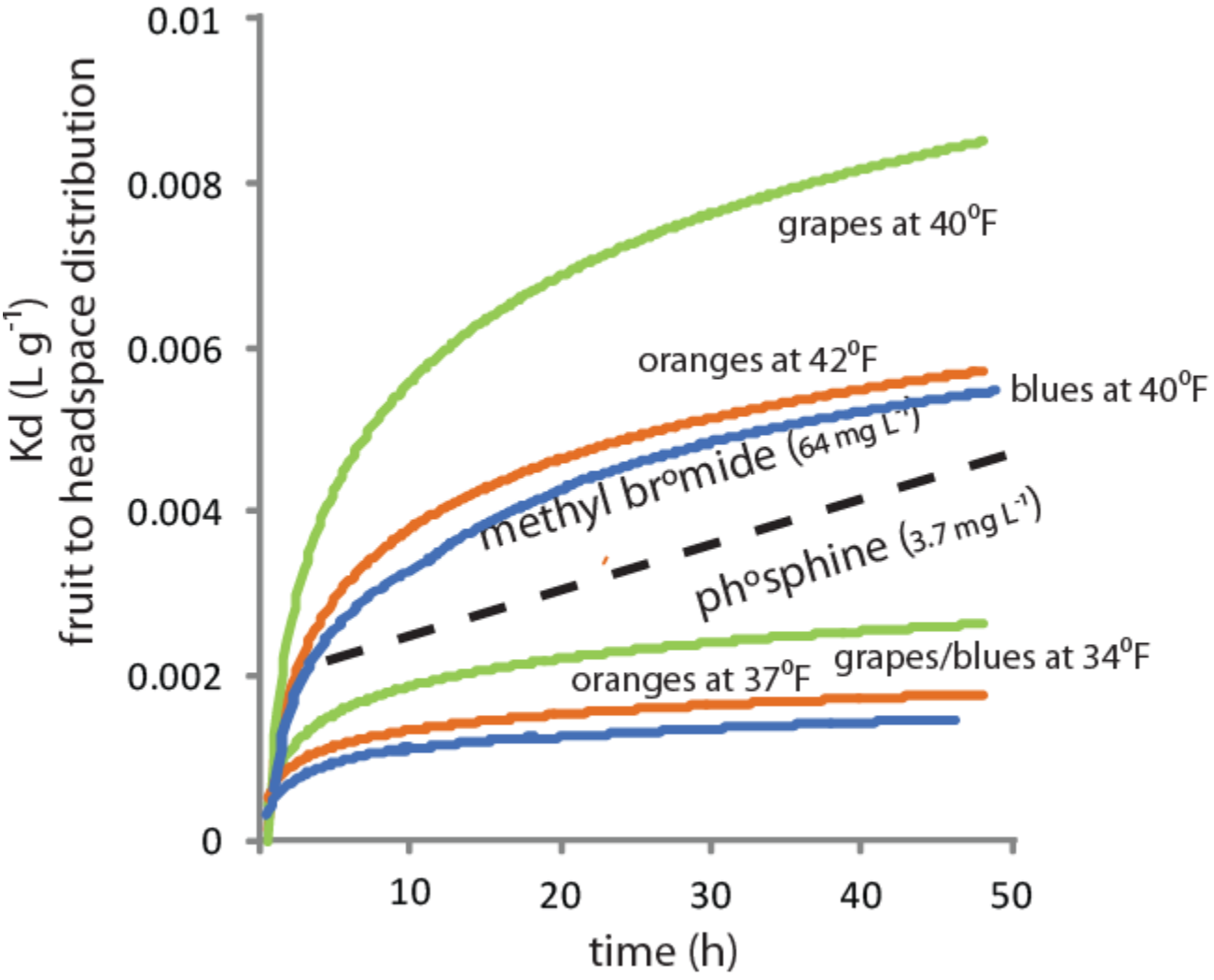

Table 1. Listing of confirmatory-scale fumigation trials conducted with phosphine on palletized loads of table grapes and blueberries at 34.0 ± 0.8 °F ($\bar{x} \pm s$) as well as and navel oranges at 37.0 ± 0.8 °F. (mg/L = g/m³ = oz./1000 ft³)

Trial #	Fruit	Applied (mg/L)	Temp. (±0.5 °C)	Temp. (±0.8 °F)	Load (%)	0 h [PH_3]	4h [PH_3]	8 h [PH_3]	24 h [PH_3]	CxT Exposure ± 5.1 ($mgL^{-1}h$)	% Sorp.
1	grapes	3.7	1.6	34.0	48.0	4.0	3.8	3.7	3.3	86.4	17.6
2	grapes	3.7	1.6	34.0	48.0	3.9	3.9	3.8	3.4	88.8	12.6
3	grapes	3.7	1.6	34.0	48.0	3.8	3.8	3.7	3.4	87.0	12.3
4	grapes	3.7	1.6	34.0	48.0	3.9	3.8	3.7	3.5	87.7	12.0
5	grapes	3.7	1.6	34.0	48.0	3.8	3.7	3.7	3.2	84.8	16.9
											15.1 (ave.)
1	oranges	3.7	2.7	37.0	48.1	3.8	3.7	3.6	3.1	82.8	18.6
2	oranges	3.7	2.7	37.0	48.1	3.8	3.7	3.5	3.2	82.7	17.8
3	oranges	3.7	2.7	37.0	48.1	3.9	3.7	3.5	3.1	82.2	19.9
4	oranges	3.7	2.7	37.0	48.1	3.9	3.7	3.4	3.1	81.7	19.0
5	oranges	3.7	2.7	37.0	48.1	3.9	3.8	3.4	3.1	82.2	19.2
											18.9 (ave.)
1	blueberry	3.7	1.6	34.0	48.1	3.8	3.7	3.6	3.1	83.2	18.4
2	blueberry	3.7	1.6	34.0	48.1	3.8	3.8	3.7	3.2	85.4	15.7
3	blueberry	3.7	1.6	34.0	48.1	3.9	3.7	3.6	3.2	84.2	17.9
4	blueberry	3.7	1.6	34.0	48.1	4.0	3.9	3.6	3.2	85.2	20.0
5	blueberry	3.7	1.6	34.0	48.1	3.9	3.8	3.6	3.3	85.4	15.4
											17.5 (ave.)

Table 2. Listing of confirmatory-scale fumigation trials conducted with methyl bromide on palletized loads of table grapes and blueberries at 40.0 ± 0.8 °F ($\bar{x} \pm s$) and navel oranges at 42.0 ± 0.8 °F.(mg/L = g/m³ = oz./1000 ft³)

Trial #	Fruit	Applied (mg/L)	Temp. (±0.5 °C)	Temp. (±0.8 °F)	Load (%)	0 h [MB]	1/2 h [MB]	1 h [MB]	1.5 h [MB]	2 h [MB]	2.5 h [MB]	3 h [MB]	CxT Exposure ±5.1 ($mgL^{-1}h$)	% Sorp.
1	grapes	64.0	4.4	40.0	48.0	60.7	60.0	59.3	58.9	58.5	57.6	57.0	176.6	6.2
2	grapes	64.0	4.4	40.0	48.0	60.0	59.6	59.4	58.6	58.2	57.5	57.0	175.9	4.9
3	grapes	64.0	4.4	40.0	48.0	62.1	61.4	60.0	59.3	58.9	58.2	57.8	178.9	6.9
4	grapes	64.0	4.4	40.0	48.0	63.5	62.4	61.8	60.4	59.2	58.7	58.5	181.8	7.9
5	grapes	64.0	4.4	40.0	48.0	60.4	59.8	58.7	58.5	58.4	57.9	57.6	176.2	4.6
														6.1 (ave.)
1	oranges	64.0	5.5	42.0	48.1	71.1	62.6	61.0	57.5	56.7	-	-	121.5	20.3
2	oranges	64.0	5.5	42.0	48.1	70.2	62.7	60.5	57.3	56.4	-	-	120.7	19.7
3	oranges	64.0	5.5	42.0	48.1	70.7	63.2	60.9	57.2	56.8	-	-	121.3	19.6
4	oranges	64.0	5.5	42.0	48.1	70.9	62.5	60.1	57.0	56.6	-	-	120.6	20.2
5	oranges	64.0	5.5	42.0	48.1	71.4	64.5	61.4	58.2	57.4	-	-	123.1	19.6
														19.9 (ave.)
1	blueberry	72.0	4.4	40.0	48.0	74.1	69.4	62.5	-	48.9	-	-	124.7	34.0
2	blueberry	72.0	4.4	40.0	48.0	75.0	66.2	61.8	-	49.2	-	-	122.9	34.8
3	blueberry	72.0	4.4	40.0	48.0	75.5	67.5	62.2	-	50.2	-	-	123.9	35.2
4	blueberry	72.0	4.4	40.0	48.0	72.4	67.4	59.9	-	51.2	-	-	121.4	32.5
5	blueberry	72.0	4.4	40.0	48.0	73.5	69.8	60.1	-	50.9	-	-	122.9	33.5
														34.0 (ave.)

Figure 3. Fumigants “off gas” (i.e., depurate) uniformly from boxed fruit over the course of cold storage with loss that follows first-order kinetic approximations given the applied doses and corresponding treatment temperature utilized in this study.

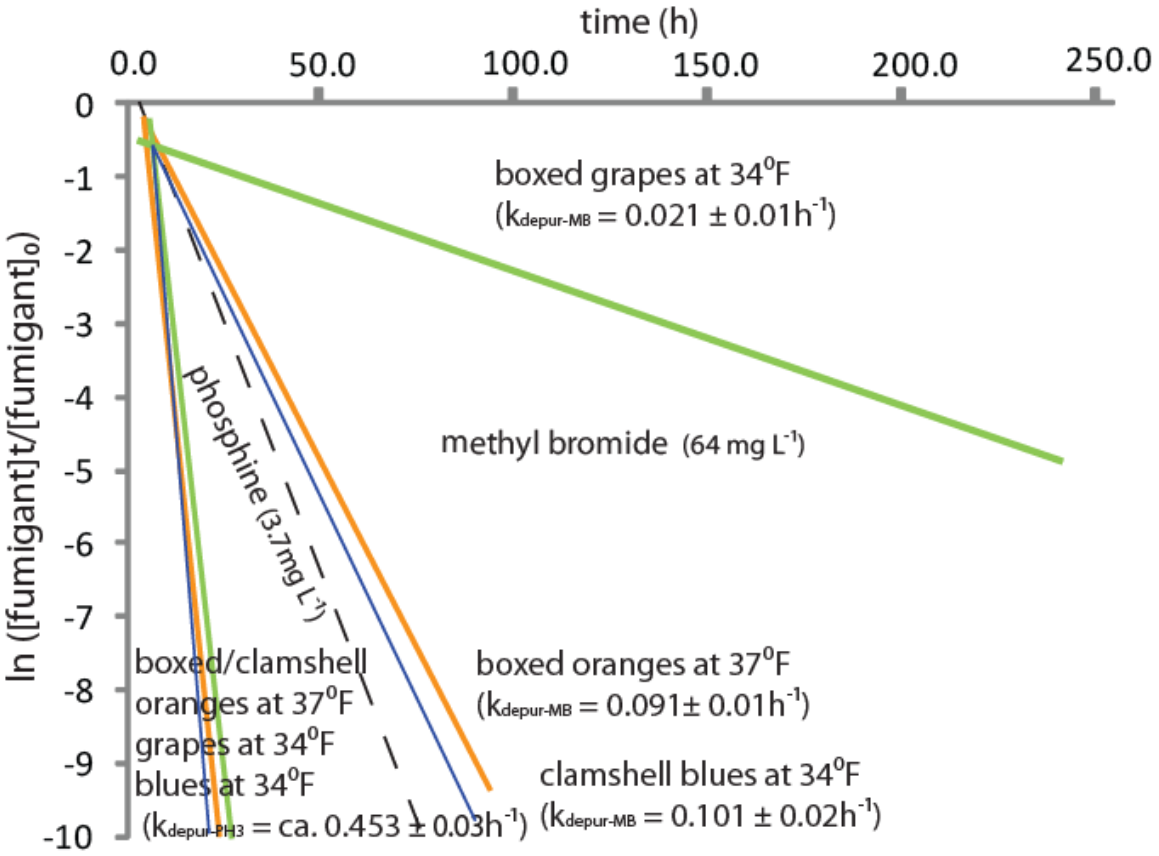

Figure 4. "Organic" (i.e., gaseous) fumigant residues in fumigated fruit decreased uniformly over the course of cold-storage at 37 °F for oranges and 34 °F for grapes and blueberries. Methyl bromide requires time-scales of days and phosphine requires timescales of hours to reach USEPA food tolerences for both methyl bromide and phosphine residues in fruit (dashed red lines).

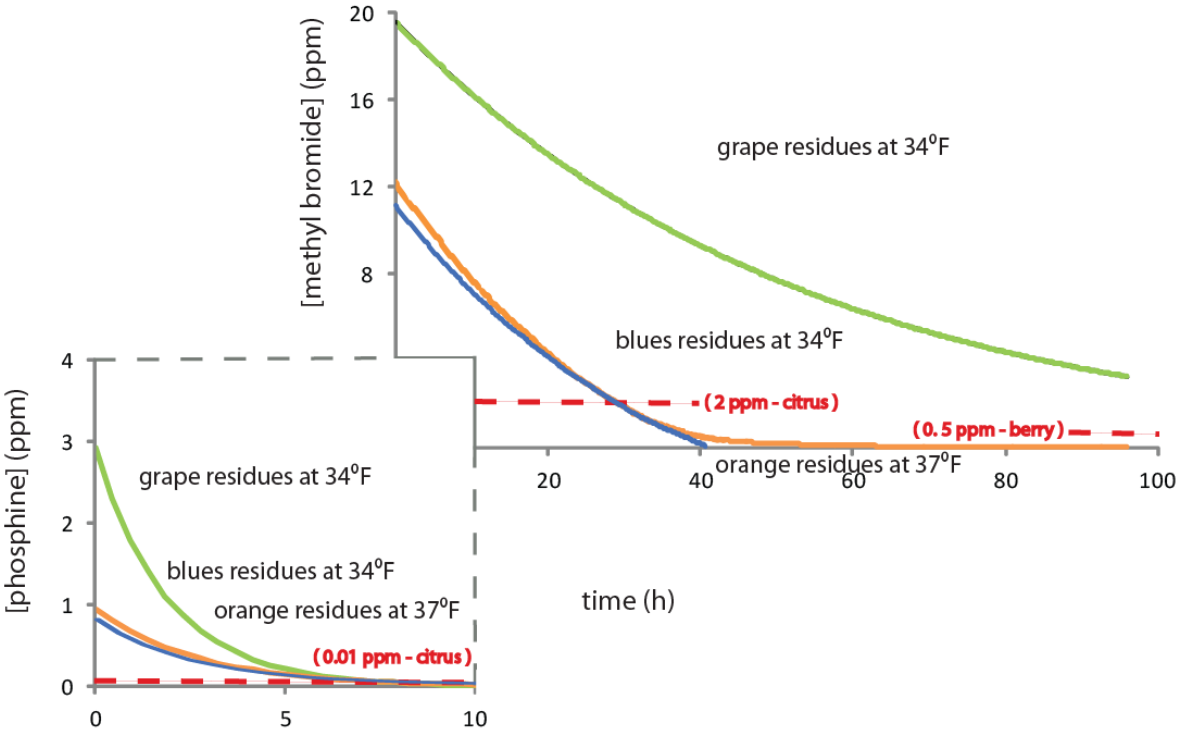

Figure 5. The rate of residue loss, via fumigant desorption/depuration from fruits, also followed the first-order kinetic model. Using the rate constant of residue loss for each fumigant, $k_{residue\text{-}fumigant}$ (h^{-1}), half-lives of residue loss were calculated for grapes ($k_{residue\text{-}methyl\ bromide}$ = 0.019 ± 0.005 h^{-1}, $t^{1/2}$ ≈ 36 h; $k_{residue\text{-}phosphine}$ = 0.371 ± 0.02 h^{-1}, $t^{1/2}$ ≈ 1.9 h), blueberries ($k_{residue\text{-}methyl\ bromide}$ = 0.073 ± 0.007 h^{-1}, $t^{1/2}$ ≈ 9.5h; $k_{residue\text{-}phosphine}$ = 0.461 ± 0.02 h^{-1}, $t^{1/2}$ ≈ 1.5 h), and oranges ($k_{residue\text{-}methyl\ bromide}$ = 0.063 ± 0.009 h^{-1}, $t^{1/2}$ ≈ 11 h; $k_{depuration\text{-}phosphine}$ = 0.45 ± 0.03 h^{-1}, $t^{1/2}$ ≈ 1.6 h) under optimal cold-storage temperatures.