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Multinomial Probabilities for Orthophosphate Isotopomers

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Abstract

It is of interest to understand how phosphorus cycles through organic pools in the environment. Much of the cycling phosphorus is in the form of orthophosphates (species derived from H_3PO_4) which are thus an important part of the global phosphorus cycle. An understanding of the flows of orthophosphate species is important in understanding the loss of phosphorus from soil and the environmental effects of using phosphorus in fertilisers. A methodology has been proposed for tracking orthophosphate flows using isotopomers of this substance. Using this methodology in practice requires the application of multinomial probabilities. This article describes the main chemistry concepts involved, derives the expected distribution of molecular weights for two models describing how elements mix in the formation of orthophosphate, and compares the models to observed data from two experiments. A simple random mixing model agrees reasonably well with the available data.

1 Introduction

Some Chemistry Background: Isotopes and Isotopomers

An understanding of the progress of orthophosphates through a natural system is important in environmental management. One way of tracking orthophosphate movements is to produce orthophosphates using water enriched in oxygen atoms with higher atomic weight. These can then be subsequently distinguished from orthophosphates from other sources, for example, by using a mass spectrometer.

Oxygen atoms with different atomic weights are referred to as isotopes, and the resulting orthophosphate molecules with different molecular weights are called isotopomers. In general, "isotopes are any of the several different forms of an element each having different atomic mass. Isotopes of an element have nuclei with the same number of protons (the same atomic number) but different numbers of neutrons" (Wikipedia, 2010). Isotopomers are compounds whose constituent atoms have the same atomic numbers, but differ in the number of neutrons. For example:

- Oxygen (O) has 8 protons and primarily consists of the isotope which also has 8 neutrons (written as ^{16}O). Another isotope, ^{18}O, has 10 neutrons and is very rare in nature.
- It is possible to purchase enriched water where a nominated percentage (e.g. 95%) of the molecules contain ^{18}O which has 10 neutrons and the remainder consists mostly of ^{16}O.
- The isotope ^{17}O, which has 9 neutrons, also occurs in nature in small quantities.
- The following are all isotopomers of orthophosphate: $H_3P^{18}O_4$; $H_3P^{16}O_4$; $H_3P^{18}O_2{}^{16}O_2$; and $H_3P^{18}O_3{}^{16}O$. All of these alternatives contain 3 H atoms, one P atom and 4 O atoms. However, the 4 O atoms consist of different numbers of ^{16}O and ^{18}O.

Producing Orthophosphate Isotopomers

Orthophosphate species can be produced in the laboratory using the following chemical reaction:

(1) $POCl_3 + 3\ H_2O \rightarrow H_3PO_4 + 3HCl$

Different orthophosphate isotopomers can be produced by using enriched water as an input to the reaction, rather than natural water.

Depending on the mixture of oxygen isotopes in the enriched water used in the reaction, the output from the reaction will include a range of orthophosphate isotopomers. Table 1 summarises all 15 possible isotopomers and their molecular masses. Note that different isotopomers sometimes have the same mass.

Table 1: Orthophosphate Isotopomers

Number	Isotopomer	Number of Atoms of:			Rounded Molecular Mass (m/z value)
		O16	O17	O18	
1	$H_2P^{16}O_4^-$	4	0	0	97
2	$H_2P^{16}O_3{}^{17}O^-$	3	1	0	98
3	$H_2P^{16}O_2{}^{17}O_2^-$	2	2	0	99
4	$H_2P^{18}O^{16}O_3^-$	3	0	1	99
5	$H_2P^{16}O^{17}O_3^-$	1	3	0	100
6	$H_2P^{16}O_2{}^{17}O^{18}O^-$	2	1	1	100
7	$H_2P^{17}O_4^-$	0	4	0	101
8	$H_2P^{18}O_2{}^{16}O_2^-$	2	0	2	101
9	$H_2P^{16}O^{17}O_2{}^{18}O^-$	1	2	1	101
10	$H_2P^{17}O_3{}^{18}O^-$	0	3	1	102
11	$H_2P^{16}O^{17}O^{18}O_2^-$	1	1	2	102
12	$H_2P^{17}O_2{}^{18}O_2^-$	0	2	2	103
13	$H_2P^{18}O_3{}^{16}O^-$	1	0	3	103
14	$H_2P^{17}O^{18}O_3^-$	0	1	3	104
15	$H_2P^{18}O_4^-$	0	0	4	105

A proportion (around 30%) of the orthophosphate (H_2PO_4) from reaction (1) decays into metaphosphate (H_2PO_3). Table 2 summarises the 9 possible isotopomers of metaphosphate, which have 7 possible values of rounded molecular mass (between 79 and 85).

Table 2: Metaphosphate Isotopomers

Number	Isotopomer	Number of Atoms of:			Rounded Molecular Mass (m/z value)
		O16	O17	O18	
1	$H_2P^{16}O_3^-$	3	0	0	79
2	$H_2P^{16}O_2{}^{17}O^-$	2	1	0	80
3	$H_2P^{16}O^{17}O_2^-$	1	2	0	81
4	$H_2P^{18}O^{16}O_2^-$	2	0	1	81
5	$H_2P^{17}O_3^-$	0	3	0	82
6	$H_2P^{16}O_1{}^{17}O^{18}O^-$	1	1	1	82
7	$H_2P^{18}O_2{}^{16}O_1^-$	1	0	2	83
8	$H_2P^{17}O_2{}^{18}O^-$	0	2	1	83
9	$H_2P^{17}O^{18}O_2^-$	0	1	2	84
10	$H_2P^{18}O_3$	0	0	3	85

A Random Mixing Model for the Distribution of Orthophosphate Isotopomers

To track the progress of orthophosphate through a system, it is necessary to know something about the relative frequencies of the isotopomers of orthophosphate. This would be greatly simplified if it could be assumed that the oxygen isotopes from the enriched water are distributed "at random" across the orthophosphate and metaphosphate molecules produced from the reaction.

Let X, Y and Z be the number of atoms of ^{16}O, ^{17}O and ^{18}O, respectively, in an orthophosphate or metaphosphate molecule. So X+Y+Z=n, where n is the number of oxygen atoms in each molecule, with n=4 for orthophosphate, and n=3 for metaphosphate. Let p_X, p_Y and p_Z be the proportions of ^{16}O, ^{17}O and ^{18}O, respectively, in the water used in the reaction (1). A large sample of (X,Y,Z) results from the output of the chemical reaction. If the oxygen isotopes are distributed at random across the orthophosphate or metaphosphate molecules, then (X,Y,Z) will be multinomially distributed with parameters (n, p_X, p_Y , p_Z).

The expected relative frequencies of the isotopomers in Tables 1 and 2 can be calculated using this assumption. We would like to compare these to the experimentally observed frequencies, to test the assumption. However, there is a further complication that the mass spectrometer can only measure relative frequencies of molecules with given masses rather than frequency by isotopomers.

Outline of this Article

This article describes two models for the expected relative frequencies of orthophosphate and metaphosphate molecules by rounded molecular weight. Section 2 sets up notation and derives the expected relative frequencies under the first model, a random mixing model. The expected relative frequencies by molecular mass are also discussed. Section 3 describes an alternative mixing model, called the "persistence model", where it is assumed that the $POCl_3$ always contributes exactly one oxygen atom and the input water contributes n-1 oxygen atoms at random to each molecule of orthophosphate or metaphosphate.

Section 4 discusses the fitting of the two mixing models using experimental data. The approach is illustrated using experimental data. The model fitting process is greatly simplified if it can be assumed that the proportion of O17 is negligible. This case is discussed in Section 5. Conclusions are stated in Section 6.

2 The Random Mixing Model

Trinomial Probabilities under Random Mixing

We let X, Y and Z be the number of atoms of ^{16}O, ^{17}O and ^{18}O, respectively, in an orthophosphate or metaphosphate molecule. So X+Y+Z=n in all cases, since each molecule contains n O's, where n=4 for orthophosphate, and n=3 for metaphosphate. Let p_X, p_Y and p_Z be the proportions of ^{16}O, ^{17}O and ^{18}O, respectively, in the water used in the reaction (1). Under the random mixing assumption, (X,Y,Z) will be multinomially distributed with parameters (n, p_X, p_Y , p_Z). This means that

(2) $$P[X = x, Y = y, Z = z] = \frac{n!}{x!\,y!\,z!} p_X^x p_Z^z p_Z^z$$

for each x, y, z = 0, 1, …, n such that x+y+z=n.

Expression (2) can be used to give the expected proportions of each of the 15 isotopomers of orthophosphate, or 10 isotopomers of metaphosphate.

Grouping by Molecular Mass

In practice the experimenter can only measure the relative frequencies by molecular mass, not by isotopomer. There are 9 possible values for the molecular mass of orthophosphate (97 to 105 inclusive) and 7 possible values for metaphosphate (79 to 85 inclusive). The expected proportions for each mass can be found by adding up the expected proportions for each isotopomer with that molecular weight.

3 Persistence Mixing Model

Let p_{X1}, p_{Y1} and p_{Z1} be the proportions of ^{16}O, ^{17}O and ^{18}O, respectively, in the input $POCl_3$ in reaction (1). These are assumed to be the natural abundances of these isotopes: $p_{X1}=0.9976$, $p_{Y1}=0.0004$, and $p_{Z1}=0.0020$. Let p_{X2}, p_{Y2} and p_{Z2} be the proportions in the input enriched water in the reaction.

It is possible that the bond between the P and O atoms in $POCl_3$ persists during reaction (1). If so, this would mean that each molecule of orthophosphate produced by the reaction contains exactly one O atom from the input $POCl_3$ and exactly 3 atoms from the input water. It is further assumed that the 3 atoms from the input water are taken at random from all of the reacting input water.

Let X, Y and Z be the number of atoms of ^{16}O, ^{17}O and ^{18}O, respectively, in an orthophosphate or metaphosphate molecule, as before. Let X_1, Y_1 and Z_1 be the number of ^{16}O, ^{17}O and ^{18}O atoms, respectively, in a molecule which come from $POCl_3$. Let X_2, Y_2 and Z_2 be the number of ^{16}O, ^{17}O and ^{18}O atoms, respectively, coming from reacting water. The alternative mixing model implies that:

$X=X_1+X_2$
$Y=Y_1+Y_2$
$Z=Z_1+Z_2$
$(X_1, Y_1, Z_1) \sim$ multinomial$(1, p_{X1}, p_{Y1}, p_{Z1})$
$(X_2, Y_2, Z_2) \sim$ multinomial$(n-1, p_{X2}, p_{Y2}, p_{Z2})$
(X_1, Y_1, Z_1) and (X_2, Y_2, Z_2) independent.

This defines the distribution of (X,Y,Z). We derive the probability function of (X,Y,Z) as follows:

$$
\begin{aligned}
P[X=x,Y=y,Z=z] &= P[X=x,Y=y,Z=z \mid X_1=1,Y_1=0,Z_1=0]p_{X1} \\
&+P[X=x,Y=y,Z=z \mid X_1=0,Y_1=1,Z_1=0]p_{Y1} \\
&+P[X=x,Y=y,Z=z \mid X_1=0,Y_1=0,Z_1=1]p_{Z1} \\
&= P[X_2=x-1,Y_2=y,Z_2=z]p_{X1} + P[X_2=x,Y_2=y-1,Z_2=z]p_{Y1} \\
&+P[X_2=x,Y_2=y,Z_2=z-1]p_{Z1} \\
&= \left\{ \begin{array}{ll} \dfrac{(n-1)!}{(x-1)!y!z!} p_{X2}^{x-1} p_{Y2}^{y} p_{Z2}^{z} p_{X1} & \text{if} \quad x>0 \\ 0 & \text{if} \quad x=0 \end{array} \right\} \\
&+ \left\{ \begin{array}{ll} \dfrac{(n-1)!}{x!(y-1)!z!} p_{X2}^{x} p_{Y2}^{y-1} p_{Z2}^{z} p_{Y1} & \text{if} \quad y>0 \\ 0 & \text{if} \quad y=0 \end{array} \right\} \\
&+ \left\{ \begin{array}{ll} \dfrac{(n-1)!}{x!y!(z-1)!} p_{X2}^{x} p_{Y2}^{y} p_{Z2}^{z-1} & \text{if} \quad z>0 \\ 0 & \text{if} \quad z=0 \end{array} \right\} \\
&= \frac{n!}{x!y!z!} p_{X2}^{x} p_{Y2}^{y} p_{Z2}^{z} \left((x/n)\frac{p_{X1}}{p_{X2}} + (y/n)\frac{p_{Y1}}{p_{Y2}} + (z/n)\frac{p_{Z1}}{p_{Z2}} \right)
\end{aligned}
$$

for each x, y, z = 0, 1, …,n such that x+y+z=n.

4 Model Fitting and Numerical Examples

Table 3 shows the results of two experiments measuring the distribution of molecular weights of orthophosphate and metaphosphate resulting from reaction (1), with two different preparation of enriched water. Preparation 1 is described in Thomas et al (2010) and preparation 2 is from Alvarez et al (2000). The results shown are the mean of 5 replicated experiments. Standard errors were calculated using the standard deviation of at five replicate analyses of the one preparation and reaction (rather than of replicate preparations) divided by the number of observations. This probably underestimates the uncertainty attached to these estimates, because only one reaction was conducted for each preparation, with replicates of the mass spectrometry measurement only. Hence these errors represent measurement variability from the mass spectrometry, and not variation in reaction conditions and inputs.

The proportions p_X, p_Y and p_Z (of ^{16}O, ^{17}O and ^{18}O, respectively) are known approximately. They are not known precisely because the isotopic signature for O in $POCl_3$ was assumed to be natural (Rosman and Taylor 1997), and because it is difficult to exclude extraneous water. As a result, it is necessary to estimate p_X, p_Y and p_Z from the observed data.

Estimates of p_X, p_Y and p_Z under the Random Mixing Model

The method of estimating p_X, p_Y and p_Z , assuming the random mixing model described in Section 2, was as follows. Let Y_{ij} be the observed proportion of

molecules or orthophosphate or metaphosphate with atomic weight i in replication j, for a given preparation. Let $\mu_i = \mu_i(p_X, p_Y, p_Z)$ be the expected proportion under the random mixing model. Estimates of p_X, p_Y and p_Z were calculated by minimising the least squares criteria:

$$\sum_{j=1}^{5}\sum_{i}\left(Y_{ij} - \mu_i(p_X, p_Y, p_Z)\right)^2 .$$

The resulting estimates are shown in Table 4. Jack-knife standard errors are also shown, obtained by recalculating the least squares estimates dropping one of the 5 replicates in each case.

Table 3: Observed Distribution of Molecular Weights from Two Experiments
(Values are means of five repeated measurements)

Ion	Prepn # 1	Prepn#2	Ion	Prepn #1	Prepn#2
$[H_2PO_4]^-$	$^{16/18}O \approx$ 60/40 [a]	$^{16/18}O \approx$ 32.8/67.2 [a]	$[PO_3]^-$	$^{16/18}O \approx$ 60/40 [a]	$^{16/18}O \approx$ 32.8/67.2 [a]
m/z	Observed proportions (%)		m/z	Observed proportions (%)	
97	15.42 ± 0.13	1.14 ± 0.04	79	23.28 ± 0.11	2.91 ± 0.04
98	2.75 ± 0.02	0.59 ± 0.02	80	4.58 ± 0.13	0.62 ± 0.26
99	36.97 ± 0.25	9.03 ± 0.14	81	43.21 ± 0.43	21.10 ± 0.20
100	2.29 ± 0.05	0.98 ± 0.01	82	1.36 ± 0.35	0.00 ± 0.00
101	30.05 ± 0.25	27.56 ± 0.10	83	21.67 ± 0.39	43.75 ± 0.33
102	0.72 ± 0.30	2.01 ± 0.02	84	1.82 ± 0.34	1.21 ± 0.31
103	9.62 ± 0.10	38.11 ± 0.25	85	4.09 ± 0.13	30.42 ± 0.22
104	0.72 ± 0.05	0.00 ± 0.00			
105	1.46 ± 0.15	20.58 ± 0.18			

a: based on preparation of enriched water

Table 4: Least Squares Estimates of p_X, p_Y and p_Z under the Random Mixing Model for Two Experiments

Ion	Prepn # 1	Prepn#2	Ion	Prepn #1	Prepn#2
$[H_2PO_4]^-$	$^{16/18}O \approx$ 60/40 [a]	$^{16/18}O \approx$ 32.8/67.2 [a]	$[PO_3]^-$	$^{16/18}O \approx$ 60/40 [a]	$^{16/18}O \approx$ 32.8/67.2 [a]
Oxygen Isotope	Estimated proportions (%) in total reaction mass		Oxygen Isotope	Estimated proportions (%) in total reaction mass	
16	63.77 ± 0.13	32.00 ± 0.19	16	62.77 ± 0.13	32.23 ± 0.23
17	1.45 ± 0.13	0.95 ± 0.04	17	2.34 ± 0.38	0.50 ± 0.18
18	34.79 ± 0.14	67.06 ± 0.21	18	34.89 ± 0.28	67.27 ± 0.16

a: based on preparation of enriched water

Estimates of p_{X2}, p_{Y2} and p_{Z2} under the Persistence Model

In this model, the problem is to estimate p_{X2}, p_{Y2} and p_{Z2} , the proportions of O16, O17 and O18 respectively in the input enriched water. Let $\mu_i = \mu_i(p_{X2}, p_{Y2}, p_{Z2})$ be the expected proportion of orthophosphate or metaphosphate with molecular weight i, under the persistence model. Estimates were calculated by minimising the least squares criteria:

$$\sum_{j=1}^{5}\sum_{i}\left(Y_{ij} - \mu_i\left(p_{X2}, p_{Y2}, p_{Z2}\right)\right)^2 .$$

The resulting estimates are shown in Table 5, along with jack-knife standard errors.

Table 5: Least Squares Estimates of p_{X2}, p_{Y2} and p_{Z2} under the Persistence Model for Two Experiments

Ion	Prepn # 1	Prepn#2	Ion	Prepn #1	Prepn#2
$[H_2PO_4]^-$	$^{16/18}O \approx$ 60/40 [a]	$^{16/18}O \approx$ 32.8/67.2 [a]	$[PO_3]^-$	$^{16/18}O \approx$ 60/40 [a]	$^{16/18}O \approx$ 32.8/67.2 [a]
Oxygen Isotope	Estimated proportions in input enriched water (%)		Oxygen Isotope	Estimated proportions in input enriched water (%)	
16	53.54 ± 0.16	20.83 ± 0.21	16	48.81 ± 0.12	21.83 ± 0.17
17	2.49 ± 0.16	4.58 ± 0.05	17	4.54 ± 0.47	6.72 ± 0.16
18	43.97 ± 0.20	74.58 ± 0.23	18	46.64 ± 0.47	71.45 ± 0.26

a: based on preparation of enriched water

Comparison of the Observed Data with the Random Mixing and Persistence Models

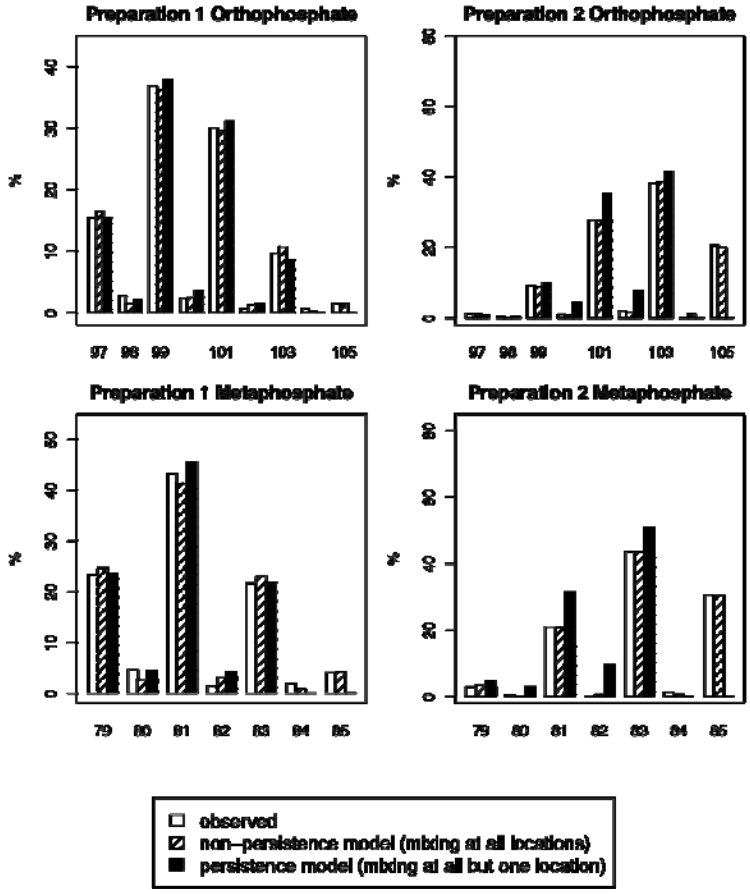
Table 6 show the fitted probabilities of the random mixing and persistence models, respectively, for each molecular weight, for orthophosphate and metaphosphate. Figure 1 shows these results, along with the observed data (mean across 5 replications). The figure shows that for preparation 1, the two models give similar fitted probabilities, both close to the observed data. For preparation 2, the random mixing model agrees closely with the observed data, but the persistence model is at odds with the data.

The persistence model implies too few molecules with the largest possible molecular weights (105 for orthophosphate, 85 for metaphosphate). This is because this model assumes that exactly one oxygen atom in the orthophosphate or metaphosphate comes from the input $POCl_3$, which is not enriched in heavy oxygen. This would make it very unlikely that all of the O atoms in the output orthophosphate or metaphosphate are heavy (^{18}O). It is clear from the preparation 2 results that the persistence assumption does not hold. The preparation 1 results are much less conclusive, because the input water was much less enriched, so that the heaviest orthophosphate and metaphosphate molecules would be rare under both models.

Table 6: Model Predictions for Two Mixing Models (parameters estimated by least squares)

m/z	Mixing in all four positions (*non-persistence hypothesis*)		Mixing in three positions (*persistence hypothesis*)	
	Preparation 1	Preparation 2	Preparation 1	Preparation 2
79	24.73	3.37	23.77	4.75
80	2.77	0.16	4.43	2.93
81	41.34	21.03	45.69	31.58
82	3.08	0.65	4.25	9.60
83	22.98	43.76	21.80	51.00
84	0.85	0.68	0.02	0.04
85	4.25	30.35	0.04	0.10
Meta Total (79-85)	100.00	100.00	100.00	100.00
97	16.53	1.05	15.31	0.90
98	1.50	0.12	2.14	0.60
99	36.12	8.79	37.85	9.82
100	2.46	0.78	3.53	4.28
101	29.58	27.64	31.14	35.18
102	1.34	1.64	1.46	7.65
103	10.75	38.61	8.54	41.47
104	0.24	1.15	0.01	0.03
105	1.46	20.21	0.02	0.08
Ortho Total (97-105)	100.00	100.00	100.00	100.00

Figure 1: Observed Percentages (Means of 5 Replications) and Model Predictions for Two Mixing Models (parameters estimated by least squares) (see Table 6 for tabular version of these results)



5 Analysis of Experimental Data assuming that the Proportion of O17 is Negligible

The analyses from Section 4 become much simpler if it can be assumed that the proportion of O17 is negligible. In this case, the distribution of the number of O18 atoms in each orthophosphate or metaphosphate molecule is binomial rather than trinomial. Moreover, there is a one to one correspondence between the molecular weight of orthophosphate or metaphosphate molcule and the number of O16/O18 atoms in the molecule. Table 4 suggests that the proportion of O17 is only around 2% or less, so that it may be reasonable to ignore this isotope.

If there is no O17, then only the odd valued molecular weights (79, 81, 83, 85, 97, 99, 101, 103 and 105) can occur. Table 7 shows the observed percentages at these molecular weights. Restricting to the odd valued molecular weights has another advantage, in that none of the molecular weights are very rare (all are >1%). This is convenient, because mass spectrometers tend to give biased measurements for rare molecular weights.

The last row of Table 7 shows estimates of the proportions of O16 and O18 in the reactant. This is more straightforward to calculate in the binomial case, so that the least squares approach described in Chapter 3 is not needed. The mean molecular weight of orthophosphate is equal to 33 plus 4 times the mean atomic weight of the oxygen atoms in this material. The mean atomic weight of oxygen atoms is equal to the proportion of O16 times 16, plus the proportion of O18 times 18. Simple manipulations then give:

Prop'n of O18 = (Mean M.Wt of Orthophosphate – 97) / 8.

Similarly, the proportion of O18 in the metaphosphate is equal to

Prop'n of O18 = (Mean M.Wt of Metaphosphate – 79) / 6.

These simple relationships were used to calculate the estimates of O16 and O18 in Table 7.

Fitted values from the random mixing and persistence models were calculated using these estimated proportions of O16 and O18, assuming that no O17 was involved. Table 8 shows these values. Figure 2 plots the observed proportions for each molecular weight against the predicted values for the two models.

Conclusions are similar to those in Section 4. The random mixing model fits the preparation 2 data reasonably well, whereas the persistence model clearly does not agree with the data, as it predicts too few molecular weights of 85 and 105. The two models are similar to each other and to the data for preparation 1. The omission of O17 from the analyses makes very little difference.

Table 7: Observed Distribution of Odd-Valued Molecular Weights from Two Experiments (Values are means of five repeated measurements)

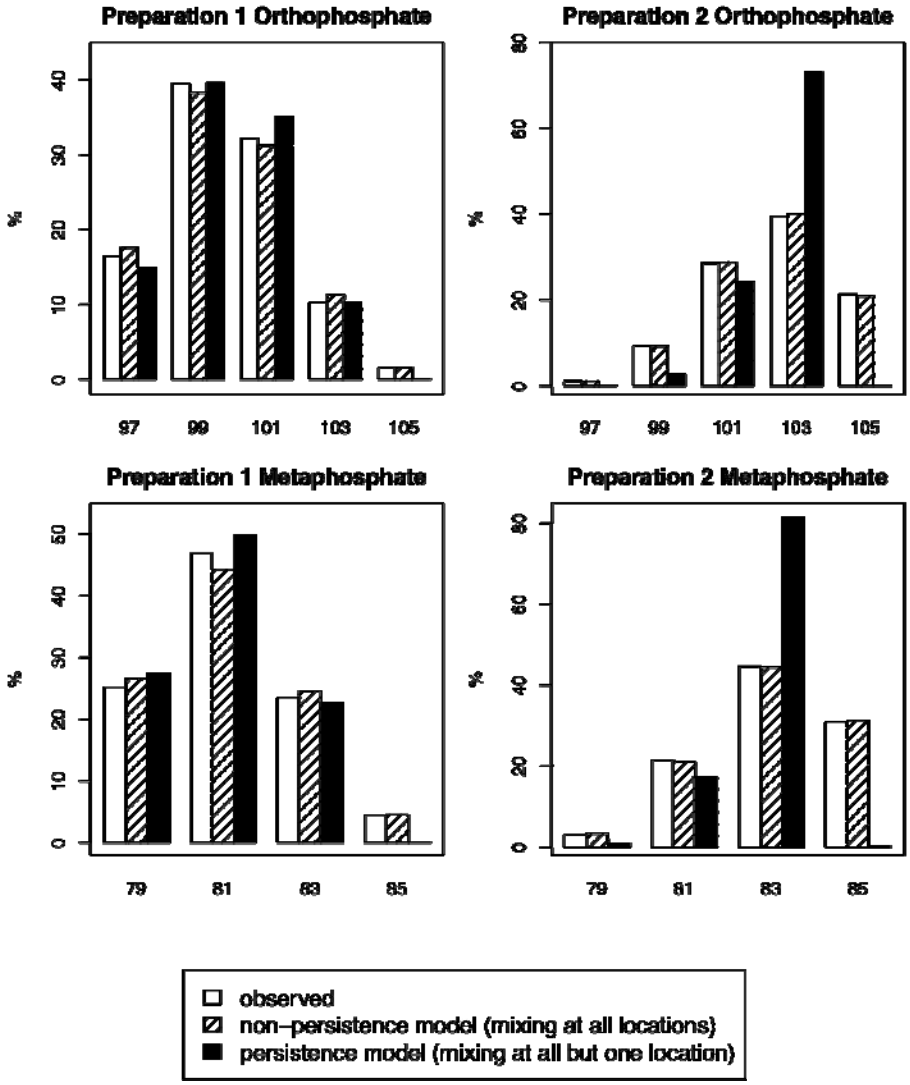
Ion	Prepn # 1	Prepn#2	Ion	Prepn #1	Prepn#2
$[H_2PO_4]^-$	$^{16/18}O \approx$ 60/40 [a]	$^{16/18}O \approx$ 32.8/67.2 [a]	$[PO_3]^-$	$^{16/18}O \approx$ 60/40 [a]	$^{16/18}O \approx$ 32.8/67.2 [a]
m/z	Observed proportions (%)		m/z	Observed proportions (%)	
97	16.49 ± 0.16	1.18 ± 0.04	79	25.24 ± 0.13	2.97 ± 0.05
99	39.53 ± 0.16	9.36 ± 0.15	81	46.84 ± 0.17	21.49 ± 0.15
101	32.13 ± 0.19	28.59 ± 0.11	83	23.49 ± 0.26	44.56 ± 0.26
103	10.28 ± 0.10	39.52 ± 0.24	85	4.44 ± 0.17	30.99 ± 0.25
105	1.57 ± 0.17	21.35 ± 0.18			
[c]Calcd $^{16/18}O$	64.8/35.2 ± 0.11	32.4/67.6 ± 0.15		64.3/35.7 ± 0.04	32.1/67.9 ± 0.09

a: based on preparation of enriched water

Table 6: Model Predictions for Two Mixing Models assuming ^{17}O is Negligible (using $^{16/18}O$ estimated ratios from Table 5)

m/z	Mixing in all four positions (*non-persistence hypothesis*)		Mixing in three positions (*persistence hypothesis*)	
	Preparation 1	Preparation 2	Preparation 1	Preparation 2
79	26.57	3.32	27.46	0.92
81	44.28	21.03	49.83	17.31
83	24.59	44.4	22.66	81.61
85	4.55	31.24	0.05	0.16
Meta Total (79-85)	100.00	100.00	100.00	100.00
97	17.6	1.1	14.94	0.1
99	38.29	9.18	39.62	2.64
101	31.24	28.76	35.05	24.07
103	11.33	40.05	10.37	73.04
105	1.54	20.91	0.02	0.15
Ortho Total (79-85)	100.00	100.00	100.00	100.00

Figure 2: Observed Percentages (Means of 5 Replications) and Model Predictions for Two Mixing Models assuming Negligible ^{17}O (see Table 8 for tabular version of these results)



6. Discussion

Orthophosphate can be produced using reaction (1). In practice, metaphosphate also results from this process. Oxygen consists almost entirely of isotope O16 in nature. If water enriched in O18 is used in the reaction, the heavy atoms in the reaction inputs appear to be randomly distributed amongst the resulting orthophosphate and metaphosphate molecules. This was confirmed by comparing observed results from two preparations with a random mixing model and an alternative "persistence" model assuming that each molecule of the input $POCl_3$ contributes exactly one atom to each output orthophosphate and metaphosphate molecule.

The fact that the orthophosphate produced in this way has a mass spectrum described by a simple model is useful, because this can be used to track the movement of phosphates in a natural system (Thomas et al 2010).

The two mixing models were complicated by the fact that O17 also occurs in small quantities in nature. This means that there is not a one to one correspondence between the molecular weight of the orthophosphate or metaphosphate and the number of O18 atoms in the molecule. The two models could still be fitted, but the analysis procedure was more complex and involved least squares estimation of the proportions of O16, O17 and O18 in the reaction mass. An alternative analysis was also conducted, which ignored the presence of O17. This was much simpler and gave similar results.

The main difference between the random mixing and persistence models was that the latter predicts too few maximally heavy molecules of orthophosphate and metaphosphate. This was clear from the preparation 2 observed data, but much less so from the preparation 1 results. This is because the input water in preparation 1 was only mildly enriched with O18, so that few maximally heavy molecules would be expected under either model. Future experimental work of this type should use water which was been sufficiently enriched so that maximally heavy molecules are not overly rare.

Another issue with the experimental data used here is that the number of replicates was small (only 5) from each preparation. Furthermore, the replication was only of the mass spectrometry measurement process, and not of the whole conduct of the experiment. As a result, most sources of experimental variation were not captured by the replication. These sources of variation could include variation in the level of enrichment of the input preparation, changes in experimental conditions such as temperature and humidity, inadvertent participation of other materials in the experiment, and incomplete participation of some of the reactants in (1). Because of this omissions, formal statistical hypothesis tests of the two mixing models were not conducted. Future experiments should include more numerous and complete replication to enable hypothesis testing. This might also allow more sophisticated modelling, parameter estimation and variance estimation.

References

Alvarez R, Evans LA, Milham PJ, Wilson MA (2000) *Analysis of oxygen-18 in orthophosphate by electrospray ionisation mass spectrometry*. International Journal of Mass Spectrometry **203**, 177-186.

Rosman KJR, Taylor PDP (1997) Isotopic compositions of the elements. *Pure and Applied Chemistry* **70**, 217-235.

Thomas, AO, Milham PJ, Morrison RJ, Clark RG and Alvarez R (2010) *Persistence of the Bond Between P and O in POCl3 During the Reaction With Water.* Submitted for publication.

Wikipedia (2010). *Isotope.* http://schools-wikipedia.org/wp/i/Isotope.htm. Downloaded 17 August 2010.