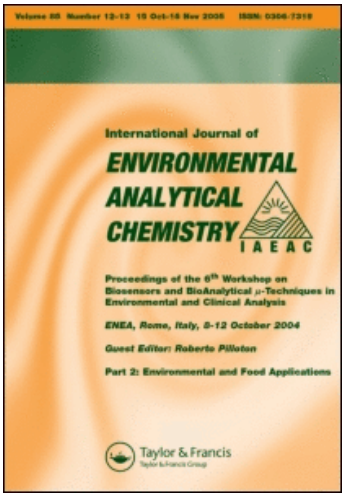

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

The Measurement of Dissolved Phosphorus Compounds: Evidence for Hydrolysis During Storage and Implications for Analytical Definitions in Environmental Analysis

Frank H. Denison[a]; Phil M. Haygarth[b]; William A. House[a]; Andrew W. Bristow[b]
[a] Institute of Freshwater Ecology, River Laboratory, Dorset, UK [b] Institute of Grassland and Environmental Research, Devon, UK

To cite this Article Denison, Frank H. , Haygarth, Phil M. , House, William A. and Bristow, Andrew W.(1998) 'The Measurement of Dissolved Phosphorus Compounds: Evidence for Hydrolysis During Storage and Implications for Analytical Definitions in Environmental Analysis', International Journal of Environmental Analytical Chemistry, 69: 2, 111 — 123
To link to this Article: DOI: 10.1080/03067319808032579
URL: http://dx.doi.org/10.1080/03067319808032579

Intern. J. Environ. Anal. Chem., Vol. 69 (2) pp. 111–123
Reprints available directly from the publisher
Photocopying permitted by license only

THE MEASUREMENT OF DISSOLVED PHOSPHORUS COMPOUNDS: EVIDENCE FOR HYDROLYSIS DURING STORAGE AND IMPLICATIONS FOR ANALYTICAL DEFINITIONS IN ENVIRONMENTAL ANALYSIS

FRANK H. DENISON[a], PHIL M. HAYGARTH[b], WILLIAM A. HOUSE[a,*] and ANDREW W. BRISTOW[b]

[a]*Institute of Freshwater Ecology, River Laboratory, East Stoke, Wareham, Dorset, BH20 6BB, UK.;* [b]*Institute of Grassland and Environmental Research, North Wyke, Okehampton, Devon, EX20 2SB, UK.*

(Received 30 April 1997; In final form 12 November 1997)

Labile organophosphorus and inorganic polyphosphate compounds have been identified as a potential source of error in the measurement of inorganic monophosphate in natural samples. To test this, a range of synthetic phosphorus containing compounds were used to prepare solutions of known composition, which were measured by three commonly used analytical methods for the determination of monophosphate in natural waters. The solutions were also measured after acidic persulphate digestion, to assess the overall recovery of phosphorus by a standard total dissolved phosphorus analysis. No significant concentration of phosphorus was detected in any of the solutions by the three monophosphate methods, when analysed within 4 h of preparation. However, when analysed after 72 h storage, about half of the selected compounds showed an increase in the monophosphate concentration. The phosphorus concentrations measured after acidic digestion were in close agreement with the formulated values for the compounds. These results indicate that the selected compounds are included in the total dissolved fraction, but may also contribute to the monophosphate fraction by hydrolysis to monophosphate in solution after storage.

Keywords: Phosphorus compounds, monophosphate, natural water, storage.

*Corresponding author. Fax No. +44-1929-462180. E-mail: W. House@ife.ac.uk

INTRODUCTION

Phosphorus (P) is generally regarded as the limiting nutrient for primary production in freshwaters and excessive loading of P has been shown to be causal in the eutrophication of freshwaters. The total P content of natural waters is distributed between various physical compartments[1], including: a dissolved fraction comprising both inorganic and organic P species; a colloidal fraction associated with both inorganic colloids such as clays and organic macromolecules including humic and fulvic acids; a particulate component including P species sorbed to particle surfaces or retained in the particle matrix and also a biological constituent associated with aquatic organisms such as algae or bacteria. Only a part of the total phosphorus pool is biologically available, i.e. that which is immediately available for biological utilisation or can be transformed into an available form by naturally occurring processes[2].

Measurements of the P content of natural waters are operationally or analytically defined, i.e. the various commonly measured P fractions are not identical to the known specific physical or chemical components of P in natural waters[1]. As the primary reason for measuring the P content of natural waters is to assess the amount of P available for biological utilisation, it is important to define which specific P compounds are being measured.

Measurement of the dissolved P content of a sample is usually performed after the physical separation of the particulate component of the sample by filtration through a 0.45 μm membrane filter. This sample fractionation is used for convenience rather than to obtain a sample wholly representative of the dissolved P fraction and the filtrate may contain significant quantities of P containing colloidal material[3]. Total Dissolved Phosphorus (TDP) concentrations are determined on filtered water samples (0.45 μm membrane) by digestion of the sample, usually by acid oxidation, to decompose the dissolved and some colloidal P species to inorganic monomeric phosphate. The resulting phosphate is then measured, usually by reacting with acidic molybdate to form 12-phosphomolybdic acid, which on reduction forms the strongly coloured phosphomolybdenum blue species which is determined spectrophotometrically[4].

Various methods are used to estimate the concentration of the inorganic monomeric phosphate component of the TDP[5]. These methods include those based on the reaction of phosphate ions with molybdate ions to form 12-phosphomolybdic acid, enzymatic methods, fluorometric methods, radiobiological assays and ion-exchange chromatography. Methods utilising the reaction of phosphate ions with molybdate ions dominate routine phosphate analyses and the most common method used for natural waters is that described

by Murphy and Riley[6]. Numerous methods utilising the phosphomolybdate complex have been described using different concentrations of acid and molybdate and different reductants and catalysts. Methods optimised for use in automated systems, such as Flow Injection Analysis (FIA), are also commonly used. These methods generally use a shorter contact time between the sample and reagents than equivalent manual methods. Measurements by ion-exchange chromatography are also used in routine analysis, and have the advantage that a number of different ions can be determined concurrently.

Phosphorus concentrations measured by different methods are often regarded as interchangeable, but due to the non-specific nature of these measurements the analytical conditions used will determine what fraction of the TDP is determined. The pH, temperature, concentration of the reagents and the contact time between the sample and reagents all influence what is measured. Indeed, it has been observed that there are statistically significant differences in the phosphate measured by different methods on the same natural water samples[7, 8].

The P fraction determined by the method of Murphy and Riley is known as Soluble Reactive Phosphorus (SRP), Molybdate Reactive Phosphorus (MRP) or Dissolved Reactive Phosphorus (DRP). SRP is assumed to be composed of largely soluble compounds which are biologically available. However, many studies have indicated that SRP is not exclusively composed of dissolved monomeric inorganic phosphates e.g. $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-} and various ion pairs such as $CaHPO_4^0$ and $CaPO_4^-$, but includes other phosphorus species[9, 10]. These include phosphorus associated with colloids either incorporated into the molecular structure or through cation bridges to anionic surface sites and possibly contributions from dissolved organophosphorus and inorganic polyphosphate compounds hydrolysed during the analytical procedure. In some natural waters, generally of low SRP concentration, a significant fraction of the SRP (25-70%) is not identical to dissolved inorganic monomeric phosphate[7, 9] and is not immediately biologically available.

The difference between the SRP and TDP concentrations of a sample is known as the Soluble Unreactive Phosphorus (SUP) and is commonly equated with organic phosphorus[1, 4]. Organophosphorus compounds play an important role in natural waters and in some waters may contribute a significant proportion of the total dissolved phosphorus fraction[11, 12]. To understand the role that organophosphorus compounds play in the cycling of phosphorus in the environment, it is important to know which methods include dissolved organophosphorus compounds in their measurements. TDP measurements are assumed to include all organophosphorus and inorganic polyphosphate compounds by their complete decomposition during acidic oxidation. It is possible that some organophosphorus compounds are included in the measurement of SRP, as it is known that

many organophosphorus and inorganic polyphosphate compounds are hydrolysed in acid conditions[13]. For example the molybdate ion, which is used in most of the common colourimetric methods, can also catalyse the hydrolysis of organophosphorus compounds[14, 15]. In addition the low pH used in colourimetric procedures is likely to reduce the sorption of phosphorus to colloids, especially from surface sites of polyelectrolytes and the edge sites of clays. The hydrolysis of labile phosphorus compounds during the analytical determination of inorganic monophosphate will result in an over-estimation of the true monophosphate concentration, and an under-estimation of the concentration of organophosphorus compounds. As SRP is commonly determined by several different methods using different analytical principals and conditions, it is possible that the extent of hydrolysis of organophosphorus or inorganic polyphosphate compounds will vary between the different methods.

The objective of this work was to compare four commonly used analytical methods for the measurement of dissolved phosphorus; a TDP method, the SRP method of Murphy and Riley, an SRP method modified for use in a flow injection analysis system, and anion exchange chromatography. This was achieved by measuring the phosphorus in solutions of known composition of a range of compounds, chosen to represent the major classes of dissolved phosphorus compounds found in natural water samples. These included compounds containing C-P, C-O-P, sugar-O-P, sugar-C-O-P, aromatic and saturated ring-O-P and P-O-P bonds. The compounds also encompassed organic molecules detected in environmental samples e.g. inositol phosphates[16, 17], adenosine 3′,5′-cyclic monophosphate[18], and also tripolyphosphate used in detergent formulations and also present in bacterial cells.

METHODS AND MATERIALS

Materials

Twelve compounds were investigated using four different methods of phosphate determination. The compounds were selected to be representative of the major classes of organophosphorus and polyphosphate compounds found in natural waters, namely: a) phosphoric esters, b) nucleosides, c) phosphonates and d) polyphosphates. α-D-Glucose 1-phosphate di-sodium salt, D-glucose 6-phosphate di-sodium salt, adenosine 5′ triphosphate di-sodium salt, p-nitrophenyl phosphate di-sodium salt, adenosine 3′,5′-cyclic monophosphate, glycerophosphate di-sodium salt, myo-inositol 2-monophosphate Di(cyclohexylammonium) salt, inositol hexaphosphate dodecasodium salt, 2-aminoethylphosphonic acid, tetra sodium pyrophosphate, pentasodium tripolyphosphate,

trisodium trimetaphosphate and potassium dihydrogen orthophosphate. These were all obtained from Sigma-Aldrich ltd. (Poole, UK) used without additional purification. Inorganic compounds were dried at 105°C for 4 hours, and organic compounds were dried in a desiccator for 2 hours prior to use. All reagents used were AR grade (BDH, Poole, UK) and analytical quality water (0.06 μS cm^{-1}) from a Purite Select Analyst HP purification system was used throughout.

Analytical Methods

The phosphorus concentration of each of the solutions was measured by four methods. These methods were calibrated using standard solutions of KH_2PO_4 (dried in an oven at 105°C for 4 h and stored in a desiccator) in the range 0.3-12 μmol dm^{-3} prepared volumetrically. The four methods were:

(1) The manual method of Murphy and Riley[6] (MR). The reagent was prepared by mixing 100 ml of sulphuric acid solution (140 ml of concentrated sulphuric acid diluted to 1000 ml in water), 40 ml of ammonium molybdate solution (15 g ammonium molybdate dissolved in 500 ml water), 40 ml of ascorbic acid solution (5.4 g ascorbic acid dissolved in 100 ml water) and 20 ml of potassium antimonyl tartrate solution (0.68 g of potassium antimonyl tartrate dissolved in 500 ml water). This mixed reagent (4 ml) was added to the sample (20 ml) and the volume adjusted to 25 ml. Absorbance was measured at a wavelength of 880 nm in a 4 cm cell after exactly 10 minutes and corrected against a reagent blank prepared from analytical quality water in place of sample.

(2) Flow-injection analysis (FIA)[19]. Reagent 1 was prepared by dissolving ammonium molybdate (5 g) in water (300 ml), adding concentrated sulphuric acid (17.5 ml) and adjusting to 500 ml with water. Reagent 2 was prepared by adding concentrated sulphuric acid (14 ml) to water (300 ml), dissolving stannous chloride (0.1 g) and hydroxylammonium chloride (1 g) in this solution and adjusting to 500 ml with water. The manifold (Figure 1) was constructed from 0.8 mm i.d. PTFE tubing. Absorbance at 690 nm was measured continuously and the absorbance peak height of each sample evaluated from the peak maximum during a window of 10-30 seconds after sample injection, minus the baseline value

(3) Anion-exchange chromatography (AEC), using a Dionex ion chromatograph, series 4500i. The sample (200 μl) was injected into the eluent stream (3.4 mmol dm-3 $NaHCO_3$/3.6 mmol dm^{-3} Na_2CO_3), passed through an IonPac AS9 - SC anion exchange column and the phosphate measured by conductivity, after suppression of the eluent conductivity by passage through a micromembrane suppressor.

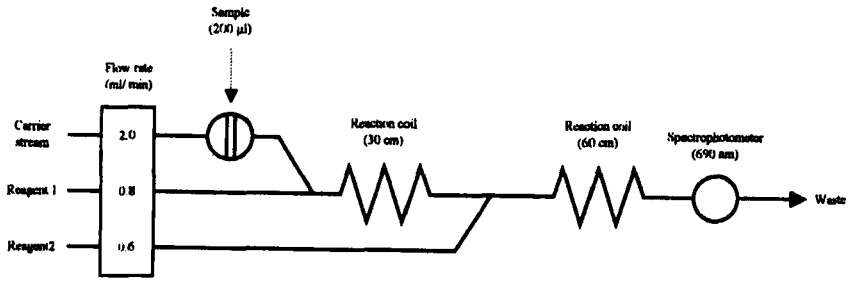

FIGURE 1 Manifold design for the Flow Injection Analysis of soluble reactive phosphate.

(4) Total Dissolved Phosphorus (TDP), was measured using a method modified from Eisenreich *et al*[4]. The reagent was prepared by dissolving potassium antimonyl tartrate (0.57 g) in 500 ml of water, adding concentrated sulphuric acid (45 ml) and mixing with sodium molybdate (8.52 g) dissolved in 400 ml of water and the volume adjusted to 1000 ml. Ascorbic acid (0.31 g) was dissolved in 50 ml of the reagent to prepare the working reagent. The sample (20 ml) was digested by adding dipotassium peroxodisulphate (0.15 g), 1 ml of 0.5 mol dm^{-3} sulphuric acid and autoclaving at 121°C, 103 kPa for 45 minutes. The concentration of phosphorus was determined by adding 1.0 ml of working reagent and the absorbance measured at 880 nm in a 4 cm cell after exactly 12 minutes. A reagent blank was prepared from analytical quality water in place of sample.

Dissolved organic carbon was measured by a TOCsinII carbon analyser. The samples were acidified and sparged by a continuous flow of carbon dioxide free air to remove inorganic dissolved carbon. The sample was then forced into an oxidation furnace at 900°C and the liberated CO_2/N_2 mixture was passed through a drying tube before reacting with hydrogen over a nickel catalyst. The dried methane was measured using a conventional Flame Ionisation Detector (FID).

Sample Preparation and Stability

Solutions of each phosphorus-containing compound were prepared with a nominal phosphorus concentration of 12 μmol dm^{-3}, with the exception of pyrophosphate which had a concentration of 20 μmol dm^{-3}. One set of solutions were measured by anion-exchange chromatography (5 replicates) and a second set was measured by both the MR and FIA methods (5 replicates for each method), all within 4 h of preparation of the solutions. The total dissolved phosphorus (5 replicates) was measured for both sets of solutions within 5 days of preparation and dissolved organic carbon (single determinations) was

TABLE I The limits of detection of the four analytical methods calculated using eqn. (2) where L_D is the limit of detection with a confidence limit of 95%.

Method	*Murphy and Riley* (MR)	*Flow Injection Analysis* (FIA)	*Anion Exchange Chromatography (AEC)*	*Total Dissolved Phosphorus (TDP)*
$L_D/\mu mol\ dm^{-3}$	0.13	0.31	0.32	0.16

measured within 2 weeks of preparation. Analysis by FIA was repeated on the second set of solutions, after 72 hours of storage in the light at ambient temperature (3 replicates). This was to assess the stability of the compounds in aqueous solution and to determine whether any phosphorus measured as monophosphate was derived from hydrolysis of the compound prior to analysis.

RESULTS AND DISCUSSION

Limit of Detection

The limit of detection for each of the methods was calculated from five replicate determinations of each of eight solutions of low concentration (<2.5 μmol dm^{-3}). The variance of samples of low concentration is assumed to be equal to that of a sample blank and visual inspection of the data shows no relationship between variance and sample concentration in the concentration range used. The standard deviations of each set of replicate determinations were combined using (1)[20],

$$S_p = \sqrt{\frac{\sum_{i=1}^{M} (s^2(N-1))_i}{M(N-1)}} \quad (1)$$

where S_p is the pooled standard deviation, s is the standard deviation of sample i, N is the number of determinations of each sample, and M is the number of samples. The limit of detection of each of the methods was calculated using (2),

$$L_D = 4.65 \times S_p \quad (2)$$

where L_D is the limit of detection with a confidence limit of 95%[20].

The limits of detection of the methods were determined using eqn. (2) and are given in Table I. The MR and TDP methods have similar limits of detection, and are lower than those of the FIA and AEC methods.

Comparison of the Methods for the Determination of Dissolved Phosphorus

No significant amount of dissolved phosphorus was detected in any of the synthetic compounds analysed by the MR, FIA and AEC methods (Table II). The mean values of the five replicate determinations were below the limit of detection except for myo-inositol 2-monophosphate which gave values of 0.26 and 0.36 μmol dm^{-3} by the MR and FIA methods respectively, and tripolyphosphate, which gave a value of 0.32 μmol dm^{-3} when analysed by FIA. The measurement of the solution containing only KH_2PO_4 was satisfactory for all three methods with values within 4% of the expected value.

Maximum concentrations of phosphorus measured in the solutions of the synthetic compounds by the three methods at a 95% Confidence Level (CL) were calculated using the data from the five replicate determinations and expressed as a % of the mean TDP concentration determined for these compounds. The maximum concentrations measured were less than 2.9, 8.2 and 5.7% by the MR, FIA and AEC methods respectively. The higher values obtained by the FIA and AEC methods were caused by the greater variance in the sets of replicate determinations and hence the poorer precision of the methods compared with the MR method. The amount of phosphorus determined from the mean values of the replicate determinations was <3.1% of the TDP content of the compounds in all cases.

Concentrations of total dissolved phosphorus (TDP) were generally in close agreement with the formulated values (Table III), with measured concentrations within ±5% of the expected values for all the compounds except for inositol hexaphosphate (85.6% recovery). A phosphorus assay by atomic emission spectroscopy for the sample batch (supplied by Sigma, Poole) gave a value of 17.3% of phosphorus by mass, 14% lower than the formulated value of 20.1%. This difference may be accounted for by the presence of a series of inositol phosphate homologues in the sample supplied and when a correction for this lower phosphorus content was applied the recovery was 99.5%. The results from the DOC analysis of the organophosphorus compounds produced carbon concentrations close to the formulated values, with agreement within ±10% of the expected concentration for all the compounds except for 2-aminoethylphosphonic acid (84% recovery).

Hydrolysis of the Synthetic Compounds

The SRP (measured by FIA) of six of the synthetic solutions increased significantly (95% CL) after storage for 72 h at ambient temperature (*ca.* 15-20°C) and in the light, indicating that hydrolysis of the compounds had

TABLE II Dissolved phosphorus measured by the three monophosphate methods. The concentration values given are the mean values of five replicate determinations performed within 4 h of preparation. The maximum phosphorus determined (at a 95% CL) is expressed as a % of the total dissolved phosphorus content measured for the compounds.

Compound	*Murphy and Riley (MR)2*		*Flow Injection Analysis (FIA)*		*Anion Exchange Chromatography (AEC)*	
	P/μmol dm⁻³	*Maximum P/%*	*P/μmol dm⁻³*	*Maximum P/%*	*P/μmol dm⁻³*	*Maximum P/%*
α-D-Glucose 1-phosphate	0.06	1.2	0.22	7.1	0.05	2.2
D-Glucose 6-phosphate	0.05	0.6	0.18	4.6	0.09	1.0
Adenosine 5′ triphosphate	0.01	0.3	0.15	3.5	0.00	0.2
p-Nitrophenyl phosphate	0.12	1.2	0.17	5.5	0.23	5.7
Adenosine 3′,5′-cyclic monophosphate	0.00	0.4	0.22	3.4	0.04	1.9
Glycerophosphate	0.00	0.2	0.16	3.1	0.02	0.6
myo-Inositol 2-monophosphate	0.26	2.9	0.36	7.8	0.17	2.6
Inositol hexaphosphate	0.00	0.2	0.17	8.2	0.02	1.0
2-Aminoethylphosphonic acid	0.01	0.6	0.02	1.4	0.06	1.0
Pyrophosphate	0.06	0.4	0.26	2.6	0.06	0.4
Tripolyphosphate	0.10	1.1	0.32	5.6	0.10	1.0
Trimetaphosphate	0.01	0.2	0.17	4.3	0.05	0.7
Dihydrogen orthophosphate	11.56	99	11.68	99	12.10	108

TABLE III Total dissolved phosphorus and dissolved organic carbon content of the synthetic compounds. The standard deviations of the five replicate determinations are shown in brackets. Standard error values are given at a 95% confidence limit, and expressed as a % of the formulated values.

Compound	*Dissolved Organic Carbon/mg dm $^{-3}$*	*Recovery of DOC (%)*	*Total Dissolved Phosphorus /μmol dm^{-3}*	*Recovery of TDP (%)*
α-D-Glucose 1-phosphate	0.83	95.9	12.12 (0.02)	101.0 ± 0.5
D-Glucose 6-phosphate	0.87	100.2	12.37 (0.11)	103.1 ± 2.6
Adenosine 5′ triphosphate	0.53	110.1	12.19 (0.23)	101.6 ± 5.3
p-Nitrophenyl phosphate	0.88	101.3	12.23 (0.09)	101.9 ± 2.1
Adenosine 3′,5′-cyclic monophosphate	1.43	99.2	12.21 (0.06)	101.8 ± 1.4
Glycerophosphate	0.41	94.2	12.28 (0.02)	102.3 ± 0.5
myo-Inositol 2-monophosphate	2.43	93.7	11.69 (0.33)	97.4 ± 7.6
Inositol hexaphosphate	0.15	100.9	10.27 (0.02)	85.6 ± 0.5
2-Aminoethylphosphonic acid	0.24	84.0	11.91 (0.27)	99.2 ± 6.3
Pyrophosphate			20.03 (0.04)	99.5 ± 0.5
Tripolyphosphate			11.72 (0.03)	97.7 ± 0.8
Trimetaphosphate			11.85 (0.10)	98.8 ± 2.2
Dihydrogen orthophosphate			11.90 (0.07)	99.1 ± 1.6

TABLE IV Phosphorus concentrations determined by Flow Injection Analysis within 4 h and after 72 h of preparation. The standard deviations of the five replicate determinations are shown in brackets. Standard error values are given at a 95% confidence limit.

Compound	*P < 4h/μmol dm⁻³*	*P 72 h/μmol dm⁻³*	*P 72 h/% ofTDP*
D-Glucose 6-phosphate	0.18 (0.14)	0.41 (0.04)	3.3 ± 1.4
p-Nitrophenyl phosphate	0.17 (0.18)	1.82 (0.09)	14.9 ± 3.1
Glycerophosphate	0.16 (0.08)	1.72 (0.14)	13.9 ± 4.8
Pyrophosphate	0.26 (0.09)	2.02 (0.06)	10.2 ± 1.3
Tripolyphosphate	0.32 (0.12)	0.83 (0.03)	6.9 ± 1.2
Trimetaphosphate	0.17 (0.12)	0.85 (0.05)	7.2 ± 1.8

occurred (Table IV). The SRP concentrations of all of the solutions of inorganic polyphosphate compounds, pyrophosphate, tripolyphosphate and trimetaphosphate increased significantly to 6.9-10.2% of the TDP concentrations. The hydrolysis of polyphosphate compounds to yield monophosphate as the final product is thermodynamically favourable, however the kinetics of the reaction in homogeneous dilute aqueous solution are slow. Various physico-chemical and biochemical factors influence the rate of hydrolysis and these have been summarised by Van Wazer[21] and by Clesceri and Lee[13]. The rate of hydrolysis of condensed phosphates is greatly increased by a) cation complexation (Ca^{2+} having a much greater effect than Na^{+}), b) lowered pH, c) colloidal gels such as hydrated oxides of Fe, Al, Co, Ni, d) the presence of phosphatase enzymes and e) increased ionic strength. The rate of hydrolysis in natural samples, even after filtration, will therefore be much greater than that observed here.

Of the organophosphorus compounds only p-nitrophenol phosphate and glycerophosphate were hydrolysed significantly to 14.9 and 13.9% of the TDP concentrations, respectively. The SRP measured in the solution of glucose 6-phosphate also increased, but only to 3.3% of the TDP concentration. There was no significant increase (95% CL) in the SRP concentrations of the solutions of myo-inositol 2-monophosphate, inositol hexaphosphate, 2-aminoethylphosphonic acid, α-D-glucose 1-phosphate, adenosine 5′ triphosphate, or adenosine 3′,5′-cyclic monophosphate indicating that these compounds are relatively stable in dilute aqueous solution. As for the polyphosphate compounds, the hydrolysis rates of organophosphorus compounds can be expected to increase substantially in natural samples, especially in the presence of enzymes.

CONCLUSIONS

No significant amounts of the synthetic compounds were determined as monophosphate by the MR, FIA or AEC methods. Maximum amounts measured

at a 95% CL as a % of the dissolved phosphorus (taken as the mean TDP value determined for the compounds) were less than 2.9, 8.2 and 5.7% by the MR, FIA and AEC methods respectively. The digestion efficiency of the total dissolved phosphorus method appeared to be good, with concentrations generally in close agreement with the formulated values.

These results indicate that the dissolved organophosphorus and inorganic polyphosphate compounds selected, do not contribute significantly to the monophosphate measured by the three analytical techniques used. However, hydrolysis during storage will contribute to changes in the monophosphate concentration, emphasising the need for rapid analysis after sampling and filtration[24, 25]. Further, these results do not exclude the determination of "labile phosphorus" associated with both small particles (<0.45 μm) and colloids, which have been identified as an important phosphorus source in many natural waters[3, 22, 23]. The present work supports the assumption that dissolved organophosphorus compounds are included in the SUP fraction, but as both inorganic polyphosphate compounds and possibly more refractory particulate (<0.45 μm) and colloidally associated phosphorus will also be included in this fraction, it is preferable to use the operationally defined term SUP rather than equate this fraction exclusively with dissolved organic phosphorus.

Acknowledgements

FHD & WAH thank the Department of Environment for funding this research as part of contract EP9/1/9/09 and the Natural Environment Research Council for their support. Thanks are also due to Lui Hepworth for analytical assistance and Phil Rowland, Gordon Irons, Ian Mckelvie and Darren Baldwin for helpful discussion and advice. PMH and AWB thank the Ministry of Agriculture Fisheries and Food, London. IGER is supported by the BBSRC.

References

[1] O. Broberg and G. Persson, *Hydrobiologia.*, **170**, 61-90 (1988).
[2] B. Bostrom, G. Persson and B. Broberg, *Hydrobiologia.*, **170**, 133-155 (1988).
[3] P. M. Haygarth, M. S. Warwick and W. A. House, *Water. Res.*, **31**, 439-448 (1996).
[4] S. J. Eisenreich, R. T. Bannerman and D. E. Armstrong, *Environ. Lett.*, **9**, 43-53 (1975).
[5] O. Broberg and K. Pettersson, *Hydrobiologia.*, **170**, 45-59 (1988).
[6] J. Murphy and J. P. Riley, *Anal. Chim. Acta.*, **27**, 31-36 (1962).
[7] P. G. W. Jones and C. P. Spencer, *J. Mar. biol. Ass.* UK., **43**, 251-273 (1963).
[8] F. H. Denison and W. A. House, unpublished results; Institute of Freshwater Ecology.
[9] F. H. Rigler, *Limnol. Oceanogr.*, **13**, 7-13 (1968).
[10] M. P. Stainton, *Can. J. Fish. Aquat. Sci.*, **37**, 472-478 (1980).
[11] F. H. Rigler, *Limnol. Oceanogr.*, **9**, 511-518 (1964).

[12] W.A. House, D. Leach, M. S. Warwick, B. A. Whitton, S. N. Pattinson, G. Rylane, A. Pinder, J. Ingram, J. P. Lishman, S. M. Smith, E. Rigg and F. H. Denison, *Sci. Total Environ.*, **194/195**, 303-320.
[13] N. L. Clesceri and G. F. Lee, *Intern. J. Air Wat. Pollut.*, **9**, 723-742 (1965).
[14] H. Weil-Malherbe and R. H. Green, *Biochem. J.*, **49**, 286-292 (1951).
[15] S. J. Tarapchak, *J. Environ. Qual.*, **12**, 105-108 (1983).
[16] W. C. Weimer and D. E. Armstrong, *Environ. Sci. Technol.*, **13**, 826-829 (1979).
[17] C. M. Clarkin, R. A. Minear, S. Kim and J. W. Elwood, *Environ. Sci. Technol.*, **26**, 199-204 (1992).
[18] D. A. Francko and R. G. Wetzel, *Limnol. Oceanogr.*, **27**, 27-38 (1982).
[19] Tecator Application Note, Determination of Reactive Phosphorus in Water by Flow-Injection Analysis. Application Note ASN 60-01/83 JM/ML. (1983).
[20] D. T. E. Hunt and A. L. Wilson, *The Chemical Analysis of Water* (Royal Society of Chemistry, 1986), 2nd ed., 683 pp.
[21] J. R. Van Wazer, *Phosphorus and its compounds*, Vol I, (Interscience, New York, 1958).
[22] H. De Haan, T. De Boer, J. Voerman, H. A. Kramer and J. R. Moed, *Verh. Inter. Verein. Limnol.*, **22**, 876-881 (1984).
[23] D. R. S. Lean, *J. Fish. Res. Board Can.*, **30**, 1525-1536 (1973).
[24] P. M. Haygarth, C. D. Ashby and S. C. Jarvis, *J. Environ. Qual.*, **24**, 1133-1140 (1995).
[25] M. D. Ron Vaz, C. A. Shand and A. C. Edwards, *Commun. Soil Sci. Plant Anal.*, **25**, 3147-3159 (1994).