Overview of Phoslock® Properties and its Use in the Aquatic Environment

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EXECUTIVE SUMMARY

Phoslock® is a modified clay developed by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) of Australia. Worldwide, Phoslock® has been applied to water bodies in over 20 countries in order to remove excess phosphate and as a blue-green algae management tool.

Phoslock® is produced through a patented ion exchange process, whereby lanthanum ions displace sodium ions within the clay matrix of bentonite and become the active component. The final product is a dry granule which can be applied to a water body both as a slurry and as granules. The removal of filterable reactive phosphorus (FRP) or soluble reactive phosphorus (SRP) by Phoslock® is attributed to lanthanum embedded within the bentonite clay which binds phosphate molecules and forms a highly stable mineral known as Rhabdophane ($LaPO_4$ nH2O). When applied to water, the lanthanum in Phoslock® will either react with phosphate anions in the water body or remain bound within the clay structure under a wide range of environmental conditions. As Phoslock® moves down through the water column, up to 95% of the FRP is rapidly removed and adsorbed onto the surface of the clay matrix. Once settled on the sediment, Phoslock® forms a very thin, permeable layer that will bind phosphate that is released from of the sediment.

In general, Phoslock® dosages are calculated specifically for each water body according to the quantity of phosphorus in the water column and the quantity of releasable phosphorus in the sediment. Application strategies can be tailored to the particular characteristics of the water body. Applications are planned and undertaken by trained staff, utilizing tailor made application equipment. With an accurate pre- and post-application monitoring approach, the effectiveness of the application for each treated water body is assessed.

Phoslock® has received certifications and approvals for use in a range of environmental jurisdictions. In 2011, Phoslock® received US and Canadian NSF/ANSI Standard 60 Certification for use in drinking water. This accreditation was provided by the Water Quality Association of the United States (WQA), an independent accreditation agency authorized by the US Environmental Protection Agency.

Worldwide, Phoslock® has been applied to more than 200 lakes. Applications have taken place both on recreational lakes as well as on water bodies with a special conservation status such as Natura 2000 lakes. These applications have had a variety of aims, including the significant reduction of phosphorus concentrations, the management of eutrophication, the achievement of a clear water state typical of oligotrophic and macrophyte dominated ecosystems, the guaranteed use of the water body for recreational activities without swimming bans and the compliance of the European Water Framework Directive (WFD) criteria. The planning, implementation and assessment of the applications have generally been undertaken in close collaboration with local environmental agencies and scientific research centres. Over the last few years, the most important results relating to the use of Phoslock® in aquatic ecosystems have been peer reviewed and published in a number of scientific articles.

To assess the potential risk of Phoslock® to organisms living in the water column and on the sediment, a large number of eco-toxicity tests with Phoslock® using sentinel water column and benthic invertebrates as well as fish species have been undertaken. The tests that have been undertaken using a suspension of Phoslock® granules or a leachate extracted according to the TCLP

method show that there is a wide safety margin associated with Phoslock® applications at typical dose rates.

The potential risk for human health after Phoslock® applications can be assessed on the basis of the available scientific literature from the use of lanthanum carbonate as an orally administered phosphate binder to treat hyperphosphatemia. Lanthanum carbonate is approved for use under the trade name, Fosrenol®, in many countries worldwide, including the United States and most European countries. Results from a large number of clinical and experimental studies undertaken as part of the approval process for Fosrenol® demonstrate that there is no identifiable risk to human health from direct ingestion of lanthanum via Phoslock® treated water or from fish consumption harvested from Phoslock® treated water bodies at normal dose rates.

Table of contents

Executive Summary **2**

1 Introduction **6**

2 Phoslock® product description **6**

2.1 Manufacturing process of Phoslock® 6

2.1.1 Cation exchange process *6*

2.1.2 Phoslock® final product *7*

2.2 Phoslock® properties 8

2.3 How does Phoslock® work 8

2.4 Environmental conditions and Phoslock® performance 11

2.4.1 pH and Anoxia *11*

2.4.2 Competition for phosphate from other ions *11*

2.4.3 Phosphate versus total (TP) or particulate phosphorus (PP) *11*

2.4.4 External versus internal phosphorous loading *12*

2.4.5 Duration of the Phoslock® treatment *12*

2.4.6 Phoslock® performance summary *12*

3 Application of Phoslock® **13**

3.1 Phoslock dosage calculation 13

3.1.1 Water column phosphorus *13*

3.1.2 Sediment phosphorus *13*

3.1.3 Quantity of Phoslock® to be applied *14*

3.2 Application and dosing strategies 14

3.3 Transport, delivery and application of Phoslock® 15

3.4 Monitoring 16

4 Approvals and certifications **18**

5 Case studies **19**

5.1 Behlendorfer See, Germany 20

5.2 Blankensee, Germany 21

5.3 Hyde Park London, UK 22

5.4 Clatto Reservoir and Loch Flemington, UK 24

5.5 Lake Rauwbraken, Netherlands 26

5.6 Lake Varese, Italy 28

6 Ecotoxicity assessment **30**

6.1 Summary of existing data from toxicity tests with Phoslock® 30

6.1.1 Zooplankton *31*

6.1.2 Fish *32*

6.1.3 Benthic organisms *32*

6.1.4 Conclusion *34*

7 Phoslock® and Human Health **35**

7.1 Bentonite and human health 35

7.2 Lanthanum and human health 35
7.2.1 Effects of lanthanum on the liver and excretion *36*
7.2.2 Effects of lanthanum on bone *37*
7.2.3 Genotoxic, mutagenic and carcinogenic potential *38*
7.2.4 Lanthanum and brain *38*
7.3 Ingestion of Phoslock® treated water 38
7.4 Exposure via fish consumption 41
7.5 Conclusion 42
References 43

1 INTRODUCTION

Phoslock® is a modified bentonite clay product containing the active ingredient lanthanum, a rare earth element. Phoslock® was developed in the nineties after rigorous scientific research work undertaken at the Australian CSIRO (Commonwealth Scientific and Industrial Research Organisation) Department of Land and Water, as a measure to remove phosphate (Filterable Reactive Phosphorus - FRP or Soluble Reactive Phosphorus - SRP) from eutrophic water bodies. The research project and the subsequent development was funded by the Western Australian State Government's Water and Rivers Commission and the Swan River Trust.

Worldwide, Phoslock® has been applied to water bodies in over 20 countries to remove excess phosphate and control algal blooms (e.g. blue green algae or cyanobacteria). The types of water bodies treated have ranged from recreational lakes and conservation sites to drinking water reservoirs and intensive aquaculture ponds.

Phoslock Water Solutions Ltd (PWS) (http://www.phoslock.com.au/) has been listed on the Australian Stock Exchange since 2005 (ASX:PHK), owns the intellectual property rights relating to Phoslock® and is the licensor for the Phoslock® technology worldwide. Phoslock® is sold in Germany under the trade name Bentophos® and under the Phoslock® trade name in all other countries.

2 PHOSLOCK® PRODUCT DESCRIPTION

Phoslock® is a management tool to control algal blooms in eutrophic water bodies. This section gives an overview of the relevant characteristics of the product related to manufacturing, properties, chemistry and application strategies.

2.1 MANUFACTURING PROCESS OF PHOSLOCK®

Phoslock® is produced through a patented ion exchange process, whereby lanthanum ions displace cations within the matrix (Figure 1) of the bentonite clay. During the manufacturing process of Phoslock®, lanthanum cations (La^{3+}) are adsorbed into sites within the bentonite mineral layers, with the result that lanthanum, which retains its capacity to bind phosphate ions, becomes the active compound.

2.1.1 CATION EXCHANGE PROCESS

The lanthanum cations are incorporated into the bentonite mineral matrix via a cation exchange process. This exchange capacity is a result of a charge imbalance on the surface of the clay mineral layers, which is balanced by surface adsorbed cations which are exchangeable in aqueous solutions. During the preparation process of Phoslock®, the lanthanum cations are exchanged with these surface-adsorbed, exchangeable cations. The embedded lanthanum ions are strongly associated with the bentonite clay and are not released as soluble lanthanum into the water when applied in aquatic environments. The lanthanum will either react with the phosphate anion in the water body or remain bound within the clay structure under a wide range of environmental conditions (DOUGLAS et al., 2000).

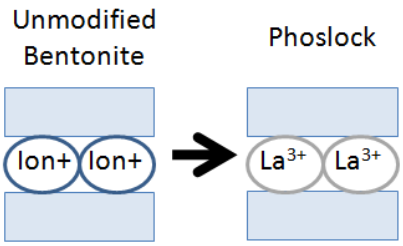


Figure 1. Principle of the cation exchange during manufacturing of Phoslock®

2.1.2 PHOSLOCK® FINAL PRODUCT

Phoslock® was originally formulated in the form of a slurry. A dry, free flowing granular form was developed in 2004 resulting in ease of transportation and reduced application costs. The Quality Control (QC) program of Phoslock Water Solutions Limited ensures that, during the manufacturing process, the lanthanum content of the Phoslock® granule (on a dry weight basis) is 50±2 mg/g i.e. 5% (±0.2%).

a b

Figure 2.Phoslock manufacture at the Phoslock® manufacturing factory (a), Dewatering and drying processes during the Phoslock® manufacturing (b).

Figure 3.Dry, free flowing granular Phoslock® (0.5 – 3 mm) and packet format: 25 kg Phoslock® bags.

2.2 PHOSLOCK® PROPERTIES

Phoslock® was originally manufactured and applied in the form of a slurry, containing 20% (w/w) of the active Phoslock®. With the introduction of its granular form, the Phoslock® formulation remained the same, however the active Phoslock® concentration was increased to more than 90% (w/w). The major properties of granular Phoslock® are listed in Table 1. By adhering to strict quality control measures, Phoslock Water Solutions Limited maintains a high concentration of the active Phoslock® consistently in the supplied product. Moreover, the low dust level and the acceptable degree of packaging stability of Phoslock® make the transportation and the application of the product convenient as well as minimising any possible health risk associated with dust levels to the personnel involved in these processes.

Table 1. Summary of properties of Phoslock® granules

Physical & Chemical Properties	Description
Phoslock® content	>90% (Bentonite content is ~95% and Lanthanum is ~5% on a dry matter basis)
Water content	8 – 10 %
Appearance	Light brown free flowing granules
Packaging stability	No deterioration of the packaging or physical appearance of the product
Size of granules	0.5 – 3 mm
Bulk density	850 – 1200 kgm^{-3}
Dust content	<1% weight 50 µm
pH	7.0 – 7.5

2.3 HOW DOES PHOSLOCK® WORK

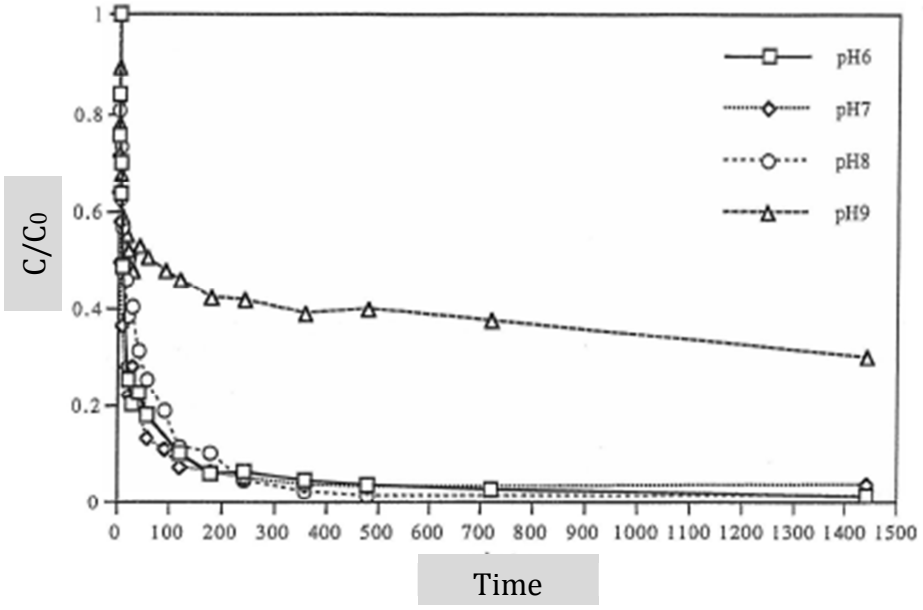
The removal of FRP (Filterable Reactive Phosphorus or Soluble Reactive Phosphorus - SRP) by Phoslock® is attributed to the highly effective ability of lanthanum to react with phosphate ions (DOUGLAS et al 2004). Removal of phosphate by lanthanum is extremely efficient and has a molar ratio of 1:1 which means that one ion of lanthanum binds with one ion of phosphate as follows:

$$La^{3+} + PO_4^{3-} \longrightarrow LaPO_4$$

Lanthanum phosphate is a highly stable, naturally occurring mineral known as Rhabdophane (LaPO4•nH2O). The stability of rare earth-anion complexes is demonstrated by their low solubility products (FIRSCHING & BRUNE 1991, FIRSCHING & KELL 1993, LIU & BYRNE 1997). The solubility product of lanthanum phosphate salts ranges from -25.8 to -24.5 in fresh water and -28.08 in seawater. This means that once the reaction has happened, lanthanum phosphate becomes a solid. The resulting solid mineral complex becomes integrated as an inert component into the natural sediments of the water body (DOUGLAS et al 1999, ROBB et al. 2003, MEIS et al. 2012, MEIS et al. 2013) and is no longer available for primary production. The nature of the bond between lanthanum and phosphate is such that it will not be broken under the pH range (pH 4-11) found in almost any natural lakes. Furthermore, the bond will not be broken under anoxic (low redox potential) conditions, which are prevalent in most lake sediments and many overlying waters (GÄCHTER & WEHRLI 1998, MEIS et al. 2012).

The reaction rate between lanthanum and phosphate is rapid (DOUGLAS et al. 2000, ROBB et al. 2003, ROSS et al. 2008). This both facilitates rapid removal of phosphate from the water column and ensures that lanthanum quickly becomes the inert and insoluble mineral Rhabdophane. Figure 4 shows the results of laboratory experiments conducted by the Australian CSIRO (DOUGLAS et al. 2000) investigating the phosphate uptake of Phoslock® under a variety of physico-chemical conditions (pH, salinity). The evolution in actual phosphate concentrations (C) over initial phosphate concentrations (C_0) shows the rapid reduction in phosphate concentrations immediately after the addition of Phoslock®.

a



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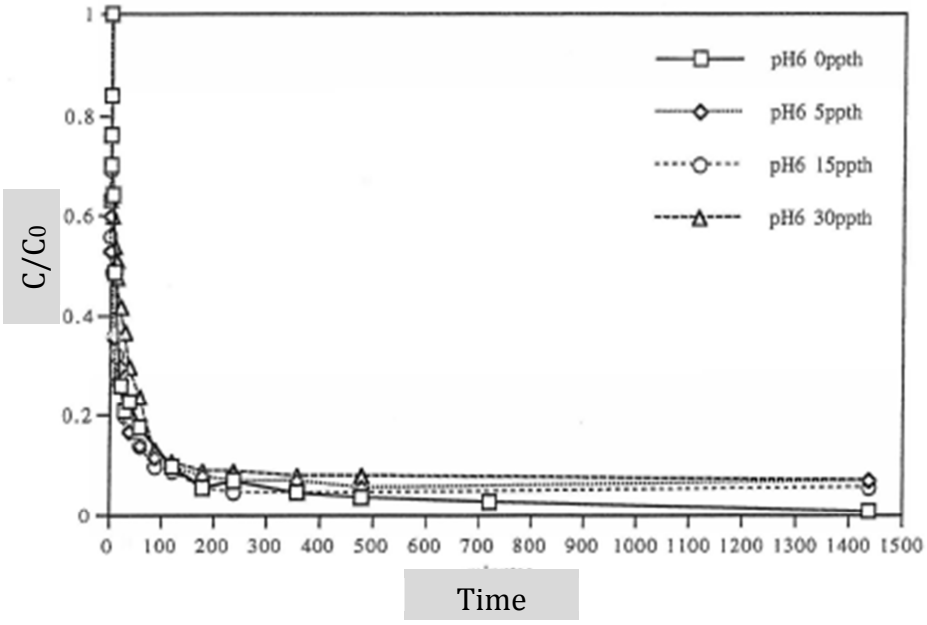


Figure 4. Lab analysis of the removal of phosphate by Phoslock® (actual concentration C over initial concentration C_0); a: removal of PO_4-P from solution over pH6-9, b: removal of P04-P from solution at pH6 at 0, 5, 15 and 30 ‰ (ppth) (NaCl equivalent) salinity (DOUGLAS et al. 2000).

During experiments conducted by the CSIRO during the development of Phoslock®, rapid and substantial removal of PO_4-P from solution occurs over the range pH 6-8 (Figure 4a). At pH 9 removal of PO_4-P after 24 hours is at a rate approximately 60% of that at pH 6 with a continued gradual decline in the residual PO_4-P concentration of approximately 12% per day. Despite this slower PO_4-P removal at pH 9, the initial PO_4-P would be removed (>99%) in approximately 3.3 days. Salinity (as NaCl equivalent) over the range 0-30 ‰ at pH6 has little effect on the removal of PO_4-P by Phoslock® (Figure 4b). There is some variation in the initial uptake rate of PO_4-P in the saline experiments relative to that at 0 ‰. However, this is rapidly overcome and there is little discernable difference in the rate of uptake of PO_4-P as a function of salinity after ca. 100 minutes.

Phoslock® granules are generally mixed with in situ water and applied to a water body as a slurry. This has the effect of creating many tiny particles of Phoslock® which sink slowly through the water column. By moving down through the water column, up to 95% of the FRP is rapidly removed and adsorbed onto the surface of Phoslock® (DOUGLAS et al. 1999). Sinking rates vary to some extent according to water chemistry. In high alkalinity waters Phoslock® settles more quickly than in low alkalinity waters (REITZEL et al. 2013). Although it can take up to a few weeks for the particles to fully settle on the sediment, the surface waters are clear within a few days. Once settled on the sediment Phoslock® forms a very thin layer which quickly becomes worked into the top sediment layer through bioturbation and other natural lake processes. As a result, Phoslock® does not act as a physical barrier, but rather a chemical barrier which, due to the affinity of Phoslock® to phosphate, will continually bind phosphate that is released from the sediment (Figure 5) as long as binding sites remain available.

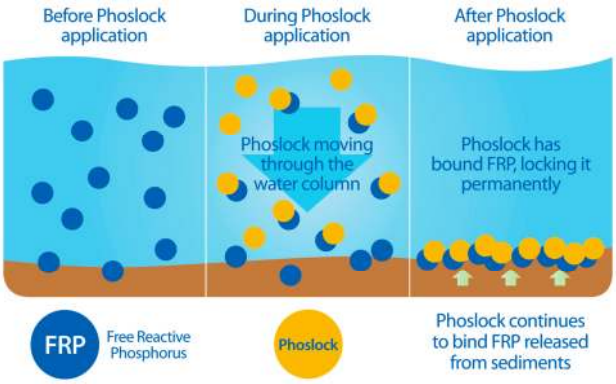


Figure 5. Illustration of the binding of Phoslock® with Free Reactive Phosphorus (FRP) in water and as it is released from sediments (Phoslock Water Solutions).

2.4 ENVIRONMENTAL CONDITIONS AND PHOSLOCK® PERFORMANCE

To a certain degree, the phosphorus binding capacity of Phoslock® is influenced by the specific conditions of aquatic environments. The following outlines the mode of action of Phoslock® in relation to varying environmental conditions.

2.4.1 PH AND ANOXIA

Phoslock® operates over a wide pH range (~ 4 to 11). The maximum adsorption capacity of Phoslock® is unaffected at pH 5–7 and starts to decrease at higher pH (HAGHSERESHT et al. 2009, GROVES 2010). This observed decline is due to the formation of the hydroxyl species of the lanthanum ions which reduces the amount of phosphate that can be bound; however it does not entirely prevent Phoslock® from working (DOUGLAS et al 2000, ROSS et al 2008). Furthermore, once bound, lanthanum or phosphate will not be released into the aquatic environment under naturally occurring environmental conditions, even when pH moves outside the optimal binding range for Phoslock®.

The uptake of FRP by Phoslock® is unaffected by the development of anaerobic and reducing conditions (DOUGLAS 2000, HAGHSERESHT 2006). Lanthanum phosphate is also stable in anoxic conditions, so phosphate will not be released even when lake sediments or overlying waters turn anoxic.

2.4.2 COMPETITION FOR PHOSPHATE FROM OTHER IONS

Lanthanum has an exceedingly high affinity for phosphate, but will also bind with other ions and compounds, including carbonate, silicate and humic acids (REITZEL et al. 2013, LUERLING & OOSTERHOUT 2013, LÜRLING et al. 2014). The performance of Phoslock® in the presence of humic acids depends on the pH of the solution. In the presence of 100 ppm humic acid, the performance of Phoslock® was not reduced significantly up to pH 7, whereas at pH 9 performance was reduced (DOUGLAS et al. 2000, ASFAR and GROVES 2009). However, the kinetics of the P uptake at pH 9 appeared to continue and Douglas et al. 2000 suggested that quantitative removal would have occurred after 9 days. Chemically, lanthanum prefers to bind with phosphate, so the lanthanum phosphate bond will form eventually, even if lanthanum temporarily binds with other ions.

2.4.3 PHOSPHATE VERSUS TOTAL (TP) OR PARTICULATE PHOSPHORUS (PP)

Phoslock® binds phosphate (PO_4^{3-}) ions. As the adsorption of phosphate onto Phoslock® is a chemical process with a 1:1 stoichiometry of lanthanum and phosphate (HAGHSERESHT et al 2009), it will not directly bind phosphorus contained within algae. Phosphorus attached to other materials (particulate phosphorus - PP) must undergo chemical or biological transformation processes before the phosphate is released and can be bound by Phoslock®. On the sediment, Phoslock® binds phosphate diffusing from the sediment pore water. It has the potential to bind phosphate released during the decomposition of organic matter on the sediment, including decaying algal cells (DOUGLAS et al. 1999, MEIS et al 2012, MEIS et al 2013).

Applications of Phoslock® to lake water where the majority of phosphorus is in the form of phosphate will therefore be highly effective and phosphate concentrations will drop quickly. On the

other hand, if most phosphorus is stored in algal cells, Phoslock® will generally work more gradually; reducing phosphorus concentrations as algae decompose. In these cases, the effects of Phoslock® applications tend to be seen a few weeks after the application, rather than immediately after an application (ROBB et al 2003, MEIS et al 2013.).

2.4.4 EXTERNAL VERSUS INTERNAL PHOSPHOROUS LOADING

One of the main aims of Phoslock® applications is to bind phosphate as it is released from lake sediments, thus forming a chemical barrier preventing diffusion of phosphate into overlying waters (DOUGLAS et al. 1999, DOUGLAS et al. 2000, ROBB et al. 2003, HAGHSERESHT et al. 2009). When Phoslock® is applied to water bodies, depending on the water chemistry, it will take a few days or weeks to sink through the water column, but will then be permanently located on the sediment (SPEARS et al. 2013). When planning Phoslock® applications, it is important to recognize that phosphorus entering lakes from external sources such as phosphorus-rich inflows or bird faeces may not be readily in contact with Phoslock® on the sediment. This spatial and temporal separation means that external sources of phosphorus must travel to the sediment (by water column mixing or the sinking and decomposition of organic matter) and be transformed into phosphate before Phoslock® has a chance to bind them. These processes may take some time and some external sources of phosphorus may never reach the sediments. This fact should be kept in mind when designing application strategies and, wherever practical, Phoslock® should be accompanied or preceded by efforts to reduce external phosphorus loadings. Where further reduction of external phosphorus inputs is impractical or unlikely to be cost effective, “top up” applications of Phoslock® may be necessary in order to ensure that phosphorus levels do not increase again following the initial application. In most cases, these “reapplications” will be significantly smaller than the initial applications.

As a result of the fact that Phoslock® is in the water column for a relatively short period of time, but thereafter on the lake sediment, Phoslock® works most effectively in lakes which have relatively low external phosphorus loads and relatively high internal phosphorus loads.

2.4.5 DURATION OF THE PHOSLOCK® TREATMENT

The longevity of the effects of Phoslock® will depend on the amount added, and thus the amount of phosphorus bound in the sediment, and the loading from external sources. Phoslock® will become saturated so beyond this point, further phosphate will not be bound (MEIS et al. 2013). Once saturated, phosphorus released from deeper sediment layers will eventually diffuse to the upper sediment layer and then be released to the overlying water. External sources may also accumulate over a period of years, providing an additional phosphorus load to the lake. Therefore the duration of the treatment depends on site specific conditions of the treated water body and the targeted phosphorus pool of the application (MÁRQUEZ-PACHECO et al. 2013).

2.4.6 PHOSLOCK® PERFORMANCE SUMMARY

To summarise the information provided in this section, the effects of Phoslock® applications will be observed most quickly when the product is applied to water bodies where the majority of water column phosphorus is in the form of phosphate and where the internal load is a relatively large proportion of the total phosphorus load to the water body. In practice this means,

1) water bodies where most of the external phosphorus sources have been removed or controlled (reduced),
2) late autumn or winter are the optimum applications times as this is when the algal standing crop is minimal, most water column phosphorus is in the phosphate form and dying algae have taken their phosphorus to the sediments.

If a summer or early autumn application is required, Phoslock® can be applied directly to the hypolimnion, which, in an eutrophic, stratified lake, will have very high phosphate concentrations. This will lead to a rapid reduction of hypolimnetic phosphorus. In this case the response by the algal community in the surface waters may not be immediate, but will trigger less phytoplankton production during the following growing seasons .

In lakes where external phosphorus inputs from the catchment remain, regular reapplications of Phoslock® are likely to be necessary.

3 APPLICATION OF PHOSLOCK®

Phoslock® is generally applied to water bodies as part of a holistic solution which includes both the supply and application of the product. Applications, especially large scale applications, are usually planned and undertaken by trained staff from PWS, its licensees or approved sub-contractors.

3.1 PHOSLOCK DOSAGE CALCULATION

Phoslock® dosing for a specific water body is always calculated based on a dataset, including all available data from lake monitoring programs as well as a "snapshot" of information collected over a short period of time prior to the application. The latter includes analysis of the most important water column and sediment parameters and provides detailed information on water and sediment chemistry (in situ parameters, ionic composition, nutrient concentration, metal content etc.), as well as an analysis of the amount of potentially releasable phosphorus in the sediment based on the Psenner fractionation method (PSENNER et al. 1988; LUKKARI et al., 2007; HUPFER et al. 2009).

Phoslock® doses are calculated according to the quantity of total phosphorus in the water column plus the quantity of releasable phosphorus in the sediment.

3.1.1 WATER COLUMN PHOSPHORUS

To obtain the phosphorus content in the water column, the measured lake water phosphorus concentration is multiplied by the lake water volume. Where phosphorus concentration varies significantly down the water column, the calculation would be based on a mean concentration.

3.1.2 SEDIMENT PHOSPHORUS

This calculation is based on the quantity of potentially releasable phosphorus in a certain depth of sediment. Potentially releasable phosphorus, analysed through Psenner fractionation (PSENNER et al. 1988; HUPFER et al. 2009), is the proportion of the total phosphorus in the sediment which comprises the loosely bound and immediately available phosphorus, the Iron and Manganese bound

phosphorus as well as the reductive releasable organic phosphorous and the phosphorous bound within organic structures such as microorganisms, detritus and humic substances.

Dose calculations are usually based on the total releasable phosphorus contained within the upper 4-5 cm of sediment. However, deeper sediment layers may also be considered for the first or later applications based on the depth of sediment which is determined to have the potential to release phosphorus into the overlying water column. Various estimates exist as to the depth of "active" sediment (BOSTRÖM et al. 1982, SØNDERGAARD et al. 1999, SØNDERGAARD et al. 2003, COOKE et al. 2005, MEIS et al. 2012) and it is between PWS and the client to agree on the exact depth of sediment to be considered.
The area of sediment to be treated is usually assumed to be somewhat less than the total lake area and is decided upon with knowledge lake bathymetry and, if appropriate, drawdown patterns.

The bulk releasable sediment phosphorus content is the quantity of releasable phosphorus contained in the total volume of sediment considered appropriate for each individual application.

3.1.3 QUANTITY OF PHOSLOCK® TO BE APPLIED

Lanthanum binds phosphate ions with a stoichiometric ratio 1:1 (HAGHSERESHT et al 2009). Laboratory tests have shown that one tonne of Phoslock® is capable of removing 34 kg of phosphate (PO_4^{3-}) or 11 kg of phosphorus (P). Based on this relationship, the recommended dosage is 100 : 1 (Phoslock® : phosphorous). With this information and a knowledge of the amount of biologically available phosphorus in the water and surface sediments of a lake, it is possible to fairly accurately calculate the Phoslock® dose for each specific water body.

3.2 APPLICATION AND DOSING STRATEGIES

Application strategies can be tailored to the particular characteristics of the water body. GPS and depth detection systems are used at all times and it is therefore possible to apply higher dosages of Phoslock® to areas of a water body where concentrations of phosphorus in the water or sediment are known to be particularly high.

It is possible to apply Phoslock® either directly onto the surface of the water body or directly into the hypolimnion. The latter approach is recommended when most of the phosphorus in a water body is located either in the sediment or the hypolimnion and when Phoslock® drift into the littoral zone of the lake is to be avoided.

Phoslock® can be added to a lake as a "one-off" application. In this case, enough Phoslock® should be added to bind virtually all of the "potentially available" phosphorus from the lake water and from the sediment. Essentially, the larger the dose (i.e. the more phosphorus removed permanently from the ecosystem), the longer and more robust the effect. An alternative to a "one off" dosage of Phoslock® could be to add Phoslock® in a staged approach. In this scenario, an initial dose would remove phosphorus from the water column and the top 4 cm of sediment and a monitoring programme would be put in place. This would indicate if and when the next dose is required. The timing of any subsequent "top up" applications will depend on the lake, external sources and the size of the first dose, but, if required, could be two or more years after the first dose. Applications of Phoslock® in this manner would be "additive", that is, the improvements from the first application will be built

upon by subsequent applications as more and more of the historically accumulated phosphorus is bound. Depending on the size of the first dose, it is also probable that later applications will be significantly smaller than the initial ones.

3.3 TRANSPORT, DELIVERY AND APPLICATION OF PHOSLOCK®

Phoslock® inventory is maintained at PWS warehouse in various locations around the world, from where it is dispatched directly to the application site (or an approved storage facility nearby) one or two days before an application. The product is generally side-loaded onto trucks and transported by road in full truck loads containing 23-24 tonnes of Phoslock®.

At the site, the product is unloaded by forklift and generally stored in a secure location undercover or under tarpaulins for 1-2 days before the application.

A number of systems exist for the application of Phoslock®. Most comprise a system of motorized coupling pontoons onto which a mixing and application system is mounted (Figure 6). The product is loaded onto the pontoon system using extended arm forklifts or telescopic loaders. Lake water is pumped through the mixing system and the Phoslock® is gravity fed through a hopper into the mixer where it is mixed into a slurry before being dispersed either onto the surface of the water body through a spray boom or injected directly into the hypolimnion.

Most application systems require three operators, with one operator steering the vessel according to a pre-determined course while the other two operators feed the product into the mixing and application system. The barge is driven in targeted transects (using Global Positioning Systems-GPS and depth sounders Figure 7b) to ensure an even dispersal of Phoslock® across the site or in the targeted areas.

Figure 6. Application of Phoslock®; pontoons with mounted mixing and application system

Large systems comprising up to 6 coupled pontoons are used for large scale applications of more than 60 tonnes (Figure 7a). These systems can hold up to 10 bulk bags and a small crane is installed on board to lift the bulk bags over the hopper through which the material is gravity fed into the mixing system.

a b

Figure 7. Large application system with coupled pontoons and crane installed on board (a), GPS system (b)

All necessary workplace safety documentation such as Risk Assessments are completed and submitted to the Client prior to the commencement of the application.

The only waste items that are generated through the applications are Phoslock® bags, the wooden pallets used for the storage and transport of Phoslock®, plastic liners, shrink wrapping used to wrap each pallet and protective cardboard pallet caps. All packaging materials are removed from the site after the application and disposed of in accordance with Local Authority Waste Regulations and authorized landfill procedures.

3.4 MONITORING

Pre- and post-application monitoring is helpful to assess and understand the effectiveness of the application. It will help determine whether internal loading is the main source of phosphorus to the lake, understanding the point at which Phoslock® has become saturated, and provide an indication as to whether there are other, previously unknown sources of phosphorus which could influence the effectiveness of the application. The monitoring can be purely chemical, to assess phosphorus concentrations, but ideally would also include some biological data, especially chlorophyll, and/or algal assessments (Figure 8). It is between PWS and the experts responsible for the specific water body to agree all particulars of the monitoring program. Inflows should also be monitored before and after applications to determine external inputs of phosphorus into the lake.

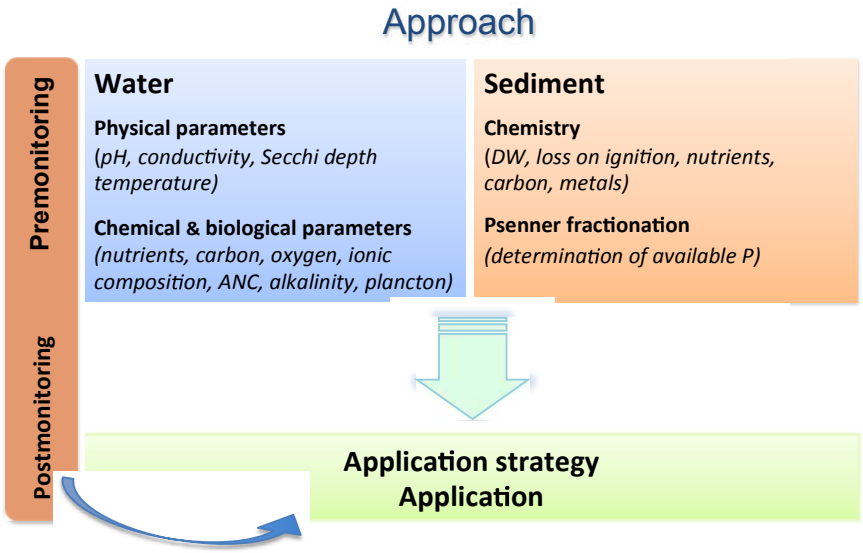


Figure 8. General approach of pre- and post-application monitoring.

The following is a typical example of a monitoring program accompanying a Phoslock® application:

Monthly lake water samples ideally taken from the open water, mid-point of the lake and taken from 1m below the surface and, in stratifying lakes, 1m above the sediment.

Unfiltered samples should be analysed for

- Total phosphorus (TP)
- Total alkalinity
- Total nitrogen (TN)
- Chlorophyll a
- Algal species composition or blue-green algal cell counts

Filtered samples should be analysed for (0.45µm filters, or equivalent)

- Soluble reactive phosphorus (SRP)
- Nitrate – nitrogen

Field measurements should include;

- Temperature and dissolved oxygen profile
- pH profiles
- Redox potential
- Secchi depth

The analysis of water samples taken near the sediment is particularly helpful in establishing the effectiveness of the application in preventing sediment phosphorus release. It also helps to differentiate between algal growth in response to external phosphorus sources and growth in response to internal sources.

The frequency of post application monitoring should be decided in consultation with the Client and can be tailored made to suit the particular water body. For example, samples could be taken every two months for two years or monthly during the summer and quarterly in the winter (or even monthly for the first year and bi-monthly for the second year, etc.)

4 APPROVALS AND CERTIFICATIONS

Phoslock® has received certifications and approval for use in a range of countries and environmental jurisdictions.

In Europe, the product can be legally imported and sold under REACH regulations (Pre-Registration No:17-2119853633-35-0000) and confirmation that Phoslock® is outside the scope of the Biocide Products Directive (BPD) (98/8/EC) was received from the UK's Health and Safety Executive in 2004.

Outside of Europe, equivalent confirmations have been received in other countries, including Australia (NICNAS Certification), the United States (Pre-manufacture Notice) and Brazil (IBAMA). In addition, a range of approvals and certifications have also been received relating to the use of Phoslock® on specific water bodies or specific types of water bodies.

Confirmation of the regulatory requirements relating to the use of Phoslock® in England and Wales was sought from the Environment Agency in 2007. As a result of this, the Policy Division of the Agency issued a Briefing Note on Phoslock® which confirmed that the Agency does not consider Phoslock® to be toxic or polluting and that Phoslock® is not a substance that requires the agreement or consent of the Agency before it can be used in water. Additional consents are however required from Natural England or the Drinking Water Inspectorate before Phoslock® can be applied to SSSI sites or sites which are used as a source of drinking water respectively.

In 2011, Phoslock® received US and Canadian NSF/ANSI Standard 60 Certification for use in drinking water. This certification ensures that Phoslock® applications to drinking water supply sources, at the maximum use rate specified on the product label, do not contribute contaminants that could cause adverse human health effects. NSF/ANSI Standard 60 is the US nationally recognized health effects standard for products which are used to treat drinking water. In addition, this certification requires annual product testing, facility inspections, quality assurance, good manufacturing practices, and product stock inspections. The United States Environmental Protection Agency and all states rely on and accept ANSI accreditations by authorized independent third party accreditation agencies, such as the Water Quality Association (WQA, http://www.wqa.org/).

In Brazil, Phoslock® has been certified by IBAMA (the Brazilian Ministry for the Environment) for import, sale and use in Brazil.

In instances where the use of Phoslock® is not covered by the above mentioned or comparable approvals, Phoslock Water Solutions, its affiliates and licensees are generally required to seek approvals to apply Phoslock® from the relevant environmental regulator. Wherever required, these have been sought and to date there have been no examples of an application failing to receive necessary approvals.

Quality Assurance

Phoslock® is manufactured in China according to PWS Australia's manufacturing protocol. The factory is certified under NSF/ANSI Standard 60. Raw materials (bentonite and the lanthanum feedstock) are supplied exclusively by US multinational companies with operations in China, both of which are accredited under the provisions of ISO 9001:2000. Details of these suppliers and their ISO accreditation certificates can be supplied upon request.

5 CASE STUDIES

Phoslock® has been applied to more than 100 lakes in Europe since 2006 and many more worldwide. The European applications have taken place in the United Kingdom, Germany, Netherlands, Poland, Italy and Finland, with the largest number of applications having taken place in Germany, followed by the United Kingdom and the Netherlands.

Most applications have taken place on relatively small recreational lakes (< 10 ha) suffering from eutrophication problems, however a number of applications have also taken place on Natura 2000 lakes with the consent of the relevant regulators. These include Loch Flemington in Scotland (treated in 2010) and the Blankensee in Germany (treated in 2010). Twelve applications have taken place on lakes with a surface area greater than 10 ha, with the largest lakes treated being Lake Kymijarvi in Finland (100 ha, 2012) and the Behlendorfer See in Germany (63 ha, 2009). By volume, the largest treatments have been the Behlendorfer See in Germany (214 tonnes, 2009), the Eichbaumsee in Germany (148 tonnes, 2010), the Mere in the United Kingdom (79 tonnes, 2013), the Serpentine in London (66 tonnes, 2012) and the Blankensee in Germany (66 tonnes, 2009). Some of the organizations which have commissioned Phoslock® applications since 2006 include:

1. The Royal Parks, UK
2. The Centre for Ecology and Hydrology, UK
3. Dundee City Council, UK
4. Wessex Water, UK
5. Water Board Brabantse Delta, NL
6. Tilburg City Council, NL
7. Het Groene Eiland Recreation Park, NL
8. City of Hamburg, DE
9. Ministry for the Environment, Schleswig Holsten, DE (Landesamt SH)
10. Ministry for the Environment, FI (Ymparisto)
11. Lahti City Council, FI
12. City of Gniezno (EU Life Plus), PL
13. The Broads Authority, UK
14. The Environment Agency, UK

The following sections present an overview of key results relating to the use of Phoslock® over the past eight years. Further details on each of these case studies, as well as a large number of other case studies, are presented on our websites at www.phoslock.com.au and www.phoslock.eu.

5.1 BEHLENDORFER SEE, GERMANY

The largest application of Phoslock® (by volume of product) to date took place in December 2009 on the Behlendorfer See near the town of Ratzeburg in Northern Germany. The Behlendorfer See is a 63 ha lake with a stable thermal stratification during summer months, a maximum depth of 15 m and an average depth of 6.20 m. The lake is frequently used for recreational purposes and is situated in an intensive farmed agricultural area. For many years the Behlendorfer See had received high inputs of nutrients. The naturally clear water conditions, typical for an oligotrophic water body, had switched to a turbid water state as a result of the regular summer blue green algal blooms and submerged macrophytes were poorly developed. As a result, the lake did not meet the ecological criteria outlined under the European Water Framework Directive. An application of 214 tonnes of Phoslock® (marketed as Bentophos® in Germany) was undertaken on the lake in December 2009 in order to permanently bind phosphate released from the sediment and break the lake's phytoplankton cycle. The material was applied to a surface area of the lake of only approximately 40 ha, which corresponded to the area of the lake which is deeper than 7m. The aim of the application was to remove 550 kg of phosphorus from the water column and 1590 kg of immediately and potentially releasable phosphorus from the upper 5 cm layer of the lake sediments.

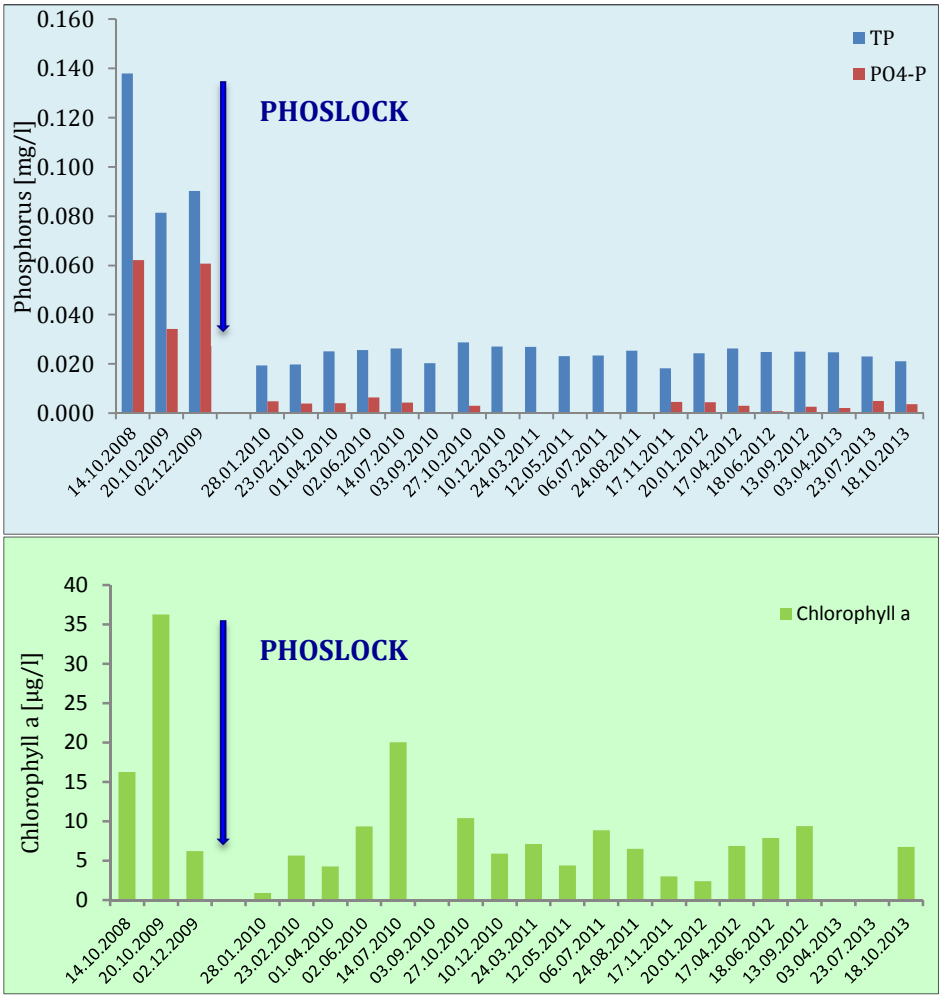


Figure 9. Phosphorus, phosphate and chlorophyll a concentrations measured in the Behlendorfer See before and after Phoslock® application in December 2009.

Since the application, the lake has been regularly monitored by the regional environmental agency, LLUR (Landesamt für Landwirtschaft, Umwelt und Ländliche Räume, the Schleswig-Holstein - Agency for Agriculture, Environment and Forestry) and the limnological institute IDN (Insitute Dr Nowak, Ottersberg). The monitoring data collected since the application indicate that phosphorus and phosphate concentrations have dropped substantially (Figure 9). Phosphorus release from the sediment has ceased and in the summer seasons following the application the summer algal biomass has been considerably lower and the Secchi depth higher. The improved light penetration in the lake has resulted in increased growth of submerged macrophytes.

5.2 BLANKENSEE, GERMANY

The Blankensee is a 22 ha shallow lake located immediately to the south of Lübeck Airport in Northern Germany. The lake has a maximum depth of 2.5 m and an average depth of 1.5 m. The lake is located within a Natura 2000 protected area and is considered under the EU's Habitats Directive to be of European significance due to its rare macrophyte community. The lake is naturally oligotrophic, but water quality in the lake had deteriorated over many years as a result of high nutrient inputs from surrounding agricultural land, waste water and the neighbouring airport. Although these inputs had been controlled, internal phosphorus loadings in the lake still remained high and the lake had frequently suffered from intensive blue green algal blooms, oxygen depletion and a substantial increase in phosphorus release from sediments. As a consequence of this, the ecosystem had shifted from macrophyte domination to phytoplankton domination.

In an effort to "switch the lake back to macrophyte domination", a decision was made by the Agency for Agriculture Environment and Forestry in the Federal German State of Schleswig Holstein (LLUR) to treat the lake in November 2009 with 66 tonnes of Phoslock®. The application of Phoslock® to the Blankensee was undertaken by the Phoslock® licensee for Germany, Bentophos GmbH. The application was designed to bind 59 kg of phosphorus in the water column and 595 kg of immediately and potentially releasable phosphorus from the upper 6 cm layer of the lake sediment.

Since the application, monitoring of the lake has continued and LLUR has reported that phosphorus concentrations have returned to their natural levels (Figure 10). The lake has reverted to a clear water state with decreased chlorophyll a concentrations (Figure 10) and ecological improvements have already been observed. These include an increase in the population of the endangered characean stonewort *Nitella flexilis*as well as the re-emergence of *Eleocharis acicularis*, an endangered species of needle spike rush.

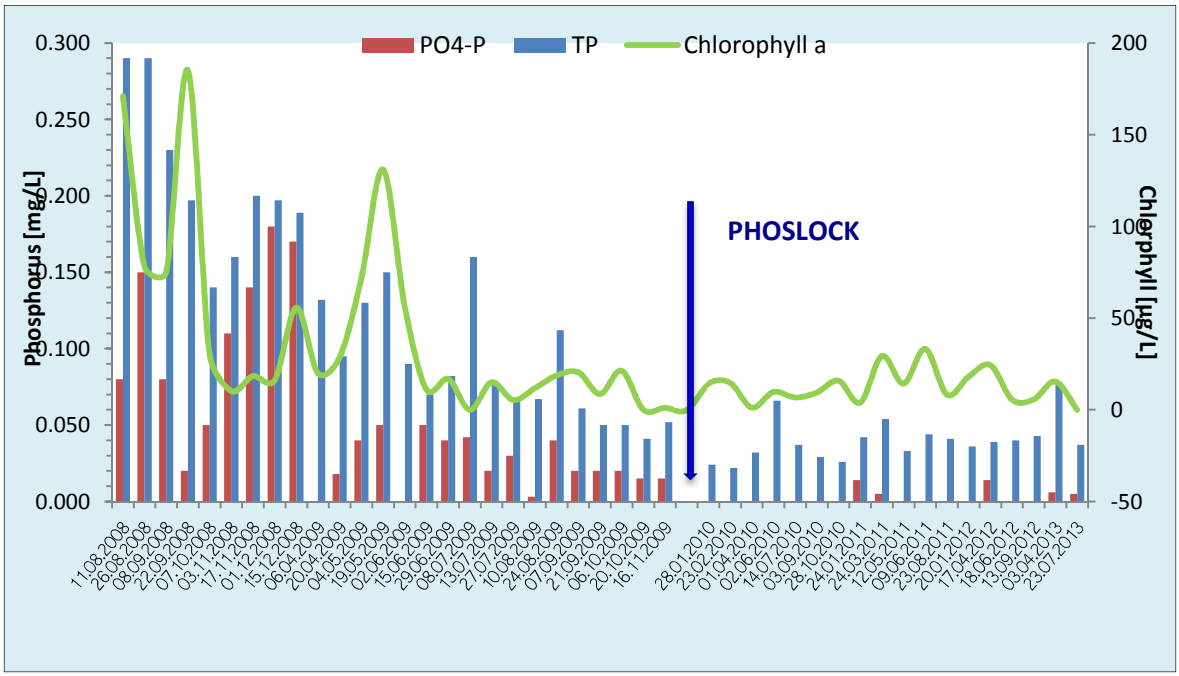


Figure 10. Phosphorus, phosphate and chlorophyll a concentrations measured in the Blankensee before and after Phoslock® application in November 2009.

5.3 HYDE PARK LONDON, UK

The Serpentine in Hyde Park and Round Pond in Kensington Gardens are among London's most famous landmarks visited by millions of people each year. Hyde Park and Kensington Gardens are located in the heart of London in the immediate vicinity of Buckingham Palace, Mayfair and Park Lane.

The Serpentine, which has a maximum depth of 4.5m and a surface area of 16 ha, is used for swimming, rowing and recreational boating and each year hosts a number of high profile sporting events. Part of the lake is located within the adjoining Kensington Gardens with this section known as Long Water. Round Pond is a shallow 3 ha lake located in Kensington Gardens next to Kensington Palace. Managing water quality in the Serpentine had always been a priority for The Royal Parks, the authority that manages Hyde Park, however, the Serpentine and the Round Pond were susceptible to blue green algal blooms during the warmer months of the year. Phoslock Europe was commissioned by The Royal Parks, to apply nearly 80 tonnes of the product in late February and early March 2012 in order to reduce phosphorus and algal levels in both lakes. In addition to Phoslock®, The Royal Parks also undertook a number of other measures aimed at improving the long-term water quality and ecology of the lakes, including aeration, fish removal, increased water exchange and the planting of reed beds.

Following the application of Phoslock® at the end of February, there was an immediate and sustained reduction in phosphorus and phosphate to < 0.02 mg/L, which is the limit of detection of the analysis method used (Figure 11).

As shown in Figure 12, chlorophyll a concentrations were also significantly lower in 2012 than they were during 2011, indicating a significant reduction in overall biomass. The clearer water and lower

phosphate concentrations in the lake during 2012 were sufficient to allow macrophytes to start to grow and for the first time in many years, an abundant growth of macrophytes was evident in the shallower areas of the lake by May. Some of the macrophytes growing in the lake needed to be cut back in July in preparation for sporting events held in the lake over the summer. The disturbance of the sediment during macrophyte removal caused a slight increase in phosphorus levels from the middle of July. Nevertheless, phosphorus concentrations in August 2012 were still 80% lower than in August 2011 and there was no bloom of blue green algae during the summer 2012.

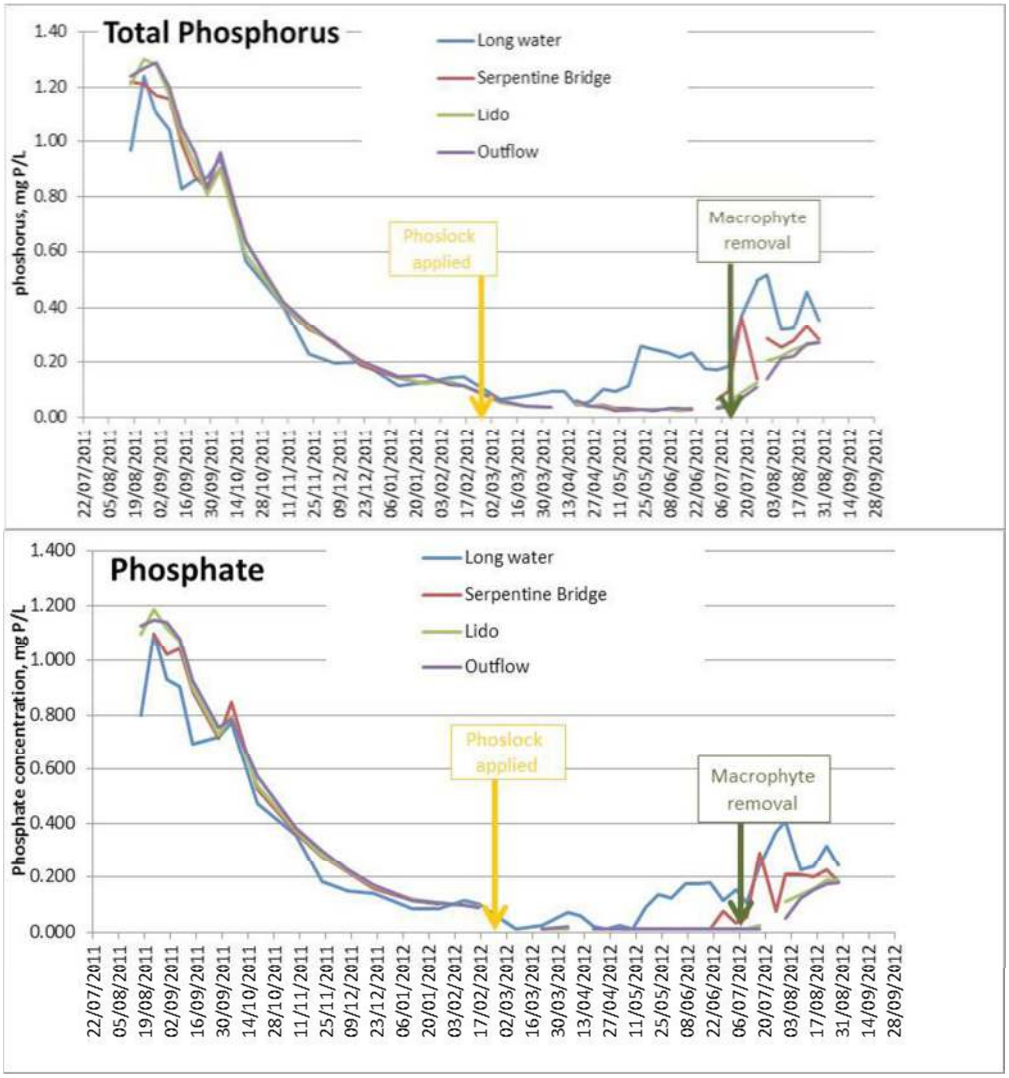


Figure 11. Average TP and PO_4-P concentrations (mg P/L) in the Serpentine. Data collated from Royal Parks and Phoslock Europe sampling. (Arrow indicates the time of the Phoslock® application and macrophyte removal)

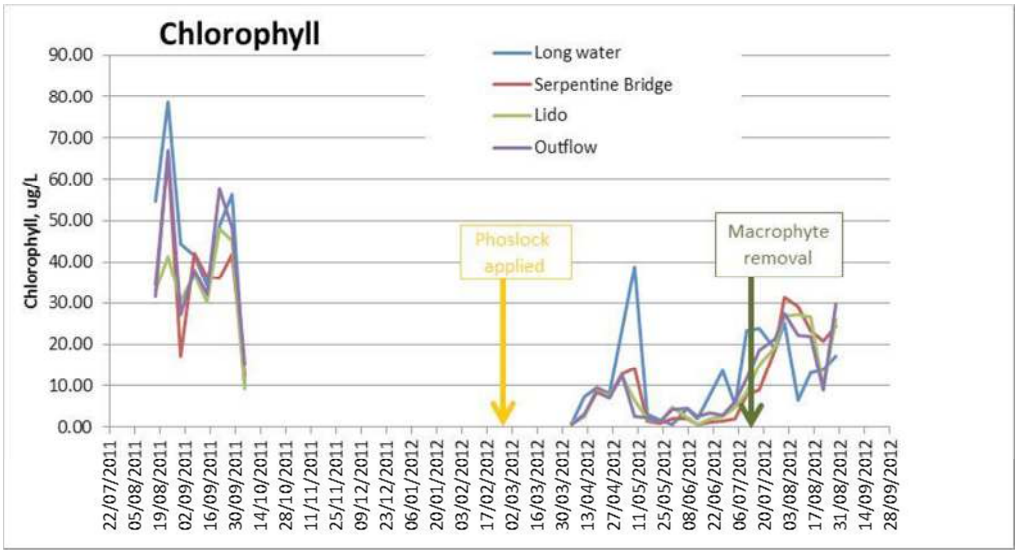


Figure 12. Chlorophyll-a concentrations (mg P/L) in the Serpentine. Data collated from Royal Parks and Phoslock Europe sampling. (Arrow indicates the time of the Phoslock® application and macrophyte removal).

5.4 CLATTO RESERVOIR AND LOCH FLEMINGTON, UK

Clatto Reservoir

Clatto Reservoir is located in the north western outskirts of Dundee. The reservoir is a popular recreational reservoir, used for various educational activities by the Ancrum Outdoor Educational Center (AOEC), and was formerly used to supply water to the city of Dundee. In recent years the reservoir has not been used for water supply, but it can still be used for this purpose in case of an emergency. The reservoir has a surface area of 9 ha, a maximum depth of 7 m and a mean depth of 2.7 m. The total water volume is around 350 000 m³.

In the years before the treatment the lake experienced large cyanobacteria blooms and had been frequently closed to recreational users. Testing undertaken by the Scottish Environment Protection Agency (SEPA) in August 2008 showed high concentrations of various species of blue green algae (including *Gloeotrichia, Coelosphaerium, Microcystis* and *Anabaena*) with concentrations ranging between 50,000 to 129,000 cells/ml (GROVES 2009), which are well above the World Health Organization (WHO) guidelines of 20,000 cells/ml (WHO 2003).

Evidence strongly suggested that the poor water quality conditions were the result of a legacy of phosphorus enrichment resulting in phosphorus release from sediments. To control the release of phosphorus from the sediment and reduce the occurrence of cyanobacteria blooms, an application of 24 tonnes of Phoslock® was undertaken on Clatto Reservoir in March 2009.

Following the application of Phoslock® the surface water (1 m) and deeper water (5 m) phosphorus (TP) and phosphate (PO_4-P) concentrations decreased considerably and average water column TP concentrations did not exceed the 35 µg/L target for more than one year (Figure 13). Nevertheless, laboratory controlled sediment core experiments conducted by the CEH (Center of Ecology and Hydrology, http://www.ceh.ac.uk/) in Edinburgh after the completion of the application indicated

that the 24 tonne application was an under dose (MEIS et al. 2012) and signs of phosphorus re-release from sediment were apparent during the second summer.

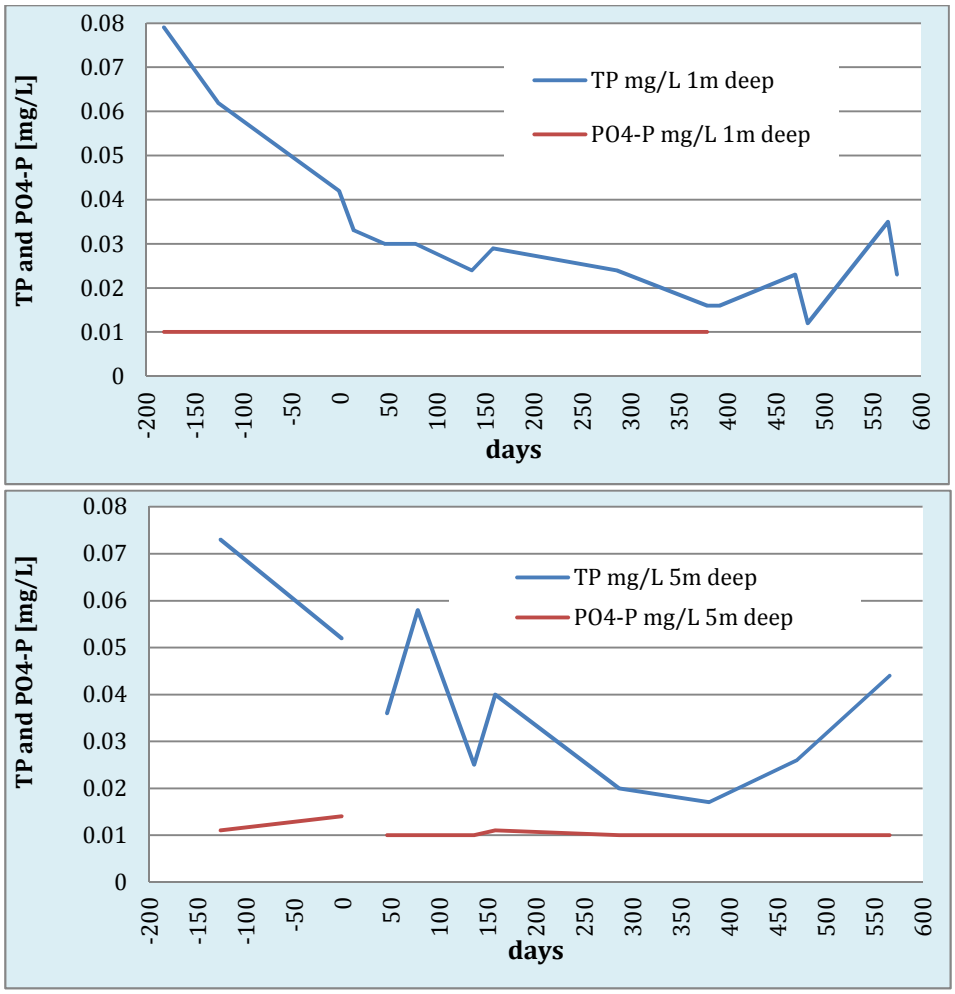


Figure 13. Phosphorus (TP, PO_4-P) concentrations from the surface (1m) and deep waters (5m) plotted against the number of days before and after the application. Application on day 0

Mean monthly (June-October) cyanobacterial abundance was significantly lower in the first season after the application (2009) than in the pre-application years of 2007 and 2008 when algal cell counts often exceeded the World Health Organisation (WHO 2003) guidelines of 20000 cells/ml (CEH Edinburgh website: http://www.ceh.ac.uk/sci_programmes/water/clattoreservoir.html, MEIS 2012). Cyanobacterial abundance increased again during summer 2010, presumably as a result of the phosphorus re-release that occurred during this year. Although cyanobacterial concentrations were relatively high in 2010, the WHO guidelines of 20,000 cells/ml were exceeded in only one month compared to the situation prior to the application of Phoslock® (MEIS 2012) and subsequent monitoring during the 5 years following the application indicate that, despite the under dose, cyanobacterial abundance has declined in all years with the exception of 2010 and algal cell counts have remained below the WHO 2003 guidelines of 20 000 cells/ml (SPEARS 2013, presentation, MEIS 2012). The phytoplankton response in Clatto Reservoir has also been consistent with WFD phytoplankton metrics (SPEARS 2013, presentation).

Loch Flemington

Loch Flemington is a high alkalinity, eutrophic loch located near the city of Inverness in Scotland. The loch has a surface area of approximately 15 ha and is shallow, with a mean depth of 0.75 m and a maximum depth of 2.35 m. The loch is situated within the Kildrummie Kames Site of Special Scientific Interest (SSSI) and is host to three European Protected Species: the Slavonian Grebe (*Prodiceps auritus*), the rare aquatic plant Slender Naiad (*Najas flexilis*) and the Great Crested Newt (*Triturus cristatus*).

The loch has a long and relatively well-documented history of cultural eutrophication problems, including regular summer blooms of potentially toxin producing cyanobacteria (BENNION et al. 2008, MEIS et al. 2013). Assessment of phosphorus loads to Loch Flemington indicated that diffuse sources were the dominant external source and that the internal, sediment-driven load to the water-column was about five times greater than the load from the catchment (MAY et al. 2001 cited in MEIS et al. 2013). The loch had become increasingly eutrophic with the result that water quality had deteriorated significantly and the conservation status of the lake was under threat.

In order to reduce water column phosphorus concentrations and sediment phosphorus release, 25 tonnes of Phoslock® were applied to Loch Flemington between 15 and 17 March 2010. The measure was undertaken as part of a restoration project initiated by the Centre for Ecology and Hydrology in Edinburgh (CEH). The application and impacts to the loch were the basis for a PhD study at the CEH. Part of this work has been published (MEIS et al. 2013, GUNN et al. 2014) and further information can be found on the CEH website (http://www.ceh.ac.uk/sci_programmes/water/lochflemington.html). The advantage of the project is the collection of pre- and post -application data specifically tailored to the Phoslock® application. The study showed that the annual average total phosphorus concentration before the application was 0.074 mg/L and following the application 0.037 mg/L. Mean summer total phytoplankton biovolume was significantly lower in 2010 and 2011 compared to pre-application estimates (MEIS 2012). Alterations in ecological structure indicated a change in state from a 'phytoplankton dominated turbid state' to a 'macrophyte dominated clear waterstate' (MEIS 2012).

5.5 LAKE RAUWBRAKEN, NETHERLANDS

Lake Rauwbraken is a bathing lake in the district Berkel-Enschot (Tilburg Municipality). It is used for swimming, daytime recreation, outdoor sports, diving activities and nature education. The lake was created in 1967 by sand excavation, has a total surface area of 4 ha, a maximum depth of 15 m and a mean depth of 6 m.

Since the mid-1990s, an increase in cyanobacteria blooms has been observed, causing bathing water quality norms to be exceeded (secchi depth, microcystin concentrations, blue green algae cell densities). Prior to treatment, the bathing area was periodically closed to the public for increasingly long periods, culminating in a 4 month swimming ban in 2007. The dominating cyanobacteria were *Planktothrix rubescens, Microcystis aeruginosa, Aphanizomenon flos-aquae, Anabaena spp*. and *Woronichinia naegeliana* (VAN OOSTERHOUT & LUERLING 2011). In April 2008, flocks and floating layers of blue green algae were detected, with these consisting primarily of the toxic *Aphanizomenon spp.. Planktothrix rubescens* appeared to be present deeper in the water column (7-10 m).

During the years prior to the treatment, the internal phosphorus load is considered to have been an important source for phosphate in the lake. Furthermore, the lake receives phosphate through groundwater and during most of the year a significant fraction of the phosphate is present in particulate form in the water column (LUERLING& VAN OOSTERHOUT 2009).

In April 2008 restoration measures were undertaken on the lake. The measures were the initiative of the Aquatic Ecology Department of Wageningen University, which proposed that the lake be treated with a combination of Phoslock® and a flocculent. The combination of the flocculent (Polyaluminiumchloride, PAC) and Phoslock® has since acquired the name "Flock & Lock". The Flock & Lock treatment was aimed at reducing the quantity of phosphate in the water column, reducing the quantity of particulate phosphorus and reducing the release of phosphate from the sediment and groundwater.

Detailed results from the treatment have been published in LUERLING & VAN OOSTERHOUT 2013 and VAN OOSTERHOUT & LUERLING 2011. In the months following the treatment, TP concentration dropped drastically and the mean summer concentration in 2008 averaged 17.5 µg P/L (LUERLING & VAN OOSTERHOUT 2013, summarised in Table 2). The treatment effectively reduced the amount of TP in the water column from on average 169 (±126) µg P/L in the years before the application to 14 (±15) µg P/L for up to 5 years after the Flock & Lock treatment (LUERLING & VAN OOSTERHOUT 2013). Post-monitoring data show that the mean summer TP concentrations were reduced from 235 µg P/L in 2006 to 17.5, 9.6, 12.4, 9.8 and 12.8 µg P/L in the years 2008 until 2012.

Before treatment the mean summer TP concentration in the hypolimnion of Lake Rauwbraken (June 21 - September 21, depths > 5 m) was 240 µg P/L. After treatment the mean hypolimnic TP concentrations during the summer months were 30 µg P/L in 2008 and 2010 whereas in 2009, 2011, 2012 the mean hypolimnic TP concentrations were below 20 µg P/L.

The treatment in Lake Rauwbraken resulted in significantly lower chlorophyll-a concentrations in the years after the application compared to the situation before the application (LUERLING & VAN OOSTERHOUT 2013). The average chlorophyll-a concentration after the Flock & Lock treatment was 3.7 (±4.5) µg/L, while the mean chlorophyll-a concentration before the treatment was 19.5 (±36.5) µg/L. The mean summer chlorophyll-a concentrations were reduced from 27 µg/L in 2006 and 28 µg/L in 2007 to 5.7, 2.3, 5.4, 2.3 and 4.9 µg/L in the years 2008 until 2012(LUERLING & VAN OOSTERHOUT 2013).

Before the treatment, Lake Rauwbraken experienced periods with very turbid water and there were water layers found at different depths with a high turbidity. The average turbidity in this period was 5.3 NTU. After the treatment, the water initially became very turbid because of the added clay, however the clay quickly settled through the water column resulting in very clear water with a turbidity of less than 1 NTU (LUERLING & VAN OOSTERHOUT 2009). The secchi depth increased steadily to more than 10 m in November 2008, at which time the sediment was visible from the pontoon bridge. In the 6 years since the application, Lake Rauwbraken has remained a crystal clear lake, open the whole year for recreational use and without the necessity to impose swimming bans (Luerling M. personal communication).

Table 2. Pre- and post treatment conditions in Lake Rauwbraken , *(Data: LUERLING & VAN OOSTERHOUT 2013)*. TP= Total phosporus concentration [µg/L], Chl-a= chlorophyll a concentration [µg/L], hypol.= hypolimnion depth >5 m.

		Pre-treatment		Post-treatment	
TP	(mean 2006-2007)	169 (±126)	(mean 2009-2012)	14 (±15)	
Chl-a	(mean 2006-2007)	19,5 (±36,5)	(mean 2009-2012)	3,7 (±4,5)	
		Swimming ban		No Swimming ban	

	Pre-treatment		Post-treatment				
	2006	2007	2008	2009	2010	2011	2012
TP (mean summer concentration)	235	29	17,5	9,6	12,4	9,8	12,8
Chl-a (mean summer concentration)	27	28	5,7	2,3	5,4	2,3	4,9

	Pre-treatment	Post-treatment				
		2008	2009	2010	2011	2012
TP (hypol. mean summer concentration)	240	30	< 20	30	< 20	< 20

5.6 LAKE VARESE, ITALY

Lake Varese is a 14.8 km^2 lake located in the foothills of the Alps in northern Italy. Lake Varese is considered one of Europe's first and most glaring examples of anthropogenic eutrophication (ZACCARA et al. 2007, CROSA et al. 2013). Its maximum depth is 25 m and the medium depth is 10.7 m. The lake's water quality started to deteriorate in the 1960s and for many years the lake was classified as hypertrophic. Improvements to water quality were observed during the 80s and 90s following the reduction of the external load through improved wastewater collection systems, however by 1998 the positive effects of this were no longer evident and the lake remained eutrophic due to its high internal phosphorus load (ZACCARA et al. 2007, CROSA et al. 2013).

Hypolimnetic water withdrawal and the injection of pure oxygen were undertaken between 2000 and 2003 in an effort to further reduce the trophic status of the lake, however neither of these measures resulted in a significant reduction in annual mean TP concentrations (SALVETTI et al. 2006, ZACCARA et al. 2007).

In 2005, the Provincial Government began considering the use of Phoslock® as a measure to reduce the internal phosphorus load in the lake. In late 2008 and following several years of lab scale trials, Phoslock Europe GmbH. and the Institut Dr Nowak were commissioned to construct a series of large-scale mesocosms in the lake so that the effect of Phoslock® in reducing phosphorus concentrations in the water column and preventing phosphorus release from the sediment could be verified in situ.

In total, 3 mesocosms made from reinforced polyethylene were constructed, with each mesocosm being 2m in diameter and 17m in length. Each mesocosm was secured to a solidly anchored floating pontoon located in 15m of water and lowered carefully from the pontoon into the sediment. 200 kg steel rings were attached to the bottom of each mesocosm so that each mesocosm would extend into the sediment. Each tube enclosed an area of 3.14 m^2 sediment and 47 m^3 of lake water (Figure 14). Following installation, Phoslock® was dosed to two of the mesocosms (M2, M3) while the other

one (M1) was used as a control. The applied dosage of Phoslock® (1150 g) was increased by 20 % over the theoretical requirement (960 g) to compensate for wall attraction effects of the tubes. The trial commenced in February 2009 and continued until October 2010, but needed to be restarted in November 2009 after a major storm event caused serious damage to two of the mesocosms. One of the treated mesocosms was damaged in a further storm event in June 2010.

The outcomes of the mesocosm trials on Lake Varese are described in detail in three independent reports which can be provided upon request by the Department of the Province of Varese (reports: YASSERI & NOWAK 2011, CROSA 2011, O.L.V. 2010; for request: http://www.provincia.va.it/ambiente-energia, http://www.provincia.va.it/Contatto). The study has been peer reviewed and published in CROSA et al. 2013.

The results demonstrate that Phoslock® had a clear effect on phosphorus concentrations in the treated mesocosm. Total phosphorus (TP) in the treated mesocosm remained around 0.025 mg / L for the duration of the trial while average TP concentrations in the lake and the control mesocosm increased to more than 0.1 mg/L following the development of anoxic conditions in the hypolimnion and the subsequent phosphorus release from sediment. Figure 15 shows the TP and orthophosphate (PO_4-P) concentrations at each depth in October 2010, the period in time when maximum phosphorus concentrations were recorded in both the lake and the untreated mesocosm M1. The situation in the treated mesocosm M3 clearly shows the effectiveness of the sediment capping with Phoslock® which inhibited the P-release from the sediment within mesocosm M3.

Figure 14. Mesocosm trial on Lake Varese, testing facility.

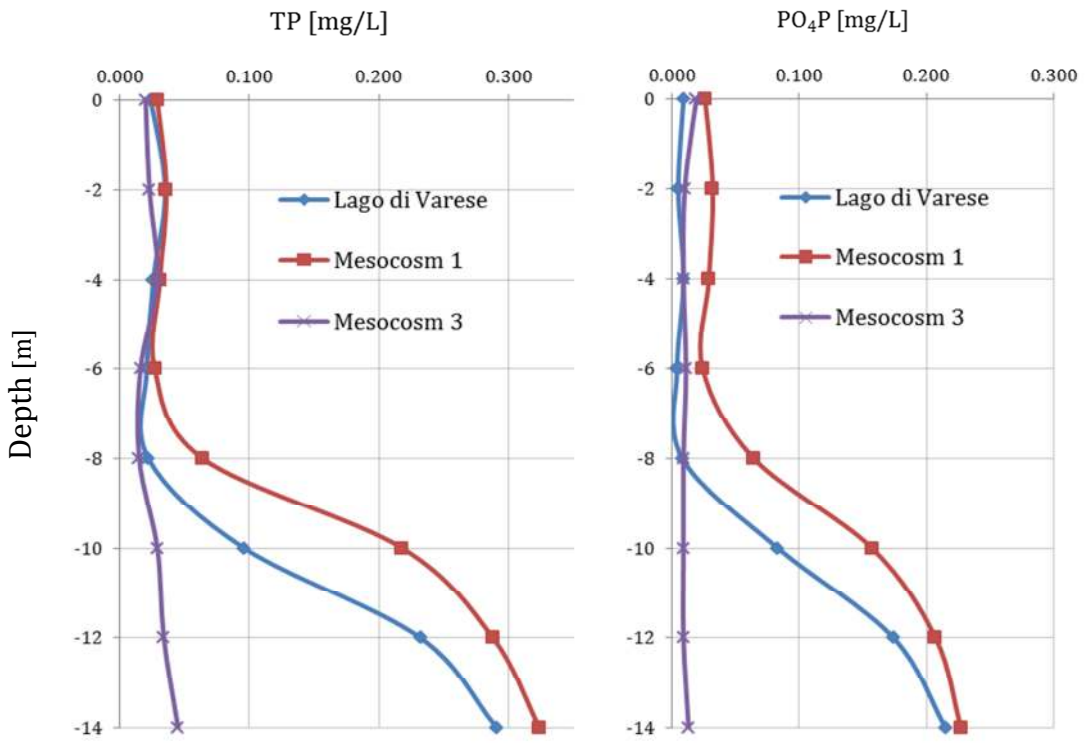


Figure 15. Depth profile of average total phosphorus (TP) and orthophosphate (PO_4-P) concentrations in the mesocosms and the lake in October 2010; mesocosm 1 (M1) untreated, mesocosm 3 (M3) treated with Phoslock®. Treated mesocosm 2 (M2) was damaged in July 2010 and data are not reported here.

6 ECOTOXICITY ASSESSMENT

Ecotoxicology is the science of contaminants in the biosphere and their effects on constituents of the biosphere, including humans (NEWMAN & CLEMENTS 2008)

Aquatic toxicology is the study of the effects of manufactured chemicals and other anthropogenic and natural materials (collectively termed toxic agents or substances) on aquatic organisms at various levels of organisation, from sub cellular through individual organisms to communities and ecosystems. Effects can cause both positive and negative deviations from previously existing circumstances, but aquatic toxicology focuses primarily on the deviations that are considered to be adverse in nature on recovery processes in biota. Adverse effects at the organismal level include both short-term and long-term lethal and sub-lethal effects (RAND et al. 1995).

6.1 SUMMARY OF EXISTING DATA FROM TOXICITY TESTS WITH PHOSLOCK®

Phoslock® can either be applied to a water body in the form of a slurry (Phoslock® granules mixed with the application water before an application) or as a direct application of granules. The potential toxicity of Phoslock® could affect the organisms living in the water column and on the sediment.

A large number of laboratory toxicity tests on Phoslock® and/or lanthanum chloride have been conducted by several independent organisations and research groups using a range of test organisms. Most of the tests used the US EPA TCLP (Toxic Characteristic Leach Protocol) method

(USEPA, 1986 cited in GROVES 2010) while a number of tests have been performed using a suspension of Phoslock® granules. The tests were performed in compliance with OECD/EEC Test Methods and in accordance with OECD Principles of Good Laboratory Practices (GROVES 2010).

A detailed delineation and discussion of the existing data from toxicity tests with Phoslock® and Lanthanum chloride using aquatic organisms is given in GROVES 2010, which is available for download on the Phoslock Europe webpage (http://www.phoslock.eu/). Therefore, in this section only a brief description of the results of toxicity tests with Phoslock® using sentinel water column and benthic invertebrates as well as fish species is provided.

6.1.1 ZOOPLANKTON

Results from nine acute and chronic toxicity studies using two different species of water fleas are summarised in Table 3. Both a Phoslock® suspension of granules and a leachate extracted by the TCLP method were used in these studies.

The responses of these zooplankton species, including mortality, reproduction and growth were evaluated throughout the Phoslock® exposures. The data show a wide margin of safety associated with Phoslock® applications at predicted use rates and environmentally relevant concentrations (cf. highest reported Phoslock® dose of 333.33 mg/L, SPEARS et al. 2013).

Table 3. Summary of results from toxicity tests with Phoslock® and sentinel zooplankton organisms. NOEC= no observed effect concentration; LOEC=lowest observed effect concentration. ✚Due to the amount of Phoslock® needed to result in impacts to biota, many studies did not test concentrations high enough to result in significant impacts, thus toxicity endpoints are reported as greater than the highest concentration tested and do not indicate the actual lowest observed effect levels

Species	Endpoint	Test duration	NOEC	✚LOEC or (EC50/LC50)	Medium	Method	Reference
Ceriodaphnia dubia	Mortality	48 h	50 mg/L Phoslock®	* > 50 mg/L Phoslock®	Natural lake water	Phoslock® solution	Ecotox 2008
Ceriodaphnia dubia	Reproduction	7 d	1mg/L Phoslock®	** > 1mg/L Phoslock®	Natural lake water	Phoslock® solution	Ecotox 2008
Ceriodaphnia dubia	Mortality	7 d	1mg/L Phoslock®	** > 1mg/L Phoslock®	Natural lake water	Phoslock® solution	Ecotox 2008
Ceriodaphnia dubia	Mortality	48 h	12 500 mg/L Phoslock®	LC50: 24500 mg/L Phoslock®	Synthetic soft water	Phoslock® TCLP	Stauber 2000
Ceriodaphnia dubia	Mortality (M)/ Repr. (R)	7 d	R: <3125 mg/L Phoslock®	M: LC50= 20500 mg/L Phoslock®	Synthetic soft water	Phoslock® TCLP	Stauber 2000
Daphnia magna	Mortality	48 h	25 000 mg/L Phoslock®	> 50 000 mg/L Phoslock®	Synthetic soft water	Phoslock® TCLP	Martin& Hickey 2004
Daphnia magna	Mortality	48 h	-	LC50: 4900 mg/L Phoslock®	Dechlor. Tap water	Phoslock® solution	Watson-Leung 2009
Daphnia magna	Mortality	48 h	-	*** > 6800 mg/L Phoslock®	Natural Pond water	Phoslock® solution	Watson-Leung 2009

Daphnia magna	Weight	5 d	100 mg/L Phoslock®	EC50: 871 mg/L Phoslock®	Artificial RT medium	Phoslock® solution	Luerling & Tolman 2010
Daphnia magna	Length	5 d	500 mg/L Phoslock®	EC50: 1557 mg/L Phoslock®	Artificial RT medium	Phoslock® solution	Luerling & Tolman 2010

* 50 mg/L was the highest concentration tested, ** 1 mg/L was the highest concentration tested, *** The 48 h LC50 of Phoslock™ for *daphnia* could not be calculated from this test since there was only 42% mortality in the highest test concentration.

6.1.2 FISH

Results from four acute toxicity studies using three different species of Rainbow fish are summarised in Table 4. Both a Phoslock® suspension of granules and a leachate extracted by TCLP method were used in these studies. Based on these data, risks to fish are to be expected to be minimal with applications of Phoslock® at standard dose rates (c.f. SPEARS et al. 2013) in aquatic environments.

Table 4. Summary of results from toxicity tests with Phoslock® and sentinel fish species. NOEC= no observed effect concentration; LOEC=lowest observed effect concentration.

✚Due to the amount of Phoslock® needed to result in impacts to biota, many studies did not test concentrations high enough to result in significant impacts, thus toxicity endpoints are reported as greater than the highest concentration tested and does not indicate the actual lowest observed effect levels

Species	**Endpoint**	**Test duration**	**NOEC**	**+ LOEC** or (EC50/LC50)	**Medium**	**Method**	**Reference**
Melanotaenia duboulayi - juvenile	Immobili-sation	96 h	50 000 mg/L Phoslock®	> 50 000 mg/L Phoslock®	Synthetic soft water	Phoslock® TCLP	Stauber 2000
Oncorhynchus mykiss - fry	Mortality	96 h	> 3125 mg/L Phoslock®	4350 mg/L Phoslock®	Synthetic soft water	Phoslock® TCLP	Martin & Hickey 2004
Melanotaenia splendida – larval fish	Imbalance	96 h	50 000 mg/L Phoslock®	EC50 >50000 mg/L	Synthetic soft water	Phoslock® TCLP	Ecotox 2006a
Oncorhynchus mykiss - fry	Mortality	96 h	-	*LC50 > 13600 mg/L Phoslock®	Natural Pond water	Phoslock® solution	Watson-Leung 2009

* 13 600 mg/L highest dose tested

6.1.3 BENTHIC ORGANISMS

When Phoslock® granules are added to water, the granules disperse as they sink rapidly through the water column before the product settles on the sediment surface and forms a permeable layer. When Phoslock® is applied as slurry, made from mixing dry granules with site water, it moves down through the water column at a slower rate and settles on the sediment surface forming a permeable layer. Both slurry and granules of Phoslock® remove FRP from water column while descending, and the permeable sediment capping layer of Phoslock® is capable of adsorbing released FRP from the sediment on its available binding sites. The thickness of the Phoslock® layer on the sediment depends on the dose rate. In the case of the recommended dose rate of 100:1(100 g Phoslock® for 1 g FRP), the sediment layer of a 2 m deep water body will be 0.2 mm thick if the concentration of phosphorus

is 0.2 mg/L (GROVES 2010). Phoslock® is a modified clay product with a density similar to that of the clay component that is already part of the natural lake sediment. Thus, the deposition of a Phoslock® layer on the sediment should be comparable to the general movement of the sediment, with the benthic organisms being able to re-establish themselves in a similar way after an application. In addition, the deposition of the Phoslock® particles occurs over several hours allowing the benthic macro-invertebrate fauna time to re-establish themselves in or over the permeable Phoslock® layer.
In this section the results from toxicity tests with one species of fresh water shrimp and four species of fresh water dwelling organisms are described.

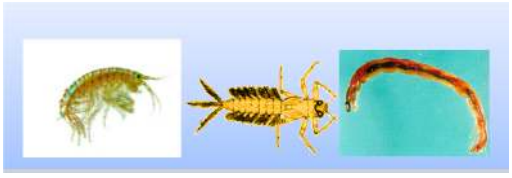
Results from five toxicity studies using the fresh water shrimp *Macrobrachium* sp. are summarised in Table 5. In these studies, a Phoslock® leachate (extracted by TCLP method) was used. The results show a wide margin of safety associated with Phoslock® applications at predicted dose rates (c.f. SPEARS et al 2013) for the tested fresh water shrimp *Macrobrachium sp.*

Table 5. Summary of results from toxicity tests with Phoslock® using fresh water shrimp *Macrobrachium sp*. NOEC= no observed effect concentration; LOEC=lowest observed effect concentration.
✚Due to the amount of Phoslock® needed to result in impacts to biota, many studies did not test concentrations high enough to result in significant impacts, thus toxicity endpoints are reported as greater than the highest concentration tested and does not indicate the actual lowest observed effect levels

Species	Endpoint	Test duration	NOEC	✚ LOEC or (EC50/LC50)	Medium	Method	Reference
Macrobrachium sp. juvenile	Survival	96 h	> 50 000 mg/L Phoslock®	EC50 > 50000 mg/L Phoslock®	Synthetic soft water	Phoslock® TCLP	Ecotox 2006b
Macrobrachium sp. juvenile	Survival	7 d	400 mg/L Phoslock®	EC50 > 800 mg/L Phoslock®	Synthetic soft water	Phoslock® TCLP	Ecotox 2006b
Macrobrachium sp. juvenile	Survival	14 d	400 mg/L Phoslock®	EC50 = 800 mg/L Phoslock®	Synthetic soft water	Phoslock® TCLP	Ecotox 2006b
Macrobrachium sp. juvenile	Survival	21 d	400 mg/L Phoslock®	EC50 = 700 mg/L Phoslock®	Synthetic soft water	Phoslock® TCLP	Ecotox 2006b
Macrobrachium sp. juvenile	Survival	28 d	400 mg/L Phoslock®	EC50 = 700 mg/L Phoslock®	Synthetic soft water	Phoslock® TCLP	Ecotox 2006b

Table 6 summarizes results from four toxicity studies using four different species of fresh water sediment-dwelling organisms. These studies used different concentrations of Phoslock® solutions. The results show that, even at high test concentrations, no significant survival or growth impacts could be observed in any of the sediment toxicity test species. This ensures that a considerable safety margin is maintained when Phoslock® is applied at the recommended dose rates.

Table 6. Summary of results from toxicity tests with Phoslock® using fresh water sediment dwelling invertebrate species; NOEC= no observed effect concentration; LOEC=lowest observed effect concentration.
✚Due to the amount of Phoslock® needed to result in impacts to biota, many studies did not test concentrations high enough to result in significant impacts, thus toxicity endpoints are reported as greater than the highest concentration tested and does not indicate the actual lowest observed effect levels.



Species	Endpoint	Test duration	NOEC	✚ LOEC or (EC50/LC50)	Medium	Method	Reference
Amphipode: *Hyaella azteca*	Growth	14 d	3400 mg/L Phoslock®	>3400 mg/L Phoslock®	Natural Pond water	Phoslock® solution	Watson-Leung 2009
Amphipode: *Hyaella azteca*	Survival	14 d	3400 mg/L Phoslock®	>3400 mg/L Phoslock®	Natural Pond water	Phoslock® solution	Watson-Leung 2009
Mayfly: *Hexagenia spp.*	Growth	21 d	450 mg/L Phoslock®	>450 mg/L Phoslock®	Natural Pond water	Phoslock® solution	Watson-Leung 2009
Mayfly: *Hexagenia spp*	Survival	21 d	450 mg/L Phoslock®	>450 mg/L Phoslock®	Natural Pond water	Phoslock® solution	Watson-Leung 2009
Midge larvae: *Chironomus zealandicus*	Survival	38 d	400 mg/L Phoslock®	>400 mg/L Phoslock®	Natural lake water	Phoslock® solution	Clearwater 2004
Midge larvae: *Chironomus dilutus*	Survival	10 d	3400 mg/L Phoslock®	>3400 mg/L Phoslock®	Natural Pond water	Phoslock® solution	Watson-Leung 2009
Midge larvae: *Chironomus dilutus*	Growth	10 d	3400 mg/L Phoslock®	>3400 mg/L Phoslock®	Natural Pond water	Phoslock® solution	Watson-Leung 2009

Note: Results published in CLEARWATER & HICKEY 2004, AUS2004-004 are not included in Table 6 as this study was based on a pre-commercial formulation of Phoslock®. A detailed review of this paper is included in GROVES, 2010.

6.1.4 CONCLUSION

Phoslock® is patented phosphorus locking technology that has been specifically formulated to decrease potential exposure to aquatic biota. Phoslock® poses a negligible to very low risk to the aquatic environment while providing a high affinity to bind and remove phosphorus that results in improvements to water quality. A review of toxicity data has shown a large margin of safety to aquatic organisms exposed to Phoslock® during and following application at typical dosage rates.

7 PHOSLOCK® AND HUMAN HEALTH

Phoslock® is a an effective P-inactivation and blue-green algae management tool, that was developed by the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia, to bind dissolved phosphorus in eutrophic water bodies. The raw materials of Phoslock® consist of an inactive clay-based carrier (bentonite) which comprises 95% of the product by weight and the active ingredient lanthanum, which makes up 5% by weight. Although lanthanum ions are strongly associated with the bentonite clay and are not released as soluble lanthanum into the water, physico-chemical properties of the water (e.g. soft water, low alkalinity, low phosphate concentrations) may however cause the release of tiny amounts of loosely bound lanthanum from Phoslock® (Spears et al. 2013). Hence, the potential risk for human health from the presence of very small amounts of soluble lanthanum in the water after a Phoslock® applications requires assessment.

This chapter summarizes, discusses and assesses the possible toxic impacts of Phoslock® to human health based on the relevant scientific literature and assessment studies. A detailed analysis and interpretation of the potential health risk for humans related to the use of Phoslock® for the reduction of phosphorus in eutrophic water bodies is given in GROVES 2009, GROVES 2011 and DAVIES 2011. All documents are available for download on the Phoslock Australia and Europe websites (http://www.phoslock.com.au and http://www.phoslock.eu/).

7.1 BENTONITE AND HUMAN HEALTH

Bentonite is not considered toxic to humans or the environment. Bentonite is used in a wide range of applications, including removal of impurities in oils, clarification in the food industry, as an additive in pet food, a filler in pharmaceutical products, an absorption and adsorption agent in protective creams, calamine lotion, wet compresses, and anti-irritants for eczema, antidote in heavy metal poisoning and in cosmetic care products such as mud packs, sunburn cream, baby powder, face powder and face creams.

The expected acute oral toxicity of bentonite in humans is very low (LD50>15 g/kg) (HSDB, 2000). Bentonite did not cause fibrosis after one year of exposure of 60 mg dust (<5 µm) in a rat study (TATRAI, 1985). Bentonite is not on the NOHSC List of Designated Hazardous Substances (NOHSC, 1999a), and based on the available information, it is unlikely to be classified as a hazardous substance in accordance with the NOHSC Approved Criteria for Classifying Hazardous Substances (NOHSC, 1999b) as it doesn't meet the criteria of a hazardous substance.

7.2 LANTHANUM AND HUMAN HEALTH

Lanthanum is a rare earth element belonging to the 'lanthanide' elements. It is the most electropositive element of the rare earth group, is uniformly trivalent, and almost exclusively engages in ionic binding (CETINER & XIONG 2008). As a hard 'acceptor', lanthanum has a preference for oxygen-containing anions. Therefore, the most common biological ligands are the carboxyl and phosphate groups ($PO4^{3-}$) with which it can form very tight complexes. These chemical characteristics form the basis for the suitability of lanthanum carbonate as an orally administered phosphate binder to treat hyperphosphatemia. A large amount of scientific literature is available on lanthanum toxicity to human health due to the fact that lanthanum carbonate ($La_2(CO_3)_3$, trade name

Fosrenol®) is used as an oral drug for humans. Fosrenol® is registered with the US patent and trademark office and manufactured by the company Shire Pharmaceuticals which owns the commercial rights to Fosrenol®. Lanthanum carbonate (Fosrenol®) is an effective non-aluminium, non-calcium oral phosphate binder for treatment of hyperphosphatemia in patients with chronic kidney disease who are undergoing dialysis (STEWART 2002, D'HAESE et al. 2003, BEHETS et al 2004a, HUTCHINSON 2009, HUTCHINSON et al. 2009, DAMMENT 2011). Fosrenol® is licensed in the following 42 countries: Australia, Austria, Belgium, Bulgaria, Canada, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, France, Germany, Greece, Hong Kong, Hungary, Iceland, Indonesia, Israel, Korea, Latvia, Lithuania, Luxembourg, Malaysia, Malta, Netherlands, Norway, Philippines, Poland, Portugal, Romania, Singapore, Slovak Republic, Slovenia, South Africa, Spain, Sweden, Switzerland, Taiwan, Thailand, United Kingdom and the USA. Tablets of 500, 750 and 1000 mg Fosrenol® are available for use in end stage renal disease patients (ESRD). Lanthanum carbonate ($La_2(CO_3)_3$, Fosrenol®) has been and is still administered daily at doses containing 375 to 3000 mg/day of lanthanum (corresponding to 750 to 6000 mg/day of $La_2(CO_3)_3$) over many years (D'HAESE et al. 2003, BEHETS et al. 2004a, BEHETS et al. 2004b, PERSY et al. 2006, HUTCHINSON et al. 2009, DAMMENT 2011). Lanthanum is reported to dissociate from its carbonate salt in acidic environments (such as that of the stomach), and therefore, regardless of the ingested form, the free La^{3+} ion is expected to be the toxicologically relevant species (PENNICK et al. 2006). Therefore, an assessment of possible risks of Phoslock® for human health based on the available evidence on the pharmacological use of lanthanum carbonate, using the drug based data is appropriate, since the critical factor in terms of toxicity of lanthanum is the presence of the soluble ion La^{3+}. Toxicity in Phoslock® treated water bodies could be predicated on the release of the lanthanum ion which will then exist in an identical form to the lanthanum ion used in the pharmaceutical preparation.

7.2.1 EFFECTS OF LANTHANUM ON THE LIVER AND EXCRETION

The bioavailability of lanthanum, administered as lanthanum carbonate, in humans is less than 0,00127± 0,00080 % (PENNICK et al. 2006, PERSY et al. 2006, BERVOETS 2009). In clinical studies with a mean lanthanum carbonate dose of 1250 mg/d, steady state was reached with plasma lanthanum levels varying around 0.6 ng/ml (SPASOVSKY et al. 2006).

Lanthanum is mainly excreted via the hepatobiliary pathway (PERSY et al. 2006). The main excretion organ for lanthanum is the liver where it is located in lysosomes and in the biliary canal but not in any other cellular organelle (BERVOETS et al. 2009). Transport and elimination of lanthanum in the liver is via a trans-cellular, endosomal-lysosomal-biliary canicular transport route (BERVOETS et al. 2009). Clinical studies over 6 years show no indications for any hepatotoxic effect of lanthanum administered as lanthanum carbonate (HUTCHINSON et al. 2008) and liver enzymes in patients did not increase during the observation period (SPASOVSKY et al. 2006, HUTCHINSON et al 2008). In a phase-III-study during which lanthanum carbonate was given to patients at doses up to 3.75 g per day for up to 3 years, no increase in liver enzyme activities was observed, nor any effect related to hepatobiliary adverse events was found (HUTCHINSON et al. 2006). The highest hepatic concentration of lanthanum ever reported after oral administration in a uremic rat model is approximately 3 µg/g wet weight (SLATOPOLSKY et al. 2005). Considering the fact that the normal concentration of other lysosomally-transported metals such as copper (Cu) or iron (Fe) are in the range of 50 and 2000 µg/g wet weight,

respectively (DAMMENT 2006a), it appears unlikely that lanthanum treatment would lead to lysosomal overload toxicity.

The extremely low urinary excretion of 0,00004 % of administered dose confirms the low systemic absorption and the negligibility of renal excretion compared with other routes of elimination (e.g. bile) (STEWART 2002).

7.2.2 EFFECTS OF LANTHANUM ON BONE

Due to the use of lanthanum carbonate as an oral administered drug in ESRD patients, a large number of studies have been conducted to determine the deposition of lanthanum in bone (BERVOETS 2003, D'HAESE 2003, BEHETS et al. 2004a, BEHETS et al. 2005, DAMMENT & SHEN 2005, SPASOVSKY et al. 2006, DAMMENT 2011). This accurate and detailed studies have been undertaken because concerns have been raised as to whether accumulation of lanthanum in bone could be associated with negative side effects similar to those previously seen with aluminium (aluminium-induced osteomalacia in dialysis patients).

In chronic renal failure (CRF) rats loaded orally with lanthanum carbonate (12 weeks and 2000 mg/kg/day), bulk bone lanthanum concentrations reached values up to 5 µg/g wet weight (BEHETS et al 2005). Lanthanum could be detected at the edge of the mineralized bone, at both actively mineralizing and quiescent sites, independent of the type of bone turnover and was also distributed diffusely in the mineralized bone matrix. Local lanthanum concentrations in bone were estimated to account only for 0.05 mol % of the calcium content in the highest lanthanum-enriched areas of the bone. This means that maximally one out of every 2000 calcium atoms might be replaced by lanthanum (BEHETS et al 2005, BERVOETS 2009). In patients treated for up to 4 years with lanthanum carbonate, total bulk lanthanum concentrations in bone remain below 10 µg/g bone (FREEMONT & MALUCCHE 2005). Compared to this, based upon a bone lanthanum concentration of 9.5 µg/g wet weight (67 nmol/g) - the highest lanthanum concentration observed in the bone of dialysis patients after 4.5 years of treatment with 2.5 to 3.0 g lanthanum/day - the molar bone lanthanum/calcium ratio would be as low as 2×10^{-5}, i.e., only 1 out of 50 000 calcium atoms would be replaced by lanthanum (PERSY et al. 2009). Hence, a physicochemical interference of lanthanum ions incorporated into the bone matrix with the mineralization process is assumed to be highly unlikely.

To address the possible development of aluminium-like bone disease, in a prospective randomized open-label study two groups of dialysis patients received lanthanum carbonate ($La_2(CO_3)_3$)or calcium carbonate($CaCO_3$) for 1 year (D'HAESE et al. 2003). Bone biopsies, performed in 63 patients in basal conditions and after 1 year of therapy, showed an increase of adynamic bone disease or an aggravation of their hyperparathyroidism in patients received $CaCO_3$, whereas those treated with lanthanum carbonate showed a reduction of the extreme form of osteodystrophy. Concomitantly, after 1 year of treatment, serum lanthanum levels reached a plateau within 12 weeks of treatment and bone levels were below 5.5 µg per wet weight (D'HAESE et al. 2003). Further, in an experimental study with rats it was demonstrated, that long-term treatment with lanthanum carbonate to control hyperphosphatemia can reduce the abnormalities of mineral and bone metabolism associated with CRF in rats (DAMMENT et al. 2011).

Since total bulk lanthanum concentration in bone is low and there is no preferential site for lanthanum to be deposited in bone, it is unlikely that lanthanum directly induces a mineralization

defect. It is demonstrated that an observed mineralization defect occurred secondary to phosphate depletion and is a consequence of administering a phosphate binder at high doses rather than being the result of a direct effect of lanthanum on bone (BEHETS et al 2004a).

7.2.3 GENOTOXIC, MUTAGENIC AND CARCINOGENIC POTENTIAL

Studies have shown that lanthanum carbonate is not genotoxic, not teratogenic, not immunotoxic nor immunosuppressive and there is no evidence that lanthanum carbonate has a carcinogenic or mutagenic potential (DAMMENt et al 2005). Results from this study provide evidence that lanthanum carbonate is safe for its intended use (i.e. Medical Product Agency – Sweden 2009, Shire Fosrenol® Product Monograph 2012) and are consistent with the good safety profile emerging from long-term studies in dialysis patients (D'HAESE et al., 2003; BEHETS et al., 2004b).

7.2.4 LANTHANUM AND BRAIN

In contrast to aluminium, lanthanum does not pass the blood–brain barrier (KATO et al., 1989, EVANS 1990, XU & LING, 1994, DAMMENT 2009, ABBOTT et al 2010). In fact, lanthanum is routinely used as a tracer to investigate the integrity of this barrier, as lanthanum ions cannot cross the plasma membrane and are excluded from passing between vascular endothelial cells in the central nervous system (CNS) by tight junctions (BRADBURY 1985, KATO et al., 1989, XU & LING 1994). In several experimental studies, lanthanum concentrations in the brain after intravenous lanthanum dosing at 30 to 300 mg/kg/day for 4 weeks or oral gavage at 1500 mg/kg/day were below the limit of quantification, i.e., <6 ng/g (PERSY et al. 2009). Furthermore, lanthanum is almost completely bound in plasma to a variety of proteins (>99.9%), effectively limiting access to some tissue compartments, especially the brain (PENNICK et al., 2006). Recent studies demonstrate that reports of deposition in rat brain (LACOUR et al. 2005) are almost certainly due to cross-contamination artefacts and highlight the need for strict quality control in animal lanthanum deposition studies, particularly if dietary administration is employed (DAMMENT et al 2006b, BERVOETS et al 2009, DAMMENT et al 2009).

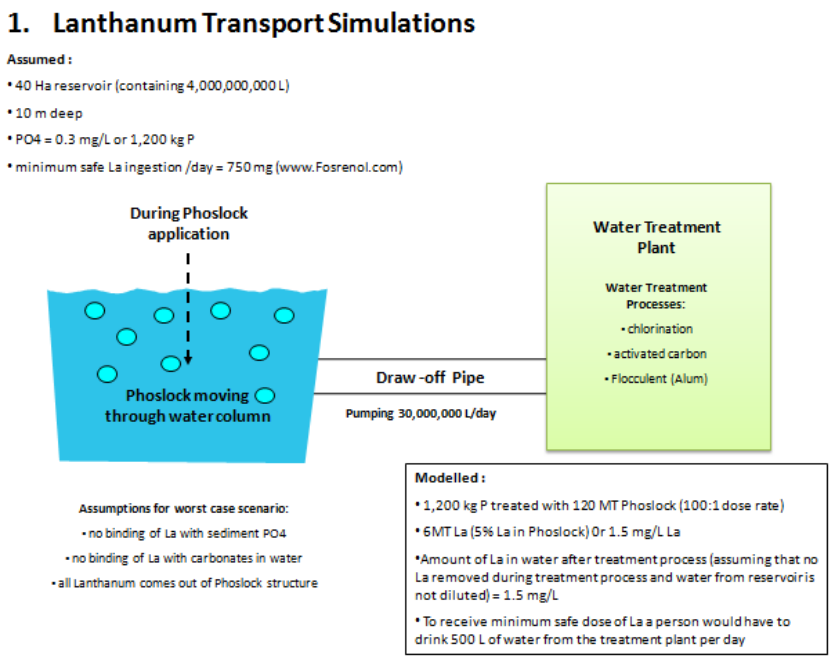
An assessment of cognitive function of patients after 2 years of treatment in a randomized open-label study comparing lanthanum carbonate with standard treatment demonstrate, that the use of lanthanum carbonate as a phosphate binder does not adversely affect cognitive function in patients compared with patients receiving the standard therapy (ALTMANN et al. 2007). Furthermore, no clinically relevant effects on the CNS were reported in a long-term follow-up study where patients received lanthanum carbonate treatment for up to 6 years (HUTCHINSON et al 2008).

7.3 INGESTION OF PHOSLOCK® TREATED WATER

In case of lanthanum ingestion via drinking even a large volume of Phoslock® treated water, there is no risk to human health. The US-FDA approved the normal human dose rate for Fosrenol® or $La_2(CO_3)_3$ (Fosrenol® website https://www.fosrenol.com/) at 750 – 3,000 mg per day. Fosrenol® contains lanthanum carbonate (2:3) hydrate with molecular formula $La_2(CO_3)_3 \times H_2O$ (on average x=4-5 moles of water) and molecular weight 457.8 (anhydrous mass). The calculated concentration of Lanthanum in Fosrenol® is 60.7%. Hence in 750 mg = 455.25 mg La; in 1,500 mg = 910.5 mg La; and in 3,000 mg = 1,821 mg La. Whereas Phoslock® contains 5% La. Therefore in an average application of Phoslock® (such as 100 mg/L) the concentration of La would equate to 5 mg La/L. This being the case,

applying Phoslock® on a reservoir at its typical dose rate of 50 ppm and assuming in a theoretical worst case scenario that 100% of lanthanum (5% La content in the product) will be leached out of the product (which will not happen because alkalinity and phosphate would "soak up any "free" La), then a person would need to drink 300 L of reservoir water per day to ingest the minimum dose of lanthanum that corresponds to the lowest Fosrenol® daily intake. To reduce plasma phosphate levels to less than 6.0 mg/dL, normally the maximum daily dose of Fosrenol® required is 3000 mg and therefore the average person would need to drink 1200 L of reservoir water per day to get the maximum dose of lanthanum that is the Fosrenol® daily intake. These large volumes of water could not be drunk by a person per day and therefore an application of Phoslock® would never deliver as much as lanthanum that a Fosrenol® tablet delivers. Therefore, even ingestion of Phoslock® treated water directly after an application would not pose a risk to human health.

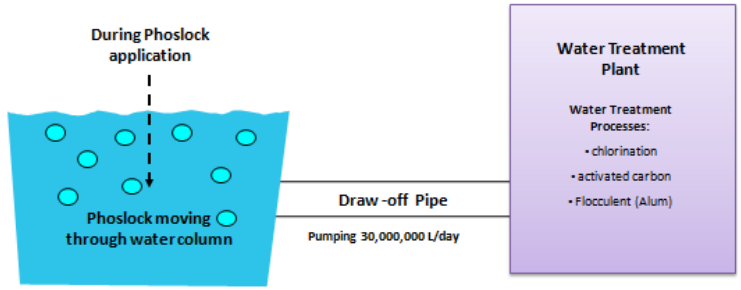
Simulations have been carried out to compare the relative concentrations of Lanthanum after a Phoslock® application to the daily allowable intake of Fosrenol® (Figure 16). The simulations below are based on there being small concentrations in the receiving water such as would be expected in the environment (Figure 2). Based on the differing P concentrations a person would have to drink 500 L – 3,000 L of water/day to ingest the same amount of Lanthanum that is in a minimum daily Fosrenol® dose of 750 mg.



2. Lanthanum Transport Simulations

Assumed :

- 40 Ha reservoir (containing 4,000,000,000 L)
- 10 m deep
- PO4 = 0.2 mg/L or 800 kg P
- minimum safe La ingestion /day = 750 mg (www.Fosrenol.com)



Assumptions for worst case scenario:

- no binding of La with sediment PO4
- no binding of La with carbonates in water
- all Lanthanum comes out of Phoslock structure

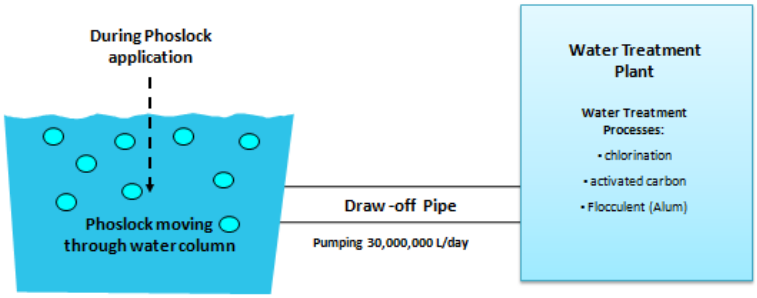
Modelled :

- 800 kg P treated with 80 MT Phoslock (100:1 dose rate)
- 4 MT La (5% La in Phoslock) Or 1 mg/L La
- Amount of La in water after treatment process (assuming that no La removed during treatment process and water from reservoir is not diluted) = 1 mg/L
- To receive minimum safe dose of La a person would have to drink 750 L of water from the treatment plant per day

3. Lanthanum Transport Simulations

Assumed :

- 40 Ha reservoir (containing 4,000,000,000 L)
- 10 m deep
- PO4 = 0.1 mg/L or 400 kg P
- minimum safe La ingestion /day = 750 mg (www.Fosrenol.com)



Assumptions for worst case scenario:

- no binding of La with sediment PO4
- no binding of La with carbonates in water
- all Lanthanum comes out of Phoslock structure

Modelled :

- 400 kg P treated with 40MT Phoslock
- 2 MT La (5% La in Phoslock) Or 0.5 mg/L La (100:1 dose rate)
- Amount of La in water after treatment process (assuming that no La removed during treatment process and water from reservoir is not diluted) = 0.5 mg/L
- To receive minimum safe dose of La a person would have to drink 1,500 L of water from the treatment plant per day

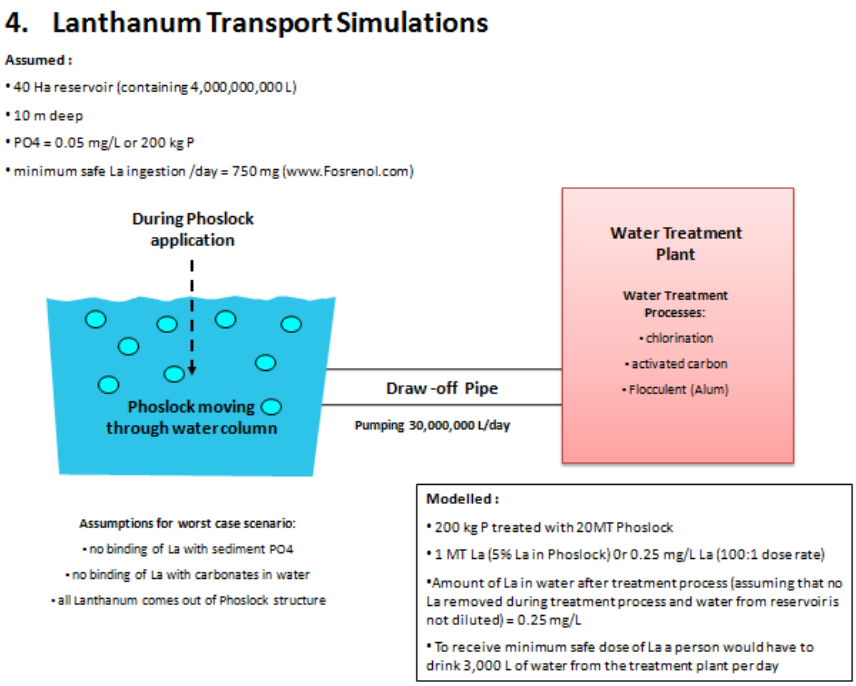


Figure 16: Simulations for 4 different phosphorus concentrations showing comparisons between the amounts of Lanthanum after a Phoslock® application relative to the minimum daily allowable intake of Fosrenol® (Phoslock Water Solutions).

7.4 EXPOSURE VIA FISH CONSUMPTION

The risk of consuming Phoslock®/lanthanum in fish harvested from Phoslock® treated water after application was shown to be negligible in a fish health investigation, after three successive applications of Phoslock® in Lake Okareka, New Zealand. The Lake Okareka fish health monitoring report (LANDMAN et al. 2007) demonstrated that trout (*Oncorhynchus mykiss***)** and koura (*Paranephrops planifrons)* accumulated lanthanum only in the liver and hepatopancreas tissue, not in the flesh/muscle following the application of Phoslock®. It was also demonstrated that lanthanum was removed from the fish liver and hepatopancreas tissues within a few months and the concentrations of lanthanum returned to baseline when the fish were sampled again one year later (before another Phoslock® application), suggesting a biological capacity to depurate lanthanum (LANDMAN et al. 2007). These results are also consistent with the findings that the main excretion route for absorbed lanthanum in humans or animals is via the liver into bile (PERSY et al. 2006, BERVOETS et al. 2009, HUTCHINSON et al. 2009). The highest concentration of lanthanum measured in the liver of male and female trout in Lake Okareka after one and two months of Phoslock® application was 1.2 and 0.8 mg/kg. Similarly, the highest concentration of lanthanum in the hepatopancreas tissues of male and female trout was 0.8 and 1.0 mg/kg respectively (LANDMAN et al., 2007). Therefore, in total the highest concentration of lanthanum in one trout was 2.0,0 mg/kg. Thus, a person would need to consume 375 kg of fish per day to ingest the minimum daily dose of Fosrenol®. Referring to the recommended maximum dosage of Fosrenol® an average person would need to consume 1500 kg of fish per day to consume the maximum dose of 3000 mg/d. These large quantities of fish would not be consumed by a person and it would therefore not be possible for an application of Phoslock® to deliver as much as lanthanum to fish body that a Fosrenol® tablet delivers.

Moreover, normally fish liver and hepatopancreas tissues are not generally consumed by humans. However, as outlined above, even consumption of large quantities of fish liver and hepatopancreas tissues harvested from Phoslock® treated water body would pose negligible risk to human health.

7.5 CONCLUSION

Bentonite is not considered toxic to humans or the environment and it is classified as a non hazardous substance in accordance with the NOHSC Approved Criteria for Classifying Hazardous Substances.

The observation that lanthanum in the liver is uniquely present in the lysosoms is in line with its elimination pathway. Results from clinical and experimental studies demonstrate the absence of any hepatotoxicity. Bone studies show heterogeneous localization of lanthanum in bone and the absence of adverse effects on bone histology in lanthanum-treated patients. No adverse effect of lanthanum treatment on cognitive functioning has been observed. Lanthanum does not cross the blood brain barrier and studies demonstrate the absence of lanthanum deposition in brain tissue.

There is no identifiable risk to human health related with a Phoslock® application to a water body including drinking water reservoirs at applicable dose rates. The margin of safety, even in a worst case scenario assuming that all lanthanum is leached out of the product after application to a water body, is substantial and sufficient to ensure that exposures from ingestion of lanthanum would always be significantly much less than the therapeutic dose used in patients with hyperphosphatemia. Risk to human health from consumption of fish harvested from a Phoslock® treated water body is negligible.

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