



**Phoslock Water Solutions Ltd.**  
**ABN 88 099 555 290**

# **Eco-toxicity Assessment of Phoslock®**

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## Table of contents

Executive Summary .....	3
1. Introduction .....	5
2. Manufacturing process of Phoslock® .....	6
3. Properties of Phoslock® .....	8
4. Trace elemental composition of Phoslock® .....	9
5. Active ingredients of Phoslock® .....	10
6. Eco-toxicity assessment.....	10
6.1. Acute (short-term) toxicity tests.....	10
6.2. Chronic (long-term) toxicity tests.....	11
7. Test organisms .....	11
8. Methods of laboratory toxicity tests of Phoslock® .....	11
9. Results and discussion of laboratory toxicity tests.....	12
9.1. Toxicity tests using Daphnia .....	12
9.1.1. Acute toxicity tests using Daphnia.....	14
9.1.2. Chronic toxicity tests using Daphnia.....	16
9.1.3. Phoslock® toxicity to Daphnia: Acute vs Chronic tests .....	17
9.2. Toxicity tests using Rainbow fish .....	18
9.2.1. Acute toxicity tests using Rainbow fish.....	18
9.3. Toxicity tests using shrimp .....	20
9.3.1. Acute toxicity tests using shrimp .....	21
9.3.2. Chronic toxicity tests using shrimp .....	21
9.4. Toxicity tests using algae .....	21
9.4.1. Acute toxicity tests using algae .....	22
9.5. Toxicity tests using sediment-dwelling organisms .....	23
9.5.1. Phoslock® toxicity to Amphipod.....	23
9.5.2. Phoslock® toxicity to Mayfly.....	23
9.5.3. Phoslock® toxicity to Midge larvae.....	24
10. Variation of results .....	24
11. Lab toxicity test vs field trial/application .....	26
12. Mitigation of Phoslock® toxicity by phosphorus and/or carbonate .....	27
13. Toxicity of Phoslock® vs toxicity of lanthanum.....	28
14. Thickness of sediment capping layer of Phoslock® and its effect on bottom-dwelling organisms.....	29
15. Conclusion.....	30
References .....	31

## List of Figures

Figure 1. Mixing bentonite clay and lanthanum chloride at Phoslock <sup>®</sup> manufacturing factory in Kunming, China.....	7
Figure 2. Dewatering and drying process during Phoslock <sup>®</sup> manufacturing at Kunming factory, China.....	7
Figure 3. Packaging dry, free flowing granular Phoslock <sup>®</sup> in manufacturing factory at Kunming, China.....	8
Figure 4. a) Dry, free flowing granular Phoslock <sup>®</sup> (2 – 4 mm), b) 25 kg Phoslock <sup>®</sup> bag...	8
Figure 5. Dose-Response curve and the determination of EC <sub>50</sub> .....	11

## List of Tables

Table 1. Summary of properties of Phoslock <sup>®</sup> granules.....	9
Table 2. Recommended ANZECC sediment quality guidelines for a range of elements (all concentrations are in ppm) (ANZECC & ARMCAZ, 2000).....	9
Table 3. Trace elemental composition of Phoslock <sup>®</sup> (all concentrations including the Lowest Level Detection (LLD) limit is in ppm).....	10
Table 4. Acute and chronic toxicity of Phoslock <sup>®</sup> and lanthanum chloride to <i>Daphnia</i> .	13
Table 5. Acute toxicity of Phoslock <sup>®</sup> and lanthanum chloride to rainbow fish.....	18
Table 6. Acute and chronic toxicity of Phoslock <sup>®</sup> to shrimp ( <i>Macrobrachium</i> sp).....	20
Table 7. Acute toxicity of Phoslock <sup>®</sup> and lanthanum chloride to algae.....	21
Table 8. Chronic toxicity of Phoslock <sup>®</sup> to sediment-dwelling organisms (amphipod, mayfly and midge larvae).....	23

## Executive Summary

Phoslock<sup>®</sup> is a modified clay product that was developed by the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia to remove phosphorus from water bodies and eliminate the chance of blue-green algal blooms. The use of Phoslock<sup>®</sup> with its active ingredient lanthanum, is a new but fast emerging effective P-inactivation and blue-green algae management tool. To assess and manage any possible adverse environmental side effects of any new product, it is crucial to: assess the eco-toxicity; ensure that the product specifications meet various regulatory standards; and ensure that it is safe for organisms contained in natural aquatic ecosystems at proposed dose rates. The objective of this eco-toxicity report is therefore to analyse and interpret historical toxicity and assessment studies using Phoslock<sup>®</sup> and the often substituted, however not comparable, lanthanum chloride. The Phoslock<sup>®</sup> toxicity assessment on human health is addressed in a separate document.

During the manufacturing process of Phoslock<sup>®</sup>, the lanthanum ions are incorporated into bentonite by using the cation exchange capacity of clay minerals. This renders the lanthanum in Phoslock<sup>®</sup> almost entirely non-bioavailable and therefore optimal over the addition of the soluble  $\text{LaCl}_3$  to a water body. After an application of Phoslock<sup>®</sup>, the lanthanum ions react with the phosphate (and other dominant anions) in the water body or remain bound within the clay structure under a wide range of physiological conditions. The only exception to the rule is in water bodies that have very low alkalinity (<20 mg/L) and/or low phosphate concentrations (<0.005 mg/L) where a small concentration of lanthanum (e.g. loosely bound or residual lanthanum) may leach out of Phoslock<sup>®</sup> (due to low anion concentrations), not being taken up by dominant anions in the water body as rapidly as in water bodies with high alkalinity and phosphate. Even in these environments, the concentration of dissolved lanthanum associated with the product is very low. Laboratory studies with granular Phoslock<sup>®</sup> demonstrated that no lanthanum was leached out within 24 h when granular Phoslock<sup>®</sup> (10 mg/L) was dissolved in Milli-Q water; and only a small concentration of lanthanum (~16 µg/L) was leached out from the 10 mg/L concentration of Phoslock<sup>®</sup> when dissolved in low alkalinity natural water or synthetic soft water.

A large number of acute and chronic eco-toxicity tests using Phoslock<sup>®</sup> and/or lanthanum chloride have been undertaken on a number of sensitive organisms and algae including: water fleas (*Daphnia*); several species of Rainbow fish; freshwater shrimp; benthic organisms such as amphipods, mayflies and midge larvae; and various species of algae. The results of these toxicity tests using Phoslock<sup>®</sup> and lanthanum (in the form of  $\text{LaCl}_3$ ) demonstrates a wide variation in responses of test organisms to Phoslock<sup>®</sup> solutions or leachates and lanthanum chloride. There are several factors that may be responsible for these variations including: differences in experimental methods used among separate experiments such as: TCLP method; the use of a suspension of Phoslock<sup>®</sup> granules or using synthetic lanthanum chloride instead of Phoslock<sup>®</sup>; different types of test media such as artificial soft water, hard water, Milli-Q water, nanopure water and natural lake or pond water; all resulting in differences in the concentration of lanthanum leaching among different tests.

During an application, once the Phoslock<sup>®</sup> granules or slurry (granules mixed with application water) are added to the water body, the Phoslock<sup>®</sup> moves down through the water column, and settles on the sediment. Therefore, it is suggested that the toxicity tests be conducted in a manner designed to simulate the application of granular Phoslock<sup>®</sup> to freshwater and utilise a range of exposure concentrations in order to encompass potential application scenarios. This can be achieved by using suspensions of different concentrations of Phoslock<sup>®</sup> granules in the toxicity tests. Results from recent toxicity studies using suspensions of Phoslock<sup>®</sup> granules that were dissolved in low and high alkalinity natural waters demonstrated no toxic effects of Phoslock<sup>®</sup> to aquatic organisms in the solution using up to 13,600 mg Phoslock<sup>®</sup>/L which is several thousand times higher than that of usual 100:1 (100 g Phoslock<sup>®</sup> for 1 g FRP) application dose rate. When applied to natural water, the lanthanum toxicity of Phoslock<sup>®</sup> significantly reduces due to phosphorus and/or carbonate uptake. Reaction of lanthanum with phosphate to the insoluble  $\text{LaPO}_4$  or with carbonate to the  $\text{La}_2(\text{CO}_3)_3$  and subsequent precipitation would result in the residual lanthanum being unavailable to aquatic biota.

## 1. Introduction

Phoslock<sup>®</sup> is a modified bentonite clay product containing the active ingredient lanthanum, a rare earth element. Phoslock<sup>®</sup> was developed during research undertaken at the CSIRO Department of Land and Water and funded by the Western Australian State Government's Water and Rivers Commission and the Swan River Trust. Phoslock<sup>®</sup> removes phosphate (Filterable Reactive Phosphorus – FRP or Soluble Reactive Phosphorus - SRP) from water. Phoslock Water Solutions Limited (PWS) manufactures and applies Phoslock<sup>®</sup> to water bodies ranging from recreational lakes, drinking water reservoirs and intensive aquaculture ponds in order to remove excess phosphate and control algal blooms.

Phoslock<sup>®</sup> has been applied to water bodies in over 20 countries in order to reduce and control the concentration of algal biomass (e.g. blue green algae or cyanobacteria). The amount of Phoslock<sup>®</sup> applied to an individual water body depends on the amount of bioavailable and total phosphorus present in the water body, hydrobiology of the water body such as inflows and runoff, sediment phosphate release as well as the chemical properties of water. As a general rule Phoslock<sup>®</sup> is applied at the rate of 100:1, i.e. 100 g Phoslock<sup>®</sup> is required to remove 1 g of bioavailable phosphorus (FRP). Before application to a water body, the Technical Team at PWS assesses the historical physico-chemical conditions of an application and calculates an applicable quantity of Phoslock<sup>®</sup> to dose for each individual water body. This is calculated by considering all aspects of the aquatic ecosystem including chemical and biological attributes.

Phoslock<sup>®</sup> consists of bentonite clay and lanthanum; bentonites are a group of clays formed by crystallisation of vitreous volcanic ash that were deposited in water. Bentonite is not considered toxic to humans or the environment. The expected acute oral toxicity of bentonite in human is very low ( $LD_{50} > 15$  g/kg) (HSDB, 2000). In addition to several other commercial uses, bentonite has been approved as a food additive in Australia (NICNAS, 2001).

Lanthanum ( $La^{3+}$ ) is not influenced by redox reactions (as in the case of  $Al^{3+}$ ), and when bound with  $PO_4^{3-}$  forms the insoluble compound,  $LaPO_4$  (Rhabdophane). Lanthanum and lanthanum salts are not on the NOHSC *List of Designated Hazardous Substances* (NOHSC, 1999a) and they are unlikely to be classified as hazardous substances in accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999b). However, it has also been found that the free/unbound lanthanum can be toxic to aquatic organisms depending on its concentration and application rate (Peterson et al., 1974). The toxicity problem of free lanthanum limited its use significantly, until an appropriate carrier that could lock the lanthanum ions into its structure was discovered in the mid 1990s by the CSIRO. Lanthanum toxicity and the availability of its free form was dramatically reduced by incorporating the lanthanum ions into the structure of a high exchange capacity mineral, such as bentonite, hence the development of the innovative product, Phoslock<sup>®</sup>.

In order to assess and manage any possible adverse environmental side effects of any product (e.g. Phoslock<sup>®</sup>), it is crucial to assess the eco-toxicity of the product and ensure

that the product specifications meet various regulatory standards. The objective of an eco-toxicity assessment or an environmental risk assessment is therefore to prove that the properties of Phoslock<sup>®</sup> and the Phoslock<sup>®</sup> product are safe for natural aquatic ecosystems at proposed dose rates.

Several independent organisations have conducted extensive laboratory and field studies on the toxicity of Phoslock<sup>®</sup> using a range of aquatic organisms and the United States Environmental Protection Agency toxicity testing criteria. Most of the results showed that Phoslock<sup>®</sup> is not toxic to aquatic organisms and humans at normal dose rates applied in to water bodies (NICNAS Public Report, 2001; Clearwater, 2004; Martin & Hickey, 2004; Ecotox report, 2006a,b; Uniquet report, 2006 & 2007; Ecotox report, 2008; Watson-Leung, 2008; Lurling et al., 2008; Lurling & Tolman, 2009). However, some results indicated that Phoslock<sup>®</sup> may be toxic to some organisms at higher dose rates in certain waters, e.g. low alkalinity.

This report, therefore, summarises the properties of Phoslock<sup>®</sup> and the results of eco-toxicity tests conducted by the CSIRO Centre for Advanced Analytical Chemistry, NIWA (National Institute of Water & Atmospheric Research, New Zealand), Ecotox Services Australasia, Institut Dr. Nowak Germany, Wageningen University Netherlands, Ontario Ministry of the Environment, Ontario (Canada) and other researchers who published their work in journals, and eco-toxicity assessments undertaken by UniQuest Pty Ltd Australia and NICNAS (National Industrial Chemicals Notification and Assessment Scheme, Australia).

## **2. Manufacturing process of Phoslock<sup>®</sup>**

During the manufacturing process of Phoslock<sup>®</sup>, modified bentonite clay and lanthanum chloride are mixed in an aqueous solution (Figure 1). The lanthanum is absorbed into sites within the bentonite and becomes the active compound that removes phosphate. The lanthanum ions are incorporated into bentonite via cation exchange in the clay. This exchange capacity is a result of a charge imbalance on the surface of the clay sheets, which is balanced by surface adsorbed cations that are exchangeable in aqueous solutions. In the preparation of Phoslock<sup>®</sup>, the lanthanum ions are exchanged with these surface-adsorbed, exchangeable cations. Lanthanum ions are strongly associated with the bentonite clay and are not released as soluble lanthanum into the water. 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 physiological conditions (Douglas et al., 2000).

Phoslock<sup>®</sup> was originally formulated in the form of a slurry. A dry, free flowing granular form was developed in 2004 (Figure 2 – 4), resulting in ease of transportation and reduced application cost. Another advantage of granular Phoslock<sup>®</sup> is that during the manufacturing process (granulation process) significant dewatering of the slurry occurs which significantly reduces the amount of residual lanthanum associated with the product. The Quality Control (QC) program of Phoslock Water Solutions Limited ensures

that, during the manufacturing process, the lanthanum content of the Phoslock<sup>®</sup> granule is  $50 \pm 2$  mg/g i.e. 5% ( $\pm 0.2\%$ ).



**Figure 1:** Mixing bentonite clay and lanthanum chloride at the Phoslock<sup>®</sup> manufacturing factory in Kunming, China.



**Figure 2:** Dewatering and drying processes during the Phoslock<sup>®</sup> manufacturing at Kunming factory, China.





**Figure 3:** Packaging dry, free flowing granular Phoslock<sup>®</sup> in the factory at Kunming, China.



a



b

**Figure 4:** a) Dry, free flowing granular Phoslock<sup>®</sup> (0.5 – 3 mm), b) 25 kg Phoslock<sup>®</sup> bag.

### 3. Properties of Phoslock<sup>®</sup>

Phoslock<sup>®</sup> was originally manufactured and applied in the form of a slurry, containing 20% (w/w) of the active Phoslock<sup>®</sup>. With the introduction of its granular form, the active Phoslock<sup>®</sup> concentration was increased to more than 90% (w/w). The major properties

of granular Phoslock<sup>®</sup> are listed in Table 1. By adhering to strict quality control measures, Phoslock Water Solutions Limited maintains a high concentration of the active Phoslock<sup>®</sup> consistently in the supplied product. Moreover, the low dust level and the acceptable degree of packaging stability of Phoslock<sup>®</sup> 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.

<b>Physical &amp; Chemical Description Properties</b>	
Phoslock <sup>®</sup> 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 the granules	0.5 – 3 mm
Bulk density	850 – 1200 kg m <sup>-3</sup>
Dust content	<1% weight 50 µm
pH	7.0 – 7.5

**Table 1:** Summary of properties of Phoslock<sup>®</sup> granules.

#### 4. Trace elemental composition of Phoslock<sup>®</sup>

Bentonite clay, which occupies ~95% of the Phoslock<sup>®</sup> content (on a dry matter basis), is not toxic to humans or the environment (LD<sub>50</sub> to human is >15 g/kg) and has been approved as a food additive in Australia (NICNAS, 2001). The potential toxicity of other trace elements in Phoslock<sup>®</sup> such as Cr, Cu, Pb and Zn and the metalloid arsenic were assessed on the basis of the ANZECC/ARMCAZ guidelines (ANZECC & ARMCAZ, 2000). The recommended ANZECC guidelines for the lower limit, provided in Table 2, signify the lowest concentration, above which the biological effects are observed and rare toxicological effects occur. On the other hand, the upper limit corresponds to the concentration above which there is a high probability of observing biological effects.

	<b>As</b>	<b>Cr</b>	<b>Cu</b>	<b>Pb</b>	<b>Zn</b>
<b>High</b>	70	370	270	220	410
<b>Low</b>	20	80	65	50	200

**Table 2:** Recommended ANZECC sediment quality guidelines for a range of elements (all concentrations are in ppm) (ANZECC & ARMCAZ, 2000).

Trace metal composition of Phoslock<sup>®</sup> granules are regularly measured for QA & QC. The most recent results are contained in Table 3 along with the lowest detection limits (LLD) used for analysis of each of the elements.

Parameter	As	Cr	Cu	Pb	Zn
LLD	3	10	6	6	4
Mean	29.75	<10	39	<6	105.25

**Table 3:** Trace elemental composition of Phoslock<sup>®</sup> (all concentrations including the Lowest Level of Detection (LLD) limit are in ppm).

## 5. Active ingredients of Phoslock<sup>®</sup>

Lanthanum (La<sup>3+</sup>) is the main active ingredient of Phoslock<sup>®</sup>. Lanthanum has been found to be toxic to sensitive species (e.g. Daphnia) depending on its concentration and application rate (Peterson et al., 1974). Although lanthanum ions are strongly associated with the bentonite clay and are not released as soluble lanthanum into the water, physico-chemical properties of water such as alkalinity, hardness and pH may influence the release of loosely bound lanthanum from Phoslock<sup>®</sup> and have the potential to cause toxicity to the aquatic ecosystem. Therefore, it is essential to measure the release/leaching rate of lanthanum in different types of water and assess the eco-toxicity of Phoslock<sup>®</sup> in order to prove that it is safe for natural aquatic ecosystems at proposed dose rates.

## 6. Eco-toxicity assessment

Aquatic Eco-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 (Rand et al. 1995). 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.

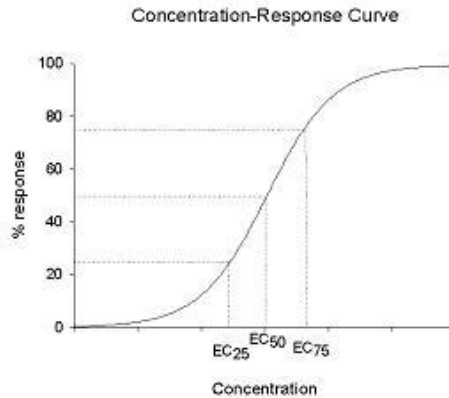
The goal of an eco-toxicity assessment is to understand the concentration of chemicals at which organisms in the environment will be affected. An eco-toxicity assessment establishes the relationship between the contaminant/s of concern and the receptor. Toxicity tests quantify the effects of a chemical which is absorbed into the body, usually via the mouth, but sometimes also through the skin or the lungs. These tests provide a database that can be used to assess the risk associated with a situation in which the chemical agent, the organisms, and the exposure conditions are defined.

There are two types of toxicity tests:

### 6.1. Acute (short-term) toxicity tests

Acute toxicity tests are rapid (2 to 4 days) procedures used to measure the concentration of a chemical that will affect the test organisms, i.e. make them sick or immobile. The most common end points of acute toxicity tests are EC<sub>50</sub> (Effective

Concentration) or LC<sub>50</sub> (Lethal Concentration) or LD<sub>50</sub>, (Lethal Dose) which is an estimate of the concentration of chemical that would produce a specific effect (response – immobilization or mortality) in 50% of an infinitely large population of the test species under the stated conditions.



**Figure 5:** Dose-Response curve and the determination of EC<sub>50</sub>.

## 6.2. Chronic (long-term) toxicity tests

Chronic toxicity tests are long-term procedures used to assess the toxic effects of a chemical on the organism for a substantial portion of its lifetime (>5 days). Chronic toxicity tests assess the effects on growth, reproduction and survival of the test organisms. The end points of chronic toxicity tests are also EC<sub>50</sub> or LC<sub>50</sub> or LD<sub>50</sub>.

## 7. Test organisms

In order to extrapolate meaningful, relevant, and ecologically significant results from aquatic toxicity tests, not only appropriate tests but also appropriate organisms should be used. Several criteria should be considered in selecting organisms for toxicity testing such as sensitivities and availabilities of organisms, representative of the ecosystem or ecologically important and easy to maintain in the laboratory. Most common species used for toxicity tests are water fleas (*Daphnia* sp.), fathead minnow, rainbow trout, shrimp, and sediment dwelling organisms such as amphipods, worms, mayflies, and midge larvae.

## 8. Methods of laboratory toxicity tests of Phoslock<sup>®</sup>

Phoslock<sup>®</sup> can either be applied to a water body in the form of a slurry (Phoslock<sup>®</sup> granules mixed with the application water before an application) or as a direct application of granules. Once applied, the Phoslock<sup>®</sup> moves through the water column until it settles on the sediment. Therefore, the potential toxicity of Phoslock<sup>®</sup> could affect the organisms living in the water column and on the sediment. Toxicity tests need to be conducted in a manner designed to simulate the application of granular Phoslock<sup>®</sup> to

freshwaters and utilise a range of exposure concentrations in order to encompass potential application scenarios and determine the concentrations that affect the aquatic organisms living both in the water column and on the sediment.

A large number of laboratory toxicity tests of Phoslock<sup>®</sup> and/or lanthanum chloride were conducted by several independent organisations and researchers using a range of test organisms. Most of the tests used US EPA TCLP (Toxic Characteristic Leach Protocol) method (USEPA, 1986), but few tests also used suspension of Phoslock<sup>®</sup> granules. Both types of tests used synthetic soft water or deionised water to prepare the Phoslock<sup>®</sup> leachate or solution. The tests were performed in compliance with OECD/EEC Test Methods and according to OECD Principles of Good Laboratory Practices.

The leachate solutions were prepared by gently mixing solid test materials (50 g of Phoslock<sup>®</sup> granules) on a rotary tumbler with 1 L of phosphorus free synthetic soft water or deionised water (Milli-Q, reverse osmosis) in a teflon bottle or plastic container for 18 hours. The solution was allowed to stand for 1 hour, and then the supernatant was siphoned off and filtered through a 0.45 µm filter (Stauber, 2000) or for some tests 40 µm filter (Martin & Hickey, 2004) before use in the toxicity tests.

For acute toxicity tests, the experiments were run for 24, 48 and 96 hours and the end point was the 50% immobilisation of test organisms (EC<sub>50</sub>). However, for chronic toxicity tests, the experiments were run up to 28 days depending on the type and species of test organism. The end points were measured as the effects on the survival and reproduction of test species (EC<sub>50</sub>).

## **9. Results and discussion of laboratory toxicity tests**

### **9.1. Toxicity tests using Daphnia**

Results from nine acute and five chronic toxicity studies using three different species of water fleas (Daphnia) are summarised in Table 4. Both Phoslock<sup>®</sup> (suspension of granules and leachate extracted by TCLP method) and lanthanum chloride were used in these studies.

No.	Species	Media	Method	Dura	EC <sub>50</sub> /LC <sub>50</sub>	NOEC	LOEC	Ref.
<b>Acute tests</b>								
1	<i>Daphnia magna</i>	Filtered tap water	LaCl <sub>3</sub> Solution	72 h	2,800 µg/L La	–	–	Peterson et al., 1974
2a	<i>Ceriodaphnia dubia</i>	Synthetic soft water	Phos TCLP	48 h	24,500 mg/L Pk 80 µg/L La	12,500 mg/L Pk 41 µg/L La	–	Stauber, 2000
2b	<i>Ceriodaphnia dubia</i>	Milli-Q water	Phos TCLP	48 h	5,000 mg/L Pk 40 µg/L La	–	–	Stauber, 2000
3	<i>Ceriodaphnia dubia</i>	Synthetic soft water	LaCl <sub>3</sub> Solution	48 h	5,000 µg/L La	2,600 µg/L La	–	Stauber & Binet, 2000
4a	<i>Daphnia carinata</i>	Filtered tap water	LaCl <sub>3</sub> Solution	24 h	484.5 µg/L La	–	–	Barry & Meehan, 2000
4b	<i>Daphnia carinata</i>	Filtered tap water	LaCl <sub>3</sub> Solution	48 h	43 µg/L La	–	–	Barry & Meehan, 2000
4c	<i>Daphnia carinata</i>	ASTM hard water	LaCl <sub>3</sub> Solution	48 h	1180 µg/L La	–	–	Barry & Meehan, 2000
5	<i>Daphnia magna</i>	Synthetic soft water	Phos TCLP	48 h	> 50,000 mg/L Phoslock	25,000 mg/L Pk	50,000 mg/L Pk	Martin & Hickey, 2004
6	<i>Daphnia magna</i>	Synthetic soft water	LaCl <sub>3</sub> Solution	48 h	23,000 µg/L La	–	–	Yasseri & Nowak, 2008
7	<i>Daphnia magna</i>	Artificial RT medium	LaNO <sub>3</sub> Solution	48 h	14,100 µg/L La	–	–	Lurling et al., 2008
8	<i>Ceriodaphnia dubia</i>	Natural Lake water	Phos Solution	48 h	* > 50 mg/L Pk > 330 µg/L La	50 mg/L Pk 330 µg/L La	>50 mg/L Pk >330 µg/L La	Ecotox, 2008
9a	<i>Daphnia magna</i>	Tap water	Phos Solution	48 h	4,900 mg/L Phoslock® 91,183 µg/L La	–	–	Watson-Leung, 2008
9b	<i>Daphnia magna</i>	Natural Pond water	Phos Solution	48 h	> 6,800 mg/L Phoslock® >14,000 µg/L La	–	–	Watson-Leung, 2008
<b>Chronic tests</b>								
1	<i>Ceriodaphnia dubia</i>	Synthetic soft water	Phos TCLP	7 d Sur.	20,500 mg/L Pk 820 µg/L La	<3,125 mg/L Pk <125 µg/L La	3,125 mg/L Pk 125 µg/L La	Stauber, 2000
2a	<i>Ceriodaphnia dubia</i>	Synthetic soft water	LaCl <sub>3</sub> Solution	7 d Sur.	510 µg/L La	–	–	Stauber & Binet, 2000
2b	<i>Ceriodaphnia dubia</i>	Synthetic soft water	LaCl <sub>3</sub> Solution	7 d Rep.	430 µg/L La	50 µg/L La	90 µg/L La	Stauber & Binet, 2000
3a	<i>Ceriodaphnia dubia</i>	Natural Lake water	Phos Solution	7 d Surv.	** > 1 mg/L Pk > 24 µg/L La	1 mg/L Pk 24 µg/L La	>1 mg/L Pk >24 µg/L La	Ecotox, 2008
3b	<i>Ceriodaphnia dubia</i>	Natural Lake water	Phos Solution	7 d Rep.	** > 1 mg/L Pk > 24 µg/L La	1 mg/L Pk 24 µg/L La	>1 mg/L Pk >24 µg/L La	Ecotox, 2008
4a	<i>Daphnia magna</i>	Artificial RT medium	LaNO <sub>3</sub> Solution	5 d Weight	800 mg/L Phoslock®	–	–	Lurling et al., 2008
4b	<i>Daphnia magna</i>	Artificial RT medium	LaNO <sub>3</sub> Solution	5 d Length	3130 mg/L Phoslock®	–	–	Lurling et al., 2008
5a	<i>Daphnia magna</i>	Artificial RT medium	Phos Solution	5 d Weight	871 mg/L Phoslock®	100 mg/L Phoslock®	–	Lurling & Tolman, 2009
5b	<i>Daphnia magna</i>	Artificial RT medium	Phos Solution	5 d Length	1557 mg/L Phoslock®	500 mg/L Phoslock®	–	Lurling & Tolman, 2009

\* 50 mg/L was the highest concentration, \*\* 1 mg/L was the highest concentration, Phos or Pk = Phoslock®

**Table 4:** Acute and chronic toxicity of Phoslock® and lanthanum chloride to *Daphnia*.

### 9.1.1. Acute toxicity tests using *Daphnia*

The US EPA investigated the nutrient inactivation efficiency of lanthanum chloride and its toxicity as early as 1974 (Peterson et al., 1974). The acute 72 h LC<sub>50</sub> for lanthanum chloride (using *Daphnia magna*) was determined as 2,800 µg/L in filtered tap water (Peterson et al., 1974). Barry & Meehan (2000) also used filtered tap water (total hardness - 22 mg/L of CaCO<sub>3</sub>) and 48 h EC<sub>50</sub> was determined as 43 µg/L La, which is 65 times lower than the value of Peterson et al. (1974). Lurling et al. (2008) used artificial RT medium and the 48 h EC<sub>50</sub> was determined as 14,100 µg/L, which is 328 times higher than the value of Barry & Meehan (2000). The exact reasons for these huge differences are unknown; however it is likely to be related to the different media used in the experiments. Although two different species of water fleas (*Daphnia magna* and *Daphnia carinata*) were used in these tests, it was unlikely that their sensitivity to the toxicants was different; however, *Ceriodaphnia* has shown much more sensitive than *Daphnia* (Krik & Gilbert, 1990). In hard water (160 mg/L of CaCO<sub>3</sub>), the toxicity of lanthanum chloride was dramatically reduced; 48 h EC<sub>50</sub> was determined as 1180 µg/L which is 27 times higher than the value of tap water EC<sub>50</sub> (Barry & Meehan, 2000). In synthetic soft water, lanthanum chloride demonstrated no/very less toxicity to *Daphnia* (Stauber & Binet, 2000; Yasseri & Nowak, 2008). Stauber & Binet (2000) determined EC<sub>50</sub> as 5,000 µg/L La using *Ceriodaphnia dubia* whereas Yasseri & Nowak (2008) determined EC<sub>50</sub> as 23,000 µg/L La using *Daphnia magna* in synthetic soft water.

CSIRO Centre for Advanced Analytical Chemistry in Australia (Stauber, 2000; Stauber & Binet, 2000) and NIWA (National Institute of Water & Atmospheric Research, New Zealand) (Martin & Hickey, 2004) used the TCLP method to extract leachate from Phoslock<sup>®</sup>. Although the lanthanum chloride dissolved in synthetic soft water demonstrated no/very less toxicity to *Daphnia* (Stauber & Binet, 2000; Yasseri & Nowak, 2008), Phoslock<sup>®</sup> leachate extracted in synthetic soft water demonstrated toxic effects to *Ceriodaphnia dubia* (Stauber, 2000). The 48 h EC<sub>50</sub> was calculated from the observed immobilisation data as 49% for filtered (0.45 µm) leachate which corresponds to 24,500 mg/L of Phoslock<sup>®</sup> and approximately 80 µg/L of lanthanum respectively (Stauber, 2000).

Phoslock<sup>®</sup> leachate extracted in Milli-Q water was found to be more toxic to *Ceriodaphnia dubia* than the soft water leachates (Stauber, 2000). However, no survival of *Ceriodaphnia dubia* in the Milli-Q water controls made the interpretation of toxicity of the filtered (0.45 µm) leachate difficult, suggesting that threshold concentrations of some ions (possibly Ca<sup>+</sup>) in the water were required for normal survival of this species. The Stauber (2000) report suggested that this species of cladoceran (*Ceriodaphnia dubia*) would not survive in water with conductivity lower than 100 µS/cm.

Martin & Hickey (2004) also used the TCLP method and extracted leachate in synthetic soft water (hardness 32 mg/L CaCO<sub>3</sub>). However, they did not measure the concentration of lanthanum in the leachate, therefore, EC<sub>50</sub> was calculated as the concentration of Phoslock<sup>®</sup>. The EC<sub>50</sub> was calculated as >100% leachate or >50,000 mg/L of Phoslock<sup>®</sup> which is more than double from the value of Stauber (2000). One difference between these tests was that the supernatant Phoslock<sup>®</sup> solution was filtered through a 40 µm nylon mesh filter (Stauber, 2000 used 0.45 µm filter) before use in the toxicity tests in

the later test (Martin & Hickey, 2004). Although EC<sub>50</sub> value was >100% leachate, there was some effect on mobility of the organism in the highest concentration (37% immobile after 48 h exposure). At this concentration, organisms became trapped in a white gelatinous precipitate, which formed during the test, and resulted in immobility and ultimately mortality. Mitigation of Phoslock<sup>®</sup> toxicity by addition of phosphorus suggested that lanthanum toxicity was significantly reduced with addition of phosphorus to the 100% elutriates.

Martin & Hickey (2004) used a 40 µm nylon mesh filter whereas the standard TCLP method suggested a 0.6 – 0.8 µm filter (USEPA, 1986). The first Phoslock<sup>®</sup> toxicity study conducted by the CSIRO, Australia (Stauber, 2000) adopted the TCLP method and used a 0.45 µm filter. NICNAS (National Industrial Chemicals Notification and Assessment Scheme), Australia (2001) interpreted the CSIRO toxicity test reports (Stauber, 2000; Stauber & Binet, 2000) for preparing an assessment report on Phoslock<sup>®</sup>. However, there was a typographical error on page 12 of the report in relation to the filter size (45 µm). This should have been 0.45 µm (not 45 µm). This is because Stauber (2000) used a 0.45 µm filter, and this was the only reference that NICNAS used in relation to the TCLP method. Martin & Hickey (2004) stated that they used the large size filter (40 µm) and referred to the NICNAS (2001) report which actually contained the typographical error. Martin & Hickey (2004) dissolved 50 g Phoslock<sup>®</sup> granules in 1 L water and the supernatant was filtered through a 40 µm nylon mesh filter. Therefore, it is likely that some Phoslock<sup>®</sup> clay particles passed through the 40 µm nylon mesh filter and caused the gelatinous precipitate at the 100% concentration (i.e. 50 g/L).

In order to investigate the toxicity of Phoslock<sup>®</sup> in the natural water where it is applied, two recent toxicity studies on *Daphnia* used suspension of Phoslock<sup>®</sup> granules in natural lake/pond water (Ecotox, 2008; Watson-Leung, 2008). Ecotox Services Australasia (Ecotox, 2008) used low alkalinity (13 mg/L as CaCO<sub>3</sub>) water with a low phosphate (0.002 mg/L) concentration collected from a drinking water reservoir (south of NSW, Australia). The 24- and 48 h EC<sub>50</sub> (with 95% confidence limits) estimated of granular Phoslock<sup>®</sup> to *C. dubia* survival was >50 mg/L (Ecotox, 2008). No significant mortalities were observed at any of the concentrations tested, and consequently the NOEC and LOEC estimates were 50 and >50 mg/L, respectively (Ecotox, 2008). At the end of experiment after 48 h, the dissolved La concentration in 50 mg/L Phoslock<sup>®</sup> treatment was measured as 330 µg/L. The highest concentration of Phoslock<sup>®</sup> used in this experiment was only 50 mg/L.

Another toxicity study was conducted by the Aquatic Toxicology Unit, Ontario Ministry of the Environment, Ontario, Canada using suspension of Phoslock<sup>®</sup> granules (not leachate) dissolved in high alkalinity Scanlon pond water (203 mg/L as CaCO<sub>3</sub>) (Watson-Leung, 2008). Results demonstrated that Phoslock<sup>®</sup> exhibited some toxicity to *Daphnia* only at very high concentrations (3,400 mg/L and 6,800 mg/L). The 48 h EC<sub>50</sub> was calculated as >6,800 mg/L (Watson-Leung, 2008). Concentration of dissolved La in 6,800 mg/L of Phoslock<sup>®</sup> treatment was measured as 14,000 µg/L. However, it was not clear why the concentration of dissolved lanthanum was lower (14,000 µg/L) in the 6,800 mg/L of Phoslock<sup>®</sup> treatment than it was in the 3,400 mg/L concentration of Phoslock<sup>®</sup> (dissolved lanthanum was 63,270 µg/L) (Watson-Leung, 2008). The highest concentration of Phoslock<sup>®</sup> used in this experiment was 6,800 mg/L.



Phoslock<sup>®</sup> had never been applied in a Canadian ecosystem prior to the toxicity study conducted by Watson-Leung (2008). Alkalinity in Canadian water is usually high (203 mg/L as CaCO<sub>3</sub> measured in Scanlon pond water during Daphnia toxicity test). Therefore laboratory toxicity testing was necessary using high alkalinity Canadian natural lake/pond water. Phoslock<sup>®</sup> application rates tested by Watson-Leung (2008) varied across the tests performed but in most cases were much greater than the proposed dose rate suggested by the Technical Division of Phoslock Water Solutions Ltd. Excessively high application rates of Phoslock<sup>®</sup> (up to 13,600 mg/L) were tested by Watson-Leung (2008) to examine the worst case scenario: such as if a concentrated pulse of Phoslock<sup>®</sup> entering the ecosystem due to equipment malfunction or human error.

### 9.1.2. Chronic toxicity tests using Daphnia

CSIRO Centre for Advanced Analytical Chemistry conducted two chronic toxicity studies on Daphnia survival and reproduction using a Phoslock<sup>®</sup> leachate (extracted by using the TCLP method) and lanthanum chloride in synthetic soft water (Stauber, 2000; Stauber & Binet, 2000). Stauber (2000) used the Phoslock<sup>®</sup> leachate and 7 d EC<sub>50</sub> for survival was calculated as 41% leachate. Based on the weight of Phoslock<sup>®</sup> used in the extraction procedure (50 g/L), and assuming a linear relationship between the mass of Phoslock<sup>®</sup> added and the elutriate/leachate composition, the 7 d EC<sub>50</sub> for filtered leachate is equivalent to 20,500 mg/L of Phoslock<sup>®</sup>. Analysis of lanthanum in the soft water leachate used for the experiment showed that 41% EC<sub>50</sub> corresponded to approximately 820 µg/L of lanthanum (Stauber, 2000). Stauber & Binet (2000) used lanthanum chloride and 7 d EC<sub>50</sub> for survival was calculated as 510 µg/L of lanthanum. The 7 d EC<sub>50</sub> for reproduction was calculated as 430 µg/L of lanthanum chloride with corresponding LOEC and NOEC were 90 µg/L and 50 µg/L respectively (Stauber & Binet, 2000).

A recent (July 2008) chronic toxicity study of Phoslock<sup>®</sup> on the survival and reproductive impairment of *Ceriodaphnia dubia* was conducted by the Ecotox Services Australasia (Sydney, Australia). The Ecotox (2008) study used low alkalinity (13 mg/L as CaCO<sub>3</sub>) natural water with a low phosphate (0.002 mg/L) concentration collected from a drinking water reservoir (south of NSW, Australia). This test was conducted by an independent consultant to fulfill the requirements of obtaining a licence for a Phoslock<sup>®</sup> application. Therefore, the highest concentration of Phoslock<sup>®</sup> was only 1 mg/L as directed by the DECC (Department of Environment and Climate Change), NSW, Australia. Results showed that there were no toxic effects of Phoslock<sup>®</sup> on the survival and reproduction of *Ceriodaphnia dubia* up to the concentration of 1 mg/L (Ecotox report, 2008). The 7 d EC<sub>50</sub>, LOEC and NOEC estimates for survival and reproduction were >100, 100 and >100%, respectively (Ecotox report, 2008). The 7 d chronic tests were subject to a complete renewal (i.e. static-renewal) on each day for the duration of the test. Sufficient test solutions were prepared on Day 0 to provide for the test solution renewals on Days 1, 2 and 3. On Day 4, fresh solutions were prepared and used to provide renewal solutions on Days 4, 5 and 6. The test was terminated on Day 7. The concentrations of dissolved La were measured for all treatments before each day of renewal. There were no significant differences between La concentrations during Days 1 – 6. Dissolved La concentrations in 1 mg Phoslock<sup>®</sup>/L solutions were 20 – 24 µg/L (measured using ICP-

MS at EnviroLab, Sydney, Australia). In the acute toxicity test (Ecotox 2008), no significant toxicity was observed up to 50 mg/L of Phoslock<sup>®</sup> in which 330 µg/L of dissolved lanthanum was measured at the end of the experiment after 48 h.

Recently (mid 2008 and early 2009), the aquatic ecology group of Wageningen University, Netherlands conducted two separate 5 d chronic toxicity tests to determine the effects of Phoslock<sup>®</sup> on the growth (length and weight) of the cladoceran, *Daphnia magna* (Lurling et al., 2008; Lurling & Tolman, 2009). The 5 d EC<sub>50</sub> were calculated as 800 mg Phoslock<sup>®</sup>/L (Lurling et al., 2008) and 871 mg Phoslock<sup>®</sup>/L (Lurling & Tolman, 2009) for weight, and 3130 & 1557 mg Phoslock<sup>®</sup>/L for length. The EC<sub>50</sub> values for weight were consistent between the two tests. However, for length, these values were significantly different. This was possibly due to the differences in health conditions of two different batches of *Daphnia* used in these tests.

### 9.1.3. Phoslock<sup>®</sup> toxicity to *Daphnia*: Acute vs Chronic tests

In the acute tests, organisms are exposed to the toxicant for a short period of time (24 – 72 h). But in the chronic tests, organisms are exposed to the toxicant for a longer period (>5 d). Therefore, it is reasonable to assume that the values of EC<sub>50</sub> for acute toxicity tests should be higher (less toxic) than the values of chronic tests. However, this general assumption or hypothesis was not reflected in the results of some toxicity tests conducted by the CSIRO (Stauber, 2000) and the values of EC<sub>50</sub> as lanthanum that was leached out from Phoslock<sup>®</sup> during TCLP extraction. In the chronic toxicity test conducted by Stauber (2000), 7 d (168 h) EC<sub>50</sub> for survival was calculated as 41% of leachate. The batch of soft water leachate used for chronic toxicity study was analysed and found to contain 2010 µg/L lanthanum, therefore, 41% would correspond to approximately 820 µg/L of lanthanum (Stauber, 2000). However, in the acute toxicity test, 2 d (48 h) EC<sub>50</sub> for survival/immobilisation was calculated as 49% of leachate (Stauber, 2000). The separate batch of softwater leachate used in that study was analysed and found to contain 163 µg/L lanthanum, therefore, 49% would correspond to approximately 80 µg/L of lanthanum (Stauber, 2000). When comparing the EC<sub>50</sub> values those were calculated as concentration of Phoslock<sup>®</sup>, the value for acute toxicity study (24,500 mg/L) was higher than the value of chronic study (20,500 mg/L) which is consistent with the assumption/hypothesis. However, when comparing EC<sub>50</sub> values of acute and chronic studies as the concentration of dissolved lanthanum, the value of acute toxicity was >10 times lower (more toxic) than chronic tests which was opposite to the assumption/hypothesis. Stauber (2000) concluded that the toxicity of the leachate was due to lanthanum, not due to the colloidal material, as removal of this fraction by ultrafiltration through a 0.1 µm membrane filter, did not reduce toxicity. Therefore, the toxicity threshold (e.g. EC<sub>50</sub>) should be calculated as the concentration of dissolved lanthanum (80 µg/L for acute and 820 µg/L for chronic), not as the concentration of Phoslock<sup>®</sup> (24,500 mg/L for acute and 20,500 mg/L for chronic). However, the exact reason for more than ten times lower value of EC<sub>50</sub> (80 µg/L) or stronger toxicity in the short-term acute toxicity test than the value of long-term chronic toxicity test (820 µg/L) was unknown and not discussed in the Stauber (2000) report. When the CSIRO toxicity studies were undertaken (Stauber, 2000; Stauber & Binet, 2000) they used Phoslock<sup>®</sup> that was formulated in the laboratory as a slurry (not under factory conditions). This may have caused the presence of residual lanthanum and variable measured concentrations.

## 9.2. Toxicity tests using Rainbow fish

Results from five acute toxicity studies using three different species of Rainbow fish are summarised in Table 5. Both Phoslock<sup>®</sup> (suspension and leachate extracted by the TCLP method) and lanthanum chloride were used in these studies.

No.	Species	Media	Method	Dura	EC <sub>50</sub> /LC <sub>50</sub>	NOEC	LOEC	Ref.
<b>Acute tests</b>								
1	<i>Melanotaenia duboulayi</i>	Synthetic soft water	Phos TCLP	96 h	> 50,000 mg/L Phoslock <sup>®</sup> > 127 µg/L La	50,000 mg/L Pk 127 µg/L La	> 50,000 mg/L Phoslock <sup>®</sup> >127 µg/L La	Stauber, 2000
2	<i>Melanotaenia duboulayi</i>	Treated tap water	LaCl <sub>3</sub> Solution	96 h	< 600 µg/L La	< 600 µg/L La	< 600 µg/L La	Stauber & Binet, 2000
3	<i>Oncorhynchus mykiss</i>	Synthetic soft water	Phos TCLP	96 h	4350 mg/L Phoslock <sup>®</sup>	<3125mg/L Phoslock <sup>®</sup>	3125mg/L Phoslock <sup>®</sup>	Martin & Hickey, 2004
4	<i>Melanotaenia splendida</i>	Synthetic soft water	Phos TCLP	96 h	> 50,000 mg/L Phoslock <sup>®</sup>	50,000 mg/L Pk	50,000 mg/L Pk	Ecotox, 2006a
5	<i>Oncorhynchus mykiss</i>	Natural Pond water	Phos Solution	48 h	> 13,600 mg/L Phoslock <sup>®</sup>	–	–	Watson-Leung, 2008

Phos or Pk = Phoslock<sup>®</sup>

**Table 5:** Acute toxicity of Phoslock<sup>®</sup> and lanthanum chloride to rainbow fish.

### 9.2.1. Acute toxicity tests using Rainbow fish

Two sub-acute toxicity tests were conducted by the CSIRO Centre for Advanced Analytical Chemistry using eastern rainbow fish, *Melanotaenia duboulayi* over a 96 h period. The tests used filtered leachate solutions of Phoslock<sup>®</sup> (TCLP method) prepared from synthetic soft water (hardness 40 - 45 mg/L as CaCO<sub>3</sub>) (Stauber, 2000) and a solution of lanthanum chloride in treated Sydney soft water (Stauber & Binet, 2000). Both experiments used a static methodology without replacement of the test media. Results of the first test showed that there was no toxicity of soft water leachate to rainbow fish; EC<sub>50</sub> was calculated as >100% leachate with the corresponding NOEC and LOEC being 100% and >100% respectively (Stauber, 2000). A separate batch of soft water leachate was prepared during the acute rainbow fish test. The concentration of lanthanum in this filtered (0.45 µm) leachate was 127 µg/L (Stauber, 2000). In the second test with a solution of lanthanum chloride in treated Sydney tap water at concentrations between (nominally) 690 µg/L and 44,000 µg/L, complete imbalance of fish was observed at all concentrations above 600 µg/L (Stauber & Binet, 2000). It was not possible to repeat the test at lower concentrations due to unavailability of additional rainbow fish. Therefore, EC<sub>50</sub> was calculated as <600 µg/L of lanthanum, with corresponding NOEC and LOEC being <600 µg/L and <600 µg/L of La respectively (Stauber & Binet, 2000). Measured concentrations of total lanthanum were 75 - 100% of nominal concentrations on Day 0. In both tests, the physico-chemical measurements of the test solutions including pH, conductivity, dissolved oxygen and temperature were within acceptable limits. Fish survival in the control was 100% (Stauber, 2000) and 90% (Stauber & Binet, 2000) over 96 h, indicating test acceptability.

During the first and second Phoslock<sup>®</sup> trial (January & April 2000) in the Canning River, Western Australia, toxicity tests using Phoslock<sup>®</sup> and juvenile rainbow fish were conducted in river water both before and after the application of Phoslock<sup>®</sup>. No toxicity to juvenile rainbow fish was observed in any Canning River water sample prior to and after the application of Phoslock<sup>®</sup> (Stauber & Binet, 2000). Although the calibration bioassay with lanthanum alone suggested that fish immobilization would occur at concentrations of lanthanum <600 µg/L, no toxicity was observed in Canning River water samples containing up to 15,000 µg total La/L in the first trial and up to 1,700 µg total La/L in the second trial (Stauber & Binet, 2000). Authors suggested that other factors in the river water, such as humic substances, were ameliorating the potential toxic effect. Lanthanum is known to strongly bind to humic substances (Clark & Chopin, 1996; Nanny & Minear, 1994).

An acute toxicity test was conducted by the NIWA (Martin & Hickey, 2004) using Phoslock<sup>®</sup> leachates/elutriates and fry of rainbow trout, *Oncorhynchus mykiss*. Results showed that the fish fry were sensitive to the Phoslock<sup>®</sup> elutriate. LC<sub>50</sub> or EC<sub>50</sub> was calculated for 24, 48, 72 and 96 h as 16.6, 8.7, 8.7 and 8.7% of elutriate respectively (Martin & Hickey, 2004). Corresponding NOEC and LOEC were calculated as <6.25% and 6.25% respectively for all durations (24, 48, 72 and 96 h) of the trial (Martin & Hickey, 2004). Based on the weight of the Phoslock<sup>®</sup> granules used in the extraction procedure (50 g/L), and assuming a linear relationship between the mass of Phoslock<sup>®</sup> added and the elutriate composition, the 96 h trout LC<sub>50</sub> or EC<sub>50</sub> was equivalent to 4,350 mg/L and NOEC was equivalent to <3,125 mg/L of Phoslock<sup>®</sup> respectively (Martin & Hickey, 2004). Toxicity to fish occurred within 48 h, and no further mortality was recorded after this time.

Martin & Hickey (2004) used a 40 µm nylon mesh filter which pore sizes are ~89 times bigger than the filter (0.45 µm) used by Stauber (2000). It was likely that some fine Phoslock<sup>®</sup> clay particles passed through the 40 µm nylon mesh filter, settled and formed white gelatinous precipitate in the test containers at all test concentrations (Martin & Hickey, 2004). Therefore, the concentration of total lanthanum was significantly reduced in the test solutions over time. After 96 h, total La in the test solutions was only 1 - 2% of the concentration at the test initiation (Martin & Hickey, 2004). Martin & Hickey (2004) suggested that the high initial total La concentrations for day 1 elutriate solution could passively be due to particle-associated material passed through 40 µm filter and turbulence caused by the fish fry in the test container. Although dissolved lanthanum was not measured during this test and it was unclear that the toxicity/death of fish was due to lanthanum or particle-associated material, however, the addition of phosphorus significantly reduced leachate toxicity in the mitigation test (Martin & Hickey, 2004). Results from the mitigation experiment suggested that dissolved lanthanum concentrations were reduced by at least 3.6 times by a 5-fold increase in phosphorus and in the containers with 100% Phoslock elutriate fish survival increased up to 100% with the addition of phosphorus (0.02 - 2.5 ppm) (Martin & Hickey, 2004).

In contrast to rainbow fish sensitivity/toxicity observed in the NIWA tests (96 h EC<sub>50</sub> was 8.7% leachate) (Martin & Hickey, 2004), up to 100% Phoslock<sup>®</sup> leachate was not toxic to fish larvae in the test conducted by the Ecotox, Australasia (Ecotox report, 2006a). Although the type of Phoslock<sup>®</sup> (granular formulation), experimental methods (TCLP

method) and test media (synthetic soft water) were similar in both tests, the results were significantly different. One possible explanation is that Ecotox (2006a) used eastern rainbow fish (*Melanotaenia splendida*) and Martin & Hickey (2004) used rainbow trout (*Oncorhynchus mykiss*). Martin & Hickey (2004) suggested that eastern rainbow fish (*M. splendida* or *M. duboulayi*) are markedly less sensitive than the rainbow trout (*Oncorhynchus mykiss*). However, no information was available on whether the TCLP leachate was filtered or what size of filter was used by Ecotox. It was also difficult to compare EC<sub>50</sub> values of these two tests in terms of the concentrations of dissolved lanthanum because Ecotox (2006a) didn't measure dissolved La at the end of the experiment.

Although rainbow trout (*Oncorhynchus mykiss*) are more sensitive than eastern rainbow fish (*M. splendida* or *M. duboulayi*) (Martin & Hickey, 2004), the Aquatic Toxicology Unit, Ontario Ministry of the Environment, Ontario, Canada demonstrated that the rainbow trout, *Oncorhynchus mykiss* showed no sign of toxicity up to the highest concentration of 13,600 mg Phoslock<sup>®</sup>/L (suspension of granules, not leachate) in pond water (Watson-Leung, 2008). The concentrations of dissolved La were not measured from the solutions used in the trout toxicity test. It can be assumed however the concentrations of dissolved La in the dilution series of Phoslock<sup>®</sup> used in the Daphnia toxicity tests will be similar where they overlap. Concentrations of dissolved La in 3,400 mg/L and 6,800 mg/L of Phoslock<sup>®</sup> treatments were 63,270 µg/L and 14,000 µg/ respectively. It can be assumed that the concentration of dissolved La in 13,600 mg Phoslock<sup>®</sup>/L (highest concentration used for trout toxicity test) would be higher than 63,270 µg/L which is much higher than the values of EC<sub>50</sub> determined by the CSIRO (127 & <600 µg/L) for rainbow fish and considerably higher than the concentrations that would ever be used in an environmental application.

### 9.3. Toxicity tests using shrimp

Results from one acute and four chronic toxicity studies using one species of fresh water shrimp (*Macrobrachium* sp) are summarised in Table 6. Phoslock<sup>®</sup> leachate (extracted by TCLP method) was used in these studies.

No.	Species	Media	Method	Duration	EC <sub>50</sub> /LC <sub>50</sub>	NOEC	LOEC	Ref.
<b>Acute tests</b>								
1	<i>Macrobrachium</i> sp.	Synthetic soft water	Phos TCLP	96 h	> 50 g/L Phoslock <sup>®</sup>	50 g/L Phoslock <sup>®</sup>	> 50 g/L Phoslock <sup>®</sup>	Ecotox, 2006b
<b>Chronic tests</b>								
1	<i>Macrobrachium</i> sp.	Synthetic soft water	Phos TCLP	7 d Survival	> 800 mg/L Phoslock <sup>®</sup>	400 mg/L Phoslock <sup>®</sup>	800 mg/L Phoslock <sup>®</sup>	Ecotox, 2006a
2	<i>Macrobrachium</i> sp.	Synthetic soft water	Phos TCLP	14 d Survival	800 mg/L Phoslock <sup>®</sup>	400 mg/L Phoslock <sup>®</sup>	800 mg/L Phoslock <sup>®</sup>	Ecotox, 2006a
3	<i>Macrobrachium</i> sp.	Synthetic soft water	Phos TCLP	21 d Survival	700 mg/L Phoslock <sup>®</sup>	400 mg/L Phoslock	800 mg/L Phoslock	Ecotox, 2006a
4	<i>Macrobrachium</i> sp.	Synthetic soft water	Phos TCLP	28 d Survival	700 mg/L Phoslock <sup>®</sup>	400 mg/L Phoslock <sup>®</sup>	800 mg/L Phoslock <sup>®</sup>	Ecotox, 2006a

Phos or Pk = Phoslock<sup>®</sup>

**Table 6:** Acute and chronic toxicity of Phoslock<sup>®</sup> to shrimp (*Macrobrachium* sp).

### 9.3.1. Acute toxicity tests using shrimp

Ecotox Services Australasia Pty Ltd conducted an acute toxicity test using juvenile freshwater shrimp (*Macrobrachium* sp.) and Phoslock<sup>®</sup> leachate (TCLP method) (Ecotox report, 2006b). Results demonstrated that the Phoslock<sup>®</sup> leachate (up to 100%) was not toxic to shrimp (Ecotox report, 2006b). The 96 h EC<sub>50</sub> (imbalance) was calculated as >100% leachate with the corresponding NOEC and LOEC being 100% and >100% respectively which was prepared from 50 g/L of Phoslock<sup>®</sup> granules (Ecotox report, 2006b). TCLP leachate was 100% sample and was used in the preparation of test treatments. No information was available on whether the TCLP leachate was filtered. Dissolved lanthanum concentrations were not measured at the end of the experiment.

### 9.3.2. Chronic toxicity tests using shrimp

Chronic toxicity tests of Phoslock<sup>®</sup> leachate on juvenile freshwater shrimp (*Macrobrachium* sp.) were conducted by Ecotox Services Australasia Pty Ltd for 7, 14, 21 and 28 days. Phoslock<sup>®</sup> leachates (modified TCLP method) were prepared by mixing a specified (50, 100, 200, 400 and 800 mg/L) weight of test material with 1 L of synthetic water for 18 hours in a rotary tumbler (without light). These leachates were allowed to settle overnight prior to use. Results demonstrated that there were some toxic effects by leachates of the highest concentration (800 mg/L) of Phoslock<sup>®</sup> granules on the survival of juvenile freshwater shrimp (Ecotox report, 2006b). EC<sub>50</sub>, NOEC and LOEC for 7 and 14 d were calculated as >800, 400, 800 mg Phoslock<sup>®</sup>/L respectively (Ecotox report, 2006b). However, EC<sub>50</sub>, NOEC and LOEC for 21 and 28 d were calculated as 700, 400, 800 mg Phoslock<sup>®</sup>/L respectively (Ecotox report, 2006b). No dissolved or total lanthanum concentrations were measured from any Phoslock<sup>®</sup> leachates.

## 9.4. Toxicity tests using algae

Results from three acute toxicity studies using three species of fresh water algae are summarised in Table 7. Both Phoslock<sup>®</sup> leachate (extracted by the TCLP method) and a suspension of Phoslock<sup>®</sup> were used in these studies.

No.	Species	Media	Method	Dura	EC <sub>50</sub> /LC <sub>50</sub>	NOEC	LOEC	Ref.
<b>Acute tests</b>								
1	<i>Selenastrum capricornutum</i>	Growth media	LaCl <sub>3</sub> Solution	72 h	450 µg/L La	<130 µg/L La	130 µg/L La	Stauber & Binet, 2000
2	<i>Pseudokirchneriella subcapitata</i>	Synthetic soft water	Phos TCLP	72 h	15,000 mg/L Phoslock <sup>®</sup>	6,250 mg/L Phoslock <sup>®</sup>	12,500 mg/L Pk	Martin & Hickey, 2004
3	<i>Scenedesmus obliquus</i>	Artificial RT medium	LaNO <sub>3</sub> Solution	72 h	450 mg/L Pk > 250 mg/L La	–	–	Lurling et al., 2008

Phos or Pk = Phoslock<sup>®</sup>

**Table 7:** Acute toxicity of Phoslock<sup>®</sup> and lanthanum chloride to algae.

#### 9.4.1. Acute toxicity tests using algae

A three day (72 h) toxicity test of Phoslock<sup>®</sup> was conducted using Milli-Q water leachates and the green alga, *Selenastrum capricornutum*. Milli-Q water leachates were prepared in 20 ml silanised glass scintillation vials containing 6 ml algal culture with medium (with EDTA). Results showed positive effects on algal growth rather than toxic effects (Stauber, 2000). Milli-Q water leachate was filtered using 0.22 µm filter. For all the leachate test solutions, the growth rate of the algae was approximately twice that of the control over 72 h suggesting that some component of the leachate acts as a growth promoter for this species. Although the lanthanum concentration was not reported for this leachate, it is assumed to be the same as *Ceriodaphnia dubia* test in Milli-Q water. Filtered leachate of Phoslock<sup>®</sup> in Milli-Q water contained 396 µg La/L (Stauber, 2000).

However, lanthanum chloride inhibited the growth of green alga, *Selenastrum capricornutum* with an apparent 72 h calibration bioassays (Stauber & Binet, 2000). The 72 h EC<sub>50</sub> was calculated from the growth data as 450 µg/L of lanthanum, NOEC and LOEC were calculated as <130 µg/L and 130 µg/L respectively (Stauber & Binet, 2000). This result must be treated with caution because precipitation of lanthanum took place over the test period. The algal test medium contained 570 µg/L PO<sub>4</sub> (which is required as nutrient for algal growth) and 15 mg/L NaHCO<sub>3</sub>. Lanthanum precipitated as phosphate or carbonate complex over the course of bioassay, leaving little phosphate or lanthanum in the solution. Phosphate concentrations in the test medium decreased from 170 µg/L to <10 µg/L in the treatments of greater than 1.2 mg/L of lanthanum. Total lanthanum concentrations on Day 0 were close to nominal concentrations. However, total lanthanum concentrations by Day 3 and dissolved lanthanum concentrations were much lower (Stauber & Binet, 2000). Although measured total lanthanum concentrations on Day 0 were used to calculate toxicity results, the EC<sub>50</sub> values should be interpreted with caution due to the losses of lanthanum over the course of the bioassay. Although dissolved lanthanum concentrations were below the detection limit (<10 µg/L), significant growth inhibition of the alga was observed even at the lowest concentrations tested. It is likely that growth inhibition was due to phosphate limitation as the removal of available PO<sub>4</sub> from the growth medium through precipitation as LaPO<sub>4</sub> (Stauber & Binet, 2000).

Acute toxicity of Phoslock<sup>®</sup> to the alga, *Pseudokirchneriella subcapitata* was conducted by the NIWA (Martin & Hickey, 2004) using the Phoslock<sup>®</sup> leachate (TCLP) method. The Phoslock<sup>®</sup> leachate was filtered using 0.45 µm membrane filter before the test solutions were prepared (although Phoslock<sup>®</sup> leachate was filtered using 40 µm nylon mesh filter for toxicity tests with *Daphnia* and fish fry). The EC<sub>50</sub> value was calculated from 72 h toxicity test as 30% elutriate which was equivalent to 15,000 mg/L of Phoslock<sup>®</sup> (Martin & Hickey, 2004). The NOEC estimate was 12.5% elutriate which was equivalent to 6,250 mg/L of Phoslock<sup>®</sup> (Martin & Hickey, 2004).

The Aquatic Ecology Group of Wageningen University, Netherlands conducted acute toxicity tests of lanthanum dissolved in nanopure water using green alga, *Scenedesmus obliquus*. From separate experiments using a suspension of Phoslock<sup>®</sup> and lanthanum, the 72 h EC<sub>50</sub> was calculated as 450 mg Phoslock<sup>®</sup>/L and 250 mg La/L (Lurling et al., 2008).

## 9.5. Toxicity tests using sediment-dwelling organisms

Results from four chronic toxicity studies using four different species of fresh water sediment-dwelling organisms (one species of amphipod, one species of mayfly and two species of midge) are summarised in Table 8. Different concentrations of Phoslock® solutions were used in these studies.

No.	Species	Media	Method	Dura	EC <sub>50</sub> /LC <sub>50</sub>	NOEC	LOEC	Ref.
<b>Amphipod</b>								
1	<i>Hyalella azteca</i>	Natural Pond water	Phos Solution	14 d Surviv.	> 3400 mg/L Phoslock®	3400 mg/L Phoslock®	>3400mg/L Phoslock®	Watson-Leung, 2008
2	<i>Hyalella azteca</i>	Natural Pond water	Phos Solution	14 d Growth	> 3400 mg/L Phoslock®	3400 mg/L Phoslock®	>3400mg/L Phoslock®	Watson-Leung, 2008
<b>Mayfly</b>								
1	<i>Hexagenia</i> spp.	Natural Pond water	Phos Solution	21 d Surviv.	> 450 mg/L Phoslock®	450 mg/L Phoslock®	> 450 mg/L Phoslock®	Watson-Leung, 2008
2	<i>Hexagenia</i> spp.	Natural Pond water	Phos Solution	21 d Growth	> 450 mg/L Phoslock®	450 mg/L Phoslock®	> 450 mg/L Phoslock®	Watson-Leung, 2008
<b>Midge larvae</b>								
1	<i>Chironomus zealandicus</i>	Natural Lake water	Phos solution	38 d Surviv.	> 400 mg/L Phoslock®	400 mg/L Phoslock®	> 400 mg/L Phoslock®	Clearwater, 2004
2	<i>Chironomus dilutus</i>	Natural Pond water	Phos Solution	10 d Surviv.	> 3400 mg/L Phoslock®	3400 mg/L Phoslock®	>3400mg/L Phoslock®	Watson-Leung, 2008
3	<i>Chironomus dilutus</i>	Natural Pond water	Phos Solution	10 d Growth	> 3400 mg/L Phoslock®	3400 mg/L Phoslock®	>3400mg/L Phoslock®	Watson-Leung, 2008

Phos or Pk = Phoslock®

**Table 8:** Chronic toxicity of Phoslock® to sediment-dwelling organisms (amphipod, mayfly and midge larvae).

### 9.5.1. Phoslock® toxicity to Amphipod

A recent chronic toxicity study (14 d) conducted by the Aquatic Toxicology Unit, Ontario Ministry of the Environment, Ontario, Canada revealed that Phoslock® was not toxic to the survival and growth of amphipod, *Hyalella azteca* up to the highest concentration (3,400 mg/L) tested (Watson-Leung, 2008). The test media contained a suspension of Phoslock® granules dissolved in pond water up to the concentration of 3,400 mg/L. Results showed that there was no significant reduction in the survival and growth of amphipods exposed to all of the test sediments and treatments when compared to amphipod survival in the control sediment ( $p > 0.05$ ) (Watson-Leung, 2008). From this study, the 14 d EC<sub>50</sub>, NOEC and LOEC can be calculated for survival and growth of amphipods as >3,400 mg/L, 3,400 mg/L and >3,400 mg/L of Phoslock® respectively.

### 9.5.2. Phoslock® toxicity to Mayfly

Phoslock® was not toxic to mayfly, *Hexagenia* spp. at the dose rate of 250:1 (250 mg Phoslock® for 1 mg FRP) applied in Canadian pond water under laboratory conditions.



Results of a chronic toxicity study (21 d) was conducted by the Aquatic Toxicology Unit, Ontario Ministry of the Environment, Ontario, Canada showed no toxicity of Phoslock<sup>®</sup> at the concentration of 450 mg/L (Watson-Leung, 2008). The test media contained a suspension of Phoslock<sup>®</sup> granules dissolved in pond water at the concentration of 450 mg/L. There was no significant reduction in the survival and growth of the mayfly exposed to the test sediments and treatments when compared to the mayfly survival in the control sediment ( $p > 0.05$ ) (Watson-Leung, 2008). The 21 d EC<sub>50</sub>, NOEC and LOEC can be calculated for survival and growth of the mayfly as >450 mg/L, 450 mg/L and >450 mg/L of Phoslock<sup>®</sup> respectively.

### 9.5.3. Phoslock<sup>®</sup> toxicity to Midge larvae

A species of midge larvae, *Polypedilum parvidum* was used for a 38 d chronic toxicity study in a suspension of Phoslock<sup>®</sup> granules (not leachate) dissolved in fernhollow spring water (New Zealand). Results showed that there were no toxic effects up to the highest concentration (400 mg/L) tested (Clearwater, 2004). The 38 d EC<sub>50</sub> was calculated as >400 mg/L of Phoslock<sup>®</sup>; NOEC and LOEC were calculated as 400 mg/L and >400 mg/L respectively (Clearwater, 2004).

Recently, Ontario Ministry of the Environment, Canada conducted a chronic toxicity study (10 d) of Phoslock<sup>®</sup> to test the survival and growth of midge larvae, *Chironomus dilutus* in a suspension of Phoslock<sup>®</sup> granules dissolved in pond water up to the concentration of 3,400 mg/L (Watson-Leung, 2008). Results showed that there were no toxic effects up to the highest concentration (3,400 mg/L) tested. There was no significant reduction in survival and growth of the midge larvae exposed to all of the test sediments and treatments when compared to the midge larvae survival in the control sediment ( $p > 0.05$ ) (Watson-Leung, 2008). The 10 d EC<sub>50</sub>, NOEC and LOEC can be calculated for survival and growth of midge larvae as >3,400 mg/L, 3,400 mg/L and >3,400 mg/L of Phoslock<sup>®</sup> respectively.

## 10. Variation of results

The results obtained from large numbers of acute and chronic toxicity tests using different species of Daphnia, rainbow fish, shrimp, algae, sediment-dwelling organisms and Phoslock<sup>®</sup> or lanthanum (as LaCl<sub>3</sub>) demonstrated a wide variation of responses of sensitive organisms to Phoslock<sup>®</sup> solutions or leachates (TCLP method) or lanthanum (Table 4 - 8). The toxicity thresholds such as EC<sub>50</sub>/LC<sub>50</sub>, NOEC or LOEC were determined either as the dissolved lanthanum or the concentrations of Phoslock<sup>®</sup> and varied significantly among different tests (Table 4 - 8). For example, the acute 48 h EC<sub>50</sub> as LaCl<sub>3</sub> for Daphnia varied from 43 µg/L in tap water (Barry & Meehan, 2000) to 23,000 µg/L La in synthetic soft water (Yasseri & Nowak, 2008). Similarly, when EC<sub>50</sub> was measured as the concentration of Phoslock<sup>®</sup>, 48 h EC<sub>50</sub> varied from >50 mg/L (50 mg/L was the highest concentration used in that test) in natural lake water (Ecotox, 2008) to >50,000 mg/L in synthetic soft water (Martin & Hickey, 2004). Lanthanum was most toxic to Daphnia in Milli-Q water (48 h EC<sub>50</sub> = 40 µg/L, Stauber, 2000) and carbon filtered tap water (48 h EC<sub>50</sub> = 43 µg/L, Barry & Meehan, 2000) compared to ASTM hard water (48

h EC<sub>50</sub> = 1180 µg/L, Barry & Meehan, 2000) or natural lake water (48 h EC<sub>50</sub> was >14,000 µg/L, Watson-Leung, 2008). Similar variation was observed in the acute and chronic toxicity test results using other test organisms such as fish, shrimp, amphipod, mayfly, worm and midge larvae (Table 5 - 8).

There are several factors that may be responsible for these variations including: the differences of experimental methods (TCLP method, suspension of Phoslock<sup>®</sup> granules and lanthanum chloride solution), different types of test media (artificial soft water, hard water, Milli-Q water, nanopure water, natural lake or pond water etc.) and different types of Phoslock<sup>®</sup> (slurry and granular formulation). All of these may have influenced the variation of lanthanum leaching from Phoslock<sup>®</sup> and the differences between toxicity thresholds.

The mode of action of Phoslock<sup>®</sup> is that granules or slurry (granules mixed with application water) added to water, moves down through the water column, and the product settles on the sediment. Therefore, toxicity tests should be conducted in a manner designed to simulate the application of granular Phoslock<sup>®</sup> to freshwater and utilise a range of exposure concentrations in order to encompass potential application scenarios. This can be achieved by using suspensions of different concentrations of Phoslock<sup>®</sup> in the toxicity tests. Some of the recent toxicity tests used suspensions of Phoslock<sup>®</sup> (e.g. Ecotox, 2008; Watson-Leung, 2008; Lurling & Tolman, 2009). However, most of the earlier tests (e.g. CSIRO toxicity tests) used Phoslock<sup>®</sup> leachates extracted by the TCLP method. The TCLP is designed to determine the mobility of both organic and inorganic contaminants present in liquid, solid, and multiphase wastes (USEPA, 1986). The TCLP may not be an appropriate method to separate lanthanum from Phoslock<sup>®</sup>. Lanthanum ions are strongly associated with the bentonite clay and remain bound within the clay structure under a wide range of physiological conditions unless reacting with the phosphate anion in the water body. However, it is possible that loosely bound lanthanum may leach out from Phoslock<sup>®</sup> depending on the characteristics of media/water in which Phoslock<sup>®</sup> is dissolved. Different types of test media (artificial soft water, hard water, Milli-Q water, nanopure water, natural lake or pond water etc.) were used for different toxicity tests. This significantly influenced the variation of lanthanum leaching and the results of toxicity tests.

Types of Phoslock<sup>®</sup> also influenced the variation of results of toxicity tests. CSIRO Centre for Advanced Analytical Chemistry used Phoslock<sup>®</sup> that was formulated as slurry (Stauber, 2000; Stauber & Binet, 2000). All other tests (on or later 2004) used Phoslock<sup>®</sup> that was formulated as dry, free flowing granular form which was developed in 2004. When in the slurry form, there was a possibility of a certain concentration of free or unbound lanthanum remaining in the solution. However, during the manufacturing process of granular Phoslock<sup>®</sup> significant dewatering of the slurry occur which significantly reduces the amount of residual lanthanum associated with the product. This has been proven through conducting a large number of laboratory experiments by EnviroLab Pty Ltd, Sydney (commissioned by Phoslock Water Solutions Ltd). Results demonstrated that no lanthanum was leached out within 24 h when granular Phoslock<sup>®</sup> was dissolved in DI water (Milli-Q water), although Stauber (2000) measured 396 µg/L of dissolved lanthanum after 24 hours from leachates prepared in Milli-Q water using slurry formulated Phoslock<sup>®</sup>. However, EnviroLab Pty Ltd, Sydney also demonstrated that a

small concentration of loosely bound lanthanum (~16 µg/L) was leached out from Phoslock® (10 mg/L) when dissolved in low alkalinity natural water or synthetic soft water. The presence of competing anions in natural water may influence lanthanum leaching from Phoslock®.

Lanthanum leaching from Phoslock® significantly varied among different batches of tests or leachates (extracted by the TCLP method) using similar types of water (e.g. synthetic soft water) or different water (e.g. Milli-Q water). For example, Stauber (2000) used two separate batches (in soft water) of leachate (TCLP method) from Phoslock® for acute and chronic tests and measured the concentrations of lanthanum that was leached out from Phoslock®. The concentrations of lanthanum in these two batches were significantly different (163 & 2010 µg/L). A separate batch of leachate in Milli-Q water contained 396 µg/L of dissolved lanthanum (Stauber, 2000). Although all of these experiments were conducted in 2000 and presumably using the same batch of Phoslock® for three separate tests/leachate extractions, the reasons for significant variation of lanthanum leaching was not discussed in the report.

Results from a large number of recent laboratory studies conducted by Phoslock Water Solutions Ltd suggested that lanthanum leaching from Phoslock® depends on the chemical properties of water (e.g. alkalinity) in which Phoslock® is dissolved. Not only the variation of the concentrations of lanthanum leaching, but the toxicity of lanthanum is also strongly dependent on the media (water) used for the study (Barry & Meehan, 2000) and significantly varied among different types of water. Barry & Meehan (2000) demonstrated that lanthanum was most toxic (>27 times) to *Daphnia* in soft tap water with an acute 48 hour EC<sub>50</sub> of 43 µg/L compared with 1180 µg/L in ASTM hard water. Stauber (2000) found that Phoslock® was more toxic when TCLP was prepared using Milli-Q water than synthetic soft water. Phoslock® toxicity was greatly reduced when tests were conducted using natural lake/pond water (Clearwater, 2004; Ecotox, 2008; Watson-Leung, 2008).

## **11. Lab toxicity test vs field trial/application**

In general, laboratory toxicity tests are conducted under controlled condition to investigate whether manufactured chemicals or products can produce any toxic effects to the aquatic organisms tested. The results of laboratory tests are considered (usually) to be the predictive of field trials. Based on laboratory toxicity test results, concentrations for field trials are selected. However, in the field trials, products are exposed to a broad spectrum of physical, chemical and biological factors that are difficult to replicate under laboratory conditions. It is unlikely that physical, chemical or biological field conditions can enhance the toxicity of a product. Rather field conditions dilute/reduce the toxicity due to several other chemical properties present in the natural water that are difficult to replicate in the lab environment. These properties (e.g. humic substances) may interfere or mitigate the toxicity of the product. The significant differences between the results of lab toxicity tests using artificial water and the tests using natural lake/pond water after a Phoslock® application in has proven this hypothesis. Laboratory toxicity tests using

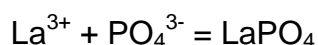
natural lake/pond water demonstrated less toxicity than those tests using tap water or artificial water.

Toxicity tests using Canning River (WA) water (after first and second Phoslock<sup>®</sup> trials - January & April 2000) and juvenile rainbow fish showed no toxicity to juvenile rainbow fish after the application of Phoslock<sup>®</sup> (Stauber & Binet, 2000). However, the lab tests that were carried out prior to tests in the field suggested that fish immobilisation would occur at concentrations of lanthanum <600 µg/L, but no toxicity was observed in Canning River water samples containing up to 15 mg total La/L in the first trial and up to 1.7 mg total La/L in the second trial (Stauber & Binet, 2000). Authors suggested that other factors in the river water, such as humic substances, were ameliorating the potential toxic effect.

A fish health investigation, after three successive applications of Phoslock<sup>®</sup> in Lake Okareka (New Zealand), demonstrated that trout and koura accumulated lanthanum in the liver and hepatopancreas tissues following the application of Phoslock<sup>®</sup> (Landman et al., 2007). However, lanthanum accumulation in the flesh of these organisms was generally low and has been measured in only a small number of specimens from each sampling period after the Phoslock<sup>®</sup> application and has demonstrated no negative effect on the health of the fish. Levels of lanthanum prior to lake dosing were low, suggesting that the interval between applications was sufficient to allow the biota to depurate the lanthanum accumulated by the previous application (Landman et al., 2007).

## **12. Mitigation of Phoslock<sup>®</sup> toxicity by phosphorus and/or carbonate**

Phoslock<sup>®</sup> has been developed to remove filterable reactive phosphorus (FRP) from water. The removal of FRP (or SRP) by Phoslock<sup>®</sup> is attributed to the lanthanum that adsorbs phosphate molecules, forming a highly stable mineral known as Rhabdophane (LaPO<sub>4</sub>.nH<sub>2</sub>O).



The majority of the lanthanum within Phoslock<sup>®</sup> is reportedly strongly bound to the clay matrix, and is therefore likely to be minimally released into the water column. The reaction with phosphate and the precipitation of insoluble Rhabdophane would likely result in lanthanum not being bioavailable to aquatic biota.

Martin & Hickey (2004) conducted a toxicity mitigation experiment by adding phosphorus in to a solution containing different concentrations of Phoslock<sup>®</sup> elutriate/leachate. Results demonstrated that the addition of phosphorus in 100% Phoslock<sup>®</sup> elutriate solutions significantly reduced fish mortality, with the highest P dose (2,500 µg/L) resulting in 0% mortality (i.e. 100% survival) after 72 h exposure (Martin & Hickey, 2004). Whereas in the 100% Phoslock<sup>®</sup> elutriates solution there was no survival of fish fry after 24 hours of exposure (Martin & Hickey, 2004). The concentrations of dissolved lanthanum also reduced significantly after the addition of phosphorus. Reduction in fish

mortality and the dissolved lanthanum concentrations for the highest dose of P indicates that when phosphorus combines with free lanthanum in the water column and precipitate as Rhabdophane, it is no longer bioavailable to the biota, therefore a reduction in toxicity (Martin & Hickey, 2004).

Lanthanum associated with bentonite clay preferentially binds with  $\text{PO}_4$ , forming a highly stable mineral, Rhabdophane. However, it can also bind with  $\text{CO}_3$  or other oxi-anions. Among all other interactions, the lanthanum phosphate bond is the strongest (approx. 300 times stronger than between lanthanum and carbonate). Carbonate competes with phosphate, therefore causes a delay in the phosphate binding of Phoslock (which requires more than 24 hours) in high alkalinity waters. Therefore, in addition to the concentration of FRP, the alkalinity of water needs to be considered in determining the dose rates of Phoslock<sup>®</sup>. In low alkalinity water, the Phoslock<sup>®</sup> dose rate should be based on available phosphate only because there is less or no potential to bind  $\text{CO}_3$  with Phoslock<sup>®</sup> active sites (i.e. lanthanum).

### **13. Toxicity of Phoslock<sup>®</sup> vs toxicity of lanthanum**

Phoslock<sup>®</sup> is a product that contains ~95% bentonite clay (including water content) and ~5% lanthanum. Bentonite is not toxic to humans or the environment (HSDB, 2000). Therefore, it is reasonable to assume that the toxic properties of Phoslock<sup>®</sup> are most likely associated with leached lanthanum from Phoslock<sup>®</sup>. Physical effect on aquatic organisms from Phoslock<sup>®</sup> clay at high concentrations may also have contributed to the chemical toxicity (Martin & Hickey, 2004). But it is highly unlikely that proposed dose rates of Phoslock<sup>®</sup> (100:1, i.e. 100 g Phoslock<sup>®</sup> for 1 g FRP) could cause any negative physical effect.

To assess the toxicity of Phoslock<sup>®</sup>, it is essential to measure the concentration of dissolved/bioavailable lanthanum in the media (e.g. natural or artificial water used for the experiment). Regulatory bodies in Australia such as the NICNAS (National Industrial Chemical Notification and Assessment Scheme) assessed the toxicity of Phoslock<sup>®</sup> and approved it for commercial application. The NICNAS and the Department of Health have considered the product “Phoslock<sup>®</sup>” itself as a non-toxic product. These organisations assessed the toxicity of Phoslock<sup>®</sup> based on the dissolved/bioavailable lanthanum in the water body after a Phoslock<sup>®</sup> application.

These regulatory bodies are concerned about the level of dissolved/bioavailable lanthanum in the water body after application regardless of the concentration of Phoslock<sup>®</sup> to be applied. Phoslock<sup>®</sup> is a product that removes  $\text{PO}_4$  from the water body. In Phoslock<sup>®</sup>, the lanthanum component is associated with the bentonite clay that binds with  $\text{PO}_4$  and forms an insoluble complex ( $\text{LaPO}_4$ ). Lanthanum can also bind with other anions such as  $\text{CO}_3$ ,  $\text{AsO}_4$ , humics etc. Therefore, the availability of dissolved/bioavailable lanthanum in a water body after the application of Phoslock<sup>®</sup> depends on the chemical composition of the water body (such as the concentration of  $\text{PO}_4$  and alkalinity/hardness) and the dose rate of Phoslock<sup>®</sup>. Before an application the Technical Team at PWS assesses the historical physico-chemical conditions of an

application and calculates separate dose rates of Phoslock<sup>®</sup> for each individual water body.

#### **14. Thickness of sediment capping layer of Phoslock<sup>®</sup> and its effect on bottom-dwelling organisms**

When Phoslock<sup>®</sup> granules are added to water, the granules swell and disintegrate as they fall rapidly through the water column, and the product settles on the sediment surface and forms a permeable layer. When Phoslock<sup>®</sup> is applied as slurry (made from mixing dry granules with the 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<sup>®</sup> remove FRP from water column while descending, and the permeable sediment capping layer of Phoslock<sup>®</sup> is capable of adsorbing the FRP on its available binding sites that released from the sediment. The thickness of the Phoslock<sup>®</sup> layer depends on the dose rate. Earlier applications of Phoslock<sup>®</sup> in the Canning River (conducted by CSIRO in 2000 & 2001) aimed to produce 0.5 – 1 mm thick sediment layer (Robb et al., 2003) to prevent the re-release of FRP from the sediment. The previous dose rate of Phoslock<sup>®</sup> (200:1, i.e. 200 g Phoslock<sup>®</sup> for 1 g FRP) suggested as a guide for average water and sediment by Phoslock Water Solutions Ltd (PWS) was capable of producing a 1 – 3 mm sediment layer. In the case of the PWS current dose rate (100:1), 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. Watson-Leung (2008) suggested that a 3,400 mg/L application of Phoslock<sup>®</sup> is needed to produce a 1 mm thick cap on the sediment in the Ontario (Canada) lakes with high alkalinity water.

Phoslock<sup>®</sup> is a modified clay product and its density is similar to that of the clay that is already present in the sediment. This means that the deposition of a Phoslock<sup>®</sup> 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 fashion after an application. In addition, the deposition of the Phoslock<sup>®</sup> particles occurs over several hours allowing some organisms (such as the macro-invertebrates) to re-establish themselves in/over the Phoslock<sup>®</sup> layer. The Phoslock<sup>®</sup> sediment layer is not a solid layer, it is permeable. Clearwater (2004) demonstrated that the Phoslock<sup>®</sup> capping layer was not toxic to bottom-dwelling organisms such as midge. After a 38 d exposure at a concentration of 400 mg Phoslock<sup>®</sup>/L (40 times higher than average dose rate of Phoslock<sup>®</sup> application), the midge demonstrated no sign of toxicity (Clearwater, 2004). Chronic toxicity tests (10 – 14 days) conducted using Canadian pond water demonstrated that the Phoslock<sup>®</sup> capping layer was not toxic to bottom-dwelling organisms such as midge, mayfly and amphipod up to 3,400 mg/L dose rate (Watson-Leung, 2008). Microbiological monitoring after the Canning River trial demonstrated that Phoslock<sup>®</sup> had no negative effect on the number of nitrate reducing and denitrifying or total heterotrophic count (THC) bacterial cells, in fact, an increase was observed in the number of denitrifying bacteria present in the sediment at two sites following the addition of Phoslock<sup>®</sup> (Franzmann et al., 2000; Hancock et al., 2000). The addition of Phoslock<sup>®</sup> had no detrimental effect on the community composition of the sediment microbiota and the rate of denitrification (Franzmann et al., 2000; Hancock et al., 2000).

## 15. Conclusion

The properties of Phoslock<sup>®</sup>, the manufacturing process, strict QA & QC guidelines and the results of a large number of toxicity studies reveal that the active ingredient of Phoslock<sup>®</sup>, lanthanum, is the only element that could cause toxic effects to aquatic organisms above certain concentrations when loosely bound lanthanum leaches out from the product as free lanthanum. Therefore, to assess the toxicity of Phoslock<sup>®</sup>, it is essential to measure the concentration of dissolved lanthanum in the application media (e.g. natural or artificial water used for the toxicity tests). It is also suggested that the toxicity tests be conducted in a manner designed to simulate the application of granular Phoslock<sup>®</sup> to freshwater and utilise a range of exposure concentrations in order to encompass potential application scenarios. This can be achieved by using suspensions of different concentrations of Phoslock<sup>®</sup> in the toxicity tests.

The results of a large number of acute and chronic toxicity tests of Phoslock<sup>®</sup> and lanthanum (in the form of  $\text{LaCl}_3$ ) using a number of sensitive organisms and algae including: several species of water flea (*Daphnia*); several species of Rainbow fish; freshwater shrimp; benthic organisms such as amphipods, mayflies and midge larvae; and two species of algae demonstrated a wide variation in responses of test organisms to Phoslock<sup>®</sup> solutions or leachates (TCLP method) and lanthanum chloride. Differences between experimental methodology and test media may be responsible for these variations. None of these tests demonstrated toxicity of Phoslock<sup>®</sup> to aquatic organisms at the proposed dose rate (i.e. 100:1). Moreover, results from recent toxicity studies using a direct application of Phoslock<sup>®</sup> granules that were dissolved in low and high alkalinity natural waters demonstrated no toxic effects of Phoslock<sup>®</sup> to aquatic organisms in the solution using up to 13,600 mg Phoslock<sup>®</sup>/L which is several thousands times higher than that of the usual Phoslock<sup>®</sup> application dose rate.

## References

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