

PHOSLOCK® TOXICITY EVALUATION

Kralingse Plas

-Final version-

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Summary

Phoslock®

In many freshwater lakes, eutrophication is an ongoing process. To reduce the availability of excess phosphorus (P) in the water column and restore water quality in such lakes, *geo-engineering* tools, such as Phoslock®, have been developed. Its development, appropriate use, and exact dose calculation are all based on solid scientific principles. Phoslock® contains 95 % bentonite clay and 5 % lanthanum. Lanthanum (La) is a naturally occurring element, also present in small quantities in European lake sediments. In Phoslock®, La is strongly associated with the bentonite clay but retains its capacity to bind phosphate. Once Phoslock® is applied to freshwaters, it disperses into small particles, binds P to form insoluble lanthanum-phosphate, and settles quickly on the lake bottom. It is not released as soluble La into the water.

The safe use of Phoslock® is related to its active ingredient (La) and also to physical effects caused by a temporary increase in turbidity in the water during application. In this review, the evaluation of risks associated with the use of Phoslock® is therefore based on the (ecotoxicological) assessment of exposed organisms of the aquatic environment and exposed terrestrial mammals including humans.

Ecotoxicological laboratory studies with aquatic organisms have been conducted with Phoslock® and soluble La-salts (e.g. LaCl_3). As soluble La-salts have different chemical properties to Phoslock®, ecotoxicological studies done with La-salts are not comparable to Phoslock® applications and, therefore, not suitable for the assessment of Phoslock® toxicity. Furthermore, these studies are often conducted under non-realistic conditions (such as the use of phosphate-free medium). Consequently, only ecotoxicological assays conducted with Phoslock® form the basis of this review.

Toxicity and biodiversity effects

During a Phoslock® application, the amount of suspended solids will increase in the short term. This temporary increase might have a short-term impact on organisms. Once Phoslock® is applied, however, it will quickly settle to the lake sediment, most of it within one day. Any potential undesired side effects can easily be prevented by dividing the application over several days.

Once Phoslock® is applied it will strongly and irreversibly bind P, hence, there will be less P available for phytoplankton and cyanobacterial growth. Thus, any observed adverse effect on algal growth is expected to occur due to phosphate limitation rather than to direct toxic effects. Phytoplankton surveys after whole lake applications of Phoslock® have shown that there are no non-target impacts on phytoplankton due to the treatment.

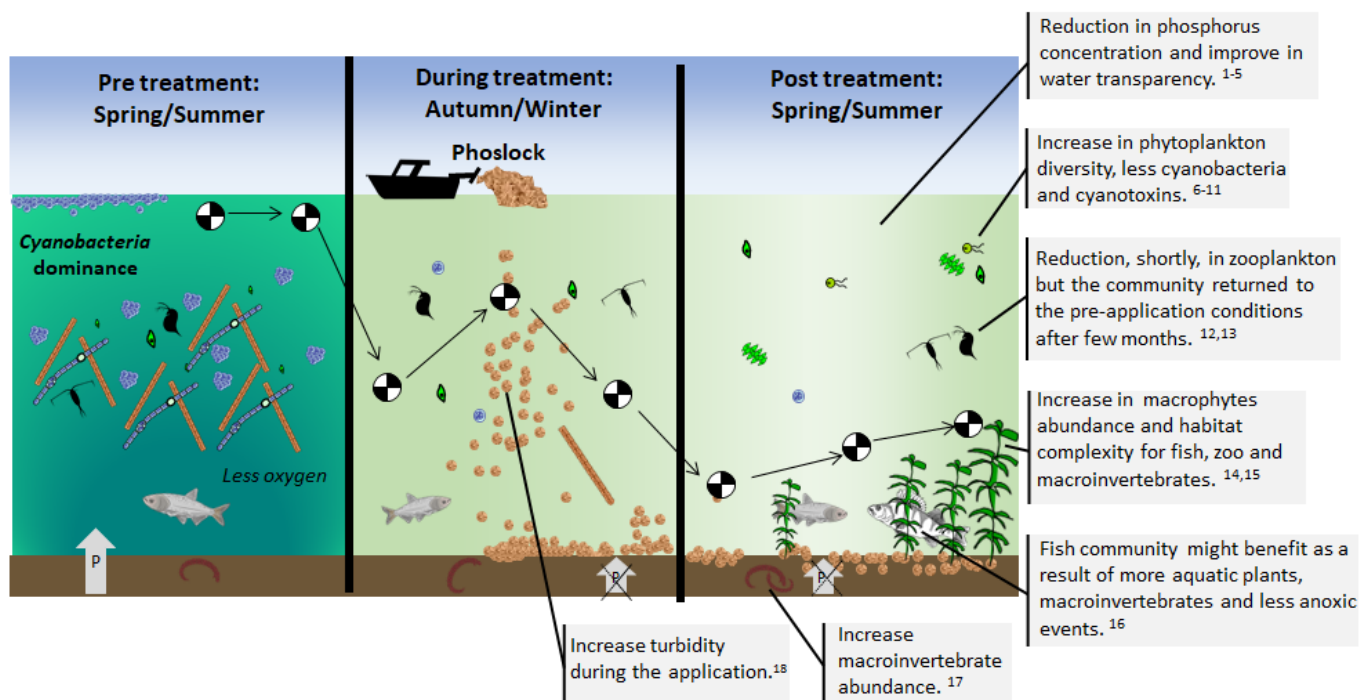
The expected shift after treatment towards more mesotrophic conditions, with less algal biomass, involves an increase in the algal biodiversity: less cyanobacteria and higher diversity of green algae and diatoms. This desired post-treatment effect is commonly observed after a strong P reduction. Changes in the trophic state of a lake will also affect other communities in the food chain.

For instance, one of the consequences of less phytoplankton is an increase in water transparency, which will positively affect macrophytes. Many field studies indeed show an increase in water transparency, followed by an increase in macrophytes after Phoslock® treatment (see figure below). A thin layer of Phoslock® on top of leaves after an application could have a potential impact on some macrophytes, however this is highly unlikely in the natural environment, where wind and waves will remove any Phoslock® from the leaves. Field studies have shown increased macrophyte coverage, accompanied by an increase in species number and colonization depths after Phoslock® treatment.

Toxicity studies undertaken on zooplankton (e.g., *Daphnia*) have shown that Phoslock® has no toxic effect when used at recommended dosages and under realistic conditions. The results also show a wide margin of safety, related to field dosages. Overall, field studies in which zooplankton surveys have been undertaken show a normal development of the zooplankton community after the application of Phoslock® (see figure below).

Likewise for tested macroinvertebrate species (e.g., amphipods and midge larvae) in toxicity studies, the application of realistic Phoslock® doses show a wide safety margin. Even at high test concentrations, no significant impacts have been observed in any of the tested species. This ensures that a considerable safety margin is maintained when Phoslock® is applied at the recommended dose rates.

Several studies have been conducted to investigate the impact of Phoslock® on community development of benthic macroinvertebrates. Some studies found no differences in macroinvertebrate communities between pre- and post-treatment. Other studies found a (short-term) effect on some macroinvertebrate groups. Changes in the macroinvertebrate community, however, can be attributed to the change in trophic state of the lake, as increased macrophyte abundance leads to increased habitat complexity, promoting macrofauna.



See Figure 4, page 23: from left to right (a) untreated system with cyanobacterial bloom in spring/summer (b) system undergoing Phoslock® treatment in autumn/winter and (c) treated system in following spring/summer.

The toxicity studies conducted on fish also show a wide margin of safety (at realistic Phoslock® doses) in the case of sentinel fish species. Based on these data, no risks to fish are expected with applications of Phoslock® at standard dose rates. The reported and expected changes in increased macrophyte coverage in the field and subsequent macroinvertebrate diversity can positively influence the fish stock, due to increased diversity in food sources, refuges and visibility.

For water-birds, exposure to Phoslock® is likely to be restricted to the consumption of, for example, macrophytes, invertebrates and fish. However, as shown in studies with humans and other mammals, it is most likely that the amount of La adsorbed by waterbirds will be very low as most of the ingested La will be excreted with the faeces. For this reason, it seems unlikely that waterbirds will suffer any toxicity caused by Phoslock® and no adverse effects on the resident avian fauna has been observed during the numerous field applications.

Pets such as cats and dogs could be exposed to Phoslock® treated water by swimming in or drinking treated water. Any risk from this exposure is evaluated from a range of data available for veterinary drugs and food additives using lanthanum carbonate. Based upon the daily oral intake concentrations of La in pet food and drugs and the peak concentrations of TLa after Phoslock® treatment, even the consumption of lake water directly after the application would not pose a risk to animal health.

Humans may be exposed to Phoslock® during recreational activities via dermal contact and oral uptake by swallowing water or by consuming fish from treated water bodies. A huge amount of data is available from mammalian toxicity studies with Phoslock® and long term clinical studies with lanthanum carbonate, an FDA approved drug ingredient that is dosed up to 3 g La/day over many years. These data show a wide margin of safety associated with Phoslock® applications at predicted dosages and environmentally relevant concentrations. Ingestion of Phoslock® treated water even directly after an application would not pose any risk to human health.

Conclusion

In summary, testresults from acute and chronic toxicity studies support the conclusion that the use of Phoslock® is safe for the aquatic environment. The low ecotoxicological profile of Phoslock® is further confirmed through several field studies in which extensive post-treatment monitoring programs have been conducted following whole lake applications. Ecosystem changes are to be expected as Phoslock® will minimize cyanobacterial (blue-green algae) nuisance by reducing the P bioavailability. In doing so, however, the base of the food chain is altered towards a more desirable (less nutrient rich) ecosystem. The use of Phoslock® as a tool for reducing eutrophication thus improves the trophic state of a waterbody, and thereby inducing a biological recovery process in formerly impaired aquatic systems.

Expected changes in the Kralingse Plas

For Kralingse Plas the calculated maximum dose of Phoslock® is within the range already applied in other systems. The application is recommended to be undertaken over several weeks, targeting different locations each day and the maximum total-La (TLa) concentration measured in the water column is expected to be similar to what has been measured in other aquatic systems (0.026 to 2.3 mg TLa/L). Based on the scientific literature discussed in this report and the Phoslock® dose calculated for Kralingse plas, a Phoslock® application to Kralingse Plas is not expected to result in any toxic effects in the aquatic organisms, nor any adverse effect on birds, pets and humans.

Samenvatting

Phoslock®

In veel zoetwatermeren is eutrofiëring (vermesting) gaande. Om de beschikbaarheid van een overmaat aan fosfor (P) in het oppervlaktewater van dergelijke meren te verminderen en de waterkwaliteit te herstellen, zijn er zogenoemde *geo-engineeringtools* ontwikkeld. Eén van deze gereedschappen is het toedienen van het product Phoslock®, waarmee de nalevering van P naar de waterlaag kan worden verminderd. De ontwikkeling van Phoslock®, het juiste gebruik ervan en de dosering zijn allemaal gebaseerd op gedegen wetenschappelijke uitgangspunten. Phoslock® bevat 95% bentonietklei en 5% lanthaan. Lanthaan (La) is een van nature voorkomend element, dat in kleine hoeveelheden aanwezig is in sedimenten van Europese wateren. In Phoslock® is La sterk gebonden aan de bentonietklei, waarbij het tevens zijn capaciteit behoudt om fosfaat te binden. Zodra Phoslock® wordt toegepast in zoet water, verspreidt het zich als kleine deeltjes, bindt het P tot het onoplosbare lanthaan-fosfaat, waarna het snel bezinkt op de bodem. Lanthaan komt bij het toedienen van Phoslock® niet als oplosbaar La in het water vrij.

De veiligheidsaspecten van Phoslock® hebben te maken met het actieve ingrediënt (La) en ook met de fysieke effecten. Fysieke effecten worden veroorzaakt door een tijdelijke toename van de troebelheid van het water tijdens de toediening. In dit literatuuroverzicht is de evaluatie van het veilige gebruik van Phoslock® dan ook gebaseerd op de (ecotoxicologische) beoordeling van blootgestelde organismen in het aquatische ecosysteem en de blootstelling aan dieren op het land inclusief de mens.

De effecten van La op aquatische organismen zijn getest in ecotoxicologische studies uitgevoerd met Phoslock® en oplosbare La-zouten (bv. LaCl_3). Aangezien oplosbare La-zouten andere chemische eigenschappen hebben dan Phoslock®, zijn studies uitgevoerd met deze La-zouten niet vergelijkbaar met Phoslock® toepassingen. Tevens zijn deze studies vaak uitgevoerd onder niet-representatieve omstandigheden (zoals het gebruik van fosfaatvrij medium). Resultaten uit dergelijke onderzoeken zijn daarom niet geschikt om de toxiciteit van Phoslock® te beoordelen. Alleen ecotoxicologische testen uitgevoerd met Phoslock® vormen daarom de basis van dit literatuuroverzicht.

Toxiciteit en biodiversiteitseffecten

Tijdens een toepassing van Phoslock® zal de hoeveelheid zwevende deeltjes korte tijd toenemen. Deze tijdelijke toename kan een korte-termijn-impact hebben op organismen. Wanneer Phoslock® eenmaal is toegediend, zal het snel bezinken op de bodem van het meer, waarbij het merendeel binnen één dag bezinkt. Mogelijke ongewenste neveneffecten kunnen gemakkelijk voorkomen worden door de toediening te verspreiden over meerdere dagen.

Wanneer Phoslock® is toegepast zal het sterk en onomkeerbaar P binden, waardoor er minder P beschikbaar zal zijn voor de groei van (blauw)algen. Waargenomen negatieve effecten op de algengroei na toediening zullen dus eerder het gevolg zijn van de beperking van fosfaat dan van directe toxische effecten. Onderzoeken aan algen na toepassing van Phoslock® in hele meren tonen aan dat er als gevolg van de behandeling geen onbedoelde effecten op de algen zijn.

De verwachte verschuiving na de behandeling van voedselrijke naar meer mesotrofe omstandigheden, met minder algenbiomassa, zal leiden tot een toename in algendiversiteit: minder cyanobacteriën (blauwalg) en een grotere diversiteit aan groene algen en diatomeeën. Dit gewenste effect wordt in het algemeen waargenomen na een sterke P-afname. Veranderingen in de voedselrijkdom van een meer zullen ook invloed hebben op andere gemeenschappen hoger in de voedselketen.

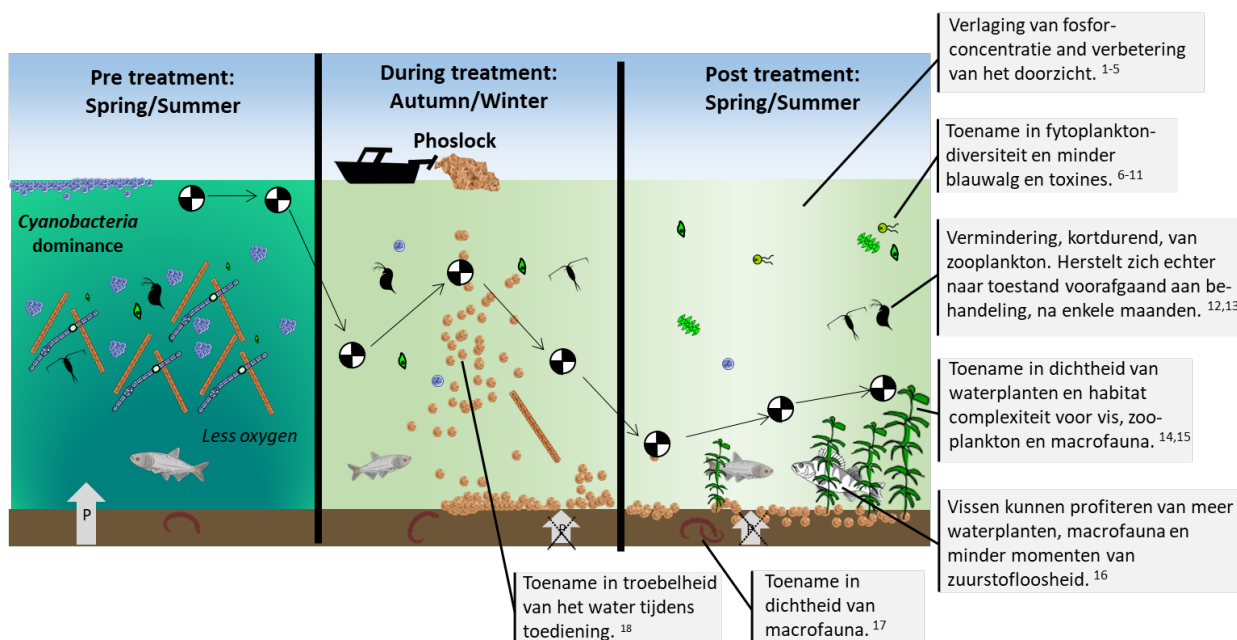
Een van de gevolgen van minder fytoplankton is bijvoorbeeld een toename van het doorzicht in het water, wat een positief effect zal hebben op de waterplanten. Uit vele veldstudies blijkt inderdaad dat na een behandeling met Phoslock® het doorzicht toeneemt, gevolgd door een toename van de waterplanten (zie figuur hieronder). Het bezinken van Phoslock® op bladeren zou sommige waterplanten enigszins negatief kunnen beïnvloeden, hoewel dit zeer onwaarschijnlijk is in een natuurlijk systeem, waar wind en golven de Phoslock® van de bladeren zullen afspoelen. Studies in het veld laten dan ook

zien dat er na de Phoslock® behandeling sprake is van een verhoogde bedekking met waterplanten, die gepaard gaat met een toename van het aantal soorten en de kolonisatiediepte.

Toxiciteitsstudies met zoöplankton (zoals *Daphnia*) tonen aan dat met aanbevolen doseringen en onder realistische omstandigheden Phoslock® geen toxische effecten veroorzaakt. De resultaten laten ook een (zeer) ruime veiligheidsmarge zien voor in het veld toegepaste doseringen. Over het algemeen bleek in veldstudies waarin zoöplankton werd onderzocht, dat na de toepassing van Phoslock® de zoöplanktongemeenschap zich normaal ontwikkelde (zie figuur hieronder).

Ook voor de in toxiciteitsstudies geteste macrofauna (zoals vlokreeften en muggenlarven) is er bij de toepassing van realistische Phoslock®-dosering sprake van een ruime veiligheidsmarge. Zelfs bij hoge testconcentraties werden geen significante effecten waargenomen bij de geteste soorten.

Verschillende studies zijn uitgevoerd om de impact van Phoslock® op de ontwikkeling van bentische macrofauna in de gemeenschap te onderzoeken. Sommige studies vonden geen verschillen in macrofauna-gemeenschappen voor en na de behandeling. Andere studies vonden een (korte termijn-) effect op sommige groepen. Veranderingen in de macrofaunagemeenschap kunnen worden toegeschreven aan de verandering van de voedselrijkdom van het meer, aangezien een toename aan waterplanten leidt tot een toename van de complexiteit van de habitat. Dit zal ook leiden tot een meer diverse macrofaunagemeenschap.



Zie Figuur 4, pagina 23: van links naar rechts (a) onbehandeld systeem met blauwalgenbloei in lente/zomer (b) systeem ondergaat Phoslock® behandeling in herfst/winter en (c) behandeld systeem in opvolgende lente/zomer.

De toxiciteitsstudies uitgevoerd met vissen laten eveneens een ruime veiligheidsmarge zien voor realistische Phoslock® doseringen. Op basis van deze studies worden geen risico's voor vissen verwacht bij toepassing van Phoslock® met standaard doseringen. De gerapporteerde en verwachte veranderingen in een toenemende bedekking aan waterplanten in het veld en de daaropvolgende macrofauna diversiteit kunnen een positieve invloed hebben op het visbestand. Er zal dan namelijk ook meer diversiteit zijn in voedselbronnen, schuilplaatsen en een beter doorzicht van het water.

Voor watervogels is de blootstelling aan Phoslock® waarschijnlijk beperkt tot de consumptie van o.a. waterplanten, ongewervelden en vissen. Echter, zoals aangetoond in studies met mensen en andere zoogdieren, is het zeer waarschijnlijk dat de hoeveelheid geabsorbeerd La door watervogels zeer laag zal zijn, aangezien het meeste ingenomen La met de uitwerpselen zal worden uitgescheiden. Om deze reden lijkt het onwaarschijnlijk dat watervogels toxische effecten zullen ondervinden van Phoslock®. Ook zijn er geen nadelige effecten op de aanwezige vogelfauna waargenomen tijdens de vele veldtoepassingen.

Huisdieren zoals katten en honden kunnen in aanraking komen met water behandeld met Phoslock® door erin te zwemmen of ervan te drinken. De risico's van deze blootstelling is geëvalueerd aan de hand van gegevens die beschikbaar zijn voor diergeneesmiddelen en voedseladditieven die gebruik maken van lanthaancarbonaat. Op basis van de dagelijkse orale inname van La in diervoeding en geneesmiddelen en de piekconcentraties van totaal-La (TLa) in water na Phoslock® behandeling, zou zelfs de consumptie van water direct na Phoslock® toediening geen risico vormen voor de gezondheid van de dieren.

Mensen kunnen blootgesteld worden aan Phoslock® tijdens recreatie in het water via huidcontact en orale opname door water in te slikken, of door het eten van vis uit behandelde wateren. Er is een enorme hoeveelheid gegevens beschikbaar uit toxiciteitsstudies met zoogdieren en Phoslock®, en klinische studies uitgevoerd met lanthaancarbonaat, een medicijn ingrediënt, gedoseerd tot 3 g/dag elementair La over vele jaren. Deze gegevens laten een brede veiligheidsmarge zien ten aanzien van Phoslock® toepassingen bij normaal gebruik en dosering, en milieurelevante condities. Inname van met Phoslock® behandeld water, zelfs direct na toediening, zal geen risico vormen voor de menselijke gezondheid.

Conclusie

Testresultaten uit acute en chronische toxiciteitsstudies ondersteunen de conclusie dat het gebruik van Phoslock® veilig is voor het aquatische milieu. Het lage ecotoxicologische profiel van Phoslock® wordt verder bevestigd door verschillende veldstudies waarbij een uitgebreid monitoringsprogramma werd uitgevoerd nadat het gehele watersysteem was behandeld. Omdat Phoslock® gericht is op het verminderen van de P-beschikbaarheid om blauwalgoverlast te minimaliseren, zijn veranderingen in het ecosysteem te verwachten. Hierdoor wordt de basis van de voedselketen veranderd naar een meer gewenst (minder nutriëntenrijk) ecosysteem. Het gebruik van Phoslock® als instrument voor het sterk verminderen van eutrofiëring verbetert dus de trofische toestand van een waterlichaam en brengt op deze manier een biologisch herstelproces op gang, van voorheen verstoorde aquatische systemen.

Te verwachte veranderingen in de Kralingse plas

Voor de Kralingse Plas ligt de berekende maximale Phoslock® dosis binnen de range van doseringen die reeds in andere systemen zijn toegepast. Het wordt aanbevolen om de toediening te verdelen over meerdere weken, gericht op verschillende locaties per dag, zodat de maximale gemeten TLa concentratie in het water naar verwachting vergelijkbaar zal zijn met wat is gemeten in andere aquatische systemen (0,026 tot 2,3 mg TLa/L). Op basis van de wetenschappelijke literatuur, besproken in dit rapport, en de Phoslock® dosis berekend voor de Kralingse Plas, worden er geen toxische effecten verwacht op de aanwezige aquatische organismen als gevolg van een Phoslock® toediening, noch enig nadelig effect op vogels, huisdieren en mensen.

Table of content

1. Introduction	1
1.1. Phoslock® and its working mechanism	1
1.2. What is lanthanum?.....	2
1.3. Toxicology - Definition of terminology	2
2. Toxicological assessment of Phoslock®	4
2.1. Ecotoxicological assessment - Aquatic biota.....	4
2.2. Exposure scenario - Waterbirds and terrestrial mammals (particularly pets).....	16
2.3. Toxicological assessment - Human exposure to Phoslock® treated water	17
3. Reality check	19
3.1. Kralingse Plas.....	19
3.2. Biological changes	20
3.3. Deferred eutrophication management.....	23
4. Conclusion	25
5. References.....	26
Appendix.....	32

1. Introduction

Phoslock® was developed in the nineties after rigorous scientific research undertaken by the Australia's Commonwealth Scientific and Industrial Research Organisation (CSIRO) in order to remove phosphorus (P) from water bodies and restore water quality in aquatic systems suffering from eutrophication (Douglas, 2002). Until 2003 Phoslock® was produced as a pre-commercial slurry formulation. A granular prototype of Phoslock® was developed in 2004 (Robb et al., 2003; Martin & Hickey 2004) while the current commercial form of the product has been produced since the end of 2006. To assess and manage any possible adverse side effects on the environment and to humans of this phosphorus remediation technique, it is crucial to understand the hazards and risks associated with its use. This report aims to outline existing information on the product and assess any risks it may pose to the aquatic environment and humans.

1.1. Phoslock® and its working mechanism

Phoslock® is a patented phosphorus locking technology containing 95% bentonite clay and 5% lanthanum (La) (Fig. 1). During the manufacturing process of Phoslock®, lanthanum cations are embedded inside the mineral layers of the bentonite clay, becoming the active ingredient. Due to the charge imbalance on the surface of the clay mineral layers, the embedded lanthanum is strongly associated with the bentonite clay and, at the same time, retains its capacity to bind phosphate (Fig. 2). Lanthanum is not released as soluble lanthanum into the water when Phoslock® is applied in aquatic environments. The lanthanum will either react with phosphate or other oxyanions (e.g. carbonate) in the water body, or it will remain associated with the clay structure under a wide range of environmental conditions (Douglas et al., 2000; Dithmer et al. 2015, 2016; Reitzel et al., 2017). Lanthanum reacts preferentially with phosphate due to the strong affinity of lanthanum with phosphate.



Figure 1. On the left, a picture of Phoslock® as a dry material and on the right Phoslock® being mixed with lake water and applied on the surface of Lake de Kuil (the Netherlands).

Once in contact with phosphate, the La in Phoslock® will chemically inactivate P through precipitation (Fig. 2). Furthermore, it will form a mineral of low solubility called rhabdophane ($\text{LaPO}_4 \cdot n \text{H}_2\text{O}$, $n \leq 3$) (Douglas et al., 2000; Slade and Gates, 1999), which over time will be transformed to monazite (LaPO_4), a mineral with even lower solubility (Dithmer et al., 2015). Phoslock® can bind phosphate under almost all redox conditions (e.g. Meis et al., 2012; Mucci et al., 2018; Noyma et al., 2016; Waajen et al., 2016), in saline waters (Mucci et al., 2020), under a wide pH range (6 to 9) (Mucci et al., 2018; Li et al., 2019), and in the presence of high concentrations of humic substances (Dithmer et al., 2016a; Li et al., 2020).

Once Phoslock® binds phosphate, the bound phosphate will not be released with redox changes, nor under natural variation in pH. Phosphate also remains bound during resuspension events (D'Haese et al., 2019; Douglas et al., 2016; Mucci et al., 2018). Theoretically, La-bound P will only be desorbed if pH drops below 4 or increases above 12 (Fig. 3A-Appendix). The P precipitated with Phoslock® will also not

be released in the presence of high amounts of DOC (Li et al., 2020). Therefore, phosphate bound to lanthanum in Phoslock® should be considered permanently unavailable for uptake by the aquatic organisms.

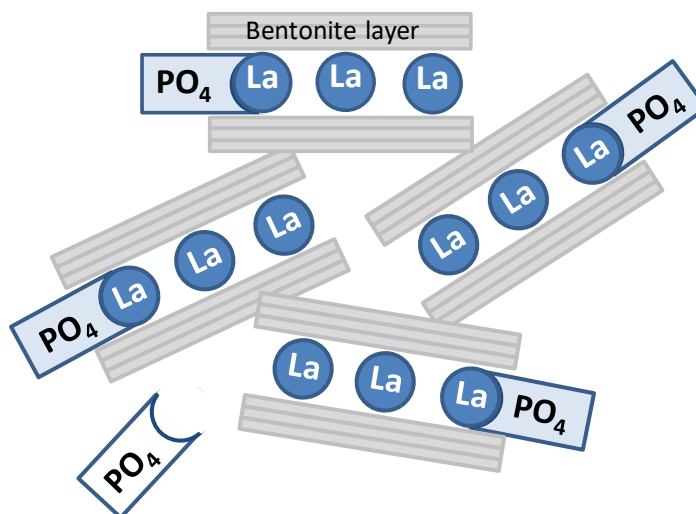


Figure 2. Scheme illustrating how the lanthanum embedded in the bentonite precipitated PO_4 without being washed out from the clay (Mucci et al., 2020).

1.2. What is lanthanum?

Lanthanum (La) is a naturally occurring element, classed in a group of elements commonly known as the rare earth elements (REEs). In the periodic table of elements, the REEs form a coherent chemical series from the atomic number 57 to 71 and include the elements yttrium (Y) and scandium (Sc). Despite the name, many REEs are more common than, for example, tin, copper and silver (Copetti et al., 2016). In the earth's upper crust, lanthanum is present at a concentration of about 30 ppm and is most commonly found in minerals such as bastnäsite and monazite (BGS, 2011). In its pure form, lanthanum is a rather soft, silvery-white metal, which tarnishes rapidly in air. The chemistry of La is primarily based on the formation of the +3 oxidation state. La forms soluble salts with chloride, nitrate and sulphate anions, whereas lanthanum carbonate, -phosphate and -oxide are highly insoluble compounds. La is also found in aquatic systems where Phoslock® has not been applied; for instance background concentrations between 8 ng/L and 8.7 $\mu\text{g/L}$ were reported from 18 groundwater and two surface water stations in the Netherlands and the reported background concentrations of lanthanum in European lake sediments range from 8 to 34 mg/kg dry weight (Cetiner et al., 2005; Meis et al., 2012; Nordberg et al., 2015; Yasseri & Epe, 2016).

1.3. Toxicology - Definition of terminology

Phoslock® is introduced into the environment as a geo-engineering tool, therefore it is essential to assess the potential risks associated with its use. Its development, appropriate use, and exact dose calculation are all based on solid scientific principles. Thus, any assessment of product safety should be described using the basic concepts of toxicological science. Before discussing the hazards and risks associated with the use of Phoslock®, the main terms commonly adopted in toxicology and used within this document will be introduced briefly:

Hazard and Risk

Hazard is defined as the inherent property of a substance to cause adverse effects under the conditions of exposure. In contrast, risk is the probability of occurrence of an adverse effect resulting from a given exposure to the substance.

Toxicological science

Toxicology is the study of how chemicals interfere with the normal function of a biological system. Accordingly, the sciences concerned with the effects of substances on ecological systems or aquatic systems are referred to as *ecotoxicology* and *aquatic toxicology*, respectively (Sullivan et al., 2005; Calow & Forbes 2014).

Dose descriptors

The key principles in toxicology are exposure, dose, and dose-response relationships. In general, a toxicological assessment of adverse effects is based on defined dose descriptors, which are determined in specific acute and chronic toxicity studies with various sentinel organisms. During these tests, the exposure effects of a given substance on the test organisms, such as death, impaired swimming ability, failure of eggs to hatch or the ability to reproduce are noted and the values for the dose descriptors are statistically derived from the test data. Commonly the following dose descriptors are used:

- LC50 dose (median lethal concentration, 50% of the test organisms die, endpoint death),
- EC50 dose (median effective concentration, 50% of the test organisms show an effect, effect endpoints vary e.g., immobilization, reduction of growth parameters),
- NOEC/NOAEL (no observed effect concentration; no observed adverse effect level)
- LOEC/LOAEL (lowest observed effect concentration; lowest observed adverse effect level)

2. Toxicological assessment of Phoslock®

The safe use of Phoslock® is related to its active ingredient lanthanum and, in the aquatic environment, also to physical effects caused by a temporary increase in turbidity in the water column (Spears et al. 2013a). Therefore the evaluation of the safe use of Phoslock® in this document is, based on:

1. The ecotoxicological assessment related to the specific features of the aquatic environment and biota to which it is introduced (chapter 2.1);
2. The evaluation of the exposure scenarios for terrestrial mammals and in particular pets (chapter 2.2);
3. The toxicological assessment related to human exposure to Phoslock® using the various services that aquatic ecosystems provide (chapter 2.3).

2.1. Ecotoxicological assessment - Aquatic biota

Phoslock® has been applied to both lentic and lotic waterbodies. Both types of aquatic systems consist of two distinct zones: a pelagic zone consisting of the open water column and a benthic zone consisting of the sediments and the bottom waters. The following section provides an assessment of the environmental safety of Phoslock® based on a dose descriptor approach and its effect on suspended solids, bioaccumulation and biodiversity in pelagic and benthic habitats.

2.1.1. Comparison of Dose Descriptors for a variety of aquatic biota- Laboratory studies

Ecotoxicological laboratory studies on pelagic and benthic organisms have been conducted with both Phoslock® and soluble lanthanum salts (Copetti et al. 2016). These different stressors have produced a high variability in the determined dose descriptors due to the higher toxicological effects caused by the different chemical properties of soluble lanthanum salts compared to Phoslock®. Ecotoxicological assays for Phoslock® should reflect conditions which are representative of an application scenario and therefore tests conducted with Phoslock® itself and not La salts should form the basis of any ecotoxicity assessment. As a result, this approach has been adopted in this document. This approach is outlined in detail in box 1A of the appendix.

Once Phoslock® is applied to freshwaters, it disperses into small particles. Lanthanum in the water column is measured analytically as either total lanthanum (TLa) or filterable lanthanum (FLa). Reitzel et al. (2017) found that Phoslock® disperses to the greatest degree in soft water lakes, producing fine clay particles with embedded La which pass through various filters with different pore sizes. In the same study, the authors also measured FLa after 30 min ultracentrifugation, however fine colloids were still detected in the filtrate, demonstrating that the measured concentrations were not truly dissolved lanthanum. As a result, the authors concluded that FLa measured after ultracentrifugation could only be considered dissolved in low alkalinity water with very low phosphate and high dissolved organic carbon (DOC) concentrations. Yet, even here, the authors suggested that the measured FLa concentrations are probably associated with humic substances and not present as La^{3+} ions (Reitzel et al., 2017).

Thus, TLa and FLa are an appropriate measure to estimate Phoslock® in the water column and assess its impact on the aquatic biota. The reported peak concentrations and the post-application range for TLa and FLa from 16 Phoslock® treated lakes are summarized in Table 1 (Spears et al. 2013a).

The following section compares the dose descriptor values from tests for a range of aquatic biota with the reported peak TLa concentration in the water after applying Phoslock®. We have focussed on total lanthanum concentrations as filterable lanthanum concentrations are low after a Phoslock® application (for more detail about FLa in Kralingse Plas, see Paragraph 3.1). The Netherlands is the only country in the world that has legislation relating to maximum permissible La concentrations (MPC) (Sneller et al., 2000) for surface water (10.1 µg FLa/L and 150 µg TLa/L) and for freshwater sediment (4700 mg TLa/ kg DW sediment). The MPC for La in surface water has been criticized in the scientific literature due to a number of methodological flaws (van Oosterhout et al., 2020). In addition, no experimental ecotoxicological tests with organisms were undertaken for the sediment data (Sneller et al., 2000).

Thus, in this report, we will discuss the effects of exposure to Phoslock® derived lanthanum based on a more recent set of ecotoxicological experiments conducted with different species in the water column and sediment (i.e., Table 2 to 7).

Table 1. Reported peak concentrations and post-application range (up to 60 months) for total lanthanum (TLa) and filterable lanthanum (FLa) in different Phoslock® applications (Spears et al. 2013a).

Location	Timing	Peak TLa [mg/L]	Peak FLa [mg/L]
Water sampled from the lake surface	during and 1 month after application	0.026-2.3	0.002-0.414
Water sampled from bottom of the lake	during and 1 month after application	0.004-0.892	0.002-0.1

Surface-Bottom post application range (up to 60 months)	Range TLa [mg/L]	Range FLa [mg/L]
Surface	<0.001-0.031*	<0.001-0.002
Bottom	<0.001-0.068*	<0.001

* 0.031 and 0.068 mg/L were measured 6 months post-application.

Bacteria

The EC50 values for the marine gram-negative bacterium *Vibrio fischeri* and nitrification rate in the sediment are 2173 and 15 times higher than the maximum reported concentration of TLa in lake water, respectively (Table 2). Thus, there are no toxic or direct impacts of Phoslock® on bacterial processes such as nitrification or the bacterial cell itself (Henriksen and Roslev, 2009 Garcia-Ordiales et al., 2019; Verweij et al., 2010). These results are also confirmed by a field survey undertaken in the Canning River after an application of Phoslock®. Sampling of both total heterotrophic bacteria and denitrifying bacteria showed no negative impact on the microbial communities in both the water and sediment, respectively (River Science 17, 2001).

Table 2. Ecotoxicity studies with Phoslock® and bacterial activity or species. EC50 values (mg of Phoslock®/L) and the corresponding TLa concentrations.

Test organisms/process	Test conditions	Endpoint	Duration	EC50 Phoslock [mg/L]	Corresp. TLa [mg/L]	References
<i>Nitrification rate</i>	Phoslock® suspens. natural pond water	NO ₂ and NO ₃	48h	686	34.3	Henriksen and Roslev, 2009
<i>Vibrio fischeri</i>	Phoslock® suspens. natural pond water	Light emission	30min	<25000	<5000	Henriksen and Roslev, 2009

Phytoplankton

It is important not to confuse indirect and direct effects when testing the toxicity of La to algae species. As La has a high affinity for phosphate, it binds quickly to any phosphate in solution, forming insoluble lanthanum phosphate, which precipitates in the culture medium. Thus, an observed adverse effect on algal growth could be due to phosphate limitation rather than direct toxic effects on the test species. This was demonstrated in a study by Petersen et al. (1974), who tested the effect of phosphate limitation in experiments conducted with the soluble lanthanum chloride (LaCl_3) salt and two green algae species (*Raphidocelis subcapitata*, formerly *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*). No significant toxic effects up to a nominal concentration of 2 mg LaCl_3/L were observed (as a soluble salt was used this corresponds to 1.1 mg ionic La).

However, tests using soluble La compounds do not reflect the unique properties of Phoslock®, which is specially designed to not release soluble La forms in the water where it is applied. As results from toxicity testing of soluble La salts overestimate the adverse effects of Phoslock® derived La, these tests are inappropriate to assess Phoslock® effect on freshwater algae and therefore are not reported in this document.

In a laboratory test with a Phoslock® suspension under constant agitation, van Oosterhout & Lürling (2013) report reduced growth of two phytoplankton species (*Scenedesmus obliquus*; *Microcystis aeruginosa*) as well as effective control of a cyanobacteria bloom (*Anabaena* sp.). The control effect is attributed to the combination of phosphate binding, light limitation and flocculation of phytoplankton cells under conditions of prolonged exposure in the experiment rather than any toxic effect. In field conditions, the effect of light limitation and flocculation is expected to be less pronounced due to the rapid settling of the Phoslock® particles. This is also confirmed by a range of data from field applications analysing the algae assemblages after a Phoslock® treatment. Several studies found a shift in the phytoplankton community structure from cyanobacteria dominance to green algae and diatoms (Robb et al., 2003; Bishop et al., 2014; Bishop & Richardson 2018; Epe, 2014; Barçante et al., 2020). Phytoplankton surveys after whole lake applications of Phoslock® demonstrate that there are no non-target impacts on phytoplankton as a result of a treatment (Robb et al., 2003; Bishop et al., 2014; Bishop & Richardson 2018; Epe, 2014; Barçante et al., 2020).

Zooplankton

Results from nine acute and chronic toxicity studies using two different water fleas species and a rotifer species are summarised in Table 3. The responses of these zooplankton species, including mortality, reproduction and growth, were evaluated throughout the Phoslock® exposures.

The results show that for *Daphnia magna*, even when a concentration up to 10 times higher than the reported peak concentration of TLa ever found in a Phoslock® application is used, no adverse effect was observed (Lürling & Tolman 2010). In two other experiments using the same species (*D. magna*) similar results were observed, the NOEC was up to 500 (Martin and Hickey, 2004), and the EC50 between 100 (Watson and Leung, 2009) and >1087 (Martin and Hickey, 2004) times higher than the reported peak concentration of TLa ever found in a Phoslock® application (Spears et al. 2013a). Thus, the values show a wide margin of safety associated with Phoslock® applications at predicted use rates and environmentally relevant concentrations (cf. highest TLa reported during a Phoslock® application was 2.3 mg La /L, Spears et al. 2013a).

Besides *D. magna*, the rotifer, *Brachionus calyciflorus* was evaluated in the presence of Phoslock®, showing that the population growth did not change even when double the maximum TLa concentration reported in a Phoslock® application was used. Overall, this indicates that there is a wide safety margin when Phoslock® is applied under realistic conditions.

During the early development phase of Phoslock® acute and chronic toxicity tests were conducted with *Ceriodaphnia dubia* using water samples from the Canning River after a Phoslock® treatment (Stauber & Binet, 2000). For this study two early formulations of Phoslock® were compared. It was demonstrated that modifications to the formulation to reduce the lanthanum release in surface water

resulted in the absence of toxicity. In this context it is important to point out that due to the amount of Phoslock® needed to impact biota, some studies did not use concentrations high enough to result in significant impacts. Thus, the respective toxicity endpoints in Table 3 are reported as being greater than the highest concentration tested and do not indicate the actual lowest observed effect levels. This was the case for the tests using the *Ceriodaphnia dubia* and therefore the EC50 values are not realistic and do not represent the actual Phoslock® concentration, which might influence the population.

Table 3. Ecotoxicity studies with Phoslock® and zooplankton species. EC50 values and corresponding TLa concentrations. Table continues on next page.

Test organisms	Test conditions	Endpoint	Duration	NOEC Phoslock [mg/L]	EC50 Phoslock [mg/L]	Corresp. TLa [mg/L]	References
Zooplankton							
<i>Daphnia magna</i>	Phoslock® Eluate, synth. soft water ²⁾	Mortality	48 h	25000	> 50000 ¹⁾	1250; >2500	Martin & Hickey 2004
<i>Daphnia magna</i>	Phoslock® suspens. dechlor. tap water	Mortality	48 h	-	4900	245	Watson-Leung 2009
<i>Daphnia magna</i>	Phoslock® suspens. natural pond water	Mortality	48 h	-	>6800 ³⁾	>340	Watson-Leung 2009
<i>Ceriodaphnia dubia</i>	Phoslock® suspens. natural lake water	Mortality	48 h	50	> 50 ¹⁾	2.5; >2.5	ECOTOX 2008
<i>Ceriodaphnia dubia</i>	River water after Phoslock® treatment	Immobility	48 h	n.s.	n.s.	>1.7 ¹⁾	Stauber & Binet 2000
<i>Brachionus calyciflorus</i>	Phoslock® suspens. WC medium	Popul. growth	48 h	100	154	5; 7.7	van Oosterhout & Lurling 2013
<i>Daphnia magna</i>	Phoslock® suspens., artificial RT medium	Weight/Length	5 d	100-500	871-1557	5-25; 43.55-77.85	Lürling & Tolman 2010

Test organisms	Test conditions	Endpoint	Duration	NOEC Phoslock [mg/L]	EC50 Phoslock [mg/L]	Corresp. TL _a [mg/L]	References
<i>Ceriodaphnia dubia</i>	Phoslock® suspens. natural lake water	Mortality	7 d	1	> 1 ¹⁾	0.05; >0.05	ECOTOX 2008
<i>Ceriodaphnia dubia</i>	Phoslock® suspens. natural lake water	Reproduction	7 d	1	> 1 ¹⁾	0.05; >0.05	ECOTOX 2008
<i>Ceriodaphnia dubia</i>	River water after Phoslock® treatment	Survival/Reproduction	7d	n.s.	n.s.	>1.7 ¹⁾	Stauber& Binet 2000

¹⁾ Due to the amount of Phoslock® needed to see impacts on biota, these studies did not test concentrations high enough to result in significant results; thus toxicity endpoints are reported as greater than the highest concentration tested and do not indicate the actual EC50 values. ²⁾ Although tests using a Phoslock® suspension is the method of choice, results from eluate tests are included for comparison. ³⁾ not high enough in the highest concentration, only 42 % of mortality.

Macrophytes

No research has precisely calculated EC50 or NOEC values for Phoslock® on macrophytes. However, laboratory experiments have shown that Phoslock® does not reduce growth in *Elodea nuttallii* and *Hydrilla verticillata* (Chrzanowski, 2012; Wang et al., 2017) and does not hamper macrophyte germination when dosing up to 5 tons per ha (Waters-Hart, 2020) (Table 4). In another experiment, growth and responses of five other macrophyte species (i.e., *Potamogeton perfoliatus*, *Myriophyllum spicatum*, *Elodea canadensis*, *Littorella uniflora*, and *Najas flexilis*) were similar between the Phoslock® treatment and the control (Waters-Hart, 2020). This study also included an additional laboratory core experiment to test the impact of a Phoslock® treatment on submerged macrophyte leaves using the four macrophyte species *Potamogeton perfoliatus*, *Myriophyllum spicatum*, *Elodea canadensis* and *Najas flexilis*. The results show evidence of Phoslock® on the leaf surfaces (which was also confirmed through scanning electron microscope (SEM) imagery with X-ray analysis). However, the only species to respond negatively to the treatment in terms of its fluorescence (lower Fv/Fm values) was *Potamogeton perfoliatus*. Although this species showed a stressed state after exposure to Phoslock®, factors that can dislodge the product from the leaf surface in natural conditions (e.g. wind, waves, fish and birds) were not present in the core experiment and need further investigation (Waters-Hart 2020). Nevertheless, the results from whole lake applications mentioned above show positive responses of the macrophyte community after a Phoslock® treatment and it can therefore be reasonably assumed that, under natural environmental conditions, negative effects on macrophytes will only be marginal as there is no indication for toxicity of Phoslock® to macrophytes.

Table 4. Ecotoxicity studies with Phoslock® and macrophyte species.

Test organisms	Test conditions	Duration	Dose used Phoslock [g/m ²]	Corresp. TLa [g/m ²]	Response	References
Macrophytes						
<i>Elodea nuttallii</i>	Phoslock® suspens. Synth. water	28d	4968.1	248.6	Plant biomass, shoot and root dry weight was not affected by Phoslock	Chrzanowski, 2012
<i>Hydrilla verticillata</i>	Phoslock® suspens. Filtered lake water	30d	Up to 20% of the sediment weight used	unknown	In a low dose (2.5% of the sediment) the biomass decrease 20%. Higher dose up to 25 % of the sediment, the biomass was not affected.	Wang et al., 2017

Macroinvertebrates

Results from toxicity studies using the freshwater shrimp *Macrobrachium* sp., the crayfish *Procambarus fallax f. virginalis* and sediment-dwelling invertebrates are given in Tables 5 and 6. The results indicated that the NOEC values for the decapod species were ten to thousand (*Macrobrachium* sp.) and two hundred (*P. fallax f. virginalis*) times higher than the reported peak concentration of TLa measured during a Phoslock® application (Spears et al., 2013a). Thus, there is a wide margin of safety (10 to 1087 times) associated with Phoslock® applications at predicted dose rates (c.f. Spears et al., 2013a).

For the studies conducted with a range of sediment-dwelling organisms, different concentrations of Phoslock® suspensions were used. The concentration of Phoslock® near the benthic habitats is expected to be higher than in the overlying water. Nonetheless, the comparison of the corresponding TLa values derived from the Phoslock® exposure dose shows a wide safety margin, both in terms of the peak concentrations for TLa in lake bottom water (Table 1) and the dose descriptor values for the tested species (Table 6). At high test concentrations (>170 mg TLa/L), no significant survival or growth impacts were observed in any of the sediment toxicity test species (Table 6). *Chironomus* sp., for instance, was not affected in the presence of 10000 mg Phoslock/L (Fig. 4A-Appendix). This ensures that a considerable safety margin is maintained when Phoslock® is applied at the recommended dose rates. As was the case previously, the amount of Phoslock® needed to result in impacts to the test macroinvertebrates species was not high enough to result in significant impacts. Thus, the respective toxicity endpoints are reported as greater than the highest concentration tested and do not indicate the actual lowest observed effect levels.

Table 5. Ecotoxicity studies with Phoslock® and juvenile freshwater decapod species. EC50 values and corresponding TLa concentrations.

Test organisms	Test conditions	Endpoint	Duration	NOEC Phoslock [mg/L]	EC50 Phoslock [mg/L]	Corresp. TLa [mg/L]	References
Juvenile Decapods							
Freshwater shrimp:							
<i>Macrobrachium</i> sp. juvenile	Phoslock® Eluate, synth. soft water ²⁾	Survival	96 h	50000	> 50000 ¹⁾	2500; >2500	Ecotox 2006
<i>Macrobrachium</i> sp. juvenile	Phoslock® Eluate, synth. soft water ²⁾	Survival	7 d	400	> 800	20; 40	Ecotox 2006
<i>Macrobrachium</i> sp. juvenile	Phoslock® Eluate, synth. soft water ²⁾	Survival	14 d	400	800	20; 40	Ecotox 2006
<i>Macrobrachium</i> sp. juvenile	Phoslock® Eluate, synth. soft water ²⁾	Survival	21 d	400	700	20; 35	Ecotox 2006
<i>Macrobrachium</i> sp. juvenile	Phoslock® Eluate, synth. soft water ²⁾	Survival	28 d	400	700	20; 35	Ecotox 2006
Crayfish:							
<i>Procambarus fallax</i> f. <i>virginalis</i>	Phoslock® suspens. Copper free tap water	Survival	96 h	10000	> 10000 ¹⁾	500; >500	Lürling unpublished data; Fasola 2010
<i>Procambarus fallax</i> f. <i>virginalis</i>	Phoslock® suspens. Copper free tap water	Growth	28 d	10000	> 10000 ¹⁾	500; >500	Lürling unpublished data; Fasola 2010

¹⁾ Due to the amount of Phoslock® needed to see impacts to biota, these studies did not test concentrations high enough to result in significant results, thus toxicity endpoints are reported as greater than the highest concentration tested and do not indicate actual EC50 values. ²⁾ Although tests using a Phoslock® suspension is the method of choice, results from eluate tests are included for comparison and due to lack of data from studies with Phoslock® suspension and shrimp.

Table 6. Ecotoxicity studies with Phoslock® and sentinel benthic macroinvertebrate species. EC50 values and corresponding TLa concentrations.

Test organisms	Test conditions	Endpoint	Duration	NOEC Phoslock [mg/L]	EC50 Phoslock [mg/L]	Corresp. TLa [mg/L]	References
Benthic Macroinvertebrates							
Amphipode:							
<i>Hyalella azteca</i>	Phoslock® suspens. natural pond water	Survival	14 d	3400	> 3400 ¹⁾	170; > 170	Watson-Leung 2009
<i>Hyalella azteca</i>	Phoslock® suspens. natural pond water	Growth	14 d	3400	> 3400 ¹⁾	170; > 170	Watson-Leung 2009
Mayfly:							
<i>Hexagenia</i> sp.	Phoslock® suspens. natural pond water	Survival	21 d	450	> 450 ¹⁾	22.5; > 22.5	Watson-Leung 2009
<i>Hexagenia</i> sp.	Phoslock® suspens. natural pond water	Growth	21 d	450	> 450 ¹⁾	22.5 > 22.5	Watson-Leung 2009
Midge larvae:							
<i>Chironomus dilutus</i>	Phoslock® suspens. natural pond water	Survival	10 d	3400	> 3400 ¹⁾	170; >170	Watson-Leung 2009
<i>Chironomus dilutus</i>	Phoslock® suspens. natural pond water	Growth	10 d	3400	> 3400 ¹⁾	170; >170	Watson-Leung 2009
<i>Chironomus</i> sp.	Phoslock® suspens.	Survival	7d	10000	>10000	500; >500	Lürling, unpublished data
<i>Chironomus zealandicus</i>	Phoslock® suspens. natural lake water	Survival	38 d	400	> 400 ¹⁾	20; > 20	Clearwater 2004

¹⁾ Due to the amount of Phoslock® needed to see impacts to biota, these studies did not test concentrations high enough to result in significant results, thus toxicity endpoints are reported as greater than the highest concentration tested and do not indicate the actual EC50 values. ²⁾ Although tests using a Phoslock® suspension is the method of choice, results from eluate tests are included for comparison

Fish

Results from five acute toxicity studies using sentinel fish species are outlined in Table 7. A Phoslock® suspension of granules, Phoslock® treated river water and a Phoslock® eluate were used in these studies. As was the case with the zooplankton and macroinvertebrates species, a wide margin of safety was associated with Phoslock® applications at predicted use rates and environmentally relevant concentrations (cf. highest TLa reported during Phoslock® application was 2.3 mg La /L, Spears et al. 2013a). For instance, even when a concentration up to 60 times higher than the reported peak concentration of TLa ever found in a Phoslock® application is used, no adverse effect was observed in fish eggs as well as in the other fish species tested (Table 7).

Based on these data, risks to fish are expected to be minimal with applications of Phoslock® at standard dose rates (cf. Spears et al. 2013a). Again, it is essential to mention that in two studies, the amount of Phoslock® used in the tests was not high enough to result in significant impacts on the fish, and an effective EC50 concentration could not be determined. Thus, the respective toxicity endpoints in Table 3 are reported as greater than the highest concentration tested.

Table 7. Ecotoxicity studies with Phoslock® and sentinel fish species. EC50 values and corresponding TLa concentrations. n.s. = not specified.

Test organisms	Test conditions	Endpoint	Duration	NOEC Phoslock [mg/L]	EC50 Phoslock [mg/L]	Corresp. TLa [mg/L]	References
Fish							
<i>Fish egg</i>	Phoslock® granules, tap water	Coagulated eggs/heart-beat/anatomy /mortality	48h	-	3000	150	Yasseri, 2008
<i>Melanotaenia splendida</i>	Phoslock® Eluate, synth. soft water ²⁾	Mortality	96 h	50000	> 50000 ¹⁾	2500; >2500	Ecotox 2006
<i>Melanotaenia duboulayi</i>	River water after Phoslock® treatment	Immobility	96h	n.s.	n.s.	>1.7 ¹⁾	Stauber& Binet 2000
<i>Oncorhynchus mykiss</i>	Phoslock® Eluate, synth. soft water ²⁾	Mortality	96 h	<3125	4350	156.3; 217.5	Martin & Hickey 2004
<i>Oncorhynchus mykiss</i>	Phoslock® suspens., natural pond water	Mortality	48 h	-	> 13600 ¹⁾	>680	Watson-Leung 2009

¹⁾ Due to the amount of Phoslock® needed to see impacts to biota, these studies did not test concentrations high enough to result in significant results, thus toxicity endpoints are reported as greater than the highest concentration tested and do not indicate actual EC50 values. ²⁾ Although tests using a Phoslock® suspension is the method of choice, results from eluate tests are included for the purpose of comparison.

Summary

In conclusion, by comparing NOEC and EC50 concentrations for various aquatic organisms with the reported TLa concentrations in lake water after an application of Phoslock®, it is apparent that even the highest TLa concentrations do not exceed the EC50 values of the various pelagic and benthic test organisms (Fig. 3). In the four studies conducted with the cladoceran species *Ceriodaphnia dubia* the respective Phoslock® concentrations used in the tests (1 mg/L and 50 mg/L, respectively) were too low to produce significant impacts to the test species. For this reason, an effective EC50 concentration could not be determined. The comparison of the reported values with other studies conducted with cladocerans indicates that higher effective EC50 concentrations can be expected, and, as a result, a sufficient safety margin could be assumed for *Ceriodaphnia dubia*. Furthermore, even though some of the studies do not report a NOEC for the Phoslock® exposure dose, the resulting EC50 concentrations are at least one order of magnitude (from 3 to 21739) higher than the measured peak TLa concentrations in lake water. Hence, based on the reported dose descriptor values for a representative range of aquatic organisms, the risk for aquatic life associated with the use of Phoslock® can be rated as low.

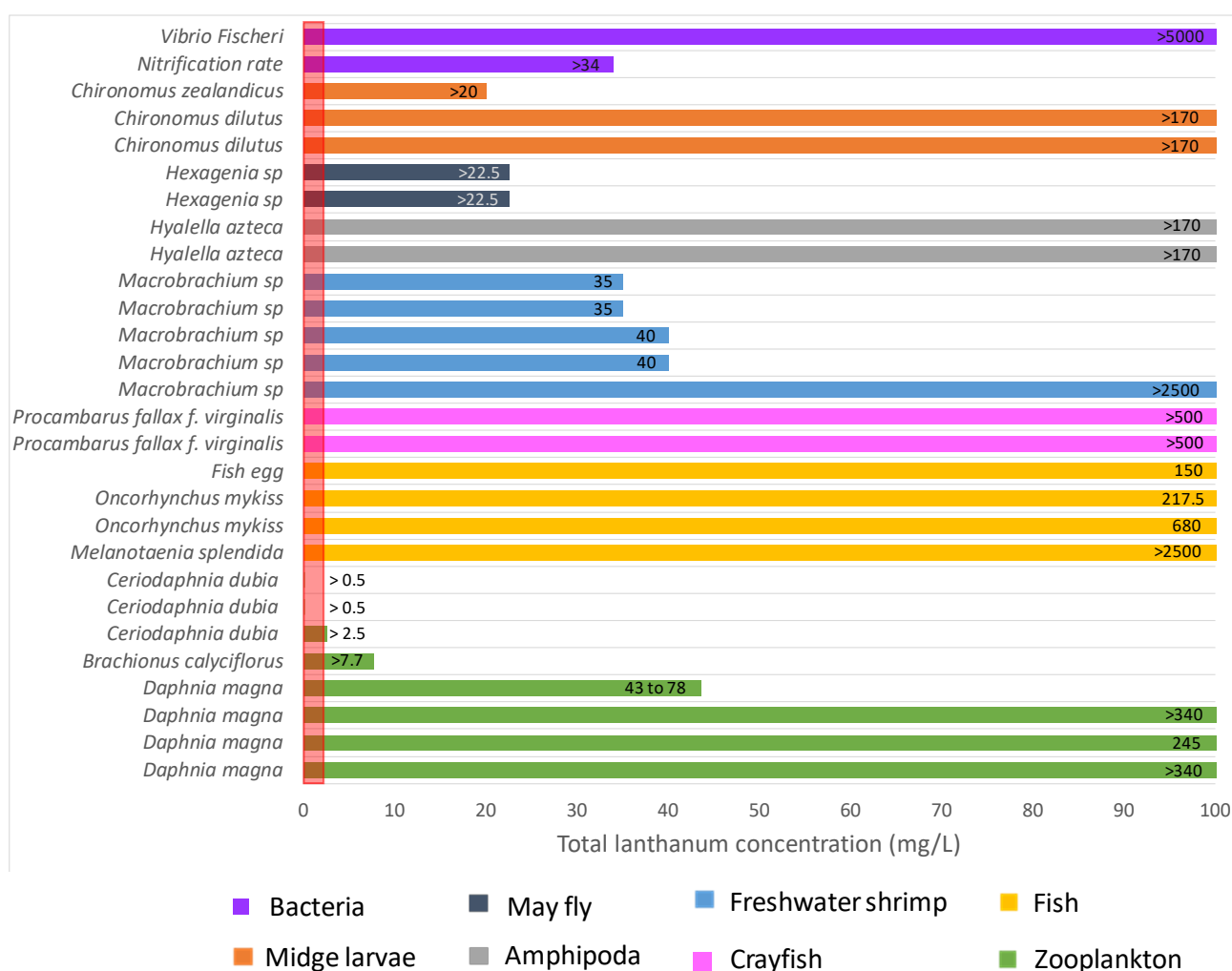


Figure 3. EC50 values indicated by numbers of lanthanum concentration (mg/L) for several aquatic organisms from different taxonomic groups (data from tables 2, 3, 4, 5, 6 and 7). The red vertical bar represents the maximum total lanthanum concentration measured during a Phoslock® application (2.3 mg T La/ L) (Spears et al., 2013a).

2.1.2. Suspended solids

The estimated range of suspended solids during an application of Phoslock® is reported to be 0.62-46.0 mg/L. This temporary increase of suspended solids during the application of Phoslock® might exert a short-term impact, especially on pelagic organisms. However, once Phoslock® is applied, it will quickly

settle to the bottom of the lake. For instance, in Lake de Kuil (the Netherlands) 58% of the Phoslock® added settled within one day. After one week and four weeks, most of the Phoslock® had settled (88% and 94% respectively) (Mucci et al., in review). Similar TLa reduction over time was observed in lake Het Groene Eiland (The Netherlands) (Lürling & van Oosterhout 2013).

Any potential effects from a temporary increase in suspended solids can easily be prevented in sensitive environments by adopting a targeted and site-specific approach, by controlling the quantity of Phoslock® applied daily, by tailoring the application strategy to each site and by avoiding applications during the growing season (Spears et al., 2013a). Application strategies should be defined once the final dose for each lake area is decided.

2.1.3. Bioaccumulation

After applications of Phoslock®, lanthanum has been detected in macrophytes, chironomids, crayfish and fish, indicating the potential for bioaccumulation of Phoslock® derived lanthanum (van Oosterhout et al., 2020; van Oosterhout et al., 2014; Waajen et al., 2017b). However, no acute or chronic effects on the studied aquatic biota have been reported and no ecotoxicological effects have been observed for up to five years after Phoslock® applications. Furthermore, lanthanum was mainly detected in the liver and hepatopancreas tissues of fish and aquatic invertebrates and the decreasing concentration patterns over time show depuration of lanthanum from the body of the aquatic biota (Landman et al., 2007; Waajen et al. 2017b).

2.1.4. Possible changes in biodiversity - Field observations

Phoslock® is used to reduce excess phosphorus in water bodies suffering from anthropogenic eutrophication in order to mitigate against the negative effects caused by cyanobacterial blooms. As a result, several studies show that the shift in the trophic state of a waterbody towards more mesotrophic conditions leads to an increase in the biodiversity after an application of Phoslock® (e.g., Barçante et al., 2020; Bishop et al., 2014; Bishop and Richardson, 2018; Epe, 2014; Robb et al., 2003).

Phytoplankton

A Phoslock® application often leads to a shift in the phytoplankton community structure from bloom-forming cyanobacteria dominance to much lower biomass of non-toxic green algae and diatoms. This reduced biomass and the increase of phytoplankton biodiversity is a desired post-treatment target and a commonly observed pattern after phosphorus reduction (Bishop et al. 2014; Schindler et al., 2016; Bishop & Richardson 2018).

Lang et al. (2016) have reported results from a field trial, documenting responses in phytoplankton community structure and abundance in Loch Flemington (UK). In this study, a Before-After-Control-Impact (BACI) analysis was used to distinguish natural variability from treatment effect and confirmed significant reductions in the magnitude of summer cyanobacterial blooms in Loch Flemington. Over the monitoring period, the phytoplankton community structure became less dominated by cyanobacteria and more functionally diverse. This resulted in continual improvements in the phytoplankton compositional and abundance metrics, which were not observed at the control site. However, this initial cyanobacterial response was not sustained beyond two years after application indicating that the applied quantity of Phoslock was insufficient to control internal P loading for longer than two years. The authors of this study emphasize that the effective control of internal P loading in Loch Flemington will require further implementation of both in-lake and catchment-based measures.

Zooplankton

Field studies in which zooplankton surveys have been undertaken show normal development of the zooplankton community after the application of Phoslock®. However, in Lake Rauwbraken (The Netherlands) a temporary disappearance of the water flea *Daphnia galeata* was observed after the combined treatment of the flocculant poly-aluminiumchloride (PAC) and Phoslock® (Floc & Lock method).

Van Oosterhout & Lürling (2011) hypothesized that the reduction was likely caused by the physical effects of the aluminium-hydroxide flocs, grazing inhibition by flocs and suspended bentonite particles, low food concentrations and the absence of predation refuge due to high water transparency and not by toxic effects of La from Phoslock®. The *Daphnia galeata* population recovered three months after the Floc & Lock treatment (van Oosterhout & Lürling, 2011). In another Dutch lake (Lake de Kuil), the zooplankton community developed normally after Phoslock® treatment (Waajen et al., 2016a). Also, Meis (2012) did not find any changes in the abundance of individuals groups after a Phoslock® application in Loch Flemington (UK).

Macrophytes

In many eutrophic freshwaters, submerged macrophyte beds disappear due to the shift of the waterbody from a clearwater state with macrophyte dominance to a phytoplankton dominated turbid state (Scheffer & van Nes, 2007). Thus, mitigation of eutrophication should not only improve the chemical water quality but also promote the re-establishment of submerged macrophyte vegetation (Waajen et al., 2016a). Aquatic macrophyte community metrics after Phoslock® applications have been reported for several lakes in Europe, USA, and Australia (Bishop et al., 2014; Gunn et al., 2014; Novak & Chambers, 2014; Spears et al., 2016). In Lake De Kuil and Lake Rauwbraken (the Netherlands), macrophytes flourished and colonisation depth increased after interventions with Phoslock® (Waajen et al., 2016a; van Oosterhout et al., in revision). In Phoslock® treated compartments in ponds in Dongen and Eindhoven, macrophytes also grew to very high biomass and coverage (Waajen et al., 2016b). In addition, in two other Dutch lakes (Groote Melanen and Kleine Melanen) after a Phoslock® application, submerged and floating macrophytes grew to higher biomass compared to previous years (Aquon, 2019a, 2019b, 2016). Also, in lake Eichbaumsee where around 10.8 tons of Phoslock per ha were applied, more aquatic plants were found afterward (Spieker et al., 2014). These case studies demonstrate that the improved water transparency observed in Phoslock® treated water bodies increases the macrophyte colonisation depth as well as the plant coverage of the lake beds, which promotes the successful re-establishment of macrophytes.

A pilot study was undertaken in a popular Austrian swimming lake, in which Phoslock® treatments were combined with active planting of Characean macrophyte species. On one occasion, Phoslock® was applied in spring after the Characeans had been planted several months previously without causing any negative effect on the plants. During this restoration project, the Characeans were successfully established in the lake, leading to an improved ecological state and an increase in the lake's recreational value (Epe et al., in prep.; Systema GmbH personal communication). The results from these case studies demonstrate that an application of Phoslock® has a beneficial effect on macrophytes due to an improvement in light conditions, geo-physical sediment properties and by counteracting the growth of benthic cyanobacteria.

Macroinvertebrates

A range of studies has been conducted to investigate the impact of Phoslock® on the resident benthic macroinvertebrate community. Bishop et al. (2014) found no differences in the pre- and post-application benthic macroinvertebrate biometrics tested (taxa richness, diversity, tolerance, functional feeding groups). Reitzel et al. (2013) found no difference in *chironomids* abundance in the control and Phoslock® treatments in a lab experiment. A study conducted in Lake Rauwbraken (The Netherlands) reports slightly increased post-treatment densities of chironomid larvae (van Oosterhout et al., 2020). In Lake De Kuil (The Netherlands), the application of a low dose flocculant (FeCl₃) combined with Phoslock® resulted in a shift of the lake from a hypertrophic to a mesotrophic state (Waajen et al., 2017a). This study shows a short-term impact on the macroinvertebrate numbers and taxa during the first month following the application. However, one year after the application, the macroinvertebrate community structure (number and taxa) exceeded the pre-application situation. The adverse impact one month after the application is most likely due to the chemical effects caused by the FeCl₃ and, to some minor extent, due to the bentonite's physical impact. The positive effects one year after the application can most probably

be attributed to the shift in the lake's trophic state. Meis (2012) found a reduction in Oligochaeta, Chironomidae and Sphaeriidae groups in Loch Flemington (UK) after a Phoslock® application. The author discussed that it could be argued that the reduction was due to an increase of La content in the sediment caused by Phoslock®; however, other studies also found a reduction in macroinvertebrate (i.e., Oligochaeta and Sphaeriidae) abundance after external nutrient load reduction (Gunn et al., 2012; Köhler et al., 2005). As a result, it is not possible to say definitively that the reduction in Oligochaeta, Chironomidae and Sphaeriidae groups in Loch Flemington was due to possible toxic effects, a reduction in nutrient availability or the change in the trophic state. However, acute tests performed with extremely high Phoslock® concentrations have shown no toxic effect, indicating that Phoslock® toxicity is low or null.

Fish

Several studies have reported increased macrophyte coverage and higher macroinvertebrate diversity after a Phoslock® treatment (Bishop et al., 2014; van Oosterhout et al., 2020; Waajen et al., 2017a; Epe et al, *in prep*). This change in the ecological structure is expected to positively influence the fish stock by creating a diversified habitat with favourable spawning conditions and improved refuge from predation. In Lake De Kuil (The Netherlands), pike biomass and mainly carp increased after the treatment. The increase of carp in the lake can be attributed to an increased appreciation of the improved water quality by fishermen which resulted in uncontrolled carp stocking. The increased biomass of pike in the lake can be directly related to the improved water clarity and enhanced macrophyte cover (Waajen et al., 2016a). Also in compartments in ponds in Dongen and Eindhoven no adverse effects on fish biomass were observed (Waajen et al., 2016b).

2.1.5. Conclusion Ecotoxicological Assessment on aquatic biota

Tests from acute and chronic toxicity studies support the conclusion that the use of Phoslock® is safe for the aquatic environment (paragraph 2.1.1). The low ecotoxicological profile of Phoslock® is further confirmed in several studies in which an extensive post-treatment monitoring was conducted after whole lake applications (Spears et al., 2016; Waajen et al., 2016a,b; 2017a,b; van Oosterhout et al. 2020; in revision) (paragraph 2.2.4). The use of Phoslock® as a tool for eutrophication management improves the trophic state of a waterbody and thereby induces a biological recovery process in formerly impaired aquatic systems.

2.2. Exposure scenario - Waterbirds and terrestrial mammals (particularly pets)

Avian fauna could potentially be impacted by short-term localised disturbances during an application, however this will be limited to a small area. In addition, applications can be timed to avoid disturbance to breeding avian species or overwintering bird species if present. For waterbirds, an exposure to Phoslock® is likely to be restricted to the consumption of aquatic organisms, e.g., macrophytes, invertebrates, and fish. It is known that the trophic transfer of compounds such as La via food uptake to higher biotic levels such as birds is assumed to be limited (Campbell et al., 2005). When given as lanthanum carbonate the oral bioavailability of lanthanum is reported to be 0.0007 % and 0.00084 % in rats and humans, respectively, and almost all administered lanthanum is excreted via the faeces, even after i.v. injection of a soluble lanthanum compound (D'Haese et al., 2019). Thus, as with humans and other mammals, it is most likely that the amount of lanthanum absorbed by waterbirds after ingestion will be excreted via the faeces (e.g., (Lürling and van Oosterhout, 2012)). In this context, it is also worth mentioning that lanthanum and other REE elements have been introduced as feed additives in livestock breeding to improve animal health and production. Amongst bird species, body weight gains have been observed in broilers, while egg production, egg-shell breaking strength and food conversion rates have increased in egg-laying hens. Overall improvements in general health and performance parameters have also been observed (Abdelnour et al., 2019). Although there is no research on the effect of Phoslock® on waterbirds, from the available information it seems unlikely that waterbirds will suffer any toxicity caused by

Phoslock®. Furthermore, no adverse effects on the resident avian fauna have been observed during the numerous field applications undertaken with Phoslock®.

Domestic animals such as cats and dogs could be exposed to Phoslock® treated water by swimming or drinking water from a treated lake. Any risk from this exposure is related to the lanthanum and can be evaluated from a range of data available for veterinary drugs and food additives using lanthanum carbonate (Lantharenol®, active ingredient lanthanum carbonate octahydrate) to prevent chronic renal malfunction in ageing animals. About the dermal and respiratory exposure pathways, Lantharenol® is not considered to pose a risk by inhalation. It is not an irritant to skin or eyes and is not a dermal sensitizer. Concerning oral exposure, the supplementation of regular feed with Lantharenol® is safe and well-tolerated by healthy animals up to a concentration of 75 g/kg complete feed. For dogs, this concentration corresponds to a daily intake of 9.3-12.4 g of Lantharenol® (corresponding to 4.3-5.7 g of elemental lanthanum). Considering that the intended dose of Lantharenol® in the feed of adult dogs and cats ranges from 1.5-7.5 g/kg complete feed, it can be concluded that this food additive is safe for adult dogs and cats at the highest dose proposed with a safety margin of at least ten (Schmid et al., 2009; EFSA 2012, EFSA 2019). Given the minimal daily intake concentration of 4.3 g that is considered to be safe and the peak concentration for total lanthanum in the water after a Phoslock® application (Spears et al., 2013a; cf. Table 1), a dog would need to drink about 2000 L of lake water per day to ingest a similar nominal safe concentration. Even when applying a safety factor of ten, the water volume that an animal has to drink is about 200 L per day, a quantity that could not possibly be consumed in a day by, for instance, a dog. Therefore, even water consumption directly after the application would not pose a risk to animal health.

2.3. Toxicological assessment - Human exposure to Phoslock® treated water

Theoretically, humans may be exposed to Phoslock® during recreational activities in Phoslock® treated water bodies. The main exposure pathways could be dermal contact and oral uptake by swallowing water during swimming or consuming fish from treated water bodies. The following summary describes the most important studies and the conclusions that can be drawn from them.

2.3.1. Toxicological studies with Phoslock® - Oral toxicity and tissue distribution patterns

In two studies conducted with Phoslock®, the potential to induce toxic effects and the gastrointestinal absorption of Phoslock® derived lanthanum was tested in rats:

- A 28-Day Repeated Dose Oral Toxicity Study in Wistar Rats was conducted with Phoslock® following the strict requirements of the OECD 407 Test Guideline and the OECD principles of GLP (Good Laboratory Practice). This study aimed to assess the possible health hazards which could arise from repeated exposure to Phoslock® via oral administration to rats over 28 days. A total of four dose groups were used in the study. According to the test guideline, the highest administered dose was 1000 mg of Phoslock®/kg body weight/day. No test-item related mortality was observed and no adverse effects of the test item were found for male and female clinical observations, functional observations, body weight development, food consumption, haematology and coagulation, clinical biochemistry, urinalysis, gross macroscopic findings at necropsy, organ weights and histopathology in all treated dose groups. Hence, according to the OECD 407 Test Guideline, the no observed adverse effect level (NOAEL) of Phoslock® is considered to be > 1000 mg/kg body weight/day (Schmid et al. 2019).
- Behets et al. (2020) conducted a study with Wistar Rats evaluating the gastrointestinal absorption and tissue distribution of lanthanum after oral administration of lanthanum chloride, lanthanum carbonate or Phoslock® over 10 weeks. Considering all possible exposure pathways, the authors evaluated the safety margins and concluded that the uptake of lanthanum through exposure to Phoslock® treated water is unlikely to pose a health risk to animals and/or humans.

2.3.2. Phoslock® human health risk assessment

As suggested by Behets et al. (2020), a further approach for a human health risk assessment for Phoslock® is to focus on its active ingredient lanthanum. Unlike aquatic ecotoxicity studies, where the chemical nature of the lanthanum added is of paramount importance, in the acid environment of the human stomach such aquatic speciation effects are minimized. All lanthanum compounds, such as lanthanum chloride, lanthanum carbonate and Phoslock®, dissociate in the acidic stomach where after liberated La precipitates with phosphate and other oxyanions (e.g. oxalate) from food. Lanthanum is an ingredient in topical formulations in concentrations of up to 30% to treat several skin disorders to reduce irritation and inflammation. Based on a comparison of these concentrations with the highest measured TLa concentration in Phoslock® treated water (Spears et al., 2013a; cf. Table 1), it can be concluded that no adverse effects are expected through dermal exposure. For oral exposure pathways, a huge amount of data from pre-clinical and clinical studies are available due to the use of lanthanum carbonate (trade name Fosrenol®, Shire Pharmaceuticals) for the treatment of hyperphosphatemia in renal failure (Stewart, 2002; D'Haese et al., 2003; Behets et al., 2004; Hutchison, 2009; Hutchison et al., 2009, 2016, 2018; Damment, 2011). Lanthanum carbonate is administered daily at doses containing 375 to 3000 mg/day of elemental lanthanum over many years. Hutchison et al. (2016) recently showed that after 10 years of lanthanum carbonate treatment in dialysis patients, corresponding to more than 850,000 person-years of worldwide patient exposure, there is no evidence that lanthanum carbonate is associated with adverse safety outcomes in patients with end-stage renal disease. The potential for any adverse effects to humans related to the use of Phoslock® in water bodies is further discussed in detail by Copetti et al. (2016) and D'Haese et al. (2019). Given an average therapeutic dose of Fosrenol® of 1500 mg per day (~900 mg lanthanum/day) without toxicity to the patients after up to 10 years of treatment, consumers would have to ingest at least 390 L of the surface water per day to attain a similar, nominally safe dose. It is not possible for such large volumes of water to be drunk by a person in a day and therefore, an application of Phoslock® would never deliver as much as lanthanum as a single Fosrenol® tablet.

Therefore, even ingestion of Phoslock® treated water directly after an application would not pose a risk to human health. A similar estimation can be carried out for the consumption of fish from Phoslock® treated water bodies. Taking the highest lanthanum concentrations measured in fish muscles (0.81 mg lanthanum/g dry weight), a person would have to consume more than 1000 kg of fish per day to reach the nominally safe recommended therapeutic dose of lanthanum in adults (D'Haese et al., 2019). Again, given this high margin of safety, it can be concluded that there are no adverse effects associated with the consumption of fish from Phoslock® treated water bodies.

2.3.3. Conclusion - Toxicological assessment for human exposure

Over the past 15 years, Phoslock® has been widely researched and scrutinized by the international scientific community and until now 92 scientific peer-review publications about Phoslock® have been published. For the human exposure assessment, a vast amount of data is available from mammalian toxicity studies with Phoslock® and long term clinical studies with lanthanum carbonate. The available data show a wide margin of safety associated with Phoslock® applications at predicted use rates and environmentally relevant concentrations.

3. Reality check

3.1. Kralingse Plas

This section aims to discuss potential direct and indirect effects a Phoslock® application might cause in Kralingse Plas based on the specific biotic and abiotic factors of the lake, the dose selected, and the scientific literature discussed above.

Phoslock® dose for Kralingse Plas

The maximum Phoslock® dose calculated for Kralingse Plas was 813 tons (Phoslock Europe, 2020); this dose will yield to around 288 mg Phoslock® per L or 7.2 tons per ha. This is a realistic dose and similar to other systems. For instance, in the paper cited above which reported peak TLa concentrations in Phoslock® treated lakes, the dosage range was 0.06 to 6.7 tons/ha (Spears et al., 2013a). Applications have also been reported in the literature where Phoslock® doses were higher: 10.8 tons/ha and 16.7 tons/ha (Novak and Chambers, 2014; Yasseri and Epe, 2016). Amongst the 16 treated lakes described in Spears et al. (2013a), Loch Flemington, Lake Blankensee and Lake Bärensee are shallow lakes and comparable to Kralingse Plas. The maximum TLa concentration (2.3 mg/L) was measured during a Phoslock application in Loch Flemington (max depth 2.5 and average depth 1m). The peak in TLa concentration will mainly depend on the application method; for instance, if the application takes place over several weeks across alternating areas (as has been proposed), the TLa concentrations are unlikely to be higher than already measured and reported by Spears et al. (2013a). The application design should be planned once the final dose for each area is defined.

3.1.1. Direct and indirect changes

Physico-chemical changes

Phoslock® will inactivate phosphate (Douglas, 2002) which can be expected to result in a reduced algal biomass, particularly cyanobacteria, in the following growing seasons. This is likely to shift the system from a eutrophic to a more mesotrophic state which in turn should result in several ecological changes (Fig. 4).

Phoslock® will increase turbidity for a short period during the application, however in the longer term water transparency should increase as a result of the reduced algal biomass, particularly during summer, and light will reach deeper in the water column (i.e., Lüring and van Oosterhout, 2013; Waajen et al., 2016; Mucci 2019). The Phoslock® application will not directly affect temperature, pH, conductivity, alkalinity or oxygen concentrations (Spears et al., 2013b; van Oosterhout and Lüring, 2013). Due to the reduction in the phytoplankton biomass as a result of reduced phosphate availability, there may be an increase in oxygen concentrations in the water column in the summer after the application due to less bacterial decay and less oxygen consumption (van Oosterhout et al., in revision) and anoxic events can be expected to be less or absent as lake productivity will be lower.

Phoslock® will increase the total lanthanum concentration in the water column. Based on a dose of 288 mg Phoslock®/L, a maximum total lanthanum (TLa) concentration of 14 mg TLa/L is theoretically possible, however this value is unrealistic as Phoslock® sinks to the bottom of the lake after application and it is not logistically possible to apply the 813 tons at the same time in all locations. Applications are usually spread over several days or weeks, targeting different locations each day. Such a strategy has also been proposed for Kralingse Plas. Therefore, the maximum concentration of total lanthanum in the water column is expected to be similar to that described by Spears et al. (2013a) in the 16 other aquatic systems (0.026 to 2.3 mg TLa/L).

Nearly all of the lanthanum applied to the lake will remain embedded within the clay matrix and not be in a soluble form. Based on the water chemistry of Kralingse Plas, estimated free La^{3+} ions concentrations are predicted to be very low (less than 6×10^{-8} mg La^{3+} /L) (Fig. 1A-Appendix) and no direct toxic effects from free La^{3+} ions are expected.

Lanthanum concentrations will also increase in the sediment of Kralingse Plas following a Phoslock® application. If the La incorporated in Phoslock were to be homogeneously mixed into the first 10 cm of the sediment following the application, the proposed dose would result in a concentration of 319 mg La/kg dry weight of sediment. Post-treatment monitoring undertaken on previous applications has shown however that lanthanum distribution is not vertically homogenous (van Oosterhout et al., 2020) and it is expected that most of the lanthanum added to Kralingse Plas will be found in the top few centimetres of the sediment in the first years. If we assume therefore that 100% of the lanthanum applied will be in the top three centimetres of the sediment, a La concentration of 1063 mg/ kg DW of sediment would be expected. This concentration does not exceed the maximum permissible concentration of lanthanum in Dutch sediments (4700 mg/ kg DW) (Sneller et al., 2000). Furthermore, it is also to be expected that the La contained in the Kralingse Plas sediments will be mixed deeper in the sediment over time. For instance, La has been found in sediment depths below 10 cm in a number of lakes treated with Phoslock® (Dithmer et al., 2016b; van Oosterhout et al., 2020), with the result that the La concentrations in the sediment become even more diluted over time and phosphorus in deeper sediment layers is bound.

It is also important to note that La has been measured in water, sediment, macrophytes and chironomid larvae in other Dutch lakes prior to Phoslock® applications (van Oosterhout et al., 2020; Waajen et al., 2017b). In the Kralingse Plas, there is already lanthanum present in the sediment as well (Data B-WARE Research Centre). An application of Phoslock® to the lake does therefore not result in the introduction of a new element to the system but merely an increase in the concentration of an existing element.

3.2. Biological changes

Phytoplankton

Phoslock® will decrease the phosphate concentration (Douglas, 2002) and this can be expected to result in a reduction in algal biomass (mainly the cyanobacterial biomass) in the ensuing growing seasons as well as a decline in concentrations of cyanotoxins (e.g., Mucci et al., in review.) and a concomitant increase in phytoplankton diversity (e.g., Robb et al., 2003; Bishop et al., 2014; Bishop & Richardson 2018; Epe, 2014; Barçante et al., 2020).

Macrophytes

The dominant macrophyte species in Kralingse Plas in 2019 were *Elodea nuttallii*, *Ceratophyllum demersum*, and *Chara globularis*. Toxic effects, reduced growth and germination issues have not been observed in any of the macrophytes tested until now, including *E. nuttallii* and *Chara sp.*, both of which are present in Kralingse Plas (Waters-Hart, 2020; Epe et al., in prep.). The abundance of *E. nuttallii* and *Chara sp.* tripled in Lake de Kuil (The Netherlands) after a Phoslock® application, while similar increases in other macrophytes species occurred in 17 other lakes where Phoslock® was added (Spears et al., 2016; Waajen et al., 2016a).

Based on the studies conducted, no toxic effects caused by Phoslock® are expected on the aquatic plants in Kralingse Plas. On the contrary, an increase in aquatic plants in the following years after the intervention is expected due to water quality improvement (i.e., reduced incidence of cyanobacterial blooms) (e.g., Waajen et al., 2016).

Zooplankton

The main group of zooplankton present in Kralingse Plas is *Daphnia sp.* (van Giels and Kleppe, 2020), and the EC50 value that has been measured for this group was 3 and > 173 times higher than the Phoslock® dose proposed for Kralingse Plas (288 mg/L). Thus, based on laboratory tests done with different zooplankton species and the Phoslock® dose chosen for Kralingse Plas, no direct toxic effects caused by Phoslock® are expected (Fig 3).

Zooplankton may be affected by an increase in water turbidity during the application; however, these effects are considered temporary and not long-lasting (i.e., van Oosterhout and Lurling, 2011;

Waajen et al., 2016). Once the algal biomass is reduced, an indirect effect through a lower availability of food (lower phytoplankton biomass) for zooplankton can be expected. This indirect effect should be contrasted however with the impact that the persistent blooms of cyanobacteria would otherwise have on zooplankton. Cyanobacteria blooms are commonly made up of large, inedible filaments or colonies that also produce toxins or grazing-repellent compounds and these traits (morphological and chemical) can act as an anti-grazing defence (Ger et al., 2016a; Lürling, 2020). Thus, a higher phytoplankton biomass does not automatically imply a higher availability of edible food or a greater zooplankton biomass (e.g., Josué et al., 2018). Cyanobacterial blooms also affect the type of zooplankton that are present. For instance, blooms typically lead to the exclusion of large generalist grazers, which are often replaced by smaller and selective grazers (Ger et al., 2016b).

Thus, it is difficult to predict what will happen with the zooplankton community in Kralingse Plas after a change in trophic state as plankton interactions are dynamic, and several factors (predation, grazing, competition, presence of macrophytes, and abiotic factors) are involved. However, based on previous whole lake applications and laboratory experiments, no toxic effects of Phoslock® on zooplankton are expected in Kralingse Plas, nor a food shortage for fish.

Macroinvertebrates

The effect of Phoslock® on insect larvae (also present in Kralingse Plas) has been evaluated using a dose 12 times higher than the one that is proposed for Kralingse Plas with no adverse effect on growth or survival rates being observed. The effect of Phoslock® on freshwater shrimp (also present in Kralingse plas as *Mysida sp.*) has been evaluated using a dose that is between 2 to 173 times higher than that proposed for Kralingse Plas, with no effect on survival being observed.

Based on the available literature and laboratory experiments, the macroinvertebrate community after a Phoslock® application may (or may not) change in Kralingse Plas. However, if a change does occur, the change will not be caused by a toxic effect but will be due to a change in water quality. For instance, if macrophyte abundance increases, as has been observed in other systems treated with Phoslock®, macrofauna abundance may also increase as a result of improved habitat complexity (Spears et al., 2016 and Waajen, unpublished data).

Fish

A reduction in the fish biomass in Kralingse Plas occurred several years ago and the biomass has remained very low since. Data from van Giels (2016) showed that in 2016 the main fish species in Kralingse Plas were perch, roach, pike, ruffe, bream and eel, totalling 12.7 kg/ha. No literature was found relating to toxicological studies concerning these specific species, although acute tests conducted on two other species of fish yielded EC50 values that were between 95 and 1087 times higher than the total La concentration expected in Kralingse Plas (2.3 mg TLa/L based on Spears et al., 2013a; Fig 3 and Table 7). Prior to a Phoslock® application, the fish population of Lake de Kuil (The Netherlands) consisted of similar species as the Kralingse Plas (perch, bream, eel, roach, pike, ruffe, besides other species such as tench, carp, and spined loach), totalling 50.2 kg/ha. No fish kill occurred during the application and five years after the Phoslock® application, the fish stock was similar in species diversity and an increase in fish biomass to 131.8 kg/ha had been observed (Fig. 2A-Appendix). Eight years after the application, the fish stock was still similar (136.9 kg/ha) (Waajen et al., 2016a). The large increase in fish stock was due to the improvement in water quality but also carp stocking by anglers (Waajen et al., 2016a). In addition, in two urban ponds in the Netherlands (Pond Dongen and Pond Eindhoven) the fish stock after a Phoslock® application developed well and increased in biomass (Waajen et al., 2016b).

Based on the field observations, acute tests done in different fish species (Table 7) and the lack of a recorded fish kill after more than 200 solo application of Phoslock®, no direct damage to the fish in Kralingse Plas following a Phoslock® application based on the proposed dose is expected. The fish community may change due to a change in trophic state; for instance, more aquatic plants/macroinvertebrates and less anoxic events, however this can be expected to create a more suitable environment for fish.

Mammals and water-birds

Kralingse Plas is a foraging area for certain bat species in the riparian zones (Grutters et al., 2018). No research has been undertaken on the effect of Phoslock® or lanthanum in bats, however no direct effects are expected as there will be no direct contact between the bats and Phoslock®. More insects are to be expected due to more underwater vegetation which can serve as a food source for some bats (Grutters et al., 2018).

The act of applying Phoslock® may cause some disturbance to fish, birds and bats due to water movement, light and sound, however as recreational activities already occur in the lake, the disturbance caused by a Phoslock® application will be limited.

Based on the Phoslock® dose proposed for Kralingse Plas and toxicological tests done with the medicine used for pets (Lantharenol®), it can be concluded that the amount of lanthanum that could be ingested by a dog or a cat would be safe even if the animal consumes 300 litres of lake water (considering a worst-case scenario of total lanthanum around 14 mg/L). Clearly, it is not possible for a dog to drink 300 litres of water per day, therefore the consumption of lake water will not pose a risk to animal health even during the application.

Human patients have been taking 900 mg La per day to treat hyperphosphatemia in renal failure without toxicity for more than ten years (Hutchison et al., 2016). A person would need to drink more than 63 litres of water from Kralingse Plas to reach a similar safe dose of Fosrenol®. This is also considering a worst case scenario that the TLa concentration is 14 mg/L. As humans are unable to drink this quantity of water per day, the proposed Phoslock dose does not pose any risks to humans.

Summary

As of 7 October 2020, Phoslock® is mentioned in the title, keywords or abstract in ninety-two peer-reviewed (accessed via Scopus). Among these 92 papers, 38 evaluate Phoslock® in the presence of live organisms however none of these 38 papers report a direct toxic effect from Phoslock® under realistic conditions and dosage.

Overall, based on the scientific literature discussed in this report and the proposed Phoslock® dose for Kralingse plas, no toxic effects on any of the aquatic organisms present in Kralingse Plas are expected to be seen as a result of a Phoslock® application. Similarly, no adverse effect on pets or humans are to be expected either.

Of the 92 papers published, 29 studies cover whole field applications or enclosure experiments using Phoslock® with most of these cases resulting in an improvement in water quality. These changes are summarized in Figure 4 and presented in more detail in Table 1A in the appendix.

The use of Phoslock® has generally resulted in a number of positive ecological changes in the treated systems with lakes moving towards a more mesotrophic state. Observed effects include a reduction in cyanobacteria and an increase in phytoplankton diversity, macrophytes and macroinvertebrates abundance. At the same, no long-term negative effects on zooplankton have been seen while fish habitats have improved.

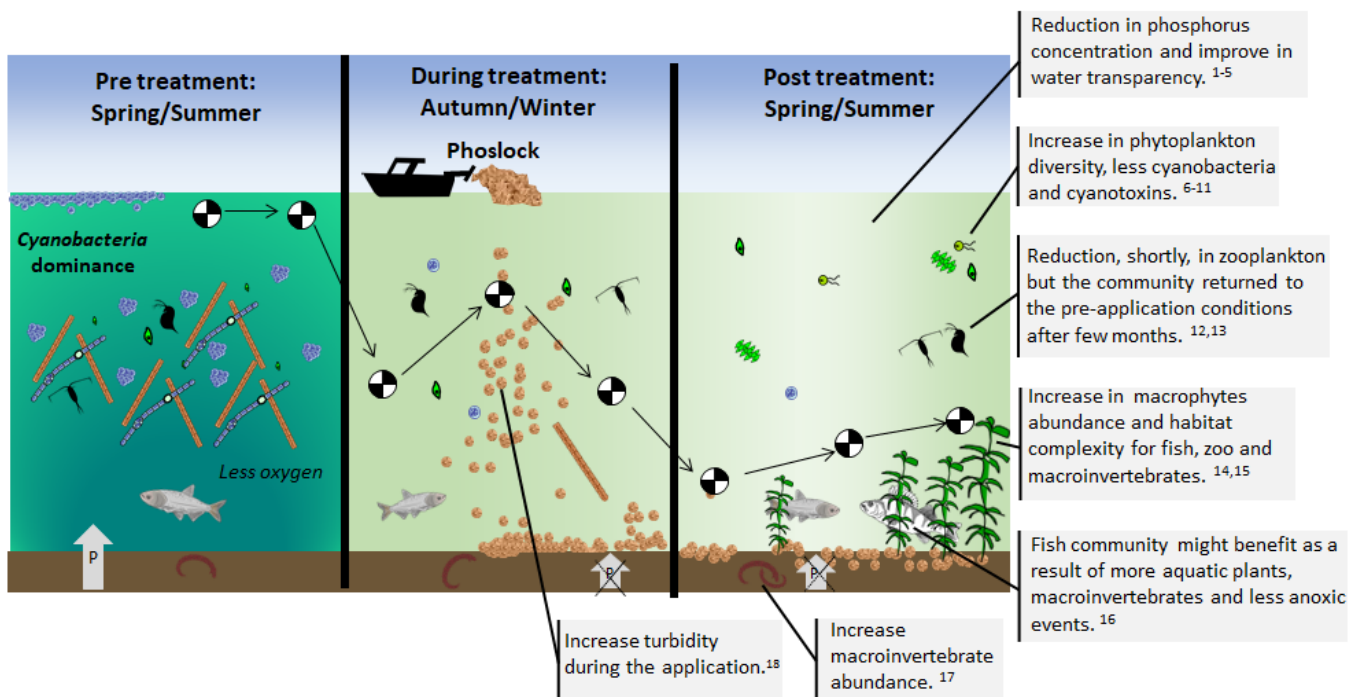


Figure 4. Scheme showing from left to right (a) untreated system with cyanobacterial bloom in spring/summer (b) system undergoing Phoslock® treatment in autumn/winter and (c) treated system in following spring/summer with clear water, less P released from the sediment, eukaryote algae dominance, more macrophytes and macroinvertebrates. Secchi disks represent water transparency. Scheme adapted from Lürding et al., (2016) and Mucci, (2019). Numbers are the references. 1 to 6: (Epe et al., 2017; Lürding and van Oosterhout, 2013; Meis, 2012; Spears et al., 2015; Waajen et al., 2016a) 6 to 11 (Robb et al., 2003; Bishop et al., 2014; Bishop & Richardson 2018; Epe, 2014; Barçante et al., 2020 and Mucci et al. in review.12 and 13 van Oosterhout and Lurling, 2011; Waajen et al., 2016a. 14 and 15: Spears et al., 2016; Waajen et al., 2016. 16: Waajen et al., 2016. 17: Waajen et al 2017.18: Mucci et al., in review).

3.3. Deferred eutrophication management

Eutrophication is threatening freshwater ecosystems worldwide by stimulating excess phytoplankton growth, triggering the risk of nuisance cyanobacteria blooms and inducing the shift of shallow lakes from clear water to a turbid state. These changes in the ecological structure in aquatic systems also include the biogeochemical processes of the lake sediments and changes in the habitat of the benthic community (Scheffer & van Nes 2007; Downing 2014; Lürding et al. 2016). The increased demand for eutrophication management measures is mainly driven by water quality, human and public health risk thresholds for cyanobacteria biomass and by the ecosystem services of waterbodies related to recreational use, drinking water supply and biodiversity conservation. Various estimates have been made for both the costs for eutrophication management as well as the costs of non-intervention, with the costs of “doing nothing” being often higher than the costs of taking pro-active measures aimed at reducing eutrophication. The costs of this ‘do nothing’ approach include the reduced values to waterfront dwellings, additional drinking water treatment costs, reduced amenity of waterbodies, removal of algal toxins and decomposition products from drinking waters, ecological effects such as fish kills, increased public health risk due to cyanobacteria toxins and tourist industry losses (Douglas et al., 2016).

Cyanobacteria blooms pose a serious health risk for humans and animals (WHO 2003). Deaths of cattle and dogs have been reported worldwide after the ingestion of cyanobacteria infested water or benthic cyanobacteria mats (Mez et al., 1997; Wood et al., 2010; Lürding & Faassen, 2013). During summer, news reports regularly refer to cases of human and animal poisoning, with the latter often having lethal consequences as was recently reported for the Lake Neuchâtel (Switzerland), where several dogs died after the ingestion of lake water (Republique et Canton de Neuchâtel, 2020). Likewise, in the Dutch

lakes Amstelmeer and IJmeer dogs died from cyanobacterial intoxication (Faassen et al., 2012; Lürling & Faassen, 2013).

In situ lake measures as part of an effective eutrophication management plan for the restoration of impaired lacustrine systems are often associated by the stakeholder community with adverse effects on the ecosystem itself and potential risks for the public health. The consequences, costs and risks related to the decision to 'do nothing' are most often not considered or underestimated. From a cost-benefit point of view an effective management of eutrophication based on a so called "system analysis" (Lürling et al., 2016) should be the method of choice and less expensive than the non-implementation of restoration measures.

4. Conclusion

The aim of this report has been to provide comprehensive information about the potential risks for the environment and public health related to the use of Phoslock® as a eutrophication management tool. Phoslock® is a lanthanum modified bentonite clay designed to bind excess phosphorus in aquatic environments suffering from eutrophication. The potential risks associated with its use are related to its active ingredient lanthanum and, in the aquatic environment, to the temporary increase of suspended solids in the water column. Data from a range of ecotoxicological studies undertaken with organisms belonging to different trophic levels demonstrate that the risk for the aquatic life associated with the use of Phoslock® can be rated low in Kralingse Plas. Extensive post-application monitoring conducted in several field studies confirms the low ecotoxicological profile in terms of bioaccumulation, biodiversity and trophic state improvement. Lanthanum carbonate is used as a food additive (Lanthanrenol®) for dogs and cats suffering from renal malfunction and thus, the risks for any adverse effects on domestic animals or other vertebrates are negligible. Human exposure to Phoslock® might occur from recreational activities or from consumption of fish from treated waterbodies. Due to the use of lanthanum carbonate (Fosrenol®) as a drug given to renal failure patients, a huge amount of data is available from the preclinical and clinical drug development phase. For the human exposure assessment, the available data show a wide margin of safety associated with Phoslock® applications at predicted use rates and environmentally relevant concentrations.

5. References

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Appendix

Box 1A - Exclusion of studies using soluble lanthanum salts

As pointed out in section 2.1.1 the use of different lanthanum compounds in ecotoxicological assays has produced a high variability in the test results, showing higher toxic effects for the soluble lanthanum salts compared to the insoluble lanthanum forms and to Phoslock® (Herrmann et al., 2016 and references therein). Furthermore, even for studies conducted with the soluble salts and using the same test species, substantial differences for the dose descriptor values were reported between the studies (Spears et al., 2013a). The discrepancy of the dose descriptor values for the soluble lanthanum compounds reported in the literature is mainly due to the implementation of different study designs and the difference in the chemical composition of the aqueous medium used in the assays (Copetti et al., 2016). Lanthanum has a strong preference for oxygen-containing anions like carbonate and phosphate with which it can form stable compounds. Therefore, the different ionic strength and ionic composition of the test medium used in the various ecotoxicological assays highly influenced the test results, even among studies conducted with the soluble salts. This inconsistency in test results with the soluble lanthanum salts does not allow a consolidated comparison with tests conducted with Phoslock® itself and was one reason why these studies were excluded in the present toxicity assessment.

In addition, with regard to the use of Phoslock® for eutrophication management, it is important to consider the speciation of any Phoslock® derived lanthanum in the natural environment to which it is introduced. Phoslock® is specifically designed to overcome any ecotoxicological effects associated with lanthanum (Robb et al., 2003). The lanthanum is strongly adsorbed within the interlayer space of the clay matrix and is not released in its ionic form in moderate to high alkalinity water. Any filterable lanthanum species in natural water is found to be in a colloidal form, either as lanthanum phosphate or associated with humic acids (Reitzel et al., 2017). Thus, due to the different chemical properties and toxicological features of Phoslock® in the aqueous environment the direct comparison with tests conducted with the soluble lanthanum salts is inappropriate and the second reason why these studies are excluded for the toxicological assessment in this report.

At least, as the early experimental formulation of Phoslock® was a liquid form which differs considerably from the actual dry granular formulation produced since the end of 2006, studies undertaken during the early development phase of Phoslock® were not considered for the present document. The sole exception to this is the study described by Stauber & Binet (2000). These authors demonstrated that after thorough testing by CSIRO, modifications to the formulation avoid excessive lanthanum leaching from the liquid form. Therefore the results from this study allows a somewhat comparability with the studies undertaken with the actual dry granular form and thus were included in the toxicity evaluation presented here.

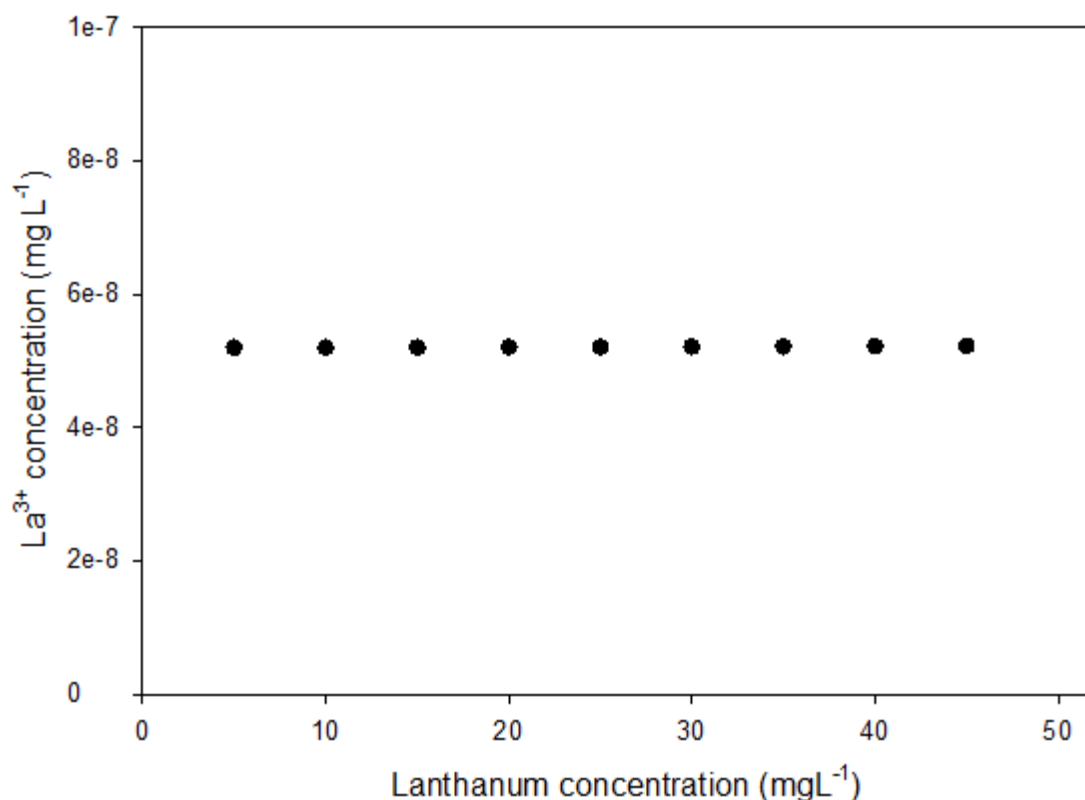


Figure 1A. free La ions concentration estimated as a function of La concentration added using CHEAQS (Verweij, 2017). The input was based on data provided by HHSK of CO₃ (170 mg/L), Ca (80 mg/L) - as a proximity of alkalinity- and PO₄ (0.6 mg/L) concentration, pH value was fixed at 7. This estimation is a worst-case scenario because we considered that 1% of the La added would be leached from the clay. The values actually measured are below 0.11% (Lürling and Tolman, 2010; NICNAS, 2001; Mucci et al., in review.). The CHEQS may also overestimate possible free La³⁺ concentration as La ions might also bind dissolved organic carbon ameliorating any possible toxic effect (Li et al., 2020; Spears et al., 2013a).

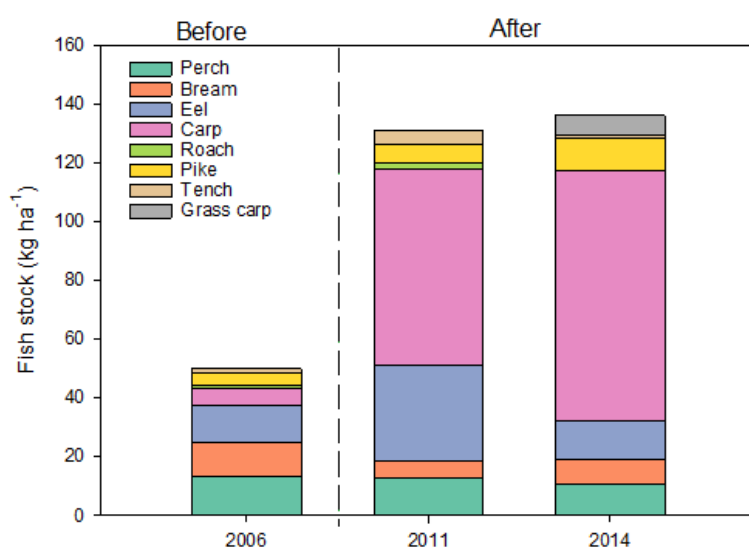


Figure 2A. Fish stock in Lake de Kuil (The Netherlands) before and after a Phoslock application (Data modified from Waajen et al., 2016a).

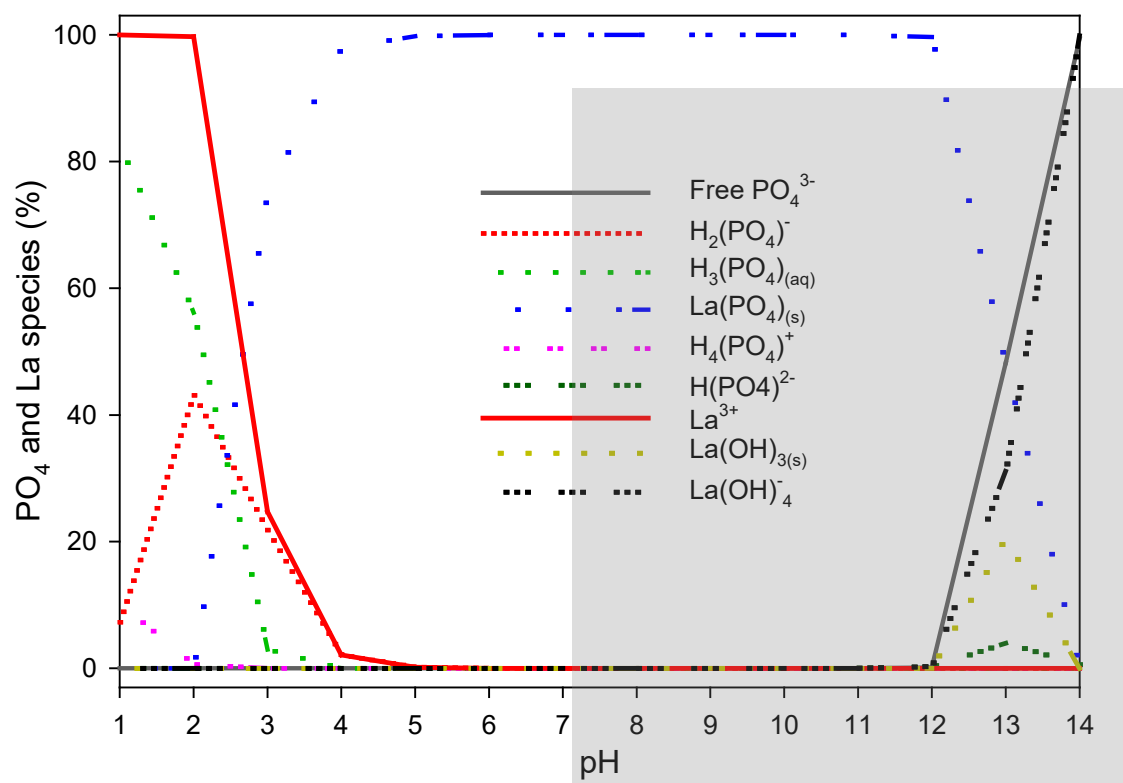


Figure 3A. La and PO₄ species (in % of abundance) at different pH predicted from chemical equilibrium modelling (CHEAQS -Verweij, 2017)) using as input 4 μM of La and P. Note that La(PO₄) is stable between pH 4 and 12. Subscript s in the legend means solids. Grey rectangle is the pH range with predominantly the insoluble La(PO₄)_(s). (graph from Mucci et al., in prep).

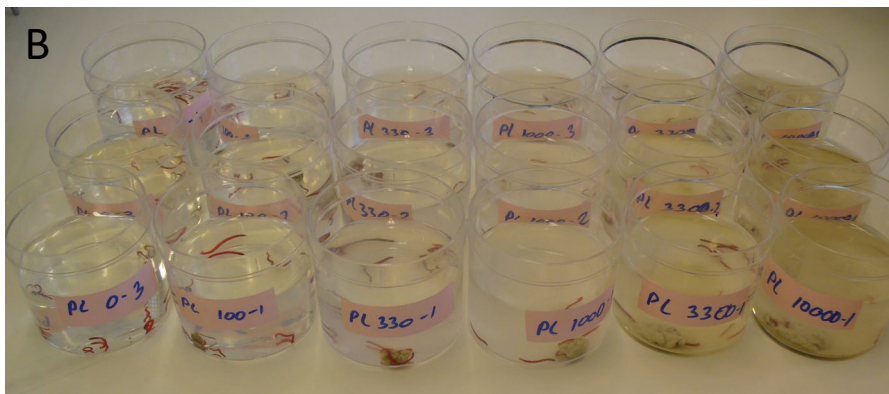
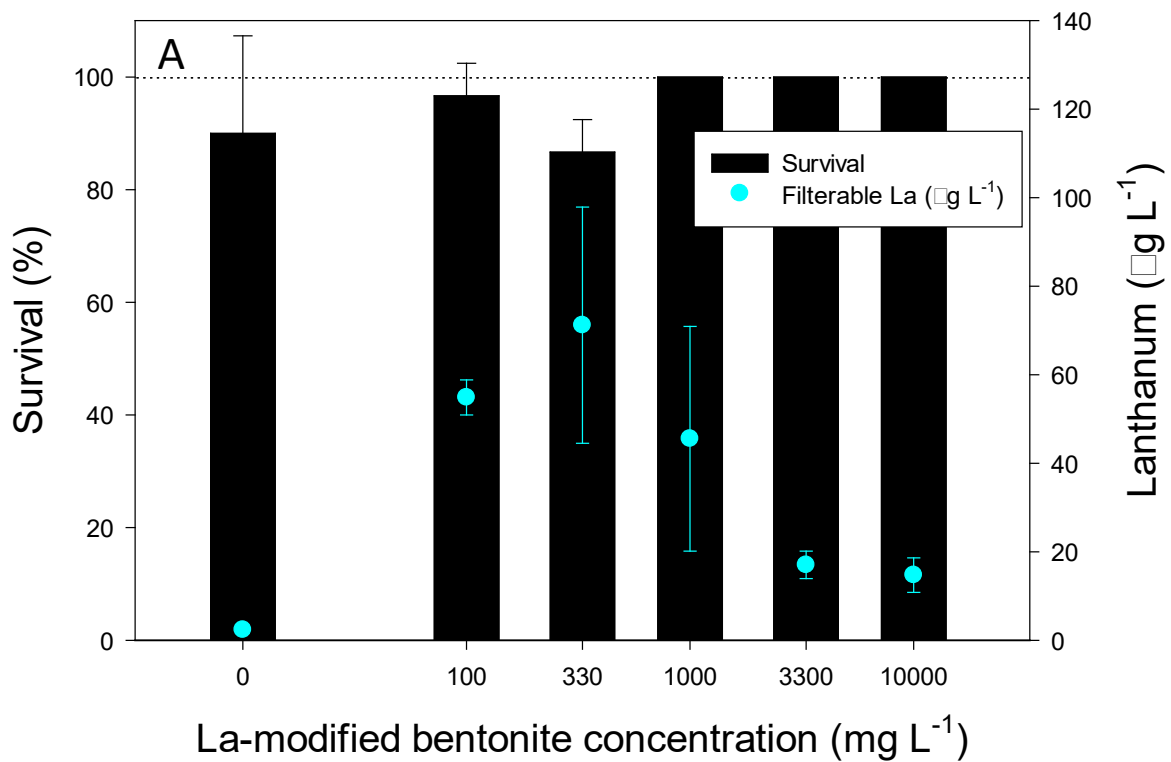


Figure 4A. (A) Experiments exposing *Chironomus* sp. to different Phoslock® concentration (100 to 10000 mg/L) for seven days. The bars represent survival rate (%) and circles filterable lanthanum concentration (µg/L). (B) picture of the experiment (Lürling, unpublished data).

Table 1A. List of all the 29 peer-reviewed studies that evaluated whole field Phoslock® application and/or enclosures experiments together with the main ecological changes observed in the studied systems. Table continues on next pages.

Reference	Main ecological changes observed
Barçante et al., 2020	Phoslock® reduced phosphorus concentration, increased Secchi disk and non-cyanobacteria biodiversity. Those changes were short due to the high large amount of nutrients that still enters in the reservoir by the tributaries. No fish kill or any adverse effect of Phoslock is mentioned.
van Oosterhout et al., 2020	It showed that after almost 10 years of Phoslock® application in lake Rauwbraken the La added was most presented in the sediment (91%), small percentage was presented in macrophytes. No fish kill or any adverse effect of Phoslock® is mentioned.
Waajen et al., 2019	Several restoration measures were implemented in Lake Kleine Melanen (Fish removal, dredging, removal trees and shrubs, construction of marsh area and sanding capping with Phoslock®). After the in-lake measures phosphorus and chlorophyll-a suffered significant reduction. Still the target of a clear water state was not completely met due the external P load caused by storm water runoff. No fish kill or any adverse effect of Phoslock® is mentioned
Bishop and Richardson, 2018	Phoslock® decreased water column total P levels and shifted mobile sediment P fractions to the residual fraction in two golf ponds. Total N/P ratios (by mass) significantly increased. Consequent changes in the algal assemblage included decreases in dominance and overall density of cyanobacteria as well as a shift away from scum-forming genera to planktonic forms. No fish kill or any adverse effect of Phoslock® is mentioned
Lürling et al., 2017	Enclosure experiment in Heesch pond showed that a combination of dredging and Phoslock® was the treatment most effected; still, Phoslock® was not able to reduce as expected the P concentration which could be connect to the presence of humic acids, however the experiment was running for 59 days. Later Dithmer et al., 2015 showed that humi acids might cause a kinetic interference so probably if the experiment were longer a further reduction in P would be expected. Also showed that the filterable La concentration was higher in the treatment with Phoslock® (However, this is not expected to be La^{3+}). Zooplankton abundance increase in all the enclosure and no adverse effect of Phoslock® was observed.
Nürnberg, 2017	The study described the application and some results from 4 Phoslock® application in Canada and gives advice that for a successful aplicaiton of Phoslock the external P lead should be smaller than the internal loading. No fish kill or any adverse effect of Phoslock® is mentioned
Epe et al., 2017	Long term (9y) monitoring showing that Phoslock® treatment in Bärensee was able to reduce total P concentration and chlorophyll-a improving water quality and allowed the lake to be used continuously for swimming and recreation acitivity. Due to the constant input of nutrients from the recreational activities small maintance applications is needed. No fish kill or any adverse effect of Phoslock® is mentioned
Waajen et al., 2017b	Phoslock® application in Lake de Kuil increased water quality and the La concentration content in macrophytes, chironomids, fish liver bone, muscle and skin. No acute and chronic effects of La accumulation were observed and human health risks are considered negligible. No fish kill or any adverse effect of Phoslock® is mentioned in the paper.

Waajen et al., 2017a	The effect of Phoslock® combined with a low dose of FeCl ₃ on macroinvertebrates were assessed by a microcosm experiment; and by evaluating the macroinvertebrate community in lake de Kuil before and after the intervention. Results show a reduction in number and taxa after one month of the application, however, after one year the macroinvertebrate numbers and taxa exceeded the pre-application situation and Gastropoda and Oligochaeta prospered. The authors affirmed that the effects one month after the treatment are most likely due to the combination of physical impacts of the use of bentonite and chemical impacts of the use of FeCl ₃ , while effects after one year are likely attributed to the shift in trophic state of the lake.
Waajen et al., 2016a	Phoslock together with fish removal reduced sediment P release and reduced chlorophyll-a concentration which established and kept a clear-water state in two urban ponds. No fish kill or any adverse effect of Phoslock® is mentioned in the paper.
Spears et al., 2016	The study evaluated 18 lakes after a Phoslock® application (some of them together with a flocculant some of them solo Phoslock). The meta-analysis showed that total phosphorus concentration decreased significantly across the lakes, also significant reduction of phosphate. In addition to reduction of summer chlorophyll-a concentration and increase in Secchi depth, relatively weak but still significant. Aquatic macrophytes species number and maximum colonization depths increased followed by a Phoslock® treatment. In general, Phoslock® application improved water quality and the authors stress the need for comprehensive pre- and post-application assessment.
Waajen et al., 2016b	Phoslock® application, together with a low dose of FeCl ₃ (as a flocculant) reduced sediment P release and effectively precipitated the cyanobacterial bloom presented in Lake de Kuil. The treatment increased secchi depth, decrease chlorophyll-a and TP, increased the macrophyte coverage. Overall, the treatment improved water quality for six summer changing the Lake to a more mesotrophic state. However, due to the constant external P load the lake the sediment P-release has gradually increased in recent years. No fish kill or any adverse effect of Phoslock® is mentioned in the paper.
Nürnberg and Lazerte, 2016	After a solo Phoslock® application in Swan lake, the TP concentration and phytoplankton biomass reduced in the year after. Secchi transparency increased and no cyanobacterial bloom nor cyanotoxin was identified. Overall, the authors observed a lack of response in the first treatment year because Phoslock® was applied late in the season (end of April and March), when the P released from the sediment was already been taken up by phytoplankton. No fish kill or any adverse effect of Phoslock® is mentioned in the paper.
Yamada-Ferraz et al., 2015	In an enclosure experiment in a reservoir, Phoslock® increased turbidity but after 12h turbidity was lower than the control. A reduction in phosphate was also observed after a Phoslock® application. An increase in phytoplankton community was also observed in the enclosure. After 29 days of treatment a reduction in richness of the benthic community was observed, the authors stressed that a natural recovery process might occur in the reservoir after the restoration period, enabling the appearance of a great diversity of organisms linked to healthier aquatic conditions. The authors also evaluate through microcosm experiments the effect on one species of zooplankton and benthic organisms, showing an effect during the treatment on <i>C. silvestrii</i> and no mortality after 984 hours. 42% of the benthic organisms died in the control which is higher than the treatment (33%), because of the high mortality rate in the control the experiment cannot conclude anything towards Phoslock® toxicity.

Dithmer et al., 2016	The study evaluated sediments from 10 European lakes treated with Phoslock®. They showed that Lanthanum phosphate mineral (rhabdophane and monazite) were formed in all of the treated lakes. Also that the ratio between La and P were above 1 and that La was vertically mixed in the sediment in a depth up to 10 cm. Phosphorus release experiment done also indicating effective control of sediment P release (2 to 9 years after the treatment).
Gunn et al., 2014	Phoslock® improved water quality in Loch Flemington (described in other study) and this paper discussed how the aquatic macrophyte community in Loch Flemington responded to Phoslock®. The Aquatic plant colonization depth increased and plant coverage of the lake bed extended as a result of Phoslock® application. However, the predominant plant was <i>Elodea canadensis</i> and species richness remains similar to previously condition. The authors discussed that the P reduction is not sufficient by itself to rapidly restore aquatic macrophyte species diversity of a lake, so the P reduction will not solve a pre-existing problem with aquatic invasive species.
Moos et al., 2014	The paper evaluate the diatoms assemblage through Paleolimnology studies in 4 system in which Phoslock was applied. The conclusion is that Phoslock reduced TP concentration in all of them and in 2 Canadian system there were a short-lived changes in the diatom assemblage while in the two Australian sites the diatom assemblage were similar to pre-application which seems that to be driven by season rainfall or climate. No fish kill or any adverse effect of Phoslock® is mentioned in the paper.
Bishop et al., 2014	Phoslock® was applied in Laguna Niguel Lake in 2011, as a result phosphorus concentration decreased, Secchi depth improved and phytoplankton diversity increased. No fish kill or any adverse effect of Phoslock is mentioned in the paper.
Novak and Chambers, 2014	The paper shows the result of two Australian river before and after a Phoslock® application. It shows that phosphorus reduced over time as a result of the treatment in both rivers. In Lower vasse river despite a TP reduction from 400 to 150 ug/L still cyano blooms persisted. The authors stressed that Phoslock® application resulted in better water quality in both periods over the period was applied and also that can provide a better environment for the establishment of macrophytes, however for certain systems other intervention to assure macrophytes transplantation might be needed for instance water level manipulation, which is the case for Canning river. No fish kill or any adverse effect of Phoslock® is mentioned in the paper.
Lürding and van Oosterhout, 2013	Lake Rauwbraken was treated with Phoslock® and a low dose of PAC in 2008 the intervention caused a reduction in phosphorus and chlorophyll-a. Also, an increase in secchi depth and water transparency. Lake Rauwbraken since the intervention has not suffered cyanobacterial blooms. No fish kill or any adverse effect of Phoslock is mentioned in the paper.
Spears et al., 2013	The paper investigated the potential for negative ecological impacts from elevated La concentrations associated with the use of Phoslock® across 16 case study lakes. Surface and bottom water peak TLa concentrations during and in the month following the application of Phoslock ranged from 0.026 mg /L to 2.300 mg/ L and from 0.004 mg/ L to 0.892 mg/ L, respectively. The authors concluded besides other thoughts that the maximum reported estimates of Phoslock® in receiving waters did not exceed the EC50 values for Phoslock reported by others and based on laboratory trials. No fish kill or any adverse effect of Phoslock® is mentioned in the paper.

Meis et al., 2013	Phoslock® was applied to Loch Flemington, and sediment cores before and after the application were collected. The paper showed that Phoslock® increased the mass of P permanently bound to the sediment (apatite bound P). Phoslock® application also caused an increase in La concentration in the sediment. The authors also discussed if a multiple smaller dose compared to a single high dose is likely to increase cost-effectiveness. No fish kill or any adverse effect of Phoslock® is mentioned in the paper.
Lürling and van Oosterhout, 2012	Lake Het Groene Eiland (clastrum) was treated with Phoslock® as a result the mean phosphate and total P concentrations were only marginally lower in the clastrum compared with the reference site. The cyanobacteria chlorophyll-a concentrations were significantly lower in the clastrum than in the reference water. The clastrum, however also experience gradual increase in P concentrations. Already after 1 year of Phoslock® application, a reapplication was needed. The authors stressed that interference with oxyanions and humic substances could negative influenced Phoslock. But these interferences were later proved to be a kinetic and given a certain time Phoslock would bind the phosphate (Dithmer et al., 2015). The authors also mentioned that external P load from birds, or groundwater rich in P could have influenced the gradual increase in P concentration. No fish kill or any adverse effect of Phoslock® is mentioned in the paper.
Lürling and Faassen, 2012	Enclosure experiment in an urban pond showed that a combination of dredging and Phoslock® was the treatment most effected. The combined treatment reduced cyanobacterial biomass, microcystin and phosphate concentration. The authors also discussed that application of Phoslock® under high humic substances needs deeper research, which nowadays this interferences was study by Dithmer et al. (2015) and showed that the interference is kinetic and does not change Phoslock® binding sites. No fish kill or any adverse effect of Phoslock is mentioned in the paper.
Meis et al., 2012	This paper studied the short term alterations in sediment composition and sediment P-fractions based on sediment cores taken 2 days before and 28 days following a Phoslock® application in Clatto reservoir. After the intervention La concentration increased in the sediment. There was an increase in residual P fraction but the P mobile did not vary significantly over a period of 28 days, it is also arguable that 28 days is still too short to see a difference in the P_{mobile} as the P is not yet available to be bound and over time the change will occur as observed in other field applications. Experimental P adsorption studies showed that Phoslock® can increase the P-sorption capacity of the sediments.
van Oosterhout and Lurling, 2011	Phoslock® and a low dose of PAC was applied in Lake Rauwbraken. The intervention reduced P and cyanobacterial biomass, also improved water transparency. They showed that right after the application <i>D. galeata</i> disappears from the water column for at least 3 months. However, after 3 months daphnids recovered to similar abundance than before the treatment. The authors discussed that several factors may have caused the disappearance of <i>D. galeata</i> following this treatment, for instance physical effects due to flocks (created by the addition of PAC), grazing inhibition by flocks and clay, very low food concentration and absence of predation refuge.
Robb et al., 2003	Phoslock® was applied in Vasse river and Canning River. In Vasse River, P and chlorophyll-a reduction were observed, however in Vasse River those changes were less clear. The authors stress that Phoslock® effectively intercepts the P released form the sediments and can strip phosphate from the water column, however, it will only work in a longer term in combination with external source control. No fish kill or any adverse effect of Phoslock® is mentioned in the paper.

Liu et al., 2009	Document not available
Kaczorkiewicz and Heese, 2008	Document not available

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