Cleaning up Pesticide Contaminated Water using Landguard™ Enzyme Technology

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EXECUTIVE SUMMARY

Orica Watercare has recently launched Landguard™, a new enzyme based technology that assists with the management of pesticide residue in water. In minutes, Landguard™ products are capable of reducing pesticide concentrations in water to levels that can take many years to occur naturally. The by-products formed are both significantly less toxic and significantly more biodegradable than the original pesticide compound.

Communities and regulators are demanding greater protection of our environment. The beneficial reuse of industrial and municipal wastewater is gaining prominence as water scarcity is acknowledged. Patrons expect sporting facilities to look their best whilst curators are keen to minimise risks associated with pesticide use. Consumers are demanding that food not only be healthy, but moreover farming practices must be sustainable. Landguard™ has considerable potential to facilitate these requirements.

The technology is based on research conducted by CSIRO Entomology (Canberra) which discovered genes from insects and soil bacteria responsible for the production of enzymes capable of breaking down a range of Organophosphate (OP) insecticides.

The first product in the Landguard™ range, Landguard™OP-A was launched in September 2004 and is being used commercially for treatment of used organophosphate insecticide livestock dips.

The performance of Landguard™OP-A has been established in a number of field trials including the treatment of waste livestock dips, spray equipment washings and contaminated irrigation run-off. Key results from the trials were a reduction in organophosphate levels of 90–99% within 30–60 minutes.

Orica and CSIRO have expanded the research program to develop and commercialise a suite of enzymes to target a broader range of pesticides classes including insecticide, herbicides and fungicides. Orica is planning to evaluate and commercialise the technology in other applications in urban, rural, commercial and municipal sectors.

The mechanism by which Landguard™ enzymes clean-up contaminated water, details of field trials and environmental implications are presented.

KEY WORDS
enzymes, pesticide, dips, rinsate, run-off

INTRODUCTION

In many circumstances pesticides are the only effective means of controlling weeds, insect, fungus, parasitic and rodent pests[1]. Application of pesticide in agriculture serves to lower the cost of production, increase crop yields, provide better quality produce and also reduce soil erosion. Pesticides are also widely used to maintain parklands and sporting facilities. Due to the toxic nature of many of these chemicals, application of pesticides also has the potential for adverse effects on human health and the environment. Known effects of some pesticides include skin irritation, sensitisation and interference with the central nervous system [2]. For years there has been vigorous debate as to the long term effects of pesticides and evidence is mounting that some pesticides maybe carcinogens [3] or endocrine disruptors[4].

Pesticides contamination of water occurs in any number of ways. Wind can cause off-target or off-farm movement of pesticide. Pesticide spray drift and the movement of contaminated soil from a field into the atmosphere can contaminate surface waters once these pesticides have returned to the ground as rain. Using excessive amounts of chemical on porous soils where there are shallow water tables can allow pesticides to leach into ground water. The disposal of contaminated wastewater generated when cleaning pesticide spray equipment, contaminated irrigation return flow and rainfall run-off are other potential sources of environmental and water contamination[3].

Changes in legislation, Integrated Pest Management (IPM) and genetically modified crops are serving to eliminate, substitute or reduce pesticide use. However, population growth, pesticide resistance and economic factors strongly suggest the continuation of, and possible growth in, pesticide use. The view of the US National Research Council is that chemical pesticides will continue to play a role in pest management for the foreseeable future, in part because the benefits of pesticide usage are high relative to risks or there are no practical alternatives [3].

Treatment of pesticide-contaminated waste is an approach that could assist to reduce the impact of pesticides on the environment. In 1976 Munnecke[4] first proposed the use of enzymes as a possible treatment method for pesticides. Nearly 30 years on and the first enzyme product for such use has been commercialised. Launched in September 2004, the first of the Landguard™ range contains as the active ingredient an Organophosphate-degrading enzyme (Landguard™OP-A). Identification of the *opd*A gene and characterisation of the Landguard™OP-A enzyme were performed by CSIRO Entomology[5] under a collaborative research agreement also involving Horticulture Australia Limited and Orica Australia Pty Ltd.

Pesticide Residue Degradation

Natural Degradation

Even the most persistent organic compounds will eventually breakdown in nature. Natural breakdown is achieved via a variety of mechanisms including microbial degradation (aerobic and anaerobic bacteria), photolysis (degradation by sunlight) and phytolysis (degradation by plants), each of which can produce different by-products. In some cases, the by-products formed via natural degradation can be more toxic than the original pesticide. Further, natural degradation can be a lengthy process meaning the pesticide compounds remain in the environment for extended periods.

Enzymatic Degradation

Enzymes are proteins that catalyse or accelerate naturally occurring biochemical reactions. The catalytic nature of the enzyme is such that a small amount can treat a relatively large volume of water. Landguard™ enzymes accelerate the breakdown of pesticides via a naturally occurring hydrolysis reaction, that is a reaction between pesticide and water.
Figure 1. shows the mechanism by which enzymes breakdown pesticides.

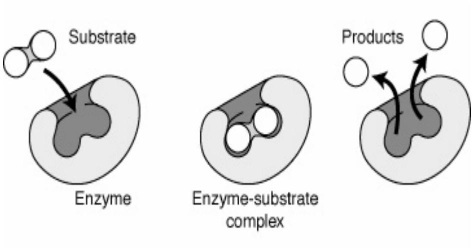

Figure1. Enzymatic pesticide degradation.

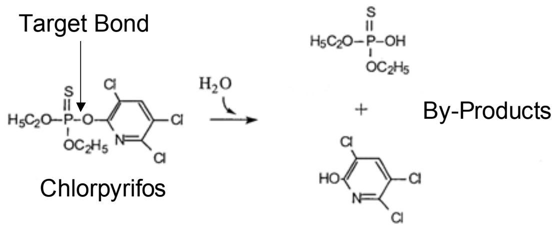

Figure 2. Chlorpyrifos hydrolysis reaction.

The reaction occurs when pesticides attach to enzymes to create enzyme–pesticide complex. A specific chemical bond within the complexed pesticide is weakened allowing water to attack and a break the pesticide bond. Many organophosphate insecticides share a common chemistry, notably a phopso–ester bond containing phosphorous and oxygen. Landguard organophosphate degrading enzyme (Landguard™OP-A) is very specific and will only attack this bond (Figure 2). This means that the by-products formed can be predicted with certainty. Importantly, in all cases the by-products are significantly less toxic and more readily biodegradable than the original pesticide.

Field Evaluations

Case Study 1. Treatment of Contaminated Spray Equipment Rinsate

A trial to establish the efficacy of the Landguard™ enzyme technology to reduce pesticide residue levels in water generated during the cleaning of pesticide spray equipment. The work was undertaken at the Tatura (Vic) research station of the Department of Natural Resources and Environment (NRE) Institute for Sustainable Irrigated Agriculture. A 600 L solution of 325 ppm methyl parathion was prepared using Folidol M500™ at the application rate recommended for pome fruit (65 mL/100 L). The solution was recirculated inside the tank (1,600L) of the pesticide sprayer (Figure 3) for 15 minutes. The entire volume was then sprayed over the designated area of the orchard (Figure 4).

Figure 3: Silvan pesticide applicator

Figure 4: Pesticide spraying at NRE

After application, the spray rig returned to the preparation area where the inside surface of the tank was rinsed by hose using 100L of water. The rinsate was recirculated inside the vat for ten minutes by operation of the recirculation pump.

Prior to the enzyme addition, a small quantity of water conditioner was dissolved in the rinsate. A Landguard™OP-A liquid concentrate was then prepared by adding 100 mL of water to the equivalent of a Landguard™OP-A rinsate treatment pack (contains 12.5g of Landguard™OP-A). The liquid concentrate was then added directly to the mixed spray tank and samples of the treated rinse water taken over a 60 minute period. The treated rinsate was subsequently transferred to a waste storage tank. Pre and post-treatment rinse water samples were sent to an external laboratory accredited by NATA for pesticide analysis and methyl parathion residue levels determined. The results obtained are shown in Figure 5 and Table 1.

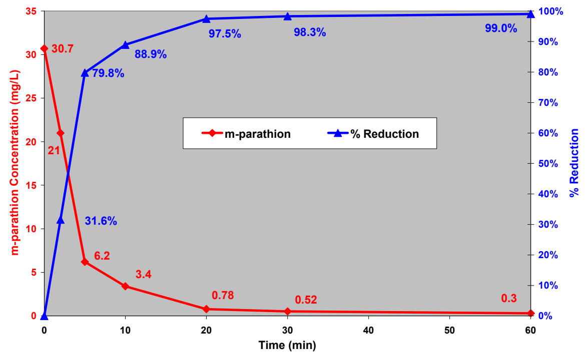

Figure 5. Reduction in residue levels.

Toxicity and Bio-degradability Comparison, m-parathion & Landguard by-products:		
Compound	Toxicity, Rat LD_{50} (mg/kg)	Bio-Degradability ($T_{1/2}$ -days)
m-parathion	3	40 days
by product	202	7 days
	∗98% reduction in toxicity	∗80% increase in bio-degradability

Table 1. Comparison of m-parathion and by-product toxicity.

Figure 5 shows the addition o f the Landguard™OP-A enzyme resulted in a rapid reduction in methyl parathion concentration from 30.7 to 0.3 ppm, a 98% and 99% reduction in 30 and 60 minutes respectively.

Table 1 illustrates the toxicity of m-parathion and the reduction in toxicity achieved by enzymatic degradation. The Rat LD_{50} is an indicator of human toxicity. It indicates the amount of a chemical that when ingested is lethal to 50% of a group of test animals and is expressed as a function of body mass, i.e mg/kg. Unintentional exposure to pesticides with an acute dose is rare, but issues from chronic long-term exposure can have effects such as allergies, headaches, etc. For comparison purposes, parathion toxicity in comparable to that of morphine [3].

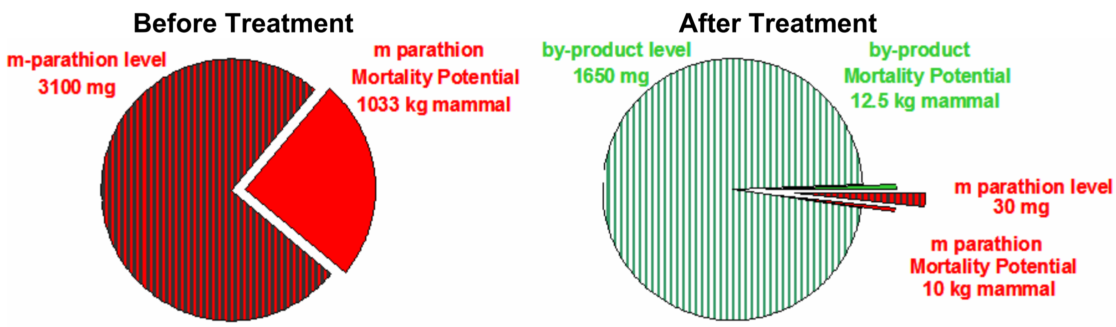

Figure 6. Environmental and Toxicity Implications of Landguard™ Treatment.

Given the volume and concentration of rinsate prior to treatment (100 L @ 30.7 mg/L respectively), the rinsate contained 3070 mg m-parathion. At these levels:

- ingestion of 0.5 L of rinsate by an infant could deliver a lethal dose
- ingestion of 7 L by a 75 Kg adult could deliver a lethal dose
- untreated rinsate constitutes a potentially serious human health issue, especially with long term chronic exposure.

As shown in Figure 6, Landguard™ treatment reduced m-parathion concentrations to 0.3 mg/L and overall m-parathion levels to 30 mg. The ingestion of this quantity of active is much less likely to result in any acute or chronic effects. The quantity and toxicity of the by-products formed is such that their formation introduces a small increase in mortality potential. However the greater biodegradability of the by-product reduces risk.

Case Study 2. Treatment of Used Livestock Dip

Sheep are routinely dipped in pesticide solutions to control lice and blowfly infestation. Dip disposal currently involves dumping onto a flat, designated area of land away from watercourses. Orica working with Time Animal Health Pty Ltd (TAH) and other contract dippers performed several trials involving Landguard™OP-A treatment of used dip. An example of a TAH plunge dip rig and shown in Figure 7 and an outline of dip process is given below.

Figure 7. Mobile dipping unit designed, fabricated and operated by Time Animal Health.

A 100 ppm solution of diazinon (an organophosphate insecticide widely used for livestock pest control) is prepared (6,000L) and sheep directed up the ramp (right) and into a cage suspended above the dip tank (centre). The cage is closed and lowered, thereby fully immersing the sheep in the diazinon dip. The cage is raised, the exit gate opened and the sheep moved into the holding pen (left). Drips from the wool fleece are captured and returned back to the dip tank. After a few minutes the exit gate of the pen is opened and the sheep walked down the ramp (far left). The dip is regularly topped up with diazinon and water to maintain a concentration in the order of 100 ppm diazinon.

A number of trials to establish the efficacy of the Landguard enzyme technology to reduce pesticide residue levels in waste diazinon insecticide dip generated during sheep delicing programmes. The data presented was obtained from work undertaken at a sheep farm in Loxton, South Australia.

After a days dipping, a liquid enzyme concentrate was prepared by dissolving the equivalent of 3, 100 g Landguard™OP-A dip treatment packs in 20 L of water. The liquid concentrate was poured over the surface of the dip and mixed into the dip solution by repeatedly raising and lowering the dipping cage for 2 minutes. Samples of treated waste dip were taken from the middle of the tank over a 60 minutes period to determine diazinon concentration as a function of treatment time.

Dirt, wool grease and other solids on sheep wash off and accumulate in the bath. The solids, known to absorb pesticides, settling out quickly and thus good mixing is essential for effective treatment. The solid fraction of the treated dip sample taken 60 min after treatment was analysed for total dissolved solids, total clay content, total organic matter and diazinon levels within the solids phase.

The samples collected were analysed by an external laboratory accredited by NATA for pesticide analysis. The results obtained are shown in Figure 8 and Table 2.

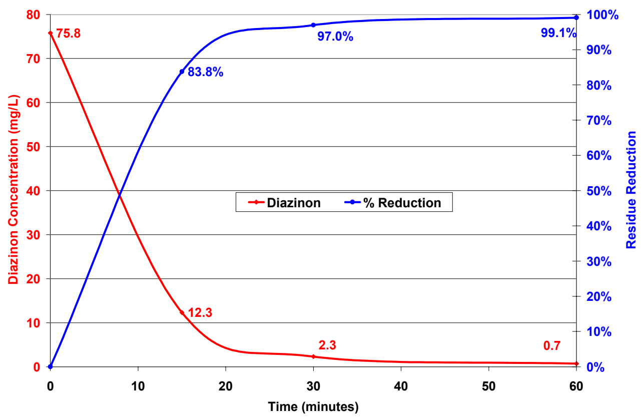

Figure 8. Reduction in diazinon concentration

Diazinon concentration in waste sheep dip (mg/L and mg/Kg)		% Reduction
Un-treated dip liquor	75.8	n.a
Treated dip liquor, 60 min	0.08	>99 %
Un-treated dip solids	110	n.a
Treated dip solids, 60 min	<0.05	>99 %

Table 2. Reduction in pesticide residue and toxicity achieved by Landguard™ treatment

The addition of 50 g of Landguard™OP-A per 1000 L waste dip reduced diazinon concentrations in the liquid phase by over 99% to 0.08 ppm within 60 min and reduced levels in the solids phase from 110 to <0.05 mg/kg. The by-products that were formed are at least 200 times less toxic to fish than the original pesticide.

The environmental implications of Landguard™OP-A dip treatment were further investigated. A preliminary study was performed to determine the fate of pesticide in soil when untreated dip is disposed of to land. Soil samples were taken from an area of the Loxton farm dedicated to disposal of waste diazinon dip. Soil samples were taken at depths of 0 – 100 mm and 100 – 200 mm and were tested to determine diazinon levels.

Diazinon concentrations in soil at a depth 0 – 100 mm was found to contain 3.31 mg/kg and 0.26 mg/kg. The woolgrower at the Loxton property reported that at least two years had passed since used diazinon dip had been discharged onto the dedicated area. The result illustrates the ability of soil to adsorb diazinon and that the disposal of untreated and concentrated waste dip can result in a highly contaminated soil.

Case Study 3. Treatment of contaminated Irrigation run off

A demonstration of the efficacy of the Landguard™ to reduce pesticide residue levels in fast flowing irrigation run-off was performed. The data presented was obtained from work undertaken on a cotton field in Narrabri NSW. The Auscott Ltd farm was chosen due to its irrigation channel system in which rain and irrigation run-off drain from the field, through a common point, back to the water source (Figure 9). The common point was important in that it facilitated treatment of all run-off with a single treatment system.

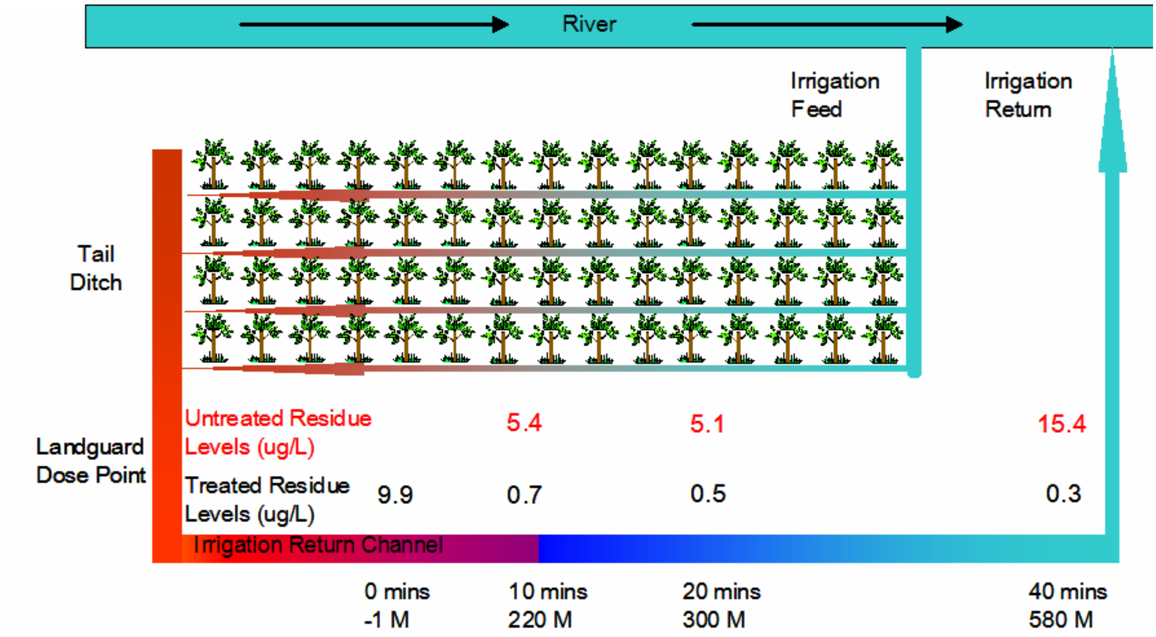

Figure 9: Layout of the cotton farm and m-parathion residue levels observed pre and post treatment.

Methyl Parathion 500™ was sprayed over 17 hectares of cotton at a rate of 2 L/ha. Twenty hours later, the field was flood irrigated. Nine hours into irrigation, appreciable flows within the irrigation return channel were observed, a timer activated (time t = 0 min) and sampling of the irrigation return water commenced. Untreated irrigation return was sampled at times t = 10, 20, 40 minutes over which time the run-off had travelled approximately 220, 300 and 580 M respectively along the return channel relative to the Landguard™ dose point.

Residue treatment commenced at t = 10 minutes. Landguard™OP-A treatment was achieved by preparing 8 L of a 0.76 g/L enzyme solution. The solution was then dosed into the irrigation return using a small pump at a rate proportional to the run-off flow rate. At a dose rate of 6 L/min and a run-off flow rate of approximately 49 L/s, the run-off was dosed for 28.5 minutes in which time some 84,000 L was treated. Treated run-off was sampled at the same sample locations used for untreated run-off at times corresponding to 0 (untreated), 10, 20 and 40 minutes post treatment.

As shown in Figure 6, appreciable residue levels were detected in untreated run-off. Residue levels were observed to vary considerable, i.e. between 5.1 and 15.4 ppb over a 40 minute period. Immediately prior to Landguard™ dosing, samples of run-off contained a residue level of 9.9 ppb. Within 10 minutes of enzyme addition, residue levels in treated run-off were measured at 0.7 ppb, a 93 % reduction in m-parathion levels. Samples taken 20 and 40 minutes after enzyme addition contained 0.5 and 0.3 ppb.

The intent of the Narrabri trial was to prove the concept that an OpdA-containing extract could effectively degrade methyl parathion in fast flowing irrigation run-off at low concentrations in the ppb level initially. The results indicate that pesticide-degrading enzymes can significantly reduce residue levels and might constitute a useful technology for the treatment of irrigation run-off. Orica is developing novel delivery systems for this application to ensure efficacy and a user friendly and cost effective solution for this critical issue.

Conclusion

The first product of the Landguard™ range, Landguard™ OP-A, has been commercialised.

The performance of Landguard™ OP-A has been evaluated in the treatment of contaminated water generated in livestock dip, equipment rinsate and irrigation run-off

Treatment of a waste livestock dip containing a liquid phase at 75.8 ppm and a solids phase at 110 ppm diazinon with Landguard™ OP-A at dose rate of 1g / 100 L resulted in a 99% reduction in liquid phase and solid phase levels within 60 minutes.

Treatment of spray equipment rinsate at 30.7 ppm methyl parathion with Landguard™ OP-A at dose rate equivalent to 12.5 g / 100 L resulted in a 99% reduction in 60 minutes.

Treatment of irrigation run-off from a cotton farm with a Landguard™ OP-A solution reduced m-parathion levels from 9.9 ppb to 0.7 ppb within 10 minutes.

These outcomes demonstrate that Landguard™ OP-A can achieve rapid and extensive degradation of the target organophosphate actives, diazinon and methyl parathion.

The work detailed here is a small part of a much larger program aimed at sourcing and commercialising enzymes against a broad range of insecticide, herbicide and fungicide actives.

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