Landguard™ OP-A - What is this novel technology?

Introduction

Landguard™ OP-A is a simple, cost-effective and reliable tool for the treatment of pesticide contaminated soil and water.

Landguard™ OP-A results in the rapid breakdown of most commonly used organophosphate pesticides. Landguard™ OP-A has been developed for the treatment of pesticide contaminated soil, irrigation run-off, effluent from agricultural processors, post harvest and stock dips, used pesticide containers and contaminated solutions arising from the washing of pesticide application equipment.

The use of Landguard™ OP-A for the treatment of organophosphate-contaminated soil and water will provide significant benefits to agriculture, the general public and the environment, due to significantly lower concentrations of these pesticides entering the environment, reduced costs associated with meeting strict regulatory requirements, maintaining access to important pesticide groups and the reduced chance of human exposure to pesticide contaminated solutions.

Background

What is Landguard™ OP-A?

Landguard™ OP-A's active ingredient is an enzyme that rapidly degrades most commonly used organophosphate pesticides, such as chlorpyrifos and diazinon. The product can be used to degrade pesticides in contaminated water and soil, and is being developed for use in treating residues on agricultural produce. Landguard™ OP-A will help to reduce the adverse effects these pesticides can have on the environment, human health and neighbouring industries such as aquaculture.

Further products are being developed that have activity on a range of pesticide classes including synthetic pyrethroids, triazines, phenyl ureas, carbamates, neonicotinoids, glyphosate, glufosinate and organochlorines.

What is an enzyme?

An enzyme is a natural organic substance (protein) produced by living organisms. Enzymes act as catalysts to speed up chemical reactions. There are many different types of enzymes, each one affecting a different chemical reaction. The enzyme within Landguard™ OP-A speeds up the natural breakdown (hydrolysis) of certain organophosphate pesticides.

Enzymes are used in a variety of ways; the most important uses include manufacturing of food and stock feed, cosmetics, medicinal products and as tools for research and development. Enzymes are also used in detergents, pulp and paper production, in textile manufacturing, leather industry, for fuel production and for the production of pharmaceuticals and chiral substances in the chemical industry.

How does Landguard™ OP-A degrade pesticides?

The Landguard™ OP-A enzyme results in the rapid conversion (hydrolysis) of an organophosphate pesticide into two compounds that are significantly less toxic (see Figure 1 and Tables 3 and 4).

For example,the two diazinon breakdown products resulting from the hydrolysis of diazinon are diethyl thiophosphoric acid and 2-isopropyl-4-methyl-pyrimidin-6-ol. These two breakdown products have been confirmed in laboratory trials and Landguard™ OP-A treated samples collected from field trials, in solutions with low and high pH.

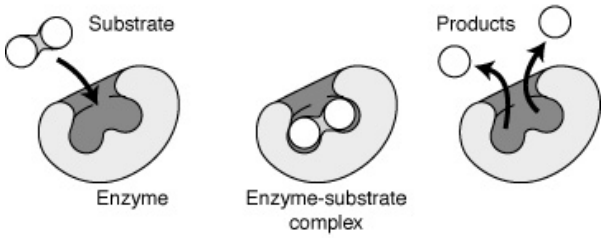

Figure 1. Enzyme catalysed hydrolysis of diazinon (substrate) to form diethyl thiophosphoric acid and 2-isopropyl-4-methyl-pyrimidin-6-ol (studies conducted by Queensland Department of Health and the Central Science Laboratory, York, UK).

Why should farmers use a product such as Landguard™ OP-A that protects the use of highly regulated and toxic pesticides such diazinon or chlorpyrifos?

There has been a marked decrease in the rate of introduction of new pesticides in recent years and this situation is unlikely to improve over the medium term. Many pesticides are also facing tighter restrictions or de-registration due to concerns over their safe use.

Existing pesticide groups must therefore be used carefully so as to prevent the development of pesticide resistance and prevent adverse environmental impacts that may necessitate their de-registration or the implementation of tighter restrictions.

The organophosphate pesticides are a highly effective, broad spectrum and costeffective group of insecticides. However, their use has been found to result in adverse impacts on the environment. Landguard™ OP-A will help minimise these adverse impacts and therefore reduce the need for tighter control over their use.

How was Landguard™ OP-A developed?

The active ingredient within Landguard™ OP-A is the opdA enzyme. This enzyme was isolated from a natural soil bacteria (*Agrobacterium tumefaciens*) collected from soil exposed to high levels of organophosphate pesticides. The bacteria had naturally evolved to utilise organophosphate pesticides as a source of energy.

Who developed Landguard™ OP-A?

Landguard™ OP-A has been developed by Orica and CSIRO. CSIRO, the Commonwealth Scientific and Industrial Research Organisation, is Australia's national science agency and one of the largest and most diverse research agencies in the world.

A number of Australian industry and farm organisations have provided funding for the development of Landguard™ products including: the Cotton Cooperative Research Centre, Sugar Cooperative Research Centre, Rice Cooperative Research Centre, Cotton Research and Development Corporation, Rural Industries Research and Development Corporation, Horticulture Australia, Australian Wool and the Australian Centre for International Agricultural Research.

Research has been conducted by a number of organisations around the world including:

- Agentase (USA), Hydrophilix corporation (USA), University of California, Stewart Agricultural Research Services (USA), CURES
- Central Science Laboratory (UK), CEMAS (UK), Orica Watercare Europe, RCC Ltd (CH)
- University of The Philippines
- Chung-Hsin University, Taiwan
- Orica, CSIRO Entomology, CSIRO Textile and Fibre Technology, Australian Centre for International Agricultural Research, Analytical Consulting Services, Agrifood Technology, Entox (Queensland Health), Agrisearch, Serve-Ag, Ecotox Services Australasia, RMIT University, and Virbac Pty Limited

Who is Orica?

Orica is a leading publicly-owned Australian company with a proud tradition of leadership, innovation, quality and safety.

Orica is one of the top 50 companies listed on the Australian Stock Exchange. The company has been in business for over 130 years, growing from a supplier of explosives to the Victorian gold fields in Australia into a multi-billion dollar company that supplies an extensive range of products and value-adding services. Our products, brands and services can be trusted for their reliability, range and quality.

Orica operates through four business platforms – Mining services, Chemnet, Chemical services and Consumer products. Each is a leader in its chosen markets and enjoys a world-class reputation.

Use of Landguard™ OP-A

What are the potential applications and uses of Landguard™ OP-A in agriculture?

Landguard™ OP-A has been developed for the treatment of contaminated soil, irrigation run-off (Table 1), effluent from agricultural processors, post harvest and livestock dips, used pesticide containers and contaminated solutions arising from the washing of pesticide application equipment.

Landguard™ OP-A is also being developed for use on crops or as a post harvest dip to remove pesticide residues on the surface of agricultural produce. Further trials are required before this use will be recommended.

Table 1. Residue Levels of Chlorpyrifos in Alfalfa irrigation run-off following treatment with Landguard™ OP-A. Trial conducted by CURES during January 2007 in California. Landguard™ OP-A treated water samples were collected 4, 8 and 12 minutes after Landguard™ OP-A was dosed into the water flow. Landguard™ OP-A treated irrigation run-off water was collected from the site (treatment 4) for toxicity testing. No toxicity was observed on Ceriodaphnia (chronic and acute), fathead minnow (acute and 7 days) and Selenastrum (chronic).

Treatment number	Landguard™ Rate (ounce/100 gallons)	Sample description	Residue, ug/L (average of 3 samples)
1	-	Control sample taken prior to Landguard™ Dosing unit	9.8
1	0.0013	Concentration 160 feet after Landguard™ dosing (4 minutes)	2.87
1	0.0013	Concentration 320 feet after Landguard™ dosing (8 minutes)	0.513
1	0.0013	Concentration 480 feet after Landguard™ dosing (12 minutes)	0.353
2	-	Control sample taken prior to Landguard™ Dosing unit	8.75
2	0.0065	Concentration 160 feet after Landguard™ dosing (4 minutes)	0.1
2	0.0065	Concentration 320 feet after Landguard™ dosing (8 minutes)	ND
2	0.0065	Concentration 480 feet after Landguard™ dosing (12 minutes)	ND
3	-	Control sample taken prior to Landguard™ Dosing unit	8.97
3	0.013	Concentration 160 feet after Landguard™ dosing (4 minutes)	0.107
3	0.013	Concentration 320 feet after Landguard™ dosing (8 minutes)	ND
3	0.013	Concentration 480 feet after Landguard™ dosing (12 minutes)	ND
4	-	Control sample taken prior to Landguard™ Dosing unit	9.29
4	0.065	Concentration 160 feet after Landguard™ dosing (4 minutes)	0.233
4	0.065	Concentration 320 feet after Landguard™ dosing (8 minutes)	ND
4	0.065	Concentration 480 feet after Landguard™ dosing (12 minutes)	ND

ND: non-detectable. Limit of detection = 0.01µg/L.

Where has Landguard™ OP-A been used commercially?
Landguard™ OP-A is currently being developed for use in the USA and Europe and is being used commercially in both Australia and the UK

Efficacy of Landguard™ OP-A

How quickly does Landguard™ OP-A work to degrade pesticides?
The Landguard™ OP-A catalysed breakdown of organophosphate pesticides occurs very rapidly, with the bulk of the breakdown occurring in the first 5 – 15 minutes (See Figure 2).

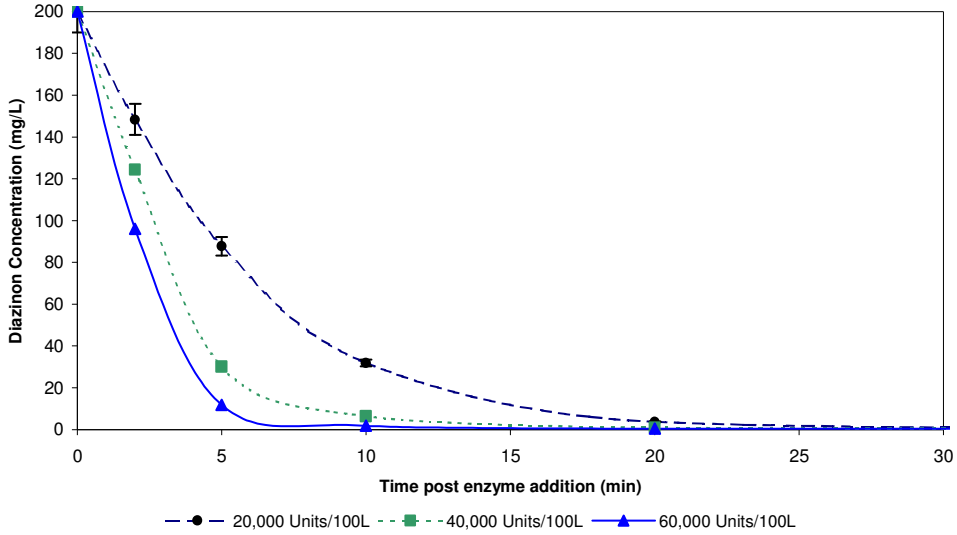

Figure 2 Degradation of diazinon over time, following the addition of Landguard™ OP-A at three application rates. 20,000 units/100L equates to approximately 0.7g product/100L. Trial conducted by Virbac Pty Ltd.

What variables affect the efficacy of Landguard™ OP-A

The primary variables influencing the efficacy of Landguard™ OP-A are:

- Dose rate of Landguard™ OP-A. Generally the higher the application rate, the faster the resulting breakdown of the pesticide.
- Treatment time. The longer the pesticide contaminated solution or soil is exposed to Landguard™ OP-A, the greater the breakdown of the pesticide. The bulk of the breakdown occurs in the first 5-15 minutes, however, further breakdown will occur for many hours or days. See Figure 2 for the rate of breakdown of pesticides over time. Landguard™ OP-A rate and treatment time can be modified to adjust the level of pesticide breakdown achieved.
- Concentration of the pesticide in the contaminated solution. Higher application rates or longer treatment times of Landguard™ OP-A are required to treat more concentrated solutions (Table 2). Economical use rates of Landguard™ OP-A will treat solutions with pesticide concentrations up to 10,000 mg/L.
- pH of the solution. The activity of the enzyme is optimal at pH's of 6-11.

- Temperature. The activity of Landguard™ OP-A is reduced at temperatures above 60°C and temperatures close to freezing.
- Mixing. Mixing may result in higher levels of efficacy.

Table 2. Laboratory study demonstrating the effect of diazinon concentration on the efficacy of Landguard™ OP-A. Higher rates or longer treatment times are required for solutions contaminated with higher concentrations of pesticides. Study conducted by Analytical Consulting Services (Study number T135).

Starting Diazinon Concentration (µg/L)	Resulting diazinon concentration (µg/L) for several Landguard™ OP-A application rates (g/L) and treatment times (hours)							
	0.0001 g/L		0.001 g/L		0.01 g/L		0.05 g/L	
	1 hour	48 hours	1 hour	48 hours	1 hour	48 hours	1 hour	48 hours
10	<0.5	<0.5	<0.5	<0.5	-	-	-	-
100	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	-	-
1,000	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	-	-
10,000	26	17	20	14	14	<0.5	-	-
100,000	37,000	51	870	<0.5	130	<0.5	19	<0.5

How do you know Landguard™ OP-A works?

Landguard™ OP-A is being sold and used successfully, and extensive laboratory and field trials conducted by Orica, CSIRO and numerous independent research organisations have demonstrated that Landguard™ OP-A consistently and reliably degrades organophosphate pesticides.

Should we not be moving away from the older pesticides such as organophosphates?

There has been a marked decrease in the rate of introduction of new pesticides in recent years and this situation is unlikely to improve over the medium term. Existing pesticide groups must therefore be used carefully so as to prevent the development of pesticide resistance and prevent adverse environmental impacts that may necessitate their de-registration.

The organophosphate pesticides are a highly effective, broad spectrum and cost effective group of insecticides. However, their use has been found to result in adverse impacts on the environment. Landguard™ OP-A will help minimise these adverse impacts and therefore reduce the need for tighter control over their use.

Environmental Impact and Fate of Landguard™ OP-A

Will Landguard™ OP-A run off the fields after treatment into surface waters or leach into groundwater?

Landguard™ OP-A is largely a mix of natural proteins and carbohydrates. These natural compounds have been shown to have minimal effect on environmental indicator species and are quickly degraded in the environment by microbial degradation, sunlight and chemical hydrolysis.

Bioaccumulation of Landguard™ OP-A is not expected given its high biodegradation potential and high water solubility.

Does Landguard™ OP-A impact on any organisms in the environment?

Landguard™ OP-A is classed as practically non-toxic to *Brachydanio rerio* (zebra fish), *Daphnia magna* (water flea) and *Scenedesmus subspicatus* (algae). The 'no observable effect concentration' (NOEC) for these three species is greater than 100mg/L. Landguard™ OP-A was found to have no inhibitory effect on activated sludge microorganisms (NOEC > 1000mg/L). Landguard™ OP-A will have a beneficial effect on the environment, due to a reduction in the adverse environmental impacts of pesticides.

Will the inert ingredients within Landguard™ OP-A impact on the environment?

All toxicity, environmental and physical properties testing has been conducted with the final freeze dried product, which includes the active enzyme plus all inert ingredients. This testing therefore evaluated both the inert ingredients as well as the active enzyme.

No chemicals are added to the final ferment media. The final freeze dried Landguard™ OP-A product contains the active opdA enzyme and debris from the bacterial cells that are used to manufacture the enzyme. This cell debris is made up of naturally occurring organic molecules such as carbohydrates, water and proteins (Table 3). These natural compounds have been shown to have minimal effect on environmental indicator species and are quickly degraded in the environment by microbial degradation, sunlight and chemical hydrolysis.

Table 3 Composition of freeze dried Landguard™ OP-A for a typical batch of product.

Property	**Specification**		**Results**
	Min.	**Max.**	%
Composition			
Ash	1.3	1.7	1.28
Carbohydrates	40	50	47.4
Chloride	0.60	0.70	0.58
Fat (by acid hydrolysis)	3	5	2.8
Magnesium	0.045	0.065	0.062
Moisture	3	5.5	3.3
Protein(As is Kjeldahl)	40	50	48.4
Total free sugars	32	42	41.05
Total Dietary Fibre	0.7	2.0	1.2
Zinc	0.035	0.055	0.045

Do the breakdown products for diazinon or chlorpyrifos created by Landguard™ OP-A treatment impact any organisms in the environment?

The breakdown products are significatly less toxic than the original pesticide (see Tables 4 and 5). The same breakdown products are created naturally when organophosphate pesticides are present in the environment, however, Landguard™ OP-A greatly speeds up the conversion of these pesticides into these less toxic compounds.

Table 4. LC 50, trout (mg/L) of diazinon and diazinon's two hydrolysis breakdown products (source: Assessment of environmental properties and effects of pesticide transformation products, final report December 2002 Cranfield Centre for Ecochemistry, Ecotox services toxicity assessment report and US EPA ECOTOX web site).

	Diazinon	Diethyl thiophosphoric acid	2-isopropyl-4-methyl-pyrimidin-6-ol
LC 50 Bluegill (Lepomis macrochirus) 96 hour (mg/L)	0.12	100	1,200
LC 50 D. magna 48 hour (mg/L)	0.0005	100	-
LC 50 Ceriodaphnia dubia 48 hour (mg/L)	0.0004	-	576.2
LC 50 Trout (mg/L)	0.53	100	1200

Table 5. Toxicity of chlorpyrifos and chlorpyrifos' two hydrolysis breakdown products.

	Chlorpyrifos	Diethyl thiophosphoric acid	3,5,6 trichloropyridin-2-ol
LC 50 fish 96 hour (mg/L)	0.041	-	1.5
EC 50 Daphnid 48 hour (mg/L)	0.0006	100	-
Long term fish toxicity (EU chlorpyrifos review, 3 June 2005)	35d NOEC = 0.00014 mg/L	-	31d NOEC = 0.0808 mg/L
LD 50 rat oral (mg/L)	149	-	800

What happens to Landguard™ OP-A after the pesticide is broken down?

After Landguard™ OP-A has completed its job, it is readily broken down in the environment by microbial degradation, sunlight and chemical hydrolysis. Landguard™ OP-A is classified as readily biodegradable (10% degradation at approximately 1 day and 95% degradation after 28 days). Bioaccumulation of the product would not be expected given its high biodegradation potential and high water solubility.

The half-life of the OpdA enzyme in sterile conditions was found to be 0.062, 13.8 and 4.5 days at pH 5, 7 and 9 respectively (tests conducted at 21 – 23°C). In non-sterile conditions the half-life of the enzyme would be expected to be less.

How can we monitor the Landguard™ OP-A enzyme in agricultural fields and the environment?

While traditional chemical substances are traditionally described by their molecular structure, enzymes, in contrast, are typically detected and described by functional parameters. Most analytical techniques used to determine enzyme levels involve the measurement of enzyme activity. The opdA enzyme can be detected using an ethyl parathion spectrophotometric assay. This assay measures the breakdown of ethyl parathion.

Who has conducted the toxicity testing?

The majority of the environmental and human toxicity testing was conducted by RCC Ltd, an independent toxicity testing laboratory based in Switzerland. Tests were conducted under GLP accreditation.

The toxicity data has been submitted to USA, UK and Australian authorities, which have reviewed the data and granted approval for the use of Landguard™ OP-A.

Occupational Health and Safety

Does the safe use of Landguard™ OP-A require any special precautions?
The MSDS and label should be read prior to use.

Landguard™ OP-A was found to have low acute toxicity via the oral and dermal routes of exposure and is not mutagenic or clastogenic. In a 28-day repeat dose oral study in rats the NOAEL was found to be ≥ 1000mg/kg bw/day. Acute oral and dermal toxicity studies in rats found the LD50 to be greater than 2000 mg/kg bw. No signs of toxicity were found. Based on the results of irritation studies Landguard™ OP-A is considered to be slightly irritating to skin and eyes and is considered to be a potential skin and respiratory sensitiser.

Landguard™ OP-A has a low vapour pressure (< 1.7×10^{-7} pa at 25°C), is not classed as "highly flammable" or "auto-flammable" and, although testing was not conducted, is unlikely to be explosive. Less than 0.26 % of the product was found to have a particle size less than 100μm.

Dust formation should be avoided. Avoid contact with skin, eyes and clothing. Wear personal protective equipment (safety glasses with side shields, gloves and long sleeved clothing). Ensure adequate ventilation. In case of insufficient ventilation, wear suitable respiratory equipment. A respirator should be worn if exposed to dust. Remove all sources of ignition.

Regulatory Issues

Is Landguard™ OP-A regulated or registered as a pesticide?

No, the product does not act on a pest, so it is therefore not classed as a pesticide. The US EPA classed the product as a new industrial chemical. A dossier has been submitted to the New Chemicals Notice Management Branch of the US EPA to obtain approval of Landguard™ OP-A. Approval by the New Chemicals Notice Management Branch was obtained during early 2006.

Can you apply Landguard™ OP-A directly to crops?

Landguard™ OP-A is being developed for use on crops and as a post harvest dip to remove pesticide residues on the surface of agricultural produce. Further field trials are required before this use will be recommended.

Manufacturing

How is Landguard™ OP-A produced?

Like the majority of enzymes currently available, Landguard™ OP-A is manufactured from bacteria. Manufacturing starts with large-scale fermentation to a yield a high volume of bacteria. Enzymes are produced inside the bacteria cells. The bacteria cells are then destroyed and broken open (lysed) to release the enzymes. In subsequent manufacturing steps the lysed bacteria cells are subjected to further purification processes using chemical, mechanical and thermal techniques (concentration, precipitation, extraction, centrifugation, filtration, chromatography). The resulting liquid enzyme concentrate is then dried to form a powder.

The final Landguard™ OP-A product is largely a mix of natural proteins and carbohydrates. The product also contains small quantities of natural fats, water and chloride, zinc and magnesium. No bacteria remain in the final product.

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