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# CSIRO Enzyme Based Bioremediation Technology

**Cameron Begley, Commercial General Manager**

**Colin Scott, Research Scientist**

**CSIRO**

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# About CSIRO

- Australia's major strategic and applied R&D organisation
- ~ Au\$1Billion p.a. (~70% Parliamentary appropriation/30% external)
- Ranks in top 1% of world science institutions in 12/22 fields
- Over 4000 science papers p.a.
- ~4000 granted or pending patents
- >100 successful spin-off companies

# Technical performance criteria

- **We discover & improve enzymes with modern molecular biology**
- **The prerequisites are demanding:**
  - **>2 logs detoxification**
  - **Preferably single step detoxification**
  - **No expensive co-factors**
  - low  $K_m$  ( $\mu\text{M}$  or ppm)
  - respectable  $k_{\text{cat}}$  ( $>100$  turnovers/minute)
  - broad substrate range within a pesticide class
  - bacterial expression ( $\geq 5\%$  yield as enzyme)
  - broad temperature range
  - broad pH range
  - shelf life in months
  - preferably proprietary platform IP
- **But are generally achievable with modern biotechnology**

# LandGuard™ - Background

## LANDGUARD™

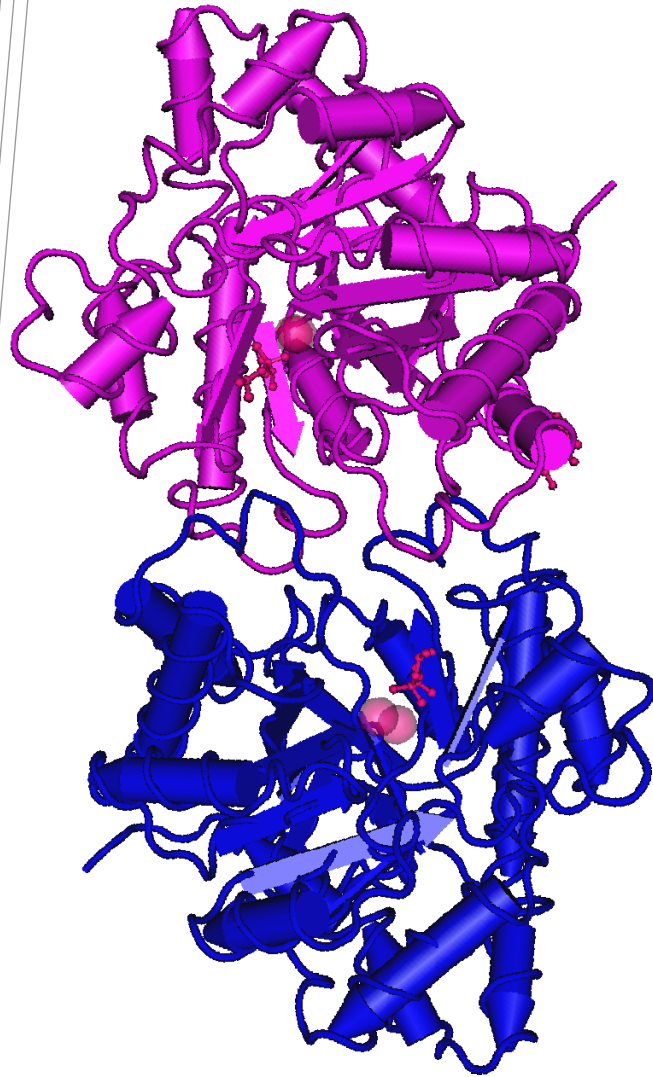
- Unique suite of enzyme technologies
- Tailored bio-remediation solutions to target specific compound classes
- Rapid acting, superior potency

## APPLICATIONS

- Potable water:
  - Municipal and point of use / entry
- Industrial water:
  - Waste water treatment for specific organic residues
- Agriculture
  - Vet pesticides remediation
  - Broad acre pesticide run off remediation
  - Commodity wash applications



# OpdA meets all the technical performance criteria



## Single step detoxification

- One step hydrolysis yielding short-lived, nontoxic products

## High catalytic efficiency

- Acts upon its best substrates at diffusion- limiting rates ( $> 2000 \text{ sec}^{-1}$ )

## Cofactor-less

- Non-diffusible metal cofactor, no added cost to enzyme production or use

## Expression and stability

- Good expression and extremely stable

## Patent position

- CSIRO proprietary

## Suitable substrate range

- Targets most major OPs in the market, except for N-alkyl (e.g. fenamiphos) and aliphatic non-vinyl OPs (e.g. dimethoate)

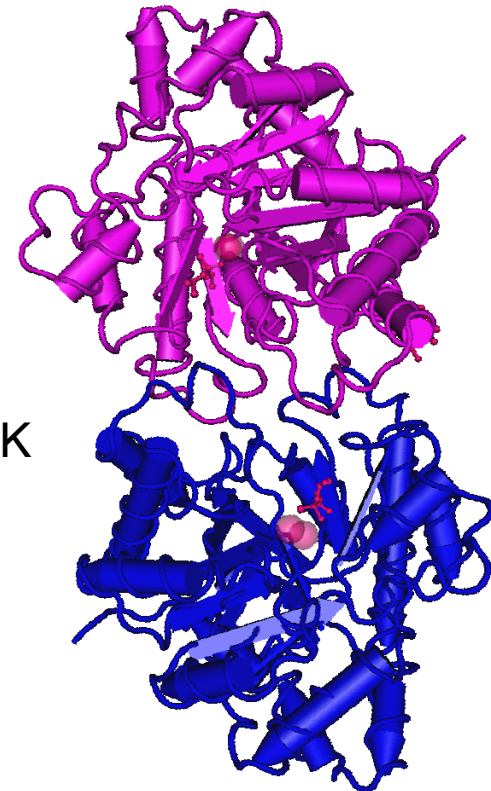
# Irrigation Run-Off Treatment

- 17 Hectares of cotton was sprayed with methyl-parathion.
- Field flood irrigated 20 hrs later
- Irrigation return channel (80,000L) treated with Landguard™ OP-A .
  - **Untreated** irrigation run-off residue levels varied between **5** and **15 ppb** over the first 40 min
  - **Treated** irrigation run-off residue levels were reduced to **0.3 ppb** prior to return to the water source
- **Repeat performance on 4000ha**



# Current Product range and status

- Landguard OP
  - Degrades a broad range of organophosphate chemistries
  - Freeze dried powder added manually (batch treatment) or via dosing system (continuous treatment)
  - Distribution partners identified in California and UK
- Landguard ZIM-A
  - Degrades fungicidal carbamates
- Landguard SP
  - Degrades a broad range of synthetic pyrethroid chemistries
- Landguard TRZ
  - Trials completed in Queensland with 1 log reduction in 4 hours in a 1 ML pond starting with 160 ppb atrazine



# LandGuard™ - Technology pipeline

- Advanced research is underway to develop similar enzymatic products for degradation of residues of other pesticides of interest:
  - Phenyl-ureas (diuron)
  - Neonicotinoids (specifically imidacloprid)
  - Insecticidal Carbamates
  - Benzimidazoles
  - Strobilurin
  - Paraquat
  - Glyphosate
  - Chlorinated hydrocarbons

## Acknowledgements:

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Thank you

For further information:

Cameron Begley  
General Manager

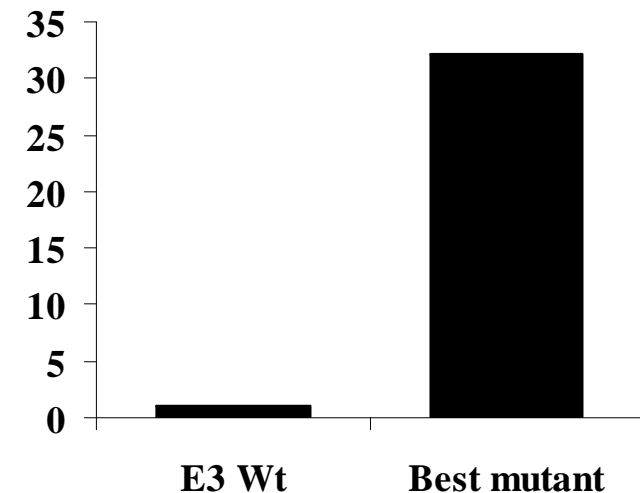
[Cameron.Begley@csiro.au](mailto:Cameron.Begley@csiro.au)



# SP performance: E3 did not initially meet all the technical performance criteria

- ✓ **Single step detoxification**
  - ✓ One step hydrolysis yielding short-lived, nontoxic products
- ✓ **High catalytic efficiency**
  - ✗ Wild-type has low activity
  - ✓ Activity improved by enzyme engineering
- ✓ **Cofactor-less**
  - ✓ No cofactor
- ✓ **Expression and stability**
  - ✓ Good expression and extremely stable
- ✓ **Patent position**
  - ✓ CSIRO proprietary
- ✓ **Suitable substrate range**
  - ✓ Targets most major SPs)
- 

**Improvement in Bifenthin degradation**



# TrzN did not initially meet all the technical performance criteria

## ✓ Single step detoxification

- ✓ One step hydrolysis yielding, nonherbicial products

## ✓ High catalytic efficiency

- ✓ Acts upon its best substrates at high rates ( $> 12 \text{ sec}^{-1}$ )

## ✓ Cofactor-less

- ✓ Non-diffusible metal cofactor, no added cost to enzyme production or use

## ✓ Expression and stability

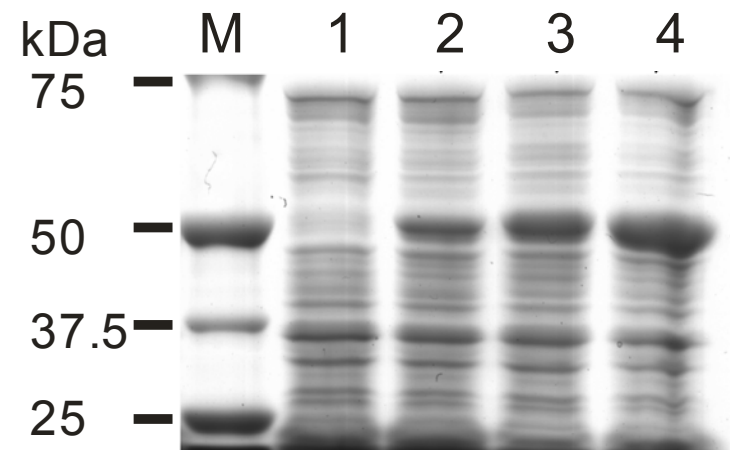
- ✗ Wild-type yields little protein
- ✓ Improved yield by enzyme engineering

## ✓ Patent position

- ✓ FTO
- ✓ Improved enzyme CSIRO owned

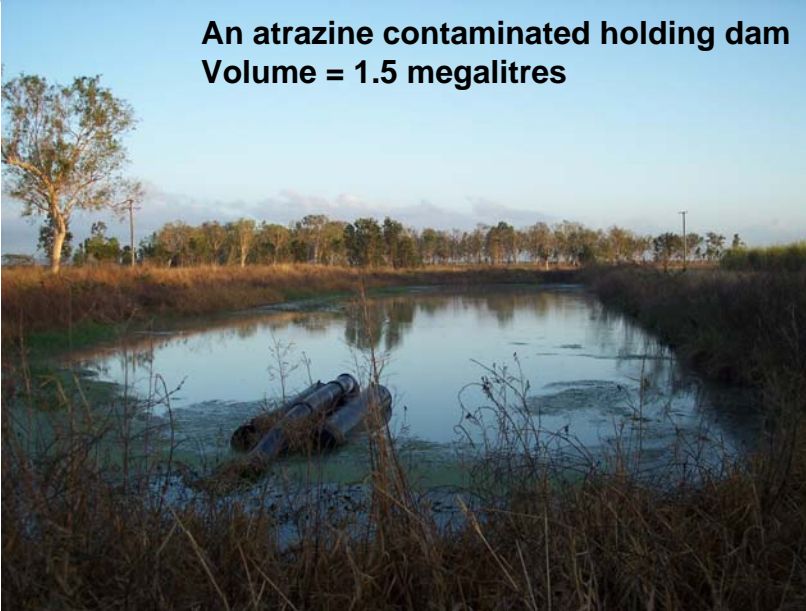
## ✓ Suitable substrate range

- ✓ Targets all major triazine herbicides

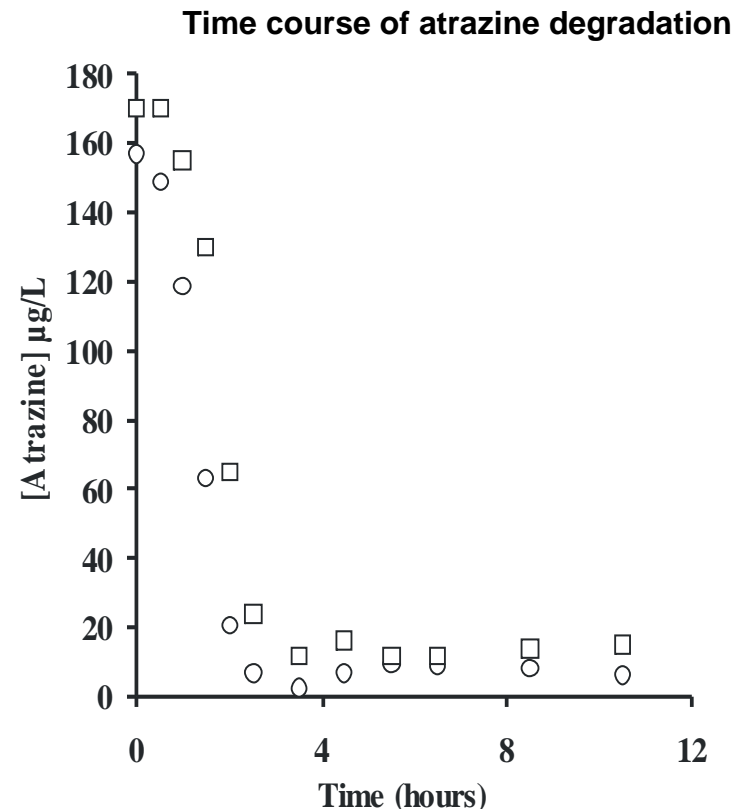


# All very good but – can we use TrzN as a bioremediant?

An atrazine contaminated holding dam  
Volume = 1.5 megalitres



Application  
of Enzyme



**>90% reduction in ~ 3 hours**

# Use Landguard OP in pesticide evaporation ponds



Addition of Landguard at low rates (200g/ML), achieves  
**>50%** reduction in profenofos

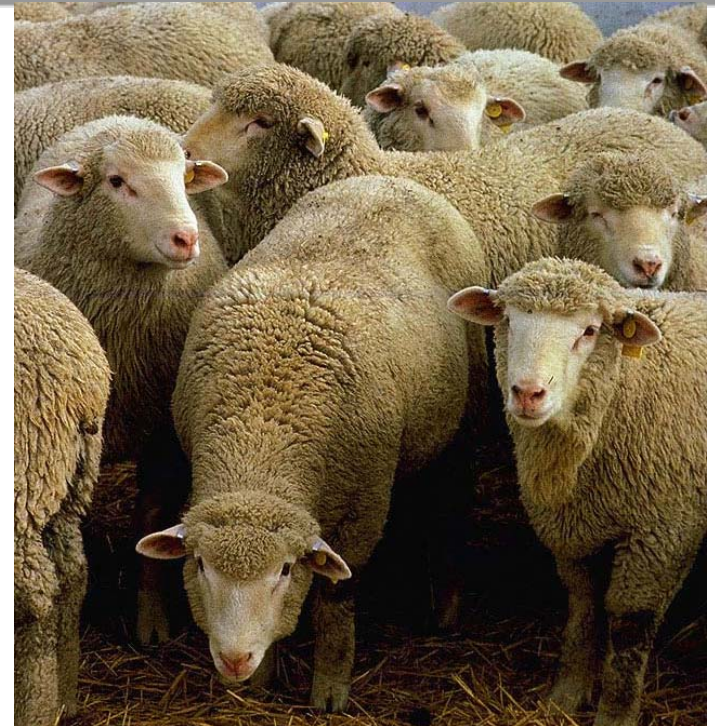
**>90%** reduction in *m*-parathion and chlorpyrifos  
**in 2 weeks**

# Treatment of contaminated spray equipment rinsate using Landguard OPA



	Methyl parathion concentration ppm and % reduction achieved by OpdA enzyme at dose rate 1g/100L						
Time (min)	0	2	5	10	20	30	60
OpdA treated	31	21(32%)	6.2(80%)	3.4(89%)	0.78(97%)	0.52(98%)	0.3(99%)

# Treatment of sheep dips using Landguard OPA



Diazinon in sludge (soil and organic matter) reduced from **90,000 to 1 ug/L** within **3 hours** following the application of Landguard OP-A at a rate of **20g/100L**

# Applications of Landguard OPA to soil following foliar pesticide applications



Landguard Dose rate g/ha	time	Diazinon Concentration in the soil (mg/kg)	Pesticide Breakdown
0	1 hr	27	
100		13.2	51%
250		6.5	76%
500		0.3	99%
500 + 1-mm rain		4.8	82%
0	7 days	34.3	
100		14.1	59%
250		1.3	96%
500		3.9	89%
500 + 1-mm rain		2.0	94%

Landguard™ OP-A sprayed onto the ground directly after foliar pesticide spraying to prevent pesticide run off into surface water following rain or irrigation.

# Using Landguard OPA for on-commodity detoxification

- Tested cucumbers, tomatoes, egg plant, bitter gourd, mangoes... (& wool)
- Tested ~10 OPs
- Low dose (2g/100L) treatment for ~ 5 minutes
- **Up to 80% OP removal**
- **But not yet consistent\***

