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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Esterase, organophosphate (Agrobacterium tumefaciens strain P230 gene opdA)

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Director
NICNAS

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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Orica Ltd (ABN 99 004 117 828) of 1 Nicholson St, Melbourne, VIC, 3000

NOTIFICATION CATEGORY
Standard: Chemical other than polymer (more than 1 tonne per year).

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication:

Chemical Composition
Introduction Volume
Identity of Manufacturer/Recipients
Manufacture Process
Enzyme Activity

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows:
Acute inhalation toxicity

As the notified chemical is an enzyme concentrate, many of the standard methods of physicochemical characterisation of chemical substances are not valid.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
CEC/619, CER/2 (renewal of permit)

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

The notified chemical is a purified non-glycosylated enzyme produced from genetically modified bacteria. The enzyme is manufactured in a liquid concentrate which is then freeze dried. Sections 2 and 3 of this report provide information on the identification of the notified chemical (OpdA enzyme) and the freeze-dried enzyme concentrate (Landguard OP-A).

CHEMICAL NAME
Esterase, organophosphate (Agrobacterium tumefaciens strain P230 gene opdA) (notified chemical)

Escherichia coli, BL21 DE3 (pET-opdA), Lysate (Enzyme concentrate)

OTHER NAME(S)
Organophosphate degrading enzyme A
OpdA
Aryldiaklyphosphatase
Phosphotriesterase
organophosphate hydrolase
phosphoric triester hydrolase

MARKETING NAME(S)
The name of the freeze-dried enzyme concentrate is Landguard OP-A

CAS NUMBER
401894-92-6 (notified chemical)

854538-40-2 (enzyme concentrate)

EC (IUB) NUMBER
The OpdA enzyme is classified by the International Union of Biochemists (IUB) with Enzyme Classification (EC) number 3.1.8.1..

MOLECULAR FORMULA
The amino acid sequence for the OpdA enzyme is shown below. The strike through section is the signal peptide of the wild type enzyme that has been removed prior to insertion into the pET vector. The section in italics is the amino acid sequence of the His tag, which is the result of gene insertion into the pET-14b vector.

MGSSHHHHHHSSGLVPRGSHM~~MQTRRDALKSAAAITLLGGLAGCASMAR~~PIGTGDLINTVRGP
IPVSEAGFTLTHEHICGSSAGFLRAWPEFFGSRKALAEKAVRGLRHARSAGVQTIVDVST
FDIGRDVRLLAEVSRAADVHIVAATGLWFDPPLSMRMRSVEELTQFFLREIQHGIEDT
GIRAGIIKVATTGKATPFQELVLKAAARASLATGVPVTTHTSASQRDGEQQAAIFESE
GLSPSRVCIGHSDDTDDLSYLTGLAARGYLVGLDRMPYSAIGLEGNASALALFGTRSW
QTRALLIKALIDRGYKDRILVSHDWLFGFSSYVTNIMDVMDRINPDGMAFVPLRVIPF
LREKGVPPETLAGVTVANPARFLSPTVRAVVTRSETSRPAAPIPRQDTER

The DNA sequence which encodes for the translated amino acid sequence is shown below. The segment in italics is the His tag region on the pET vector and the strike through is the signal peptide on the opdA wild type gene that has been removed. The wild type gene was isolated from *Agrobacterium tumefaciens* strain P230. This bacterium was collected from soil repeatedly exposed to organophosphate pesticides.

atgggcagcagc catcatcatc atcatcacag cagcggcctg gtgccgcgcg gcagccatat
~~*gatgcaaacg agaagagatg cacttaagtc tgcggccgca ataactctgc tcggcggctt*~~
~~*ggctgggtgt gcaagcatgg cccga*~~ccaat cggtacaggc gatctgatta atactgttcg
cggccccatt ccagtttcgg aagcgggctt cacactgacc catgagcata tctgcggcag
ttcggcggga ttcctacgtg cgtggccgga gtttttcggt agccgcaaag ctctagcgga
aaaggctgtg agaggattac gccatgccag atcggctggc gtgcaaacca tcgtcgatgt
gtcgactttc gatatcggtc gtgacgtccg tttattggcc gaagtttcgc gggccgccga
cgtgcatatc gtggcggcga ctggcttatg gttcgacccg ccactttcaa tgcgaatgcg
cagcgtcgaa gaactgaccc agttcttcct gcgtgaaatc caacatggca tcgaagacac
cggtattagg gcgggcatta tcaaggtcgc gaccacaggg aaggcgaccc cctttcaaga
gttggtgtta aaggcagccg cgcgggccag cttggccacc ggtgttccgg taaccactca
cacgtcagca agtcagcgcg atggcgagca gcaggcagcc atatttgaat ccgaaggttt
gagcccctca cgggtttgta tcggtcacag cgatgatact gacgatttga gctacctaac
cggcctcgct gcgcgcggat acctcgtcgg tttagatcgc atgccgtaca gtgcgattgg
tctagaaggc aatgcgagtg cattagcgct ctttggtact cggtcgtggc aaacaagggc
tctcttgatc aaggcgctca tcgaccgagg ctacaaggat cgaatcctcg tctcccatga
ctggctgttc gggttttcga gctatgtcac gaacatcatg gacgtaatgg atcgcataaa
cccagatgga atggccttcg tccctctgag agtgatccca ttcctacgag agaagggcgt
cccgccggaa acgctagcag gcgtaaccgt ggccaatccc gcgcggttct tgtcaccgac
cgtgcgggcc gtcgtgacac gatctgaaac ttcccgccct gccgcgccta ttccccgtca
agataccgaa cgatga

STRUCTURAL FORMULA
Not applicable

MOLECULAR WEIGHT
The molecular weight of the OpdA enzyme was predicted to be 40,710.37 Dalton (40.7 KDa) by in silico analysis and experimentally determined to be 40,947.7 Dalton (Kotsonis, 2004).

SPECTRAL DATA

ANALYTICAL METHOD	UV/visible and infrared (IR) spectrometry.
Remarks	IR and UV/vis spectra were provided for three batches of the notified chemical.
TEST FACILITY	Orica (2005a, 2005b, 2005c)

METHODS OF DETECTION AND DETERMINATION

While chemicals are described by their molecular structure, enzymes, are typically characterised by functional parameters such as specific activity, Michaelis-Menten constant (Km), turnover number (Kcat) and pH and temperature optima.

METHOD	*Determination of Specific activity (pesticide specific)* A dilute sample of the notified chemical is thoroughly mixed with a dilute sample of ethylparathion. Hydrolysis of ethyl parathion was measured spectrophotometrically by monitoring the production of 4-nitrophenol at 401nm. See section 6 for results.
Remarks	Every mole of ethyl parathion hydrolysed yields one mole of 4-nitrophenol. One unit of enzyme activity is defined as the amount that hydrolyses 1 μmole of ethyl parathion per minute at 25ºC, in 50 mM Tris.HCl pH 8.0, with a starting concentration of 100 μM ethyl parathion.
TEST FACILITY	
METHOD	*Determination of Kinetic constants Km and Kcat (pesticide specific)* Hydrolysis of coumaphos, coroxon and dMUP (O,O-dimethyl 4-methyl umbelliferyl phosphate) were monitored by measuring the formation of fluorescent products. Hydrolysis of parathion, methyl parathion and paraoxon were measured spectrophotometrically by monitoring the production of *p*-nitrophenol at 405nm. Hydrolysis of fenthion was measured spectrophotometrically by determining the loss of fenthion at 252nm. Hydrolysis of phosmet and malathion was measured by quantifying the formation of thiol groups produced during hydrolysis. Hydrolysis of diazinon and chlorfenvinphos were measured using a radiometric partition assay. All assays were performed in 50mM Tris-HCL (pH 8.0) at 25°C.
Remarks	K_m is a measure of the affinity of an enzyme for a substrate. k_{cat} represents the maximum number of molecules of substrate which can be converted into product per enzyme molecule per unit of time
TEST FACILITY	Horne et al (2002)
METHOD	*Determination of pH Optima* The rate of organophosphate hydrolysis in the pH range 3-9 was assayed using 10 μM coumaphos as the substrate. Evolution of the fluorescent product was measured over time, and rate of hydrolysis at each pH was calculated.
TEST FACILITY	Scott (2005a)
METHOD	*Determination of DNA and bacterial content* Samples of the notified chemical were plated directly onto LB agar plates supplemented with 100 μgmL^{-1} ampicillin. Colony forming units (CFUs) were counted after 16 hours growth at 37ºC. The total nucleic acid content of the samples was estimated using the spectrophometric method (absorbance at 260nm using a Molecular Devices SpectraMAX 190). The concentration of transformable DNA was determined by transforming 50 μl aliquots if Top10 chemically competent cells with 10 μl of 500 $mgml^{-1}$ heat inactivated notified chemical. PUC18 was used as a positive control, and ddH_2o as a negative control. Ten-fold serial dilutions of the transformations were plated on LB agar supplemented with 100 μgmL^{-1} ampicillin and incubated at 37ºC for 16 hours, CFUs were then counted.
TEST FACILITY	(Scott 2005b)

3. COMPOSITION

CHEMICAL COMPOSITION

The concentration of the OpdA enzyme in Landguard OP-A will be less than 10% or less than 35,000 enzyme units/g and the product will contain 40 – 50% proteins, 40 – 50% carbohydrates, 3 - 5% fat and < 1% chloride magnesium and zinc.

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS

As a result of processing of the fermentation media (Homogenisation, PEI treatment and filtration) no live modified *E. coli* are detected in the notified chemical (Orica 2005a, Orica 2005b, Orica 2005c and Scott 2005b) and there is a 99% reduction in transformable DNA and 98% reduction in total nucleic acid residues (Scott, 2005b). Transformable DNA accounts for only 0.0000001% of the total nucleic acid content. (Scott, 2005b)

4. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS

The notified chemical will be manufactured within Australia

MAXIMUM INTRODUCTION VOLUME OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS

The following volumes relate to the freeze-dried enzyme concentrate containing the notified chemical.

Manufacture

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	< 10	< 10	< 10	< 10	<10

Use within Australia

Year	*1*	*2*	*3*	*4*	*5*
Tonnes – total use within Australia	< 2	< 2	< 2	< 2	< 2

USE

Landguard OP-A containing <10% OpdA enzyme (notified chemical) will be used for the treatment of solutions contaminated with certain organophosphate pesticides. The enzyme catalyses the hydrolysis of certain organophosphates to reduce the concentration of active pesticides. Landguard OP-A will be used by users of pesticides and processors of agricultural produce, in the agricultural, turf, ornamental and pest control industries. The use of Landguard OP-A can broadly be divided into the three categories with approximate usage as follows:

Treatment of used insecticide dips used to treat insect pests in sheep and other livestock and companion animals: approximately 25% of total sales.

Treatment of pesticide contaminated wash solutions from pesticide application equipment used in a variety of industries including cotton, sugar cane, turf, ornamentals, vegetables, cereals, oilseeds, domestic and industrial pest control, fruit and vines: approximately 60% of total sales.

Treatment of pesticide contaminated solutions from the washing of agricultural produce such as fruit and vegetables or processing equipment: approximately 15% of total sales.

5. PROCESS AND RELEASE INFORMATION

5.1. Distribution, transport and storage

PORT OF ENTRY
The notified chemical will be manufactured in Australia

IDENTITY OF MANUFACTURER/RECIPIENTS
The manufacture of the notified chemical and the freeze dry process will occur in Victoria, Australia.

TRANSPORTATION AND PACKAGING
Following manufacture, the notified chemical will be packaged into a fully enclosed sterile 200 L stainless steel transfer vessel and transferred to the freeze-drying facility by road.

The freeze dried notified chemical will either be packaged in 330 mL polyethylene terephthalate (PET) jars containing 100 g, 125 mL PET jars containing 12.5 g or 250/500g foil bags. The notified chemical will be transported to distributors of the product by road, rail and air. The quantity transported will vary from 37.5 kg to 505 kg. From the distributors, the product will normally be transported by road to the end user. The quantity of Landguard OP-A transported from the distributor to the end user is expected to vary from 37.5 g to 12 kg.

5.2. Operation description

Manufacture of the liquid concentrate

Production method

The production of the OpdA enzyme by recombinant *E. coli* takes place during fermentation of the *E. coli*. The ferment media then passes through a number of processing steps, including homogenisation, centrifugation, filtration, concentration and sterile filtration. These steps are designed to extract the OpdA enzyme from the host microorganism (*E. coli*), remove unwanted biological debris, reduce the volume of the extract and remove *E. coli* to levels below the level of detection. Stabilised liquid concentrate is pumped through a final filtration train before finally flowing into the enclosed stainless steel transfer vessel. The process is largely automated and is carried out in contained systems. All genetic manipulations during preparation of the recombinant *E.Coli* involving open containers or potential aerosol formation are carried out in a class 2 biological safety cabinet.

Sampling

During fermentation samples of the *E. coli* culture are taken to monitor growth. To sample, an 80 mL Schott bottle is screwed onto the sample port. With a series of valve operations and by making use of 100-150 kPa pressure in the fermentation vessel, fermentation culture flows into the Schott bottle. The sample line is isolated, the Schott bottle containing 80 mL of culture unscrewed from the port and the lid quickly screwed on. After sampling the sample line is steam cleaned to prevent contamination. The culture is sampled up to 24 times per batch.

The first method of sampling during bioprocessing involves operating a ball valve and draining up to 100 mL of extract into a sample container. The second method involves piercing a septum with a needle and drawing off up to 25 mL of extract in a syringe. Three samples of homogenate, a sample post-dilution and a sample post-PEI treatment are taken using the ball valve method. Up to 16 samples are taken during centrifugation via a septum. Three samples are taken during depth filtration using the ball valve method. During the concentration step sampling via a septum can occur up to 6 times, and the final sterile filtered extract is sampled once, also via a septum.

Equipment maintenance, decontamination and cleaning

If a filter blocks mid-processing, the pump is stopped and valves either side of the housing are shut so as to isolate the filter. Silastic tubing is used to connect the housing drain to a container with a 0.2 µm filtered air vent. The housing is drained into the container and the contents pumped back into the tank immediately upstream. The housing is then flushed with water so as to remove residues, and the flushings recycled to the tank upstream. The blocked filter is then replaced.

Maintenance workers may be involved in the following tasks: seal lubrication, replacement of pumps and agitators, modification of pipes or pipe fittings, instrument calibration, servicing of homogeniser and centrifuges.

After fermentation the fermenter is steam sterilised at 121ºC for 1 hour and then treated with 0.1M NaOH at 60ºC for 12 hrs. Bioprocessing equipment is flushed with 0.1M NaOH at 70°C and rinsed several times. Effluent from decontamination and cleaning cycles is treated as it is transferred to bulk waste storage. Prior to being discharged to sewer the pH of the bulk waste is neutralised and samples are analysed by a NATA accredited laboratory for total dissolved and suspended solids, pH, total nitrogen, nitrate and nitrite, total kjeldahl nitrogen, ammonia nitrogen, BOD, total oxidised sulphur, total sulphide, sodium, chloride and GMOs.

Freeze-drying and Packaging

After arrival at the freeze driers, the transfer vessel is unloaded from the truck with a fork lift and wheeled into the freeze-drying chamber room. The liquid concentrate is dispensed from the transfer vessel onto trays using measuring containers via a valved outlet located at the base of the transfer vessel. The trays are then placed into the freeze-drying chamber and cooled to -30°C. Freezing takes 2-4 hours. Over a three day period a vacuum is applied and the temperature of the chamber is gradually increased. On completion of freeze-drying trays containing the freeze-dried material are inverted over drums with poly plastic liners. The majority of the product falls into the drums, to remove the remaining material the trays are scraped into the bag with a spatular. Empty trays are then transferred to the wash room and immersed and gently scrubbed in a detergent and alkaline salts wash, rinsed in clean water and then washed in 70% alcohol. Effluent is discharged to sewer.

After filling the drums the poly plastic liners are tied closed to minimise moisture entering the product. The drums containing the freeze-dried product are transferred to the packing room. The product at this stage is in loosely packed clumps. To break up these loose clumps a 5 mm sieve is placed over an empty 200 L drum with plastic liner. The product is poured into the sieve using a measuring cylinder and gently pushed through the sieve using a spatula to break up the larger particles. The desired quantity of powder is then manually weighed in a beaker and poured into the PET jar packaging with the use of a funnel. The air space above the powder in the container is flushed with nitrogen and sealed. To accommodate increased production, the freeze driers aim to have completed the installation of automated and enclosed equipment to mill and pack the freeze-dried enzyme concentrate in the near future.

End Use

Treatment of animal pesticide dips – dipping contractors

On completion of dipping a dipping contractor will open a container of Landguard OP-A and pre-dissolve the product by manually pouring the required amount of the Landguard OP-A into a container of water in a ratio of 100 g/5 L. This solution is then poured directly into the organophosphate contaminated solution and mixed. The entire contents of a container of Landguard OP-A will be used on the day of opening. The used packaging will be thoroughly rinsed and the rinsed solution added to the pesticide contaminated solution. Mixing will involve raising and lowering the dipping cage (automated process) or the use of an item such as a garden rake, which is also used during the addition of the organophosphate on commencing dipping. After at least one hour the treated solution will then be disposed of. Typically, 4,000 – 6,000 L of pesticide contaminated solution will be treated at any one time, although a maximum of 12,000 L may be treated. Therefore, the user will typically be using 200 – 300 g (600 g maximum) of the Landguard OP-A at any one time.

Treatment of animal pesticide dips – farm workers

A farm worker will use Landguard OP-A using the same methods employed by the dipping contractors.

Treatment of pesticide contaminated wash solutions from pesticide application equipment – spray contractors

A contractor will first pre-dissolve the required amount of Landguard OP-A in water in a ratio of 12.5 g/500 ml or 100 g/5 L, by manually pouring the product into a container of water. This solution is then manually poured into the organophosphate contaminated solution and mixed using the sprayers agitation system or an implement such as a garden rake. Treatment of the contaminated solution may occur in the spray tank or the pesticide contaminated solution may first be pumped to a holding tank and then treated. The entire contents of a container of Landguard OP-A will be used on the day of opening. After at least one hour the treated solution will then be disposed of. Typically, 100 – 600 L of pesticide contaminated solution will be treated at any one time. Therefore, the user will typically be using 12.5 – 600 g of Landguard OP-A at any one time.

Treatment of pesticide contaminated wash solutions from pesticide application equipment – farm workers
A farm worker will use Landguard OP-A using the same methods employed by the spray contractors.

Treatment of pesticide contaminated solutions from the washing of agricultural produce or processing equipment
The organophosphate contaminated solution will be drained into a separate holding tank. A worker will then pre-dissolve the required amount of Landguard OP-A in water in a ratio of 100 g/5 L, by manually pouring the product into a container of water. This solution is then manually poured into the organophosphate contaminated solution and mixed. The entire contents of a container of Landguard OP-A will be used on the day of opening. After at least one hour the treated solution will then be disposed of. Typically, 4,000 – 10,000L of pesticide contaminated solution will be treated at any one time. Therefore, the user will typically be using 200 – 500g of Landguard OP-A at any one time.

Research and Development
Field and laboratory efficacy studies will continue to be conducted with the notified chemical to generate data to demonstrate the use of the product to potential users. These studies involve the application of the freeze-dried enzyme concentrate to either field collected organophosphate pesticide contaminated solutions or solutions spiked with known concentrations of organophosphate pesticides.

5.3. Occupational exposure

Number and Category of Workers

Category of Worker	*Number*	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Production of liquid concentrate – production staff	6	4	5 days x 48 weeks = 240
Production of liquid concentrate – maintenance workers	3	4	12
Production of liquid concentrate – staff involved in site inspections and auditing	6	1	4
Transport of liquid concentrate	10	1.5	2 days x 48 weeks = 96
Freeze-drying and packaging	5	3.5 #	3 days x 48 weeks = 144#
Storage and transport workers	4,000	-	-
Retailers	2,400	-	-
Research and development	25	4	10
Analytical chemists	20	2	48
Users – Treatment of wash water from pesticide application equipment – farm workers.	10,000	1.5	4
Users – Treatment of wash water from pesticide application equipment – contractors.	2,500	1.5	20
Users – treatment of pesticide contaminated solutions from washing of agricultural produce or processing equipment	150	1.5	24
Users – Treatment of animal insecticide dips – farm workers.	4,000	1.5	0.5 (1 day every second year) - 1
Users – Animal dip contractors. Treatment of animal insecticide dips	30	1.5	50

Figures quoted for freeze-drying and packaging workers are per batch using the current manual sieving and packaging techniques. Milling and packaging will require automation before production volumes can be increased, as is planned for 2006. Automation of these production steps is expected in the near future.

NB. Figures quoted for other production, transport and storage workers are based on maximum production which is expected in 2009. Figures quoted for retailers and users are for worst case and plateau sales, which are expected to be achieved in 2008.

Exposure Details

Transport, storage and distribution

It is not expected that the workers involved in the transport, storage or distribution of Landguard OP-A will have any direct contact with the notified chemical, except in the case of an accident. The nature of the packaging, and the standard operating and emergency procedures minimise the likelihood of a loss of containment and worker exposure during a transport incident.

Manufacture of the Liquid Concentrate

Production Staff

The production process is largely automated and carried out in contained systems and as such exposure to the notified chemical during manufacture and purification steps is expected to be negligible. Exposure during sampling could potentially occur if sampling is not conducted correctly, through aerosols or spillage.

Exposure to the notified chemical could occur during sample analysis. Sample analysis involves sample dilution (5,000 - 50,000 fold dilution), addition of chemical reagents and absorbance measurements. Typically all samples taken during a batch are analysed for turbidity, which takes approximately 2 minutes. A protein assay takes approximately 5 minutes per duplicate sample and the enzyme activity takes approximately 8 minutes per triplicate sample.

Production staff are required to wear, as a minimum, the following protective clothing: safety boots, full length cotton pants, a lab coat, ear protection and safety glasses. For activities outside of the control room that involve touching equipment production staff wear latex gloves. During sampling workers also wear a face shield. A Class II biological safety cabinet is used for general microbial-based dealings. The testing of enzyme activity, which involves the preparation of pesticide solutions, is conducted in a fume hood.

Equipment maintenance, decontamination and cleaning

Workers may also be exposed to the liquid concentrate while changing blocked filters. Up to one mid-production filter change per 5 batches may be required. A filter housing can hold up to 20 L of extract and 40L of water is used for rinsing. Changing a filter takes approximately 30 minutes.

Decontamination and cleaning of the fermenter and bioprocessing equipment is largely automated and carried out in contained systems. Steam and sodium hydroxide denature and hydrolyse residual biological molecules and destroy bacterial cells. Therefore, operator exposure to the liquid concentrate during decontamination and cleaning of the fermenter and bioprocessing equipment is thought to be negligible.

All equipment is decontaminated using steam and sodium hydroxide solutions by production personal before being worked on by maintenance workers and as such exposure to the notified chemical is expected to be negligible.

Staff are required to wear, as a minimum, the following protective clothing: safety boots, full length cotton pants, a lab coat, ear protection and safety glasses. For activities outside of the control room that involve touching equipment production staff wear latex gloves. During changing of filters workers also wear a face shield. Heat resistance gloves are used during steam sterilisation.

Freeze-drying and Packaging

Workers involved in freeze-drying have the potential to come into contact with the liquid concentrate and freeze dried powder. Five personnel may be directly involved in the freeze-drying operation; two will dispense the liquid concentrate and two workers and a manager may monitor the operation. The same people then remove the product from the freeze-drying chamber and wash the drying trays. Two of these workers are involved in the sieving and packaging of the product.

Dermal and possible ocular exposure to drips spills and splashes of the liquid concentrate could occur during the manual transfer to the shallow drying trays and the insertion of these trays into the freeze-

drying chamber. Worker exposure during the freeze-drying process is not expected as this is conducted in a sealed unit. Dermal and inhalation exposure to the freeze-dried enzyme concentrate could occur during the filling of the drums, the washing of the trays and the sieving and packaging processes.

Workers involved in the freeze-drying and packing operation wear disposable full length overalls with hood, safety boots, dust masks, goggles and latex gloves. A local air extraction unit is positioned above the sieving and packing operation to extract any dust that may be produced from these operations. The room used for the washing of trays and equipment is installed with air extraction. All other rooms are supplied with filtered air and are operated under positive pressure.

End Use
Treatment of pesticide dips and wash solutions.
Dermal and inhalation exposure to neat notified chemical could occur during addition of the notified chemical to water. Dermal exposure to up to 2.5% enzyme concentrate could also occur during the addition of the diluted solution to the pesticide solution and to up to 0.09% during disposal of the treated pesticide solution.

With the possible exception of users from agricultural processing operations, end users should be frequent users of agricultural chemicals and be AGSAFE accredited. At the time of using Landguard OP-A these end users will be attired for the handling of organophosphate pesticides, typically protective clothing, rubber boots, hat, impervious gloves and goggles and in some cases a full face-piece respirator with combined dust and gas cartridge. Workers involved in the treatment of pesticide contaminated solutions from the washing of agricultural produce or processing equipment are unlikely to be wearing protective equipment suitable for the handling of organophosphates at the time of using Landguard OP-A, due to the lower concentrations of pesticides present in the solutions. The label for the notified chemical advises users to wear suitable protective clothing, dust mask and gloves.

Research and Development
An individual study will involve the use of less than 50 g of freeze-dried enzyme concentrate and will take less than 4 hours potential contact time with Landguard OP-A or solutions containing Landguard OP-A. Up to 20 analytical chemists may be involved in the analysis of samples. There may be up to 1000 samples analysed per year. Each sample will be less than 500ml in volume and contain traces of up to 0.5g of the enzyme concentrate. Workers involved in research and development and analytical chemistry will be equipped to handle organophosphate pesticides as per the pesticide MSDS. Overalls, gloves, and face masks/dust masks are worn. All sample preparation in laboratory studies are conducted in fume hoods.

5.4. Release

RELEASE OF CHEMICAL AT SITE
Manufacture of Liquid Concentrate
Release of the chemical into the environment from the manufacturing facility may occur via liquid, solid and gaseous discharge streams. Of the waste released, the majority is to sewer, with small amounts of solids disposed of to land fill and negligible quantities in gaseous emissions. Control measures involving filtration, high temperature and high pH ensure the product in the effluent is either inactivated or degraded prior to release and no live GMOs are released. The following release estimates are per batch.

Liquid Waste
Bioprocessing of the fermentation culture yields liquid concentrate of 180 L. Sources of liquid wastes follow (in brackets an estimate for the total volume of waste is given, followed by an estimate for the percent of total enzyme units per batch for this waste stream):

- desludge from centrifugation (< 300 L, < 43%)
- residual culture in fermenter and bioprocessing equipment and samples (< 100 L, < 8%)
- permeate stream from concentration (< 1815 L, < 0.3%)
- residual in transfer vessel (< 0.5 L, < 0.05%)

All liquid waste streams and tanks that contained waste or biological material are first treated with a

warm (50ºC) solution of 0.1M NaOH which is then transferred to the Biological Waste – Ultra High Temperature (BW-UHT) unit. The high pH of the cleaning solution irreversibly denatures and hydrolyses the enzyme and other biological molecules. Treatment by the BW-UHT involves pumping the liquid waste out of the vessel and through a series of heat exchangers that heat the liquid in the line to above 70ºC. The material is held at this temperature for 1 minute as it flows through a residence tube, before being cooled to 35ºC and then transferred directly to a bulk Waste Storage tank. When the bulk waste storage tank is close to being full of liquid wastes, waste water from operation and back flushing of the RO unit, the contents are transferred to the Waste Neutralisation tank. The pH of the waste water is lowered from pH 11 – 12 to pH 6 – 8 and then discharged. Samples of the waste water are analysed by a NATA accredited laboratory and the results reported to City West Water as per the trade waste agreement.

Solid waste
Solids are also released into the environment with the disposal of used filters. On completion of a batch up to five depth filters, one Milligard (0.65 μm) and a Durapore (0.22 μm) sterile filter are disposed of.

Negligible quantities of waste are present on the depth filters after use. Close to 100% activity is recovered during depth filtration. In the worst case a 2.2% decrease in the activity was observed during depth filtration. A 2.2% drop in activity equates to a loss of approximately 10.7 M Units.

Milligard (0.65 μm) and Durapore (0.22 μm) filters supplied by Millipore are very low binding pharmaceutical grade filters. Product losses associated with these filters are due to entrapped liquid. After processing of liquid concentrate containing 470 M Units, there is estimated to be 100 mL of entrapped liquid on the filters, containing approximately 0.3 M Units of enzyme.

After removal from their housings, the filters are autoclaved at 121ºC and 1 atmosphere pressure for 70 minutes. Elevated temperature irreversibly denatures the OpdA enzyme and other biological molecules and kills any bacteria that may be present. Following autoclaving the solid waste is loaded into a general waste bin and sent to land fill.

Gaseous emissions
The quantity of gaseous emissions from the manufacturing process is thought to be negligible. A 0.2 μm filter heated to 60°C is used on the fermentation exhaust as a control. The 60ºC setting prevents water vapour from condensing on the hydrophobic filter.

Tanks involved in downstream processing are vented to the atmosphere. Liquid entering the tank displaces air from the head space above the liquid. The air exits through the tank vent and travels by conduit to an extraction fan that discharges the air outside the facility. A one way valve also on the tank draws air from the room and into the tank during discharge of the liquid contents. The combination of the vent and one-way valve serves to protect the operators. Though the discharge point is not heated nor is there a filter installed, the air flow rate is estimated to be quite small at 20 m^3 over a 36 hour period. Unlike the fermentation exhaust, air flow in and out of the tanks is not done through an air sparger and so aerosol formation will be minimal.

Freeze-drying and Packaging
The chemical may be released from the freeze drying facility via three points.

- Clean room sink: At this point up to 50 g/batch of the freeze-dried powder may be released in water to general sewer at a concentration of approximately 1 g/L following the washing of freeze drying trays, measuring containers, spatulas and sieve. Discharge of waste water via the clean room sink and into the sewer constitutes the majority of the chemical released from the facility. The trays and equipment are soaked in a solution of alkaline salts and detergents. The trays and equipment are then rinsed with water and 70% ethanol. Whilst these chemicals are used for cleaning purposes, they have the added benefit of inactivating the product before discharge to sewer.
- Dust collected on the filter from the local air extraction unit: At this point up to 5 g/batch may be collected and disposed of to general waste.
- Material remaining on poly plastic liners: Liners are disposed of to general waste. At this point up to 5 g/batch may be released. Cleaning solutions (detergents and alkaline salt) are applied to the

liner before disposal into the sewer.

In total up to 60 g of the freeze dried powder is estimated to be released per batch during freeze drying and packaging.

RELEASE OF CHEMICAL FROM USE

During use there may be some accidental spillage during the manual pouring of the notified chemical from its 125 or 330 mL PET jar into the container of water and then into the contaminated solution. The extent that this occurs is unclear, but it is unlikely to be more than 5% of the introduced volume.

After use, Landguard OP-A will be released with the treated pesticide contaminated solution, with a concentration ranging from 5 g/100 L to 100 g/100 L. The submission indicates that Orica will be recommending that users of Landguard OP-A continue to dispose of their treated pesticide solutions as per current best practice for the disposal of pesticide contaminated solutions and in accordance with state legislation. Orica will promote Landguard OP-A as improving or building on current best practice rather than replacing current accepted management practices.

The rate of Landguard OP-A used, volume of pesticide contaminated solution treated and the frequency of use at a site will vary for each user. The following are worst case estimates for each use type.

Treatment of animal pesticide dips

The notifier indicates up to 12,000 L of diazinon dip solution can be used to treat sheep, and that a user may dip sheep with a diazinon insecticide formulation on a particular site up to once every second year. Further it is stated more typical dip volumes are 4,000 – 6,000 L, with dipping using diazinon occurring once every third year at a site. However, it is known that over 33% of the Australian flock is treated with diazinon (APVMA, 2005), and therefore in a worst case situation use and disposal every year would take place. The maximum recommended rate of the Landguard OP-A is 100 g/2000 L of organophosphate dip solution. Therefore up to 600 g of Landguard OP-A may be released to the surrounding land with the treated waste diazinon dip solution per site every year.

Treatment of pesticide contaminated washings from pesticide application equipment

The notifier indicates that the largest spray equipment used for applying organophosphate pesticides have a 12,000 L spray tank. At worst, 1% of this volume may remain after the application of the pesticide, which may then be diluted up to 5 fold during the equipment washing procedure resulting in 600 L of contaminated solution. The maximum recommended rate of Landguard OP-A is 100 g/100 L of contaminated solution. Therefore, up to 600 g of Landguard OP-A may be released with the treated pesticide solution. The maximum number of times per season equipment may be washed following the use of an organophosphate at a given site is said to be 20 times. This worst case may occur at aerial agricultural contractors wash down sites in cotton growing regions. Therefore, up to 12 kg of Landguard OP-A may be released at a site. It is current practice for aerial agricultural spray contractors to dispose of their contaminated solutions to evaporation ponds. However, the notifier indicates that more typically a user will treat up to 600 L of diluted pesticide contaminated solution after each application with 12.5 g/100 L, with up to 4 applications of an organophosphate occurring per year, resulting in up to 300 g of Landguard OP-A being released to pesticide evaporation pits or surrounding land at a site per year.

Treatment of pesticide contaminated solutions from the washing of agricultural produce or processing equipment

Waste water generated from the washing of agricultural produce is typically disposed of to the surrounding land, sewer or into an on-farm irrigation return channel. The maximum rate of the chemical in this use situation will be 100 g/2,000 L. Up to 10,000 L of water may be used for the washing of agricultural produce such as fruit and vegetables. This water is replaced with a frequency of up to once per week. This wash water may be disposed of up to 48 times a year. It is possible that up to 24 of these wash solutions may be contaminated with organophosphate pesticides. Therefore, up to 12 kg of Landguard OP-A may be released to the surrounding land at a site/year.

5.5. Disposal

Disposal of waste liquid concentrate resulting from spills during manufacture will be added to the production facility's waste stream, up-stream from the biological waste ultra high treatment unit, from where it will be treated as described above.

The entire contents of a container of Landguard OP-A will be used on the day of opening. Users will be advised to thoroughly rinse each container after the contents are used and the rinse solution emptied into the treated pesticide solution. Minimal chemical is therefore expected to be released with the packaging which can then be disposed of to general waste (most likely to be land filled).

The MSDS contains adequate instructions for the containment and clean up of both small and large spills from use. These should be recovered mechanically, and small spills should be disposed of as per normal use, and large spills using a licensed waste disposal company (after advice from the manufacturer).

Disposal of treated pesticide contaminated solution is dealt with above.

5.6. Public exposure

It is not expected that the general public will face significant exposure to the liquid or freeze dried enzyme concentrate during manufacture, storage, transport, use and disposal of Landguard OP-A, except in the case of accident. Orica's internal standard operating procedures and the material safety data sheet supplied with the chemical have adequate instructions for the clean up and disposal of any accidental spills or releases of the chemical. Engineering controls and standard operating procedures prevent any significant release of the liquid concentrate or freeze dried powder from the production facilities.

6. PHYSICAL AND CHEMICAL PROPERTIES

The following physicochemical properties were conducted on the freeze-dried enzyme concentrate Landguard OP-A.

Appearance at 20ºC and 101.3 kPa Yellowish brown powder

Melting Point/Freezing Point > 400ºC

METHOD	OECD TG 102 Melting Point/Melting Range. EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks	Determined by Differential Scanning Calorimetry. The notified chemical freeze-dried concentrate did not melt under the conditions of the test. No endothermic reaction could be observed. After the experiment the sample had lost approximately 53% of its mass and the sample was still solid and black.
TEST FACILITY	RCC (2005a)

Boiling Point > 400ºC at 101.325 kPa

METHOD	OECD TG 103 Boiling Point. EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks	Determined by Differential Scanning Calorimetry. No endothermic peaks were detected from which boiling could be deduced. After the experiment the test item was a black powder.
TEST FACILITY	RCC (2005a)

Density 1350kg/m^3 at 19.6ºC

METHOD	OECD TG 109 Density of Liquids and Solids. EC Directive 92/69/EEC A.3 Relative Density.
Remarks	Density was determined using a gas comparison pycnometer with helium 46 used as the reference gas.
TEST FACILITY	RCC (2005b)

Vapour Pressure < 1.7 x 10^{-7} kPa at 25°C (estimated)

METHOD OECD TG 104 Vapour Pressure.
EC Directive 92/69/EEC A.4 Vapour Pressure.

Remarks The vapour pressure of the notified chemical freeze-dried concentrate was calculated using the Modified Watson correlation and based on a boiling point value of > 400 °C.

TEST FACILITY RCC (2005c)

Water Solubility 154 g/L at 20°C

METHOD OECD TG 105 Water Solubility.
EC Directive 92/69/EEC A.6 Water Solubility.

Remarks The water solubility was determined using the Flask Method with concentrations determined gravimetrically. The water solubility stated is the mean of measured results for six samples.

TEST FACILITY RCC (2005d)

Surface Tension 34.6 mN/m at 20.5°C

METHOD OECD TG 115 Surface Tension of Aqueous Solutions.
EC Directive 92/69/EEC A.5 Surface Tension.

Remarks The surface tension was measured by means of a tensiometer using the ring method at a concentration of 1 g/L. The notified chemical is surface active.

TEST FACILITY RCC (2005e)

Hydrolysis as a Function of pH Highly unstable in pH 5 buffer, unstable at pH 6.7-9

METHOD An in-house method was used to determine the effect of pH on the stability of the OpdA enzyme.

Landguard OP-A was added to 4 sterile buffered solutions and samples collected from the solutions were assayed for enzyme activity using the ethyl-parathion assay (see above) at 0, 0.1, 0.25, 1, 3, 7, 15, 22, 35, 43, 58, 88 and 119 days.

pH	*T (°C)*	*$t_{½}$ (days)*
5 (acetate)	21-23	0.062
6.7 (acetate)	21-23	6.4
7 (phosphate)	21-23	2.6
7 (Tris)	21-23	13.8
9(Tris)	21-23	4.5

Remarks The most dramatic drop in enzyme activity was observed with the pH 5 buffer, which had no detectable activity after 6 hours. A rapid drop was also observed in the pH 9 buffer over the first 6 h, but this then became relatively slow. The observed higher decay rate in the phosphate pH 7 buffer was not unexpected as phosphate is known to interfere with OpdA enzyme activity. Likewise acetate does not buffer well at pH7 and these two buffers were not considered appropriate for the study.

Under sterile conditions the degradation of the OpdA enzyme is thought to be due to a combination of hydrolysis, enzyme unfolding, loss of metal ions and breakdown by proteases.

TEST FACILITY Orica (2005d)

Partition Coefficient (n-octanol/water) Not determined

Remarks The notified chemical is introduced in a complex mixture of other biological

molecules such as proteins, complex carbohydrates, fats and inorganic salts. Therefore this property would be difficult to measure and would not provide meaningful data. However, the water solubility would indicate that most would partition into the aqueous phase.

Adsorption/Desorption Not determined

Remarks This property would be difficult to measure and would not provide meaningful data as the notified chemical is introduced in a complex mixture of other biological molecules such as proteins, complex carbohydrates, fats and inorganic salts. While the water solubility would indicate mobility in soils and sediments, this may be offset by the large molecular size of the enzyme, which also is likely to be unstable in soil. It is also surface active which will reduce potential mobility.

Dissociation Constant Not applicable

Remarks The notified chemical is introduced in a complex mixture and a meaningful result could not be obtained.

Particle Size

METHOD European commission, Directorate general X11-JRC, Science Research and Development-Joint Research Centre. "Particle Size Distribution, Fibre Length and Diameter Distribution" Guidance document, ECB/TM/February 1996.

Range (μm)	*Mass (%)*
> 2000	9.12
1000-2000	27.65
500-1000	33.32
250-500	18.75
125-250	10.89
75-125	0.26
<75	0

Remarks Determined using the sieve method. Two tests were conducted.

Inhalable fraction: 0.26%
Respirable fraction: 0%

TEST FACILITY RCC (2005f)

Flash Point Not determined

Remarks The notified chemical is introduced as a complex mixture of non-volatile components and as such a flammable vapour is not expected to be evolved.

Flammability Limits Not highly flammable

METHOD EC Directive 92/69/EEC A.10 Flammability (Solids).
Remarks The test item could be ignited with a flame during the preliminary test. In contact with the ignition source the test item glowed and turned black and the flame changed colour to orange. The test item was still burning without contact with the ignition source, and after 3-6 seconds the flame extinguished. Therefore, a main test was not conducted.

The test item is not "highly flammable" according to the criteria of the guideline
TEST FACILITY RCC (2005g)

Autoignition Temperature > 400ºC

METHOD 92/69/EEC A.15 Auto-Ignition Temperature (Liquids and Gases).

92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.

Remarks Two small exothermic heat effects were observed (at ~ 230 ºC and 350 ºC) but both with a maximum temperature below 400ºC. These effects were not due to self-ignition of the test item. The test item was black and carbonised at the end of the measurement.

TEST FACILITY RCC (2005h)

Explosive Properties Not determined

Remarks The notified chemical is introduced as a complex mixture, however, it is not expected to have any chemical functional groups that would infer explosive properties

Reactivity

Remarks Based on the results of the melting point, boiling point and autoignition temperature, the enzyme concentrate appears not to be stable at temperatures > 230 ºC. The thermal stability of the OpdA enzyme has been studied (see Thermal Stability)

Enzyme Activity

METHOD See Methods of detection and determination for method details.

Remarks The enzyme activity was provided for three batches, the specific activity values has been requested as confidential but are reported as < 35,000 enzyme units/g.

TEST FACILITY Orica (2005a, 2005b, 2005c)

Catalytic Constants (Km & Kcat)

METHOD See Methods of detection and determination for method details.

Remarks The K_m (μM) values for particular pesticide active ingredients for the OpdA enzyme are as follows:

- Coumaphos: 8.3±1.8
- Coroxon: 15.9±1.9
- Paraoxon: 242±61
- Parathion: 92.6±6.4
- Diazinon: 51.9±4.5
- Parathion methyl: 61.2±2.3
- Phosmet: 208.3±13.2
- Fenthion: 148.6±17.2
- dMUP: 66.0±9.1

The k_{cat} (min^{-1}) values for the OpdA enzyme for particular pesticide actives are as follows:

- Coumaphos: 12.4±0.6
- Coroxon: 22.7±0.1
- Paraoxon: 33.5±0.5
- Parathion: 21.9±2.0
- Diazinon: 65.2±6.7
- Parathion methyl: 94.2±0.8
- Phosmet: 0.1±0.002
- Fenthion: 1.63±0.01
- dMUP: 81.7±9.1

TEST FACILITY Horne et al (2002)

Thermal Stability

METHOD The thermal stability of the OpdA enzyme was evaluated by incubating an sterile enzyme concentrate solution at 10ºC and 22ºC and assaying for enzyme activity over a period of 105 days. Enzyme activity was assessed using the ethyl-parathion assay (see methods of determination)

T (°C)	*$t_{½}$ <days>*
10	19.5
22	11.9

Remarks The OpdA enzyme was found to be relatively stable at 10 and 22ºC. At both temperatures, an initial rapid decrease in enzyme stability was observed, followed by a more gradual decline. Degradation would be expected to be much faster in the environment in no-sterile conditions.

TEST FACILITY Orica (2005e)

7. TOXICOLOGICAL INVESTIGATIONS

The following toxicological studies were conducted on the freeze-dried enzyme concentrate Landguard OP-A.

Endpoint and Result	*Assessment Conclusion*
Rat, acute oral	low toxicity, LD50 > 2000 mg/kg bw
Rat, acute dermal	low toxicity, LD50 > 2000 mg/kg bw
Rat, acute inhalation	not determined
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	slightly irritating
Skin sensitisation – mouse local lymph node assay (LLNA)	evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOAEL 1000 mg/kg bw/day
Genotoxicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro chromosome aberration test	non clastogenic
Genotoxicity – in vivo	not determined

7.1. Acute toxicity – oral

TEST SUBSTANCE	Landguard O-PA
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method. EC Directive 92/69/EEC B.1tris Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/HanRcc:WIST (SPF)
Vehicle	Purified Water
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	3 females	2000	0
II	3 females	2000	0

LD50	> 2000 mg/kg bw
Signs of Toxicity	There were no deaths or test substance-related clinical signs or remarkable body weight changes during the study period.
Effects in Organs	Congested lungs were observed in one animal and grey-white focus/foci on the left kidney was observed in another animal.
Remarks - Results	As no clinical signs were recorded during the 14 day observation period and only isolated abnormalities were recorded, the macroscopic findings were considered to be within spontaneous background alterations and were not considered to be test item related. The LD50 cut-off estimated using the flow chart in Annex 2d of the OECD TG423 would be 5000 mg/kg bw
CONCLUSION	The notified chemical concentrate is of low toxicity via the oral route.
TEST FACILITY	RCC (2005i)

7.2. Acute toxicity – dermal

TEST SUBSTANCE	Landguard OP-A
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test.

	EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal) – Limit Test.
Species/Strain	Rat/HanRcc:WIST (SPF)
Vehicle	Purified water
Type of dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	5 per sex	2000	0/10

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	Slight general erythema was observed in all 5 males on test day 2 and 3 and 1 male additionally showed slight crusts from test day 4 to 10. Yellow discolouration was observed in all 5 females on test day 2 and 3 and therefore no local signs were assessable. Slight scales were seen in two females either from test day 4 to 11 or from test day 6 to 10. One female additionally showed slight crusts from test day 9 to 11.
Signs of Toxicity - Systemic	There were no deaths or systemic signs of toxicity or remarkable body weight changes during the study period.
Effects in Organs	Grey-white focus/foci on the left side of the kidneys were observed in two males and congested lungs seen in one female.
Remarks - Results	As no adverse clinical signs were recorded during the 14 day observation period and only isolated abnormalities were recorded, the macroscopic findings were considered to be within spontaneous background alterations and were not considered to be test item related.

CONCLUSION The notified chemical concentrate is of low toxicity via the dermal route.

TEST FACILITY RCC (2005j)

7.3. Acute toxicity – inhalation

Not determined. Enzymes are regarded as respiratory sensitisers (European Commission, 2002) and as such inhalation exposure to the notified chemical is expected to be avoided.

7.4. Irritation – skin

TEST SUBSTANCE Landguard OP-A

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.
EC Directive 92/69/EEC B.4 Acute Toxicity (Skin Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	Test substance was moistened with purified water before application.
Observation Period	7 days
Type of Dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	0	0	1.67	2	3-6 days	0
Oedema	0	0	0	0	0	0

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.

Remarks - Results	Very slight erythema was observed in one animal at the 1 hour observation. Well defined erythema as seen in a further animal from the 1 to the 48 hour observation and persisted as a slight erythema at the 72 hour observation. All signs of irritation had reversed by day 7. The test item caused no deaths, or staining or corrosive effects on the skin. No clinical signs were observed. Body weights were within the normal range of variability.
CONCLUSION	The notified chemical concentrate is slightly irritating to the skin.
TEST FACILITY	RCC (2005k)

7.5. Irritation – eye

TEST SUBSTANCE	Landguard OP-A
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion. EC Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Observation Period	10 days
Remarks - Method	No significant protocol deviations

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	1.67	1.0	1.33	2	7-9 days	0
Conjunctiva: chemosis	1.0	0.33	0	2	3-6 days	0
Conjunctiva: discharge	0	0.33	0	2	1 day	0
Corneal opacity	0	0	0	0	0	0
Iridial inflammation	0	0	0	0	0	0

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.

Remarks - Results	In addition to the signs of irritation noted above moderate reddening of the sclerae was present in all animals from the up to the 48 hour observation and slight to moderate reddening persisted in all animals at the 72 hour observation. No abnormal findings were observed in the cornea or iris of any animal. No deaths, corrosion, staining or clinical signs were observed. Body weights were within the normal range of variability.
CONCLUSION	The notified chemical concentrate is slightly irritating to the eye.
TEST FACILITY	RCC (2005l)

7.6. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE	Landguard OP-A
METHOD	OECD guideline 429: Skin sensitisation: Local Lymph Node Assay EC Directive 2004/73/EC B.42 Skin Sensitisation: Local Lymph Node Assay
Species/Strain	Mouse/ CBA/CaHsdRcc(SPF)
Vehicle	Propylene glycol/water (3/7, v/v)
Remarks - Method	Deviations from protocol: The notified chemical was tested at a maximum concentration of 10%. No rationale was provided as to why higher concentrations were not tested. No rationale was provided as to why acetone/olive oil (4:1 v/v), dimethylformamide and methyl ethyl ketone (preferred vehicles to propylene glycol) were not used as the vehicle. There were not other significant protocol deviations.

RESULTS

Concentration	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
2.5	394	2.0
5.0	927	4.7
10.0	2252	11.4
Positive Control		
5	664	2.4
10	1007	3.6
25	3169	11.2

Remarks - Results	A dose response relationship was observed. An EC3 value of 3.4 % (w/v) was determined for the notified chemical. All treated animals survived the scheduled study period. No clinical signs or signs of local toxicity at the ears were observed in any of the animals. The positive control confirmed the sensitivity of the test.
CONCLUSION	There was evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical concentrate.
TEST FACILITY	RCC (2005m)

7.7. Repeat dose toxicity

TEST SUBSTANCE	Landguard OP-A
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents. EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).
Species/Strain	Rat/HanRCC:WIST (SPF)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28days Dose regimen: 7 days per week Post-exposure observation period: None

Vehicle	Bidistilled water
Remarks - Method	No significant protocol deviations. Doses selected based on a 5 day dose range-finding study.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
I (control)	5 per sex	0	0
II (low dose)	5 per sex	50	0
III (mid dose)	5 per sex	200	0
IV (high dose)	5 per sex	1000	0

Mortality and Time to Death

No mortality was observed during the treatment phase.

Clinical Observations

No substance-related clinical signs were observed during the treatment period. There was no significant difference in body weight gain and food and water consumption in treated animals when compared to controls. Statistically significant differences noted in the functional observations (increased forelimb grip strength and increased and decreased locomotor activity) were either not dose related and considered incidental or were due to low control values and considered not to be related to the test substance.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Clinical Chemistry

There were considered to be no test-substance related changes to any of the clinical chemistry parameters. For the significant differences noted in the group II males (decreased aspartate aminotransferase and phosphorus levels), the levels determined remained within the range of historical control data and therefore were considered to be of no toxicological relevance.

Haematology

There were considered to be no test-substance related changes to any of the haematological parameters. For the significant differences noted in the group II males (increased red cell volume distribution width, increased haemoglobin concentration distribution width and increased activated partial thromboplastin time) and group III females (increased relative reticulocyte levels), were either not dose related and considered incidental or the levels determined remained within the range of historical control data and therefore were considered to be of no toxicological relevance.

Effects in Organs

Organ Weights

Statistically significant increased relative liver weight was noted in group II (9.8%, $P<0.05$) and group IV (9.8%, $P<0.05$) males and statistically increased relative kidney weight was noted in group IV males (8%, $P<0.05$) compared to controls. A similar increase of 3% (but not significant) in relative liver weight was noted in group IV females. Statistically significant increased absolute ($P<0.01$) and relative ($P<0.05$) thymus weight and absolute spleen weight ($P<0.05$) was noted in group II females. A similar effect was not observed in treated males.

Macroscopic Findings

There were no remarkable necropsy findings.

Histopathology

Minimal centrilobular hepatocellular hypertrophy was observed in 2 of 5 group IV males. All other findings were regarded to be incidental and to represent spontaneous changes occurring in rats of this strain and age.

Remarks – Results

The small (<10%) increase in relative liver weight and was regarded to represent a metabolic adaptation and can be correlated with the minimal hepatocellular hypertrophy observed microscopically. The observed increase in kidney weight was not accompanied by any test-substance related histopathological change and therefore not considered to be of toxicological relevance. In the absence of a dose response and associated histopatological changes, the differences in organ weights observed in group II females were considered to be

incidental.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 1000 mg/kg bw/day in this study, based on the absence of treatment related effects.

TEST FACILITY RCC (2005n)

7.8. Genotoxicity – bacteria

TEST SUBSTANCE Landguard OP-A

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
EC Directive 2000/32/EC Annex 4D
Pre incubation procedure

Species/Strain *S. typhimurium*: TA1535, TA1537, TA98, TA100, TA102
Metabolic Activation System S9-Mix from phenobarbitone/β-napthoflavone induced rat liver.
Concentration Range in Main Test Test 1
a) With and without metabolic activation: 3 - 5000 µg/plate (TA98, TA100), 33-5000 µg/plate (TA1535, TA1537, TA102)

Test 2
a) With and without metabolic activation: 156.3 - 5000 µg/plate (All strains)

Vehicle Deionised water
Remarks - Method The preliminary toxicity experiment was conducted on strains TA100 and TA98. The results of this test were used as part of test 1.

Deviations from protocol
2-Aminoanthracene was used as the sole indicator of the efficacy of the S9-mix.
The following positive controls were used in the absence of S9-mix:
4-nitro-1, 2-phenylene diamine (TA1537 and TA98)
methyl methane sulphonate (TA102)

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 5000	> 5000	> 5000	negative
Test 2		> 5000	> 5000	negative
Present				
Test 1		> 5000	> 5000	negative
Test 2		> 5000	> 5000	negative

Remarks - Results Reduced background growth was observed in strains TA98 and TA 100 with and without activation in test 1. No reduction in background growth was observed in the remaining strains in test 1 or in test 2. No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation.

The test substance did not cause a marked increase in the number of revertants per plate of any of the tester strains either in the presence or absence of activation. Positive controls confirmed the sensitivity of the test system. Although for some strains the number of colonies was lower than the historical control ranges this is not considered to impact the

outcome of the study.

CONCLUSION The notified chemical concentrate was not mutagenic to bacteria under the conditions of the test.

TEST FACILITY RCC (2005o)

7.9. Genotoxicity – in vitro

TEST SUBSTANCE Landguard OP-A

METHOD OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
EC Directive 2000/32/EC Annex 4D Mutagenicity - In vitro Mammalian Chromosome Aberration Test.

Species/Cell Line	Chinese Hamster V79 cells
Metabolic Activation System	S9-Mix from phenobarbitone/β-napthoflavone induced rat liver.
Vehicle	Deionised water
Remarks - Method	No significant protocol deviations. Dose selection for the main tests were based on toxicity data and the occurrence of precipitation in the preliminary test.

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period (hours)*	*Harvest Time (hours)*
Absent			
Preliminary Test	39.1, 78.1, 156.3, 312.5, 625, 1250, 2500, 5000 (a + b)	4 (a), 24 (b)	-
Test 1	31.3, 62.5, 125*, 250*, 500*, 1000	4	18
Test 2a	19.5, 39.1*, 78.1*, 156.3*, 312.5, 625	18	18
Test 2b	78.1, 156.3*, 312.5, 625	28	28
Present			
Preliminary Test	39.1, 78.1, 156.3, 312.5, 625, 1250, 2500, 5000	4	-
Test 1	31.3, 62.5, 125*, 250*, 500*, 1000	4	18
Test 2	39.1, 78.1*, 156.3*, 312.5*, 625*, 1250	4	28

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	1250 (4 hr), 312.5 (24 hr)			
Test 1		1000	500	negative
Test 2a		625	> 625	negative
Test 2b		312.5	> 625	negative
Present	1250			
Test 1			500	negative
Test 2			> 1250	negative

Remarks - Results In the absence and presence of S9 mix, cytotoxicity indicated by reduced cell numbers and/or mitotic indices and/or low metaphase quality was observed in all experimental parts. In both tests, in the absence and presence of S9 mix, at least the highest applied concentrations were not scorable for cytogenetic damage.

In both tests, no biologically relevant increase in the number of cells carrying structural chromosomal aberrations was observed after treatment with the test item. Three statistically significant increases ($p<0.05$) were

observed, but these were within the laboratories historical control range (0.0 – 4.0% aberrant cells, exclusive gaps) and therefore were not regarded as biologically relevant.

No biologically relevant increase in the frequencies of polyploid metaphases was found after treatment with the test item as compared to the frequencies of the controls.

In test 1, in the absence and the presence of S9 mix, after treatment with the test item the frequencies of endomiotic cells were increased (0.0 – 2.9%) compared with the respective controls (0.1 – 0.3%) indicating that the test substance has the potential to inhibit cell cycle progression in V79 cells. A distinct increase (2.9% endomiotic cells) was observed after 4 hours treatment with 250 µg/mL in the absence of S9. Increases frequencies of endomiotic cells were not observed in test 2.

In both tests, EMS (300 and 400 µg/ml, respectively) and CPA (1.4 µg/ml) were used as positive controls and showed distinct increases in cells with structural chromosome aberrations.

CONCLUSION The notified chemical concentrate was not clastogenic to Chinese hamster V79 cells treated in vitro under the conditions of the test.

TEST FACILITY RCC (2005p)

7.10. Genotoxicity – in vivo

Not determined due to negative results obtained in the chromosome aberration and reverse mutation assays.

8. ENVIRONMENT

8.1. Environmental fate

8.1.1. Ready biodegradability

TEST SUBSTANCE	Landguard OP-A
METHOD	OECD TG 301F Ready Biodegradability: Manometric Respirometry Test. EC Directive 92/69/EEC C.4-D Biodegradation: Determination of the "Ready" Biodegradability: Manometric Respirometry Test
Inoculum	Aerobic activated sludge from a waste water treatment plant (ARA Ergloz ll, Fullinsdorf, Switzerland).
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	COD & BOD
Remarks - Method	There were no significant protocol deviations. The sludge was washed twice with tap water by centrifugation and the supernatant liquid phase was decanted. A homogenised aliquot of the final sludge suspension was weighed, dried and the ratio of wet to dry weight was calculated. Based on this ratio, calculated amounts of wet sludge were suspended in test water to obtain a concentration equivalent to 4 g dry material per litre. During holding the sludge was aerated at room temperature. Prior to use the sludge was diluted with test water to a concentration of 1 g per litre. This diluted activated sludge was used as inoculum to give a final concentration of 30 mg dry material per litre.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
7	90.5	7	80
14	101.5	14	88.5
21	105.5	21	95
28	108 (95*)	28	93

*corrected for nitrification

Remarks - Results	The percent biodegradation of the test item was calculated based on the chemical oxygen demand of 1.42 mg O_2/mg test item. The biochemical oxygen demand of the test item in the test media significantly increased from exposure day 1. Ten percent degradation occurred at approximately 1 day. At the end of the 28 day exposure period the mean biodegradation of the test item was 108% without nitrification taken into consideration. Since nitrate concentrations were measured in the test flasks at the end of the test, nitrification must have occurred. Therefore, the oxygen consumption of the test item flasks was corrected for the oxygen consumed by nitrification. The results above clearly indicate that the 10-day window criterion, i.e. > 60% degradation must occur within 10 days of reaching 10%, was reached (if fact within the first few days). In the procedure controls, sodium benzoate degraded by an average of 89% by exposure day 14 and 93% by day 28, confirming the suitability of the activated sludge and the test method.

No degradation of the test item occurred in the abiotic control (poisoned with mercury dichloride) under the test conditions within 28 days.

In the toxicity control, containing both the test item and sodium benzoate, no inhibitory effect on the biodegradation of sodium benzoate was observed. Therefore the test item did not have an inhibitory effect on the activated sludge micro-organisms at the tested concentration of 100 mg/L.

CONCLUSION The notified chemical concentrate can be classed as readily biodegradable.

TEST FACILITY RCC (2005q)

8.1.2. Bioaccumulation

Bioaccumulation of the notified chemical is not expected given its large molecular size, high biodegradation potential and high water solubility.

8.2. Ecotoxicological investigations

8.2.1. Acute toxicity to fish

TEST SUBSTANCE Landguard OP-A

METHOD OECD TG 203 Fish, Acute Toxicity Test – semi static, limit test.
EC Directive 92/69/EEC C.1 Acute Toxicity for Fish - semi static, limit test.

Species	*Brachydanio rerio* (zebra fish)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	None. Results are based on a nominal concentration of 100 mg/L.
Remarks – Method	There were no significant protocol deviations.

A semi static test procedure with renewal every 24 hours was selected to keep the concentration of the test item in the test medium as constant as possible during the test period of 96 hours. The test medium consisted of reconstituted water (analytical grade salts dissolved in deionised water). The pH of the test medium was 7.7 – 7.9. Due to the complex nature of the test item no analytical work was conducted, but at this pH at least 20% degradation may be expected (and probably more due to the lack of sterile conditions) over 24 hours.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
0	-	7	0	0	0	0	0
100	-	7	0	0	0	0	0

LC50	> 100 mg/L at 96 hours.
NOEC	100 mg/L at 96 hours.
Remarks – Results	In the control and in the test medium of 100 mg/L all fish survived until the end of the test and no visible abnormalities were observed in the test fish.

The test medium appeared to be a clear solution at the start of the test

medium renewal periods, but at the end, turbidity and a small quantity of fine particles was observed floating on the surface and lying at the bottom of the aquarium. This turbidity may have been due to bacterial growth.

CONCLUSION The notified chemical concentrate is practically non-toxic to *Brachydanio rerio* (zebra fish).

TEST FACILITY RCC (2005r)

8.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Landguard OP-A

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test – static, limit test.
EC Directive 92/69/EEC C.2 Acute Toxicity for Daphnia – static limit test.

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None.
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	None. Results are based on a nominal concentration of 100 mg/L.
Remarks - Method	No significant protocol deviations.

The test medium consisted of reconstituted water (analytical grade salts dissolved in purified water). The pH of the test medium was 7.5 – 7.8.

Due to the complex nature of the test item no analytical work was conducted. Appreciable degradation (> 50%) may be expected over the 48 hour test period.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0	-	20	0	0
100	-	20	0	0

LC50	> 100 mg/L at 48 hours
NOEC	100 mg/L at 48 hours
Remarks - Results	One daphnia in the control was immobile after 48 hours, but this is within the 10% allowed by the guideline. No remarkable observations were made concerning the appearance of the test medium during the first 24 hours. At the end of the test homogeneous turbidity was observed, possibly caused by bacterial growth.

CONCLUSION The notified chemical is practically non-toxic to *Daphnia magna*.

TEST FACILITY RCC (2005s)

8.2.3. Algal growth inhibition test

TEST SUBSTANCE Landguard OP-A

METHOD The study followed the procedures indicated in the following guidelines and recommendations:
OECD TG 201 Alga, Growth Inhibition Test.
EC Directive 92/69/EEC C.3 Algal Inhibition Test.

Species *Scenedesmus subspicatus*

Exposure Period	72 hours
Concentration Range	100 mg/L (nominal)
Auxiliary Solvent	None
Water Hardness	24 mg $CaCO_3$/L
Analytical Monitoring	None. Results are based on a nominal concentration of 100 mg/L.
Remarks - Method	There were no significant protocol deviations. The test medium was synthetic water, prepared according to the test guidelines. Due to the complex nature of the test item no analytical work was conducted.

RESULTS

Biomass		*Growth*	
EbC90 mg/L at 72 h	*NOEC* mg/L	*ErC90* mg/L at 72 h	*NOEC* mg/L
> 100	*100*	*> 100*	*100*

Remarks - Results	The mean algal cell density in the test medium of 100 mg/L was nearly identical with or even slightly higher than that in the parallel control culture throughout the duration of the test. Microscopic examination of the algal cells after 72 hours showed no difference between algae growing in the test medium and control. No remarkable observations were made concerning the appearance of the test medium. The pH ranged between 7.9 to 8.5.
CONCLUSION	The notified chemical concentrate is practically non-toxic to *Scenedesmus subspicatus.*
TEST FACILITY	RCC (2005t)

8.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Landguard OP-A
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test. EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test.
Inoculum	Aerobic activated sludge from a waste water treatment plant (ARA Ergloz ll, Fullinsdorf, Switzerland), treating predominantly domestic waste water.
Exposure Period	3 hours
Concentration Range	1000 mg/L
Remarks – Method	There were no significant protocol deviations. 3,5-Dichlorophenol was used as a positive control.
RESULTS	
IC50	> 1000 mg/L
NOEC	1000 mg/L
Remarks – Results	The oxygen consumption rates of the two controls differed by only 1.0%. The test item had no inhibitory effect on the respiration rate of the activated sludge after the incubation period of 3 hours at the test item concentration of 1000 mg/L. The respiration rate at the test concentration was even increased by 53% compared to the controls. This may be because the test item was an additional food resource for the activated sludge microorganisms.

The 3 hour EC50 of the positive control was calculated to be 18 mg/L, confirming the suitability of the activated sludge used.

CONCLUSION The test item was found to have no inhibitory effect on activated sludge microorganisms.

TEST FACILITY RCC (2005u)

8.3. Metabolite investigations

8.3.1. Study of metabolites from diazinon degradation

TEST SUBSTANCE Landguard OP-A

METHOD

Remarks - Method

Diazinon is an organophosphate insecticide widely used in sheep dips and will be a key target chemical for breakdown using the notified enzyme prior to disposal. The effectiveness of Landguard OP-A (enzyme concentrate) in breaking down diazinon was examined over a 24 hour period at both pH 6.0 (acidic) and 9.0 (alkaline), under unstated conditions but these were presumably ambient. The buffers were prepared by adjusting ammonium acetate (10 mM) in HPLC grade water by the addition of acetic acid and ammonia, respectively. The starting diazinon concentration was < 50 ppm and after 30 minutes and 24 hours samples were taken and analysed using liquid chromatography/mass spectrometry (LC-MS/MS). The notified chemical was added at a rate of 0.1 g/L of diazinon solution, with thorough mixing ensured.

In addition an enzyme treated diazinon solution, stated to be highly turbid water with an initial concentration of 80 mg/L from the Loxton field trial, was examined by the same methodology.

RESULTS

Remarks - Results

In the laboratory trial no diazinon was detectable (estimated detection limit 0.1 mg/L, therefore > 99.8% conversion assuming a 50 mg/L starting concentration) after 30 minutes or 24 hours, whereas the diazinon control did not show any decrease in concentration. Two breakdown products only could be detected at both times and buffers and were identified as diethylthiophosphoric acid and 2-isopropyl-4-methylpyrimimid-6-ol. These are formed by hydrolysis of the P-O bond of diazinon attached to the aromatic ring, and are the initial degradation products as identified in the literature, including the APVMA review (http://www.apvma.gov.au/chemrev/diazinonenv.pdf). Note, however, that authentic samples were not obtained to allow for an absolute identification, and that the yield of the transformation was also not determined.

No diazinon was detectable (at least 99.9% conversion) in the field trial sample, and again the two above degradation products were the only metabolites identified, though again no authentic samples were used, or yield determined.

CONCLUSION The notified chemical hydrolyses diazinon to two detectable degradation products formed by hydrolysis of the P-O bond attached to the aromatic ring. No other products were detectable.

TEST FACILITY Entox (2005a)

8.3.2. Study of metabolites from chlorpyrifos degradation

TEST SUBSTANCE	Landguard OP-A
METHOD	
Remarks - Method	Chlorpyrifos is an organophosphate used in the control of a wide range of insect pests on fruit, vegetables, oilseeds, cotton, cereals, pasture etc., and is an example of a pesticide that will be targeted for use in treating of contaminated wash solutions from application equipment. The effectiveness of Landguard OP-A (enzyme concentrate) in breaking down chlorpyrifos was examined over a 24 hour period under identical conditions to the laboratory degradation of diazinon above. No field treated sample was examined in this case.
RESULTS	
Remarks - Results	No chlorpyrifos was detectable (estimated detection limit 0.1 mg/L, therefore > 99.8% conversion assuming a 50 mg/L starting concentration) after 30 minutes or 24 hours, whereas the chlorpyrifos control did not show any decrease in concentration. Again two breakdown products only could be detected at both times and buffers and were identified as diethylthiophosphoric acid and 3,5,6-trichloropyridin-2-ol. These are also formed by hydrolysis of the P-O bond of chlorpyrifos attached to the aromatic ring, and are the initial degradation products as identified in the APVMA review (http://www.apvma.gov.au/chemrev/chlorenv.pdf). Note, however, that again authentic samples were not obtained to allow for an absolute identification, and that the yield of the transformation was also not determined.
CONCLUSION	The notified chemical hydrolyses chlorpyrifos to two detectable degradation products formed by hydrolysis of the P-O bond attached to the aromatic ring. No other products were detectable.
TEST FACILITY	Entox (2005b)

8.3.3. Study of metabolites from methyl parathion degradation

TEST SUBSTANCE	Landguard OP-A
METHOD	
Remarks - Method	Methyl parathion is an organophosphate used in the control of a wide range of insect pests, and is another example of a pesticide that will be targeted for use in treating of contaminated wash solutions from application equipment. The effectiveness of Landguard OP-A (enzyme concentrate) in breaking down methyl parathion was examined over a 24 hour period under identical conditions to the laboratory degradation of diazinon above. Again no field treated sample was examined in this case.
RESULTS	
Remarks - Results	No methyl parathion was detectable (estimated detection limit 0.1 mg/L, therefore > 99.8% conversion assuming a 50 mg/L starting concentration) after 30 minutes or 24 hours, whereas the methyl parathion control did not show any decrease in concentration. Again two breakdown products only could be detected at both times and buffers and were identified as dimethylthiophosphoric acid and 4-nitrophenol. These are also formed by hydrolysis of the P-O bond of methyl parathion attached to the aromatic ring, and are the initial degradation products as identified in the APVMA review (http://www.apvma.gov.au/chemrev/pmenv.pdf). Note, however, that again authentic samples were not obtained to allow for an absolute

identification, and that the yield of the transformation was also not determined.

CONCLUSION The notified chemical hydrolyses methyl parathion to two detectable degradation products formed by hydrolysis of the P-O bond attached to the aromatic ring. No other products were detectable.

TEST FACILITY Entox (2005c)

8.4. Efficacy investigations

8.4.1. Diazinon breakdown studies

TEST SUBSTANCE OP1 Clenz (notified chemical liquid concentrate)

METHOD

Remarks - Method The rate of breakdown of diazinon solutions when freshly prepared in the laboratory and also from samples of dip wash collected from the field was studied using the notified chemical which at the time was a liquid extract (rather than freeze dried material) named OP1 Clenz was studied.

A 200 ppm stock solution was prepared using the commercial formulation Virbac Jet dip and tap water and aliquots of 20 mL were dispensed into glass beakers and stirred. Volumes of 25, 50 and 75 µL of OP1 Clenz (equivalent to 200, 400 and 600 units per L solution) were added to these and samples taken at a number of times over 60 minutes, with GC used to quantify the diazinon concentration. Dose response curves were generated. Again the storage temperature was not indicated, and it is assumed these were ambient laboratory conditions.

Livestock dippers similarly prepared a 200 ppm solution of diazinon in accordance with the label instructions for Virbac Jet dip. Samples of dip wash were taken at various intervals over 2 hours from the commencement of the dipping process, and on return to the laboratory filtered through a Whatman #4 filter to remove large particulate matter. The stirred filtrate in beakers was treated with OP1 Clenz at a rate of 20 µL per 20 mL (equivalent to 160 units per L dip wash, note less than those above). Again samples were taken over 1 hour and analysed for diazinon by GC, and dose response curves generated.

RESULTS

Remarks - Results The rate of breakdown of the diazinon solutions prepared in the laboratory for the lowest and highest addition of OP1 Clenz are tabled below. The intermediate strength solutions gave an intermediate rate. After 60 minutes only 0.15-0.4% of initial remained, with the graphs of the dose response curves provided clearly illustrating the much more rapid rate this was achieved at the 2 higher strengths. However, even at the lowest strength there was > 98% degradation in 20 minutes.

	Diazinon concentration <ppm> after treatment with:	
	25 µl OP1 Clenz/20 ml diazinon solution (200 UNITS OpdA/L diazinon solution)	*75 µl OP1 Clenz /20 ml diazinon solution (200 UNITS OpdA/L diazinon solution)*
Time		
0 minutes	200	200
2 minutes	148.3	96.1
5 minutes	87.7	11.9
10 minutes	31.9	1.8
20 minutes	3.7	0.5

50 minutes	0.8	0.3

The rate of breakdown of diazinon dip solutions obtained after the dipping process commenced and then treated with OP1 Clenz at a low strength in the laboratory are tabled below for the beginning and the end of the 2 hour dip sampling. Again samples obtained at intermediate times in the dipping process degraded at an intermediate rate. After 50 minutes <0.2% of initial remained from clean samples, whereas this level was achieved within 2 minutes for samples taken 2 hours after dipping had been in progress.

Diazinon concentration <ppm> at the following times after commencement of dipping:

Time after addition of OP1 Clenz	*Initial*	*180 Minutes*
0 minutes	115.9	43.5
2 minutes	110.0	0.3
5 minutes	75.5	-
10 minutes	23.0	-
20 minutes	0.8	-
30 minutes	0.4	-
60 minutes	0.3	-

CONCLUSION

The notified chemical efficiently breaks down diazinon both from freshly prepared laboratory solutions, and from filtered samples of dip wash commercially prepared. The rate depends on the strength of the solution and the amount of enzyme added, but was >98% in 20 minutes at 200 ppm diazinon and a range of 160 - 600 units of enzyme per L of solution/dip.

TEST FACILITY

Orica (2005f)

8.4.2 Lake Bolac (VIC) field sheep dip treatment trial

TEST SUBSTANCE

Landguard OP-A

METHOD

Remarks - Method

Diazinon is commonly used in Australia for the treatment of lice and blowflies on sheep, which are dipped in 4000-10,000 L tanks containing diazinon solutions. This trial describes the results of experiments at Lake Bolac in western Victoria to evaluate the optimal Landguard dosing rate for the degradation of waste dip solution.

The trial involved a portable cage plunge dip of capacity 10,000 L in which 7,000 sheep, with 4 weeks wool growth since shearing, had been dipped at a target dose of 100 ppm diazinon. After this time the dip volume was 8,000 L and had a moderate amount of sludge on both the surface and in the base (the latter was viewed after the dip had drained).

In a replicated trial the right amount of Landguard (2.5, 5 and 10 g/100 L equivalent to 25,750, 51,500 103,000 IU/100 L respectively) was dissolved in water via vigorous shaking to form a 2% w/v solution and added to each of the nine 10 L containers (3 replicates per concentration) and the contents mixed for 2 minutes by repeated turning upside down and returning to the upright position. In addition to the controls, hydrated lime at 300 g/100 L was also added to 3 containers.

Samples were taken via a tap located at the bottom of the containers after 30 minutes (0, 30 and 60 minutes for the controls), and after the pH and

temperature was recorded sufficient 40% perchloric acid was added and swirled for 1 minute to achieve a pH of 2 to denature the enzyme and prevent further breakdown of diazinon. After returning to pH 7 – 7.4, each sample was analysed for diazinon (details of the method are not provided).

In an unreplicated trial the required amount Landguard (800 g at 10 g/L or 103,000 IU/100 L) was dissolved into 10 L of water and this solution was then uniformly added to the dip tank and the contents mixed for 2 minutes by raising and lowering the cage simulating typical dipping of sheep. This mixing was indicated to have been excellent. After 30 and 60 minutes 2 L samples were taken in 10 L containers after the dip solution had been mixed for 30 seconds by raising and lowering the cage. Again the pH and temperature were recorded and the diazinon content measured as above.

RESULTS

Remarks - Results

The pH of all the treated waste sheep dip solution samples remained at 7.4, indicating excellent buffering capacity of the dip solution, and the temperature also remained unchanged. The diazinon content in the control that had been pH treated as for the dip samples was initially 35.7 mg/L (ppm) and remained 33 ppm after 60 minutes, indicating no breakdown under the conditions of the test. By contrast the diazinon content in all treated waste dip solutions was <0.01 ppm, indicating >99.97% breakdown efficiency within 30 minutes.

This was also the case for the unreplicated trial using the actual dip solution. In addition the diazinon concentration of the solid component of the dip solution was measured after 30 minutes at the treatment rate of 10 g/L (highest strength for laboratory solutions). This fell from 420 to 0.2 mg/kg indicating a 99.9% reduction. It also indicates that the majority of the diazinon had partitioned into the solid matter present at the bottom of the dip during the dipping process.

By contrast the hydrated lime treatment after 41 hours standing still had a mean diazinon content of 34.3 ppm, which was not statistically different from that of the control sample. Two of the 3 replicates were actually higher than the control at 45-46 ppm, with the 3rd 12 ppm (standard error of 11.1). It was concluded that hydrated lime, which is often added at this concentration for treatment of waste post-harvest fruit and vegetable pesticide dips, has little effect on diazinon concentrations in spent sheep dip solutions.

CONCLUSION

The notified chemical efficiently degrades diazinon (>99.97% in 30 minutes) in moderately contaminated spent sheep dip solutions (about 35 ppm diazinon) both in laboratory samples and in the actual dip itself at a range of Landguard additions. It also degrades diazinon present in the solid component of the dip, but slightly less efficiently over the 30 minutes, possibly due to the more intractable nature of the solids, and the very much higher concentration (420 mg/kg).

TEST FACILITY

Orica (2005g)

8.4.3. Gundagai (NSW) field sheep dip treatment trial

TEST SUBSTANCE

Landguard OP-A

METHOD

Remarks - Method

This trial describes the results of experiments at Gundagai in New South Wales to evaluate the optimal Landguard dosing rate for the degradation of waste dip solution.

The trial involved a portable cage plunge dip of capacity 5,500 L in which 800 sheep, with 3 weeks wool growth since shearing, had been dipped at a target dose of 200 ppm diazinon. After this time the dip volume was 4,000 L and had a moderate amount of sludge on both the surface and in the base (the latter was viewed after the dip had drained).

The replicated trial was carried out as for Lake Bolac above except 2 further rates of Landguard addition (1 and 20 g/100 L equivalent to 10,300 and 206,000 IU/100 L, respectively) were also tested. In addition the effect of hydrated lime was not compared.

Sampling and work up was identical to the Lake Bolac trial described above. Further, in an unreplicated trial the required amount Landguard (200 g at 5 g/100 L or 51,500 IU/100 L) was similarly added to the dip tank and the contents mixed for 2 minutes. However, in this case mixing was indicated to have been poor.

RESULTS

Remarks - Results

The pH of all the treated waste sheep dip solution samples varied between 7.6 – 7.9 from an initial pH of 8.0, and as above the temperature remained unchanged. The diazinon content in the control was initially 54.3 ppm and had risen to 64.3 ppm after 60 minutes, indicating no breakdown under the conditions of the test. However, in contrast to the above the diazinon content in all treated waste dip solutions was still measurable after 30 minutes ranging from 47.0 ppm (21% breakdown) at 1 g Landguard/100 L to 0.76 ppm (98.7% degradation) at 20 g Landguard/100 L.

This was also the case for the unreplicated trial using the actual dip solution which measured 9.6 ppm diazinon (84% breakdown) after 30 minutes and 2.0 ppm (97% breakdown) after 60 minutes. In addition the diazinon concentration of the solid component of the dip solution was measured at the treatment rate of 20 g/100 L (highest strength for laboratory solutions). This fell from 372 to 40 mg/kg after 30 minutes indicating a 97.6% reduction. It again indicates that the majority of the diazinon had partitioned into the solid matter present at the bottom of the dip during the dipping process.

CONCLUSION

In this case the notified chemical less efficiently degraded diazinon in moderately contaminated but higher strength spent sheep dip solutions (about 54.3 ppm diazinon), both in laboratory samples and in the actual dip itself. Efficiency was greater at higher strength Landguard additions. It also degraded diazinon present in the solid component of the dip, again slightly less efficiently compared with solutions with the same dose rate of addition over the 30 minutes, possibly due to the more intractable nature of the solids, and the very much higher concentration (372 mg/kg). The report suggests that the slower rate may be due to differences in the starting concentrations concludes that longer treatment times may be required under some circumstances.

TEST FACILITY

Orica (2005h)

8.4.4. Loxton (NSW) field sheep dip treatment trial

TEST SUBSTANCE

Landguard OP-A

METHOD

Remarks - Method

This trial describes the results of experiments at Loxton in South Australia to evaluate the optimal Landguard dosing rate for the degradation of waste dip solution.

The trial involved a portable swim through dip of capacity 4,000 L in which 1200 sheep, with 3 weeks wool growth since shearing, had been dipped at a target dose of 300 ppm diazinon. After this time the dip volume was 3,000 L and had a low amount of sludge on the surface and a moderate amount in the base (the latter was viewed after the dip had drained).

The replicated trial was carried out as for Gundagai above except for the lack of 1 and 2.5 g/L additions and 3 further rates of Landguard addition (25, 30 and 50 g/100 L equivalent to 257,500, 309,000 and 515,000 IU/100 L respectively) were also tested.

Sampling and work up was identical to the Lake Bolac trial described above. For the unreplicated trial the required amount Landguard (750 g at 25 g/100 L or 257,500 IU/100 L) was similarly added to the dip tank and the contents mixed for 2 minutes. In this case mixing was manual and indicated to have been satisfactory.

RESULTS

Remarks - Results

The pH of all the treated waste sheep dip solution samples remained at 8.4, indicating excellent buffering capacity of the dip solution, and the temperature also remained unchanged. The diazinon content in the control was initially 73 ppm and had risen to 78.3 ppm after 60 minutes, indicating no breakdown under the conditions of the test. Again the diazinon content in all treated waste dip solutions was still measurable after 30 minutes, ranging from 5.9 ppm (92% breakdown) at 5 g Landguard/100 L to 0.21 ppm (99.7% degradation) at 50 g Landguard/100 L. Breakdown was slightly more efficient than in the Gundagai trials, but still significantly less than at Lake Bolac.

This was also the case for the unreplicated trial using the actual dip solution which measured 0.41 ppm diazinon (99.5% breakdown) after 30 minutes and 0.079 ppm (99.9% breakdown) after 60 minutes. In addition the diazinon concentration of the solid component of the dip solution was measured at the treatment rate of 25 g Landguard/100 L. This fell from 110 to 5.37 mg/kg after 30 minutes indicating a 95.1% reduction, but was <0.05 ppm if not pH treated after 30 minutes and the reaction allowed to go to completion (actual time not stated). Note that in this case the diazinon partitioned only slightly more preferentially into the solid matter present at the bottom of the dip during the dipping process.

In addition two soil samples were taken from a location on the Loxton farm where waste sheep dip solution had been released on the same area over a period of 3 years, but not for the past 2 years. The first soil sample taken from a depth between 0 - 10 cm contained 3.31 mg/kg diazinon, where a second taken from 10 – 20 cm contained 0.26 mg/kg diazinon. This is said to indicate that diazinon is persistent in the soil under these conditions, but no initial or earlier results are available to indicate the amount of degradation over time.

CONCLUSION

Again the notified chemical less efficiently degraded diazinon in moderately contaminated but higher strength spent sheep dip solutions (about 73 ppm diazinon), both in laboratory samples and in the actual dip itself. Efficiency was again greater at higher strength Landguard additions. It also degraded diazinon present in the solid component of the dip, again slightly less efficiently compared with solutions with the same dose rate of addition over the 30 minutes, probably due to the more intractable nature of the solids.

TEST FACILITY Orica (2005i)

8.4.5. Co-factor for Organophosphate treatment and dose rate for diazinon degradation

TEST SUBSTANCE Landguard OP-A

METHOD

Remarks - Method

In preliminary laboratory studies it was identified that at high concentrations (said to be in the order of 400 mg/L) Landguard delivered undesirably low breakdown efficiencies (around 40%) at an economically viable dose. Incomplete degradation (after 30 minutes) was also a feature in the studies above at diazinon spent dip concentrations of >50 ppm, particularly with low dose additions. In the laboratory studies it was found that during the organophosphate pesticide breakdown the solution pH decreased over time due to the increase in the concentration of an acidic breakdown product (note this does not seem to have been the case in sheep dip trails above where the pH was always >7.4). To rectify this problem a number of additives were screened for their potential to improve the efficacy of Landguard.

Using a commercial sheep dipping formulation, a 400 mg/L diazinon solution was made up with tap water and 250 mL aliquots were transferred to sample jars. The pH was recorded, and then the potential additives [calcium carbonate, chloride and hydroxide; glycine pH 9 buffer; magnesium chloride and hydroxide liquid; non-ionic surfactant (BS 100); potassium carbonate and bicarbonate; sodium acetate and carbonate; and vegetable oil] mixed into the samples and the pH recorded again. Landguard was then added and then after 30 or 60 minutes the pH recorded and the sample quenched with 40% perchloric acid as outlined above.

RESULTS

Remarks - Results

The initial screen was carried out with an additive dose rate of 5 g/100 L solution (except that potassium bicarbonate was at 10 g/100 L and the glycine pH 9 buffer at 10 mM, and there were 50 g/100 L additions for sodium acetate and carbonate as well), and a Landguard dose rate of 2500 IU/L. After 30 minutes pH ranged from 5.5 to 10, with most in the acidic range. The breakdown efficiency for Landguard alone was only 38%, and that for the additive solutions ranged between 13-92%, but it was clear that the best performances were in basic solutions (best was at pH 10 with 50 g/100 L sodium carbonate addition). Interestingly the addition of vegetable oil and the non-ionic surfactant (BS 100) reduced the breakdown efficiencies considerably (13-14% after 30 minutes), highlighting that oils and surfactants hinder the enzyme reaction.

From these results potassium, sodium and calcium carbonates as well as calcium hydroxide were chosen as candidates for further work. This was carried out at additive dose rates between 5 and 50 g/100 L, but at the same Landguard dose rate except for one occasion (potassium carbonate at 5 g/100 L where it was 1250 IU/L). While calcium hydroxide was the best performer at the lowest additive rate (87% breakdown after 30 minutes), the efficiency (42%) dropped significantly at the highest additive rate. This was attributed to the high pH (11.7) where Landguard performs poorly as the enzyme stability is low above pH 11. While sodium carbonate performed well in the range of additive rates, release of water containing sodium could be detrimental to soil health, and of the remainder potassium was chosen over calcium carbonate as it had the superior performance.

Further studies on this additive in the range 5-30 g/100 L and with a Landguard dose rate of between 625 and 2500 IU/L, indicated that the optimum conditions were 20 g/100 L and 2500 IU/L, which after 60 minutes resulted in a pesticide breakdown efficiency of 95%. Other combinations had an efficiency of between 42-85% except for 96% in the 30 g/L dose rate at 2500 IU/L. However, this had a final pH of 9.1 as opposed to 7.5 for the lower additive rate, the latter being more desirable for land disposal. The adjusting of the water pH from 6-9 to between 9.5 – 10.6 of potassium carbonate is seen as an advantage as it means variations in the initial pesticide solution pH will have a negligible effect on breakdown efficiency.

CONCLUSION

To improve pesticide breakdown efficiency, particularly in acid solutions and/or when the diazinon concentration is high (> 400 ppm), potassium carbonate addition at a rate of 20 g/100 L with Landguard rates of about 2500 IU has been concluded to be the best set of conditions.

TEST FACILITY

Orica (2005j)

8.4.6. Dose response with Landguard on 500 mg/L chorpyrifos for recreational turf rinsate treatment

TEST SUBSTANCE

Notified chemical

METHOD

Remarks - Method

Chlorpyrifos based pesticides are widely used by the recreational turf industry in Australia and around the world to control various pests on turf. Therefore a golf course rinsate sample was collected from a sprayer which had just applied a commercial 500 EC formulation at a concentration of 10,000 mg/L and returned with approximately 2 L of unused spray solution. The tank was then rinsed with an unspecified quantity of water and a sample taken from the bottom of the tank. This had a chlorpyrifos concentration of 345 mg/L, which had dropped to 240 mg/L after storage in the fridge at 4^{o}C for 2 weeks. Spiking this with further chlorpyrifos resulted in a concentration of 500 mg/L. After examination of chlorpyrifos product labels and discussions with golf course superintendents and representatives of recreational turf bodies around Australia, this concentration is stated to have been selected as the worst case concentration that would be encountered in the field.

The modified field sample was then buffered with 50 g Water Conditioner (potassium carbonate - see above and below) per 100 L, and then treated with 5, 10, 20, 30, 40, 50, 60 or 100 g of Landguard per 100 L of pesticide rinsate for 10 minutes and 1, 6 and 12 hours, respectively, with the pH before and after treatment recorded. After treatment each sample was quenched with 40% perchloric acid as described in 8.4.2 above. The chlorpyrifos content was then analysed using an identified assay.

RESULTS

Remarks - Results

The results show that after 10 minutes the breakdown efficacy clearly was less than desired, with a maximum of 66% at the 100 g/100 L Landguard loading (range 16-66%, with a clear trend with the Landguard rate). Even after 60 minutes this was still not optimal, ranging from 35-98%, though breakdown in all solutions with $\geq$50 g Landguard/100 L was $\geq$96%. After 6 hours the range was 94-99.5%, and after 12 hours it was 95-99.8%.

The report argues that these results would be worst case as formation of by products during storage in the fridge would have resulted in weak competitive inhibitors that can slow the activity of the enzyme. This would

not be the case with the treatment of "fresh" rinsates under normal practice.

CONCLUSION ≥95% breakdown efficiency can be achieved in 1 hour for solutions treated with 50 g/100 L Water Conditioner (potassium carbonate) and ≥50 g/100 L Landguard. This rate can also be achieved after 6 hours with ≥20 g/100 L Landguard, or after 12 hours with 5-100 g/100 L Landguard.

TEST FACILITY Orica (2005k)

8.4.7. Use of Landguard on rinsates in evaporation ponds

TEST SUBSTANCE Notified chemical

METHOD

Remarks - Method The disposal of rinsates from the cleaning down of aircraft used for aerial application presents problems, and one solution commonly used, particularly in the cotton growing areas is the use of evaporation ponds. The rinsate is collected and stored in plastic lined ponds and the liquid recirculated by either spraying or running the rinsate over the exposed plastic liner to ensure good evaporation occurs.

A draft sheet entitled Landguard Pesticide Clean-up, Environmentally responsible Organophosphate Remediation for aerial operators contains the results of testing the efficacy of breakdown of 3 organophosphates commonly used on cotton, but contains very little detail.

RESULTS

Remarks - Results Landguard at a rate of 1, 2 or 4 g/1000 L evaporation pond solution was added to methyl-parathion at a concentration of 235 mg/L, chlorpyrifos at 10 mg/L or profenophos at 35 mg/L, respectively. For methyl parathion breakdown was in the range of 20-43% after one week, but was 99-99.95% after 3 and 4 weeks. The rate was slower for chlorpyrifos, with 2-25% degradation after 1 week, 93-96.4% after 3 weeks, and >95.5% after 4 weeks. For profenophos it was slower still, with only 68-71% breakdown after 4 weeks.

CONCLUSION The sheet recommends that a Landguard rate of 2 g/1000 L is needed for the satisfactory remediation of organophosphates in evaporation ponds over 3 weeks, Water Conditioner (potassium carbonate) is also needed at a rate of 25 g/1000 L to ensure the maximum benefit of the enzyme is obtained.

TEST FACILITY Orica (2006)

9. RISK ASSESSMENT

9.1. Environment - Risk assessment of the notified chemical (active enzyme)

9.1.1. Environment – exposure assessment

Environmental release from several areas will occur during production of the product concentrate but all liquid waste streams are to be treated in a Biological Waste – Ultra High Temperature unit etc to ensure that no biological material remains before release to the sewer. Some release to the sewer will also result during the freeze-drying process from the washing of trays and equipment. These will be soaked in a solution of alkaline salts and detergents prior to washing with water and 70% ethanol, all of which are expected to help inactivate any residues of the product prior to discharge to the sewer. Any remaining material is not expected to survive the sewer processes since it has been shown to be readily biodegradable.

The main environmental release will result from the use of the product. While there may be some losses due to spills, these are expected to be minor compared with release from treated pesticide solutions. Since as an enzyme it catalyses the process and is not in itself consumed a worst case assumes that none is lost during treatment (i.e. it is not denatured itself by the prevailing conditions during treatment of dips/solutions – note no data are available for this aspect). The notifier estimates that the maximum released to a site may be up 600 g per treatment or a maximum of 12 kg of Landguard per year from treatment of pesticide application equipment or spent post-harvest dip solutions, following repeated disposal on the same site. Assuming this worst case with disposal over 1 hectare the concentration in the top 5 cm of soil would be 17.1 mg/kg.

There are no terrestrial toxicity data with which to compare this estimate to determine whether a hazard exists. However, these are likely to be very much worst case as the enzyme is likely to degrade in soils (no data for soils but it is readily biodegradable with > 90% degradation in 7 days) and it is likely that the bulk of the enzyme will be destroyed between each disposal event, and the actual level would be much lower (probably < 5 mg/kg).

In the case of use on spent sheep dips disposal is only likely after each yearly treatment of sheep for lice or blow flies and in this case a maximum of 600 g per treatment would be the worst case. Making the same assumptions as above the concentration in soil would be 0.85 mg/kg, which would also be the case for single disposals at a site from the other uses.

No deliberate aquatic release is expected from the proposed use pattern and therefore a Predicted Environmental Concentration (PEC) calculation cannot be performed. Aquatic exposure is also possible from run-off from disposal sites, but again this is expected to be limited as the water solubility should mean that the enzyme will soak into the soil, where it is expected to break down.

9.1.2. Environment – effects assessment

Results for toxicity to fish, aquatic invertebrates and algae all indicates a low aquatic toxicity with LC/EC50s all > 100 mg/L. Based on this a Predicted No Effect Concentration (PNEC) of > 1 mg/L can be derived using an assessment (safety) factor of 100 since tests have been carried out at 3 trophic levels. The enzyme has also been shown not to be inhibitory to sewage micro-organisms up to a level of 1000 mg/L.

9.1.3. Environment – risk characterisation

Limited, if any, aquatic exposure will result from the proposed use of the notified chemical, and therefore a Risk Quotient (RQ = PNEC/PEC) cannot be determined. Further it is practically non-toxic to 3 trophic levels of aquatic organisms, and therefore the risk is expected to be low and acceptable.

The risk to terrestrial organisms (including invertebrates) from disposal of treated solutions cannot be quantified as there are no appropriate toxicity data. However, this will be to discrete and small parcels of land, and any effects on organisms before the notified chemical degrades will be overcome by migration from adjacent non treated areas.

The risk to both aquatic and terrestrial organisms from the disposal of treated pesticide solution has yet to be conducted.

9.2. Environment – Risk assessment of the disposal of treated pesticide solution/dips

9.2.1. Environment – exposure assessment

As noted in section 5.4, the notifier will be recommending that users of Landguard OP-A continue to dispose of their treated pesticide solutions as per current best practice for the disposal of pesticide contaminated solutions and in accordance with state legislation. Landguard OP-A will be promoted as improving or building on current best practice rather than replacing currently accepted management practices. Therefore it is relevant to assess the risk from disposal of treated pesticide solutions, including the relative risk compared with current practice, almost all of which is understood to be disposal to flat land or to evaporation ponds.

As also noted there are 3 main uses proposed, treatment of used sheep and other animal pesticide dips, treatment of contaminated washings from pesticide application equipment (including material disposed of to evaporation ponds) and treatment of contaminated solutions from the washing of agricultural products or processing equipment. As practices are different in each of these cases they will be treated in turn.

9.2.1.1. Spent pesticide dips

The draft directions for used livestock dips indicate that diazinon is the sole target and that 100 g of Landguard OP-A, dissolved in 5 L of water, and 400 g of Water Conditioner (potassium carbonate) in 10 L of water should be added per 2,000 L of spent dip. The water conditioner should be added first by dispersal across the surface, followed similarly by the enzyme. The dip should be thoroughly mixed for at least 5 minutes (there are no directions as to how this should be achieved) and then allowed to react for at least 60 minutes, before disposing of the treated dip over an area of land dedicated to the disposal of treated waste dip solutions that is flat and does not drain to rivers, dams or other watercourses.

While the rate of addition of potassium carbonate is clearly proportional to the most efficient rate (20 g/100 L) derived from the co-factor study (Section 8.4.5. above), this is more difficult to compare for the Landguard addition as the activity is described in terms of IU in this study. The notifier has clarified that the batch used in this trial had an activity of 22,235 units/g on the day of freeze drying. Earlier batches had much lower activity, eg the batch used for the 3 sheep dip field trials above only had an activity of 10,300 units/g, whereas product to be sold will have up to 35,000 units/g due to improved production conditions. Assuming the maximum latter activity 100 g of Landguard in 2000 L is equivalent to 1 g/20 L or 35,000 units/20 L or 1750 units/L, which by comparison of the rates in the co-factor study suggests about 85% conversion after the minimum 60 minutes.

However, the co-factor study was conducted at a diazinon concentration of 400 mg/L, whereas the literature (Sherwood *et al*, 1999) indicates that spent dip concentrations are unlikely to be > 100 mg/L. This is supported by the trials summarised above, where even in the dip charged at 400 mg/L the concentration was around 73 ppm when spent (the others were 35.7 and 54.3 ppm, respectively). Thus the results for this trial should be viewed as worst case in the risk assessment. Re-examining the data and taking into account that the strength for the commercial product will be 3X greater (see above), it may be concluded that around 97.5% would be degraded in 30 minutes. Data for 60 minutes were not generated but a conservative worst case would be a minimum of 98% conversion under the draft directions for use.

From the above a worst case Potential Environmental Concentration (PEC) scenario for diazinon and its metabolites following treatment and dip disposal to land can be constructed as follows. Spent dip solution containing 100 ppm diazinon is treated as per the draft directions for use, resulting in a maximum remaining concentration of 2 ppm. The treated dip is at the maximum size of 12,000 L, and is disposed of over an area of 0.1 ha of land. The size of this disposal site is taken on a proportional basis from data published by Levot *et al* (2004). At this concentration there would be 24 g of diazinon remaining in the solution, and disposal at a rate of equivalent to 240 g/ha would result in a concentration of 0.17 mg/kg soil in the top 10 cm of soil based on a soil density of 1.4 g/cm^3.

Assuming diazinon is split quantitatively and efficiently into 2 roughly equal halves, the maximum concentrations in spent dip of diethylthiophosphoric acid and 2-isopropyl-4-methylpyrimimid-6-ol would be 50 ppm. A similar calculation to the above results in a worst case soil concentration of 4.3 mg/kg of each metabolite in the top 10 cm of soil.

The notifier has clarified that use to detoxify sheep dip sludges will not be specifically targeted. These contain much higher pesticide concentrations, and historically are often intractable (hard to break up and remove). The notifier has indicated that they have not encountered anyone that is still using an in-ground dip, so have not been able to determine if the enzyme could be used in situations with very high levels of highly contaminated compacted sludge sitting on the bottom of the dip. All farmers they have encountered have been using a mobile dip tank or a shower dip, and though in-ground dips do still exist, they are very rare. More importantly where they are still used, the dips are pumped out after each use, because of the OHS concerns etc related to shovelling out large quantities of sludge. The data provided in the three trials show that > 95% reduction in the solid component of the mobile dips was achieved after 30 minutes, indicating that a separate risk assessment of this phase in portable dips is not required.

9.2.1.2. Spray equipment wash water

The draft directions for treatment of pesticide spray equipment wash water indicates diazinon and chlorpyrifos are the sole targets and give a number of options for additions depending on whether rapid or relatively slow deactivation is sought. For the latter (called Landguard Overnight with a claimed > 95% reduction in OP residues within 12 hours) 100, 200 or 300 g Landguard OP-A are added to 200-400, 600-800 and 1000-1200 L solution, respectively. For the former (Landguard Rapid with a claimed > 95% reduction in OP residues within 1 hour), 200 or 400 g Landguard OP-A should be added to a 200-400 and 600-800 L solution (there is no larger option).

The maximum amount of water should be added to the dilute unused spray mix, ie to tank capacity. In all cases 50 g of Water Conditioner (potassium carbonate) should be added per 100 L of tank washings directly to the agitated spray tank (the notifier has clarified that the higher rate, 50 versus 20 g/100 L, needs to be used because the organic matter and clay in the sheep dips buffer the dip solution, and as sheep dips typically have lower levels of pesticides). This is to be followed by the correct amount of Landguard OP-A (see above) dissolved in water at a rate of 100 g/2 L, and added to the agitated tank within 5 minutes of preparation. After the minimum standing time the treated tank wash water should be disposed of over an area of land dedicated to the disposal of treated equipment wash water that is flat and does not drain to rivers, dams or other watercourses.

The draft label indicates that dose rates and residue level reductions of > 95% are based on an unused spray mix volume of ≤ 5% of the spray tank volume, ie a maximum of 20 L residual for a 400 L sprayer, or 50 L residual for a 1000 L sprayer. The water carrier to active ratio also needs to be > 25:1, ie the pesticide should be applied in > 300 L water/ha, and the organophosphate application rate should be no greater than the maximum label rate. Finally should there be other chemicals or adjuvants in the mix the notifier or supplier should be contacted for advice.

Data provided in the submission for this type of use are the efficacy study summarised in 8.4.6 and the study of metabolites from diazinon and chlorpyrifos (8.3.1 and 8.3.2 above). Considering the former study, the amount of Landguard to be added in the field ranges between 25-50 g/100 L for the 12 hour treatment, and 50-100 g/100 L for the 60 minutes treatment. Taking into account that the enzyme activity in the trial was about 2/3 of that expected for production batches this translates to about 37.5-75 g/100 L and 75-150 g/100 L, respectively. Comparing this with the results in the trial described in Section 8.4.6 (noting that the concentration of potassium carbonate is the same), it may be concluded that 99 and 98% would have broken down after 12 hours and 60 minutes, respectively.

In the latter studies the notified chemical at a rate of 0.1 g/L was added to solutions of <50 ppm chlorpyrifos and diazinon in ammonium acetate buffers of pH 6 and 9, and >99.8% conversion of the pesticide was achieved within 30 minutes. This is a lower rate of addition than proposed

in the field, which ranges between 0.5-1.0 g/L for the Landguard Rapid and 0.25-0.5 g/L for Landguard Overnight. Offsetting this is the much lower concentration of the pesticide, which in general leads to much quicker degradation, even though, as noted above, the production batches used for laboratory and field experiments generally had lower (roughly 2/3) enzyme activity than product that will be sold.

Thus it is agreed the claimed > 95% conversion on the draft directions for use should be achievable in the field under the label conditions, and that at least 98% may be expected. The need to seek advice from the notifier/supplier should there be other chemical or adjuvant in the mix may well seem to be quite restrictive, as diazinon and chlorpyrifos formulations are expected to contain organic solvents, surfactants etc and spray adjuvants are also often used, and the vegetable oil and non-ionic surfactant in the co-factor trial (Section 8.4.5) significantly reduced breakdown efficiencies. However, the trial described under Section 8.4.6 was undertaken on a chlorpyrifos 500 EC (emulsified concentrate) product, which would have included these additives, and the notifier has clarified that ionic surfactants are not an issue at the recommended rates of the enzyme addition or pesticide concentrations.

Looking at the use pattern in the interim APVMA review of chlorpyrifos (http://www.apvma.gov.au/chemrev/chlorenv.pdf) suggests a number of uses could leave high concentrations in the spray tank. This would include Pest Control Operator use on termites where an emulsion of 10 g/L (20 g/L in the tropics) is used direct. The highest other rate for this kind of use would appear to be 4 kg/ha for lawn and turf maintenance. Assuming this is applied in > 300 L of water/ha (as required by the draft directions for use), the maximum concentration would be 13.3 g/L, or 13,300 ppm.

Use in cotton at the maximum rate of 1.5 kg/ha would also be at relatively high concentrations particularly as ULV sprays by aircraft, where the products are diluted very little if at all. Aerial use as an EC would typically be in 20 L of water (or 25 g/L assuming the common 500 g active/L in products). Similar concentrations could result from treatment of cereals and pasture by air, but in both cases ground application would be as a more dilute spray since 80-100 L of water per hectare is often used, as it would for vegetable treatments. Use in orchards would also be at relatively dilute levels given the maximum is 100 g/100 L (or 1 g/L or 1000 ppm), except for one relatively minor use in citrus (200 g/100 L).

Similar considerations arise for diazinon where use by both pest control operators and in the broadacre and horticultural industries (though not as extensive as many of these uses may be removed from labels, see http://www.apvma.gov.au/chemrev/diazinonenv.pdf) are possible.

To assist with the risk assessment the notifier has clarified that the current enzyme will be restricted to the following situations:

- where concentrations of the OP pesticides are below 500 mg/L in the contaminated solution or where dilution to this level is possible;
- where the target OP pesticide is the primary environmental issue; and/or
- situations where time is not a concern (eg pesticide evaporation ponds)

As a consequence for equipment rinsate the recreational turf industry and evaporation ponds will be the main initial marketing focus in Australia. Hence the use of the word “Turf” on the draft Directions of Use. The notifier had indicated that the following considerations were used to develop the "turf" label (in conjunction with the industry):

- 15,000 mg/L present in left over spray solution (application rate of 6 L/ha of chlorpyrifos 500 EC with a water volume of 200 L/ha)
- 3 L of spray solution remaining at the end of spraying with equipment fitted with a 200 L tank (1.5% remaining in spray lines and bottom of tank). This worst case figure was estimated after speaking with golf curators, agronomists and spray equipment manufactures.
- left over solution diluted to the capacity of tank (as per directions on the draft label), leading to 225 mg/L, which is significantly less than the 500 mg/L that was used in the laboratory trial described above (Section 8.4.6).

Nevertheless a worst case Predicted Environmental Concentration (PEC) should be derived on

the maximum tested in the laboratory, and under the maximum conditions described on the draft directions for use. Thus 50 L is diluted with water to 1000 L to give a 500 ppm solution, which is treated giving a minimum of 98% conversion. This would leave a maximum of 10 ppm solution, or 10 g in the 1000 L, which is disposed of to 0.01 ha (10 m X 10 m, proportionally based on the dip example above) of land. Disposal at a rate of equivalent to 1000 g/ha would result in a concentration of 0.71 mg/kg soil in the top 10 cm of soil based on a soil density of 1.4 g/cm^3.

Assuming chlorpyrifos or diazinon are split quantitatively and efficiently into 2 roughly equal halves, the maximum concentrations in spent dip of diethylthiophosphoric acid and 2-isopropyl-4-methylpyrimimid-6-ol (from diazinon) or 3,5,6-trichloropyridin-2-ol (from chlorpyrifos) would be 250 ppm. A similar calculation to the above results in a worst case soil concentration of 17.9 mg/kg of each metabolite in the top 10 cm of soil.

9.2.1.3. Use on evaporation ponds

The notifier has clarified that the target will be evaporation ponds used by the aerial agricultural industry in cotton growing areas. Because of the formulation types (ULV) and high concentration of pesticides in the equipment rinsate from aircraft tanks, there is a need to add the enzyme directly to the evaporation ponds rather than the aircraft's tank. The notifier will be recommending considerably lower rates in this situation, given that a fast reaction time is not required.

The notifier has provided a draft document entitled Treatment of Pesticide in Evaporation Ponds and Rinsate Tanks. The targets will be chlorpyrifos, diazinon and methyl parathion, and the following Landguard OP-A rates are given for "slow degradation":

Volume (L) Tank/Pond	*Dose Rate Landguard™ OP-A*	*Dose Rate Water Conditioner*
20,000 L	4 g	500 g
100,000 L	20 g	2.4 kg
250,000 L	50 g	7 kg
500,000 L	100 g	13 kg
1,000,000 L (1 ML)	200 g	25 kg

The draft instructions indicate that the Water Conditioner should be pre-dissolved in water at a rate of 10 kg/10 L, and the solution introduced near the suction point of the operating recirculation pump to ensure the potassium carbonate is dispersed throughout the pond or tank. The pump should be run for at least 1 day to ensure good mixing. Alternatively it can be added directly to the pond, but must be spread fairly evenly.

Next the required amount of Landguard should be pre-mixed with water in a ratio of 100 g/2 L and the enzyme solution slowly introduced to the evaporation pond or tank at the suction point, and be allowed to continue to mix for several hours with the agitation pump going. The pump should be operated for at least 4 hours each day, and Landguard/Water Conditioner should be added each week during the spraying season while contaminated solutions containing the target pesticides are continually being added to the pond.

The rates of addition of Landguard of 0.2 g/1000 L is 10% of that recommended following the trial described in Section 8.4.7 above, and while the potassium carbonate rate at 24-28 g/1000 L is similar, it is therefore impossible to estimate the percentage reduction over the 3-4 week period. However, further contaminated solutions, probably also containing pesticides which are only poorly or not broken down by Landguard, will continually be added to the ponds during the spraying season, and therefore it is redundant to estimate the efficacy of individual or overall treatments. Further the reaction will be taking place in situ within the evaporation pond, with no need to dispose of the treated solution to land, though at the end of the season there may be a need to dispose of a highly concentrated brew as per current practice. As a result no PEC can be estimated for this use.

9.2.1.4. Washing of agricultural produce or processing equipment

No draft specific directions for use for treatment of contaminated solutions from the washing of agricultural produce or processing equipment have been provided, but the notifier has clarified that there will be a separate set of directions for these uses. Directions will be similar to the "livestock" directions, although potassium carbonate will not be required. The big difference between these uses and equipment rinsate and dips is the significantly lower concentration of pesticide. At these concentrations of pesticide the enzyme is highly active and it is indicated non detectable levels of the target pesticides are readily achieved. It is accepted that the above 2 situations will represent the worst case PECs.

9.2.1.5. Treatment of spent post-harvest dip solutions

Use is not proposed at this time for treatment of spent post-harvest dip solutions, for which fenthion and dimethoate are the OPs most commonly used in Australia. The current enzyme has lower activity on these two actives at economical use rates and will not be recommended. However, the notifier will have a variant in the future that is active on both actives at economical use rates.

9.2.2. Environment – effects assessment of pesticides in solutions/dips

No toxicity data for the target pesticides which, at least initially, are diazinon and chlorpyrifos, have been provided. However, these are readily available from the comprehensive reports undertaken as part of the detailed chemical reviews of these two active constituents by the Australian Pesticide and Veterinary Medicines Authority (APVMA), and which have been published on the APVMA's web site. Methyl Parathion is also targeted, but only in evaporation ponds where the estimation of a PEC has not been deemed relevant above.

For diazinon, the risk assessment was based on a 48 h EC50 to *Daphnia magna* of 0.96 µg/L (http://www.apvma.gov.au/chemrev/diazinonenv.pdf), which though not the most sensitive end point was the most reliable. Including an assessment factor of 10, the Predicted No Effect Concentration (PNEC) would be 0.1 µg/L. There were limited data for soil invertebrates with an earthworm 14 day EC50 of 130 mg/kg, with the observations from field trials that there was unlikely to be significant mortality of earthworms at 20 mg/kg in soil. In field studies diazinon had no effects on spiders at rates up to 1000 g/ha, though for rove beetles no adults emerged from pupae when sand was treated at 2500 g/ha. Diazinon was also shown to have no effect on soil microbial processes at up to 80 mg/kg in soil.

In the case of chlorpyrifos (http://www.apvma.gov.au/chemrev/chlorenv.pdf), the most sensitive end point was again a 48 h EC50 of 0.015 µg/L to *Daphnia magna*. This may have reflected an increased bioavailability for the EC formulation tested, as the next most sensitive end point was a 96 h EC50 of 0.045 µg/L to the mysid shrimp, known to be a very sensitive marine species. However, the aquatic end point mostly used was from a mesocosm study where arthropod populations suffered sharp reductions at 0.3-3 µg/L, and needed 2-4 weeks to recover. Therefore again the PNEC will be assumed to be 0.1 µg/L.

There are a number of end points available for testing of earthworms' sensitivity to chlorpyrifos, with acute (14 day) LC50s ranging from 104-1174 mg/kg, and two EC50s based on reproduction, both of which were 112 ppm. However, a test on soil dwelling beetles in the laboratory at 960 g/ha showed no beetles hatched at this rate. While at greater than field rates chlorpyrifos seems to have some effects on soil micro-organisms, which take some time to stabilise, a recent study on soil microbial populations by determining total viable cell numbers on nutrient media gave an LC50 to bacteria of 300 ppm on the third day. Fungi, however, were much more sensitive, being totally inhibited even at 1 ppm.

While there will be some residual pesticide disposed of to land, the main constituents will be the metabolites of the enzyme process. As noted in Sections 8.3.1 and 8.3.2, these have been identified as diethylthiophosphoric acid and 2-isopropyl-4-methylpyrimid-6-ol or oxypyrimidine (from diazinon) and 3,5,6-trichloropyridin-2-ol or TCP (from chlorpyrifos), respectively. The APVMA report for diazinon notes only that (hydr)oxypyrimidine has an EC50 of 118 mg/L to the bacteria *Photobacterium phosphorem* when tested in a Microtox system (cf 10.3 mg/L for diazinon itself).

For chlorpyrifos an older (pre-1970) study on TCP gave a 96 h LC50 range of 0.75-4.85 mg/L on 3 species of fish. More recent testing has provided 96 h LC50 results ranging from 12.5-58.4 mg/L from a further 3 fish tests, and 48 h EC50s of 3.13 and 10.4 mg/L in two tests to *Daphnia magna*. The chlorpyrifos report also notes some effects of TCP on soil microbes at rates between 500-1000 mg/kg (but not at 10 mg/kg), based on a slowing in the degradation rate over time in one laboratory soil.

There are no data for diethylthiophosphoric acid in either of the reports, but the literature (Sinclair and Boxall, 2003) lists 2 results of 96 h LC50s to fish of 100 mg/L and a daphnia 48 h EC50, also of 100 mg/L. This reference also confirms the significantly lower toxicity of the organic metabolites, listing a fish 96 h LC50 of 1200 mg/L for the pyrimidinol formed from diazinon, and a fish 96 h LC50 of 1.5 mg/L for TCP. The lower toxicity is well accepted and corresponds with the loss of the organophosphate "toxicophore".

9.2.3. Environment – risk characterisation

In Section 9.2.1 Predicted Environmental Concentrations (PEC) have been calculated for two scenarios, and the environment risk resulting from these are estimated in turn below.

9.2.3.1. Spent pesticide dips

In this case the worst case PEC would result in a concentration of 0.17 mg diazinon/kg soil in the top 10 cm of soil within the area of land where the treated spent dip has been disposed. While there are limited data for soil invertebrates this is well below the level of 20 mg/kg where effects in the field on earthworms were not observed, and also below the 80/mg/kg where soil microbiological processes were not affected. Further degradation of the residual diazinon would still occur, with the literature (Levot et al, 2004) indicating a half-life of about 9 days when spent but untreated dip wash was disposed of to land.

On the other hand the worst case PECs for the two degradation products diethylthiophosphoric acid and 2-isopropyl-4-methylpyrimimid-6-ol results in soil concentrations of 4.3 mg/kg of each metabolite in the top 10 cm of soil. In this case there are very limited soil/terrestrial toxicity data available with which to compare this estimate to determine whether a risk exists. This is limited to an EC50 of 118 mg/L to the bacteria for the latter (cf 10.3 mg/L for diazinon itself). However, the much lower general toxicity of these metabolites, including to aquatic organisms, should ensure that the estimated worst level is not toxic to the expected limited range of soil organisms which may inhabit the disposal site.

No deliberate aquatic release is expected from the proposed use pattern and therefore a Predicted Environmental Concentration (PEC) calculation was not performed. Aquatic exposure is also possible from run-off from disposal sites, but again this is expected to be limited if the site is properly bunded. The maximum concentration of diazinon in the spent treated dip is 2 ppm, which is well above the PNEC of 0.1 μg/L, emphasising the need for the disposal site to be properly bunded. Likewise the treated solutions have a maximum of 50 ppm each of diethylthiophosphoric acid and 2-isopropyl-4-methylpyrimimid-6-ol. While this is below the 96 h LC50s to fish and 48 h EC50 to daphnia of 100 mg/L for the former and the fish 96 h LC50 of 1200 mg/L for the latter, this would not be the case if an assessment factor of 100-1000 is applied due to the limited data, again emphasising the need for proper bunding to prevent run off.

As noted above, the notifier will be recommending that users of Landguard OP-A continue to dispose of their treated pesticide solutions as per current best practice for the disposal of pesticide contaminated solutions and in accordance with state legislation.

In the APVMA's draft environmental assessment report for diazinon, (http://www.apvma.gov.au/chemrev/diazinonenv.pdf), the following disposal instructions are recommended on an interim basis:

"Dispose of used dip solution and sludge over an area of dedicated and bunded flat land, away from watercourses and any drainage areas etc that could contaminate watercourses, and restrict access to humans and stock for a period of at least 3 months".

These recommendations are appropriate for spent dip solutions treated with Landguard, noting that the diazinon concentration will be <2% of current practice.

9.2.3.2. Spray equipment wash water

In this case the worst case PEC would result in a concentration of 0.71 mg diazinon or chlorpyrifos/kg soil in the top 10 cm of soil within the area of land where the treated spent dip has been disposed. This is still well below the levels at which effects on soil organisms may be expected for diazinon. Based on the two earthworm reproduction EC50s of 112 ppm, and the soil micro-organism data, no effects might also be expected from chlorpyrifos, except that a risk to fungi cannot be ruled out, as these were totally inhibited even at 1 ppm. However, this would only occur in the disposal site, and re-colonisation from outside the bunded area would occur once chlorpyrifos levels drop.

On the other hand the worst case PECs for the three degradation products diethylthiophosphoric acid, 2-isopropyl-4-methylpyrimid-6-ol (from diazinon) and 3,5,6-trichloropyridin-2-ol (or TCP from chlorpyrifos), respectively, results in soil concentrations of 17.9 mg/kg of each metabolite in the top 10 cm of soil. Again for diazinon this is still below the EC50 of 118 mg/L to the bacteria for the latter (cf 10.3 mg/L for diazinon itself), and taken together with the much lower general toxicity of these metabolites, including to aquatic organisms, should ensure that the estimated worst level is not toxic to the expected limited range of soil organisms which may inhabit the disposal site.

For the metabolite TCP formed from chlorpyrifos, the only information suggests some effects on soil microbes at rates between 500-1000 mg/kg, again well above the worst case PEC. TCP appears to be more toxic to aquatic organisms that the other two metabolites, with LC/EC50s in the range 0.75-58.4 mg/L, which suggests soil invertebrates may also be more sensitive and it is difficult to rule out a risk. However, again this will be limited to the actual site, which is expected to be bunded and quite limited in size (10 X 10 m as estimated above), with re-colonisation likely to occur once TCP levels drop.

Unlike disposal of spent sheep dips, which is generally only expected to take place at shearing once per year, there will be a need to dispose of spray equipment wash water throughout the spraying season, which the notifier accepts may be up to 20 times. This may be on consecutive days if the applicator is unable to complete a task within one day, or on repeat spraying with either diazinon or chlorpyrifos. If as expected this would be disposed of to the same area of land, the soil concentrations would be higher than estimated above, even taking into account degradation of diazinon or chlorpyrifos between disposal.

According to the APVMA report repeated use is only expected for diazinon with vegetables (http://www.apvma.gov.au/chemrev/diazinonenv.pdf). As this is not a targeted use, and the half-life in field studies ranged from 3-16 days, with one at 27 days, and for the principal metabolite between 7-24 days, accumulation in soil is not expected to be of concern. For chlorpyrifos (http://www.apvma.gov.au/chemrev/chlorenv.pdf), field half-lives in soil were somewhat longer (between 2-8 weeks), though repeat use in the cotton industry, where spray equipment wash water will be disposed of to evaporation ponds, is more likely than in the turf industry. Examination of representative labels for the latter reveals that there are no specific instructions for repeat spraying, with the application generally occurring when pests or signs of infestation appear, with (in a couple of instances only) follow up treatments as required. Given the relatively small areas of turf expected to be treated, it will be assumed disposal of spray equipment wash water, at least containing the target pesticides diazinon and chlorpyrifos, is not likely to occur on consecutive days, or even weeks.

Again no deliberate aquatic release is expected from the proposed use pattern and therefore a Predicted Environmental Concentration (PEC) calculation was not performed. Aquatic exposure is also possible from run-off from disposal sites, but again this is expected to be limited if the site is properly bunded. In this case the maximum concentration of diazinon or chlorpyrifos in the treated solutions is 10 ppm, which is well above the PNECs for both of 0.1 µg/L, again emphasising the need for the disposal site to be properly bunded. Likewise the treated solutions have a maximum of 250 ppm each of diethylthiophosphoric acid and 2-isopropyl-4-methylpyrimimid-6-ol or 3,5,6-trichloropyridin-2-ol. This is only below the fish 96 h LC50 of

1200 mg/L for 2-isopropyl-4-methylpyrimimid-6-ol, again emphasising the need for proper bunding to prevent run off.

There are no specific instructions for the disposal of spray equipment wash water for either diazinon or chlorpyrifos in the respective APVMA environmental assessment reports. The AG Labelling Code (http://www.apvma.gov.au/MORAG_ag/vol_5/ag_labelling_code.pdf) also contains no general instructions for this, with the closest being the following for formulations which are not diluted with water before use (ULV, ready to use etc.):

> Triple or preferably pressure rinse containers before disposal. Dispose of rinsings in a disposal pit specifically marked and set up for this purpose, clear of waterways, desirable vegetation and tree roots.

Given this the following instructions, amended from those for diazinon spent dips (above), are recommended:

"Dispose of spray equipment wash water over an area of dedicated and bunded flat land, away from watercourses and any drainage areas etc that could contaminate watercourses, and restrict access to humans and stock".

This is expected to largely conform to current practices, except that bunding is not guaranteed. This would be required in view of the above risk assessment.

9.3. Human health

9.3.1. Occupational health and safety – exposure assessment

Manufacture of the Liquid Concentrate

Due to the largely automated and contained nature of the manufacture process, and treatment of the equipment prior to maintenance, negligible exposure to the notified chemical is expected for the majority of workers. Workers involved in sample analysis have the potential to be exposed to be the enzyme concentrate (concentration notified chemical < 10%) via dermal and ocular routes from contact with drips spills and splashes. However, exposure is expected to be low due to the small samples involved (< 100 mL), the short sampling times (< 8 minutes) and the use of PPE (lab coat, safety glasses, latex gloves). Workers may also be exposed to the enzyme concentrate during the changing of blocked filters, however, exposure is also expected to be low due to the low frequency of exposure and use of PPE.

Freeze-drying and Packaging

Dermal and possibly ocular exposure to the notified chemical could occur during the transfer of the liquid concentrate to the shallow drying trays. The estimated dermal exposure to the enzyme concentrate is 420 mg/day, based on EASE model (EASE) using reasonable worst case defaults for the exposure scenario 'manual addition of liquids' (European Commission, 2003). Therefore, for a 70 kg worker and a 100% dermal absorption factor, systemic exposure is estimated to be 6 mg/kg bw/day. Worker exposure during the freeze-drying process is expected to be negligible. Exposure would be limited by the use of PPE.

Dermal and inhalation exposure to the freeze-dried enzyme concentrate could occur during the filling of the drums, the washing of the trays and the sieving and packaging processes. The estimated dermal exposure is 84-840 mg/day, based on the EASE model (EASE) using the following inputs: dusty solid, non-dispersive use, direct handling (LEV not effective), intermittent contact (2-10 events per day) and assuming an exposed surface area of 840 cm^2 (hands only). Therefore, for a 70 kg worker and a 100% dermal absorption factor (based on the high molecular weight and low log P_{ow}), systemic exposure is estimated to be 0.12 – 1.2 mg/kg bw/day. If local exhaust ventilation is effective dermal exposure is estimated by the EASE model to be very low. Exposure would be limited by the use of PPE.

The estimated atmospheric concentration of freeze-dried enzyme concentrate due to dust is 5-50 mg/m^3, based on EASE model (EASE) using the following inputs: dry manipulation, non-fibrous and LEV absent. Therefore for a 70 kg worker, assuming an inhalation rate of 1.3 m^3/hour, 3.5 hour exposure time and 0.26% inhalable fraction, inhalation exposure is estimated

to be 0.00014-0.0014 mg/kg bw/day. In the presence of effective local exhaust ventilation, inhalation exposure is estimated by the EASE model to be 0.0005-0.0014 mg/kg bw/day. Exposure would be limited by the use of dust masks.

Inhalation and dermal exposure to dust would be further minimised by the presence of engineering controls (LEV (sieving, packing and washing operations), positive pressure (all other rooms))

End Use
Although inhalation and dermal exposure to the freeze-dried enzyme concentrate could occur during manual addition to water, exposure is expected to be low due the method of the addition (tipping contents not weighing), the low frequency of exposure < 24 days per year, the low % of inhalable particles and for most workers the level of PPE being worn due to the pesticide solutions being treated. The level of PPE is likely to be lower for workers involved in the treatment of pesticide solutions from the washing of agricultural produce or processing workers however, the MSDS recommends the use of protective clothing, dust mask and gloves.

Following addition to water/contaminated pesticide solution, inhalation exposure is not considered to be a possible route of exposure. Dermal exposure will be further reduced by the low concentration of the enzyme concentrate in these solutions (< 2.5% (aqueous solution), <0.09% (pesticide solution)

9.3.2. Public health – exposure assessment

The enzyme concentrate is not supplied to the public and not direct contact is expected as a result of the manufacturing, freeze-drying and end use processes, therefore negligible exposure is expected.

9.3.3. Human health – effects assessment

Acute toxicity.
The notified chemical is considered to be of low acute toxicity via the oral and dermal routes.

Irritation and Sensitisation.
Based on the results of irritation studies in rabbits, the notified chemical is considered to be slightly irritating to skin and eyes. In a mouse local lymph node assay (LLNA), the notified chemical induced delayed contact hypersensitivity and as such is considered to be a potential skin sensitiser. The EC_3 value for the notified chemical is equal to approximately 3.4 % (w/v). Enzymes are reported to be potent inhalation sensitisers in concentrated form. Any enzyme preparation, independent of its source should be considered to be a respiratory sensitiser unless proven otherwise by unequivocal experiments (European Commission, 2002).

Repeated Dose Toxicity (sub chronic).
In a 28 day study in rats, the No Observed Effect Level (NOAEL) was established as 1000 mg/kg bw/day based on the absence of adverse treatment related effects.

Mutagenicity.
The notified chemical was negative in an Ames test and an in vitro chromosome aberration test. The notified chemical is not considered to be a potential mutagen.

Hazard classification for health effects.
Based on the available data, the notified chemical is classified as a hazardous substance in accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC 2004).

R42 May cause sensitisation by inhalation
R43 May cause sensitisation by skin contact

9.3.4. Occupational health and safety – risk characterisation

Systemic Effects
Exposure to the enzyme concentrate (containing < 10% notified chemical) during freeze-drying

and packaging operations was estimated to be 7.2 mg/kg bw/day. Based on a NOAEL of 1000 mg/kg bw/day, derived from a 28-day rat oral study the margin of exposure (MOE) is calculated as 140. MOE greater than or equal to 100 are considered acceptable to account for intra- and inter-species differences. Therefore, the risk of systemic effects using modelled worker data is acceptable for workers involved in freeze-drying and packaging operations. Exposure to the notified chemical is considered to be greatest for workers involved in the freeze-drying process and as such the risk of systemic effects for all workers is considered to be low.

Irritation/Sensitisation
The enzyme concentrate (containing < 10% notified chemical) is considered to be a slight eye and skin irritant and a skin and respiratory sensitiser.

Manufacture of the liquid concentrate
Due to the largely automated and contained nature of the manufacture process, and treatment of the equipment prior to maintenance, negligible exposure to the notified chemical is expected for the majority of workers and as such the risk of skin sensitisation is also considered to negligible. Where skin contact could occur (sample analysis, change of filters) the risk of irritant effects to the skin and eyes and the potential for skin sensitisation would be reduced by the use of PPE (lab coat, safety glasses, latex gloves). As inhalation exposure to the notified chemical is not expected, the risk of respiratory sensitisation effects is considered to be low.

Freeze-drying and Packaging
For workers involved in the transfer of the liquid concentrate to the shallow drying trays the risk of irritant effects to the skin and eyes and the potential for skin sensitisation would be reduced by the use of PPE (full length overalls, safety glasses, latex gloves). As inhalation exposure to the notified chemical is not expected, the risk of respiratory sensitisation effects is considered to be low. However, sensitisation cannot be ruled out in sensitive individuals.

Similarly during the sieving and packing of the freeze-dried enzyme the risk of irritant effects to the skin and eyes and the potential for skin sensitisation would be reduced by the use of PPE (full length overalls, safety glasses, latex gloves). Although inhalation exposure is also possible during this process, the risk of sensitisation is considered to be low due to the low % of inhalable particles (<0.26%), use of engineering controls (LEV) and use of PPE (dust masks). However, sensitisation cannot be ruled out in sensitive individuals.

End Use
The risk of irritant and sensitisation effects is considered most likely during the transfer of the enzyme concentrate to aqueous solutions due to the lower concentrations present following dilution and the fact that inhalation exposure is no longer a potential route of exposure. As the majority of workers will be wearing PPE suitable for treating pesticide solutions (protective clothing, impervious gloves, goggles and respirator) the risk of adverse irritant and sensitisation effects is considered to be low. Exposure and hence the risk of irritant and sensitisation effects is considered to be greatest for workers involved in the treatment of pesticide solutions from the washing of agricultural produce or processing equipment due to a reduced level of PPE. The label and directions for use state that the product may cause sensitisation by inhalation and skin contact, advise the use of suitable protective clothing, dust mask and gloves and advise that the worker avoid contact with skin and eyes and the breathing of dust. Therefore the risk is considered to be low provided the directions for use are followed. Sensitisation cannot be ruled out in sensitive individuals.

9.3.5. Public health – risk characterisation

As exposure to the notified chemical is considered to be negligible, the risk to public health is also considered to be negligible.

9.4 Genetic Modification Issues

The notifier has obtained a licence form the OGTR to produce commercial quantities of the notified chemical (DNIR licence number 136/2002). The risk assessment and risk management plan (OGTR, 2002) addressed issues such as risks from genetic modification and risks of

unintentional release. The risks to the health and safety of people and the environment from the manufacture of the notified chemical were assessed to be low. The risks from unintentional release were controlled by a proposed risk management plan.

The host bacterium for expression, *E. coli* BL21 DE3 is non-pathogenic to humans (Chart et al, 2000) and the genetic modification does not alter this status (OGTR, 2002).

As a result of processing of the fermentation media (Homogenisation, PEI treatment and filtration) no live modified *E. coli* are detected in the final product (Orica 2005a, Orica 2005b, Orica 2005c and Scott 2005) and there is a 99% reduction in transformable DNA and 98% reduction in total nucleic acid residues (Scott, 2005). The most recent method of production removes 99.997% of total and >99.999% of transformable DNA respectively.

The plasmid DNA contains an ampicillin antibiotic resistance gene (*bl*a), in addition to the *OpdA* organophosphate degrading enzyme. Intact plasmid DNA has the potential to be incorporated in the gene pool of bacteria and therefore persist in the environment. Although, recombinant plasmid DNA is not a genetically modified organism (GMO), the release of the plasmid into the environment could result in its uptake by bacteria, thus generating GMOs unintentionally. This occurrence (transformation) is well documented and known to occur in soil. If the plasmid is taken up by bacteria, it may also be transferred to other species of bacteria by further transformation, or by transfection or conjugation (transfer by bacteriophages or bacteria-directed DNA transfer respectively). In the presence of selective pressure for an activity encoded by the plasmid (antibiotic resistance or organophosphate degradation in this case), bacteria containing the plasmid could become a significant part of the (perturbed) ecosystem.

The concentration of the intact (transformable) plasmid in the product is extremely low and from the literature, the likely frequency of uptake of the plasmid by bacteria in the environment is also likely to be extremely low. Because of the high frequency of antibiotic-resistant bacteria already present in the environment it appears unlikely that there would be any negative impact on the environment from localised selection for bacteria able to metabolise organophosphate pesticides that also express ampicillin resistance.

Ampicillin is not among the top 10 antibiotics by human prescription volume for Australia and ampicillin resistance in human pathogenic bacteria is not listed as a major concern (JETACAR, 1999). Of healthy Humans, 19% were shown to harbour *E. coli* cells in their intestine conferring resistance to ampicillin (DANMAP, 1997). In human clinical *E. coli* isolates, approximately 35% are resistant to ampicillin (Kresken et al, 1999). Bacterial resistance genes exist naturally at low frequency for most families of antibiotics derived from bacteria (JETACAR, 1999). In the unlikely event that the *bla* gene was transferred to bacteria as a result of the use of Landguard OP-A it would only join the small population of already resistant bacteria, therefore the risk to public health is considered to be low.

10. CONCLUSIONS – ASSESSMENT LEVEL OF CONCERN FOR THE ENVIRONMENT AND HUMANS

10.1. Hazard classification

Based on the available data the notified chemical is classified as hazardous under the NOHSC *Approved Criteria for Classifying Hazardous Substances*. The classification and labelling details are:

R42 May cause sensitisation by inhalation
R43 May cause sensitisation by skin contact

and

As a comparison only, the classification of notified chemical using the Globally Harmonised System for the Classification and Labelling of Chemicals (GHS) (United Nations 2003) is

presented below. This system is not mandated in Australia and carries no legal status but is presented for information purposes.

	Hazard category	*Hazard statement*
Skin sensitiser	1	May cause allergic skin reaction
Respiratory sensitiser	1	May cause allergic or asthmatic symptoms or breathing difficulties if inhaled

10.2. Environmental risk assessment

The notified chemical is not considered to pose a risk to the environment based on its reported use pattern.

10.3. Human health risk assessment

10.3.1. Occupational health and safety

Manufacture of the liquid concentrate

There is Low Concern to occupational health and safety under the conditions of the occupational settings described.

Freeze-drying and Packaging/End Use

There is Moderate Concern to occupational health and safety due to the risk of respiratory sensitisation. This concern is reduced by the low concentration of inhalable particles, the use of engineering controls (freeze-drying and packing) and recommended PPE/directions for use.

10.3.2. Public health

There is Negligible Concern to public health when used in the proposed manner.

11. MATERIAL SAFETY DATA SHEET

11.1. Material Safety Data Sheet

The MSDS for Landguard OP-A provided by the notifier was in accordance with the NOHSC *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC 2003). It is published here as a matter of public record. The accuracy of the information on the MSDS remains the responsibility of the applicant.

11.2. Label

The label for Landguard OP-A provided by the notifier was in accordance with the NOHSC *National Code of Practice for the Labelling of Workplace Substances* (NOHSC 1994). The accuracy of the information on the label remains the responsibility of the applicant.

The second dot point under the disposal instructions in the draft Directions for use for both the Used Livestock Dip and treatment of Pesticide Spray Equipment Wash-water should be modified as follows

"Ensure the disposal area is flat ***and bunded***, and does not drain ….".

12. RECOMMENDATIONS

REGULATORY CONTROLS

Hazard Classification and Labelling

- The Office of the ASCC, Department of Employment and Workplace Relations (DEWR), should consider the following health hazard classification and safety phrases

for the notified chemical:
- R42/43 May cause sensitisation by inhalation and skin contact
- S22 Do not breathe dust or S23 Do not breathe spray as appropriate
- S24 Avoid contact with skin
- S37 Wear suitable gloves

- Use the following risk phrases for products/mixtures containing the notified chemical:
  - Conc $\geq$ 1% R42/43

Health Surveillance

- As the notified chemical is a skin and respiratory sensitiser employers should carry out health surveillance for any worker who has been identified in the workplace risk assessment as having a significant risk of sensitisation.

CONTROL MEASURES
Occupational Health and Safety

- Employers should implement the following engineering controls to minimise occupational exposure to the notified chemical during freeze-drying and packaging:
  - Local Exhaust Ventilation

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the enzyme liquid concentrate:
  - Work practices to minimise skin contact and generation of spray.

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the enzyme freeze-dried concentrate:
  - Work practices to minimise skin contact and generation of airborne dust.
  - Collect and dispose of spilt material without exposing workers to dust
  - Transfer of the Landguard O-PA to water should be conducted in a manner to minimise dust generation i.e. transfer should take place in protected areas or in low wind conditions and away from the breathing zone and the height at which the Landguard O-PA is dropped into the container of water should also be kept to a minimum.

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical *during handling of the enzyme liquid concentrate:*
  - Coveralls;
  - Impervious gloves; and
  - Eye protection

  during handling of the enzyme freeze-dried concentrate:
  - Coveralls;
  - Impervious gloves;
  - Eye protection; and
  - Dust Mask

  Guidance in selection of personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

- A copy of the MSDS should be easily accessible to employees.

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances*, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Disposal

- The notified chemical should be disposed of as described under Emergency procedures below.

Emergency procedures

- Exclude unprotected persons from area of spill. Wear suitable protective clothing, dust mask and gloves. Avoid the generation of dust. Clean up spills promptly to reduce risk of dust developing. Provide adequate ventilation. Recover spilled material mechanically and transfer it into clean and dry containers for disposal. To avoid the generation of dust, vacuum or gently shovel up the spilt material. For disposal of small quantities (< 5kg) of spilt or expired product, dissolve 1 part powder in 10 parts water and discharge in water or soil that is suspected to contain organophosphate contamination, as per directions for use. For the disposal of large quantities of spilt or expired material (> 5kg), seek advice from the manufacturer or dispose using a licensed waste disposal company. For small quantities remaining on surfaces wash down with low pressure water or mop up with a damp cloth.

12.1. Secondary notification

The Director of Chemicals Notification and Assessment must be notified in writing within 28 days by the notifier, other importer or manufacturer:

(1) Under Section 64(1) of the Act; if
- A new production or source organism is used; or
- the temperature optimum/range or pH optimum/range is changed so that handling, or inactivation techniques might need to be changed; or
- there are changes to the manufacturing process that have the potential to alter the toxicity, ecotoxicity and biodegradability profile of the notified enzyme and its concentrate.

or

(2) Under Section 64(2) of the Act:
- if any of the circumstances listed in the subsection arise.

The Director will then decide whether secondary notification is required.

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