final report

Project code: **FLOT.111**

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Rapid quality assurance tests for persistent chemical contaminants in animal feeds

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2. Summary of Project Achievements

Project title: Rapid quality assurance tests for persistent chemical contaminants in animal feeds

Project aims:
1. Convert existing laboratory ELISAs for persistent insecticides into a rapid, multiresidue field screening test kit
2. Develop a rapid screening test for organophosphate and carbamate insecticides either based on an ELISA (detecting common structures within the target pesticides) or by cholinesterase enzyme inhibition
3. Validate the performance of the tests with individual feed grains and with compound feeds, containing typical additives. After achievement of these aims, we will interact with the feed industry on the validation of prototype tests, and liaise with test kit manufacturers to ensure that the kits are available in a timely, on-going manner to industry.

Background:
Currently there is no systematic lot-by-lot testing of feed grains or of feeds by the Australian industry. Some feed industry companies test occasional lots of raw materials but this is done on a sporadic basis. "Market basket" type surveys provide some statistical data by analysing a few samples of each of a range of commodities, but they do not have a trace-back element. The lack of systematic testing for persistent chemicals was a factor in both of the contaminated-meat crises in recent Australian history - the organochlorine crisis in the mid-1980's and the more recent chlorfluazuron (Helix) crisis. With an increase in feedlotting in the Australian beef industry, the relevance of testing of feeds / feed grains has increased. Indeed, the last major contamination problem arose from contamination of feed (Helix-contaminated cotton trash) rather than contaminated grazing land. Without a more formal and systematic testing regiment, the risk of further "residue crises" from time-to-time is high.

The research aimed to provide a test method for screening for each of the persistent insecticides that may potentially contaminate Australian feed grains. A single test (or at most, two tests performed with the single feed / grain extract) will also indicate the potential source of contamination by identifying the chemical group to which the contaminant belongs. By directly involving the U.S. company EnviroLogix, the tests were projected to be commercially available immediately after the conclusion of this project, and during the latter half of the project, industry trials of the test/s were to be carried out with key feed industry users. The Australian feed grains industry (and meat industries) will benefit from having a unique quality assurance tool, which can also be used to assist in the promotion of Australian feed grains and Australian beef and other livestock products as "clean".

Project achievements:
The project has resulted in several immunoassay tests for screening persistent insecticide residues in feed grains, including (1) persistent chemicals which could cause potential residues in meat (organochlorines, type 2 synthetic pyrethroids, benzoylphenylureas, methoprene), (2) chemicals which have moderate to high animal toxicity (organophosphates and carbamates). The tests can be applied to the analysis of animal feed grains and were meant to be able to perform in field situations. (3) Field trials of prototype test kits have been carried out as planned. Eleven sites across NSW, Queensland and WA were chosen for the field trials. The feedback from these trials indicated that such immunoassay tests would be useful for the quality assurance regarding absence of pesticides in composite feeds. However, although the newly developed rapid test system did provide an alternative to current laboratory testing procedures, reservations were expressed that the development of the current rapid tests were still too technically sophisticated and not yet adequate to allow for their widespread use by the grains industry.

Project outputs:

The detailed outputs of the project should be assessed from the milestones set. The summary of the project outputs is as following:

- Immunoassay rapid tests were developed for persistent insecticides in grain feed. To complement the rapid analysis, a simple and rapid extraction method which can be performed in field was also developed.
- Prototype ELISA kits in potential commercial test formats as strip-wells have been prepared and demonstrated to the feedgrain receival agents and feed-lots in NSW, Queensland and WA by the research team. These tests include the full range of compounds originally targeted, although an attempt to develop a new generic organophosphate ELISA test failed because the haptens prepared failed to elicit a satisfactory antibody. However, cholinesterase enzyme inhibition test for OPs has been adapted into rapid format. Because of the withdrawal of the U.S. company, EnviroLogix, in the second year of project, the aim to prepare test kits in a single multi-test format using immunochromatography (ICT) failed and a more modest output from the project as rapid strip-well tests was negotiated with GRDC. These strip-well tests were

prepared in as simple a format as possible, using disposable plastic pipettes and chemical reagents dispensed in dropper bottles.

- The detailed feed-back from industry of their assessment of the tests and their need for such products was obtained. The feed-back obtained shows that such tests are needed by the grains industry, but the current format is still too technical, and more user-friendly format, such as ICT format, is desirable. This feed-back has been useful in making recommendations from the project.

Industry benefits:
Benefits will apply to grains used for feeds as well as compound feeds. The main target industry for the tests is beef cattle, although all industries which utilise feed grains (dairy cattle, pigs, chicken, and aquaculture) will benefit.

Unit benefit - economic benefits
In a preliminary study to this project, Mr Ian Black (SARDI) conducted an independent economic assessment of the use of rapid tests for chemical contaminants of grains at sites of stockfeed manufacture and/or grain receival. Such an analysis involves several assumptions, because contaminant testing is essentially a form of insurance. The main risk associated with persistent agrochemicals is the appearance of detectable residues in meat or milk. His analysis assumed that 70% of feedstuff costs in Australia are of grain origin, and that average annual costs of feed materials are around $1300 million. A "contaminant incident probability analysis" indicated that the break-even investment cost for measuring contaminants tests for feed grains in Australia was between $ 13-16 million per year. If tests were made compulsory within the feeds industry, the industry could pay up to $75 for a 30 tonne truckload while for non-compulsory testing, cost of up to $50 per truckload could be borne. It is expected that a single test per truckload would usually be performed. A target price of up to $ 25 for a test for a panel of contaminants is available, well under the maximum acceptable cost from both the economic analysis and industry survey.

Scale of system to which benefits apply
The increasing use of grain in animal feeds is a growing end-use, accounting for the utilisation of between 1/4 and 1/3 of the Australian grain crop. The main grain end-uses of different cereals are:
- Wheat - almost 2 million tonnes - approximately 10 % of Australian wheat production (i.e. 50 % of the domestic usage) is for feed;
- Barley - between 2-3.5 million tonnes - 60-70 % of Australian production is for feed, on average half of this is exported;
- Maize - 50 -70 % of Australian production of 0.2-0.3 MT is used for feed;
- 90 - 98 % of Australian production oat production of 1.5 MT, sorghum production of 0.6-1.6 MT and triticale production of 0.2 - 0.3 MT is used for feed. About one-third of the sorghum used is exported.
- Feed uses are important for the non-cereal crops. Virtually all of the 1-1.5 MT of lupines are used for feed; on average half of this is exported. The majority of the 0.4 MT of field peas grown annually are also used for animal feed. In addition, oilseed meals are important feedstuffs.

Other benefits:
The first benefit of the research (to the animal industries) will be accrued nationally. International use of the test methods will facilitate their acceptance in international grain trading as well as providing a royalty stream on kit sales.
While the project is focussed on development of rapid semi-quantitative field tests, we have had to typically develop an accurate but fully quantitative laboratory version of the test and then “reverse engineer” the test to a field version. As a spillover benefit, this project will ensure the availability of the quantitative, laboratory based assays for groups wanting to analyse large numbers of samples. A second spillover benefit is that the project will provide more users with "hands on" experience in ELISA technology. This will enable users to carry out other ELISA assays such as those for mycotoxins, that are either already commercially available or are to be developed in other projects involving Sydney University and the CSIRO (e.g. an ACIAR-sponsored project).

3. Attachments
The following attachment is included:

- Summaries of the results of questionnaires submitted to feed-grain manufacturers and feed-lots regarding the effectiveness and the suitability of the ELISA strip-well tests that were demonstrated in Queensland, NSW and WA.

4. Conclusions, recommendations & other R&D opportunities

Provide a summary of

a. *Major conclusions*

This 2-year project has provided benefits in the form of a simple means of monitoring feed grains such as wheat, sorghum and barley for pesticide contamination at MRL's . Sensitive immunoassays (ELISA) previously developed at CSIRO Plant Industry and several new tests developed at the University of Sydney for the main groups of pesticides have been modified for use in a strip-well test for monitoring the quality of grain. Two new assays for carbaryl and methoprene which were unavailable before were also developed during the life of this project. The modified objectives set in the proposal after the withdrawal of EnviroLogix have been achieved, and strong interaction with the agricultural feed-grains industry during the latter stages of the project has resulted in better understanding of the market needs. The potential to commercialise the test kit materials produced from this project is still quite high.

In field demonstrations and user trials of the simple tests presented as strip-wells at 11 sites the following feed-back was received:

(i) Such tests were almost universally considered as desirable. The tests were seen as providing a more satisfactory alternative (field kit) to the use of gas chromatographic analysis (laboratory tests) because of the greater convenience / speed of analysis and the possibilities for management and trace-back of residues (e.g. confirming with-holding periods).

(ii) The strip-well tests chosen as achievable outputs, while considered suitable for achieving quality assurance by experienced personnel (where speed in tests of less than 10-15 min is not imperative), are still too slow to be used as a means of accepting or rejecting individual truck-loads of grain.

(iii) The Grains Pool of WA has indicated possible interest in using such tests for a major Quality Assurance (QA) program in 2001-2002. Analysis of composite samples of up to five grain crops at over a 100 receival sites in WA was foreshadowed, to be conducted using the strip-well tests at the University of Sydney. This would be with a view to further development of the tests to be employed in the future more directly by the grains industry. The Grains Pool contrasted this pro-active method of QA with the current procedure of the National Residue Survey providing information after grain had been exported too late to take remedial action.

b. Recommendations

The methods and strip-well tests developed in this project have significant potential to provide (contamination) protection for the grains and related industries as well as providing a guarantee for grain quality. However, the withdrawal of the commercial partner EnviroLogix severely limited the extent of progress towards commercial ELISA kit development. This was probably exacerbated by the withdrawal of J. Skerritt and A. Hill as researchers during the project's life. However, with hindsight, it is unlikely that the objective proposed of a single immunochromatography (ICT) strip test capable of screening up to five pesticide groups at once could have been achieved. EnviroLogix had proposed an intermediate stage of ICT development within the time of the project involving diflubenzuron alone. In consideration of the more limited outcome, the following recommendations are made:

(i) A strong positive outcome of the project was affirmation by industry (grain and feedlot) participants of the need and the convenience of such simple tests. We recommend that this interaction with potential users of the tests in industry be continued. In addition, liaison with researchers and potential kit suppliers be encouraged to develop simplified kit formats more appropriate to industry needs. Demonstrations of pilot test kits have indicated key factors in the acceptability of the kits and their potential range of application. For example, demonstration of strip-well test kits in WA by IR Kennedy provided strong positive feedback regarding the potential of the kits to provide quality assurance. It is recommended that this process be continued as part of any plan to further develop the technology to commercial formats.

(ii) The user surveys have shown that a major impediment to widespread adoption of the test kits is the need to develop a more "user-friendly" format allowing easier decision-making. The opinion is that the current strip-well tests still demand too high a degree of technical skill, despite as much

simplification as possible. Extended funding of an additional period (of say 18 months- 2 years) to provide a more rapid user-friendly product is recommended. This should be done in collaboration with an Australian commercial partner, such as ELISA Systems or C-Qentec.

c. Other R&D opportunities that emerged during the course of the project

The requirements of a rapid, non-laboratory test format are that they can be performed in under 15 minutes without laboratory facilities or sophisticated equipment, and provide a visual readout of results in a form that is also compatible with low-cost readers, if quantification is required. There are four possible formats, including: 1. rapid microwell strip assay, 2. antibody-coated tube, 3. dipstick or 4. immunochromatography (IC) formats. We use format 1 as prototype test format at present, since it is not subject to licensing of the format and provides a direct comparison with the current microwell forms of the assay. Formats 3 and 4 provide a simpler method and faster result, and may be more amenable to simultaneous multiresidue screening. Format 4 is the simplest and most elegant. The feedbacks from our industry demonstration show that although the current prototype format is simple and fast compared to the traditional analytical methods, farmers would be more likely to adopt such technology if a more "user-friendly" format such as IC format was available. More developmental research is clearly needed to develop IC format.

5. Milestones

Milestone no.	*Description and criteria*	*Planned Achievement Date*
7	Assess performance with individual feed components and compound feeds. Criterion: High recoveries of spikes of target agrochemicals into a range of feed grains and mixtures.	March 2001
Indicate how the milestone was achieved. If the milestone was not achieved, please indicate why This milestone has been achieved in time. Barley, sorghum and wheat have been assessed with immunoassay, including matrix effect studies and recovery studies.		

Milestone no.	*Description and criteria*	*Planned Achievement Date*
8A	Technology transfer to commercial partner. Criterion: Subject to commercial agreement, make available details of the production of prototype to EnviroLogix of other potential partners for commercial production of grainstest kits.	January 2002
Indicate how the milestone was achieved. If the milestone was not achieved, please indicate why This extra-project milestone should be achieved in time. Prototypes of the test kits have all been produced, and negotiation with potential commercial partners (e.g. EnviroLogix, ELISA Systems, C-Qentec) can now be conducted.		

Milestone no.	*Description and criteria*	*Planned Achievement Date*
9	Industry demonstrations and trials of new tests. Criterion: Kits successfully trialled by at least 10 industry users.	June, 2001
Indicate how the milestone was achieved. If the milestone was not achieved, please indicate why This milestone has been achieved in time. Industry demonstration of prototype kits has been completed. Eleven feedgrain receival firms across over NSW, Queensland and WA have participated in this trial.		

Milestone no.	*Description and criteria*	*Planned Achievement Date*
10	Final report submitted to GRDC. Criterion: Report received by GRDC.	30 September 2001
Indicate how the milestone was achieved. If the milestone was not achieved, please indicate why See this report		

6. Achieved outputs

6.1 Output 1

Description	*Delivery date*	*Output code*
Production of prototype test kits for screening persistent chemical contaminants in animal feeds	30 June, 2001	P1

Indicate the intended users of the output

T1. The main target industry for the tests is feed-grains for beef cattle, although all industries which utilise feed grains (dairy cattle, pigs, chicken, and aquaculture) can also use these kits.

Indicate how the output has, and will continue to be, promoted and adopted leading to the expected outcome (benefits)

During the two-year period of this project, the Research Team undertook to consult with an industry Advisory Group each 6 months regarding the needs of the various industry partners and the direction, progress and commercialisation of the project. Material was circulated describing the objectives, methodology, milestone achievements and expected outcomes of the project. The meetings of the Advisory Group were held in Canberra on December, 1999, June, 2000 and February 2001. One major focus of these meetings was the targets for the sensitivity of the tests. Communication between researchers and the feed grain industry was also conducted as part of the project, with involvement of Mr Tony Shorter of CSIRO. Test kits have been demonstrated to the potential farmer users during the last six months of this project.

A pilot trial for pesticide residues as part of grain quality assurance process in Western Australia has been suggested, and the trial may commence this December using immunoassay kits produced from this project to measure the pesticide residues in grain crops including wheat, barley, oats, canola and lupins. Immunoassay tests would provide the users with quality assurance very soon after grain is received, allowing action to be taken before export, if contaminated samples are found.

Indicate whether the output contains any third party owned technology and any implications this might have for the commercialisation of the output

EnviroLogix Inc expressed interest in commercialisation of the outcomes of the project when the application was originally proposed from CSIRO. This process was envisaged to involve the following three phases:

1. The RESEARCH required the development of antibodies and to extend tests to a research prototype stage. Performance in-house with feed components and feeds containing spiked and / or incurred residues was then investigated. The University of Sydney/CSIRO has substantially undertaken these tests' with the resources described in the attached project budget, and with University of Sydney/CSIRO's in-kind contribution.

2. The DEVELOPMENT of the research prototype into a commercial product. This includes manufacture trials, stabilisation trials, packaging trials, development of manufacture standard operating procedures, QA and QC protocols. This needed to be done by the manufacturer and would have occupied several of their development staff, as well as specialised manufacture equipment. EnviroLogix estimated that their cost of development for the full panel of tests in both formats would be US $102,000 (A $ 166,000). This was effectively money that EnviroLogix would have expended for this project within their own development laboratories (not the partners research laboratories). These resources are no longer available.

3. INDUSTRY trials. An additional commitment to be made by EnviroLogix was the provision of US $ 20,000 ($A 32,600) of test prototypes to the Australian Feeds Industry within specified deadlines. They were to fund the manufacture and shipping to Australia. From this GRDC project budget, we in the two CSIRO Divisions undertake the industry demonstrations and trials throughout Australia, and do validating instrumental analyses to confirm positives.

Unfortunately, EnviroLogix withdrew from the project (advised on February 23, 2001), claiming they were unable to commit the financial and human resources required because of the pressure of other work. Advice will be sought from Mark Bennett of the Business Liaison Office, University of Sydney, as to whether EnviroLogix should be given to option to fulfil legal obligations they owe to according to their agreement with CSIRO, licensing them to use the immunodiagnostic technology and to promote its use in Australia.

In view of the withdrawal of EnviroLogix, substantial additional resources were no longer available to the project.

However, a significant part of the project's objectives were achieved, including industry feedback concerning the potential acceptance and market for these tests. EnviroLogix can be asked to exercise any option they may possess for their commercialisation, or to assist a mechanism to obtain another Australian commercial partner.

Detail the commercialisation strategy for the output during and post the project if relevant, including the involvement of all commercial parties and their inputs (financial or otherwise)

There would be opportunities for commercialisation of these tests. At this stage, no specific arrangements with commercial companies have been made. However, investigation to find appropriate manufacturers such as ELISA Systems in Brisbane or C-Qentec based in Sydney can be conducted, and new arrangements will be made as appropriate.

If the output was not achieved during the course of the project, indicate the reasons why

The original output of this project was a set of immunological materials, for EnviroLogix to prepare the final commercial product using simple test formats to be agreed on. A strategy for these test formats was prepared following the mid-2000 Industry Advisory Committee meeting and suggested to EnviroLogix.

Unfortunately, EnviroLogix then withdrew from the project as mentioned above.

The research work in this project at the University of Sydney and in CSIRO has been completed on schedule. The withdrawal of EnviroLogix removed a substantial and essential component of the infrastructure required to bring this project to completion as proposed. It required that a new strategy be designed in order that tests can become available for use in the Australian grains industry. So it was decided to prepare prototype test kits as strip-wells rather than in ICT format in CSIRO/University of Sydney, allowing testing under field conditions, as part of this strategy. Industry feedback, some worthwhile market assessment and prototype kit demonstration have been achieved as part of the new output.

6.3 Management of Intellectual Property (IP)

Provide a summary of any IP strategies undertaken or planned to facilitate the protection and / or commercialisation of the project's realised outputs

The immunoassays for cyclodiene and the DDT family organochlorines and synthetic pyrethroids have been commercially licensed to EnviroLogix Inc. Thus, even though significant research and development work has been done in this project, it would be necessary to make new arrangements with EnviroLogix to allow licensing of the development of assays for these persistent insecticides to other companies. However, University of Sydney/CSIRO jointly own the IP for the development of other assays, including carbaryl, methoprene and part of that for diflubenzuron. We recommend that the following schedule be adhered to regarding commercialisation of the project's outputs.

- A business plan be prepared, acting on advice received from organisations such as the Grains Pool of WA (Peter Portmann), Graintrust (Garry Goucher), GRDC (John Thorne/Mike Taverner), Business Liaison Office University of Sydney (Mark Bennett), CSIRO Plant Industry (Lindsay Adler/Kevin Gale) interacting with firms such as ELISA Systems or C-Qentec based in Australia. This plan would aim to provide a funding mechanism to further develop the tests to a commercial format with the desirable properties of speed, sensitivity and ease of use. From the University of Sydney's viewpoint, the main requirement is to provide a salary plus operating expenses for 1-2 years for Dr Shuo Wang so that this development can be facilitated.
- Simultaneously, EnviroLogix be approached with a view to obtaining their cooperation in this venture, for example by a sub-licensing agreement that might yield them a royalty where their IP is employed. The possibility of using immunoreagents prepared at alternative sites using methods in the public domain (e.g. the Post Harvest technology Institute in Ho Chi Minh City) can also be investigated.

Provide a list of all scientific or technical papers published, and any patents filed

Wang, S. Hill, A., and Kennedy, I. R. (2001) Rapid on-site immunoassay for diflubenzuron in grains. *Analytica Chimica Acta* (In Press).

7. Expected Outcome (benefits)

7.1 Description

a) Specify any outcome (benefits) achieved during the project
b) Specify the expected outcome (benefits) post project

The project has resulted in several prototype test kits for screening persistent insecticide residues in feed grains. A market survey of industry users by an independent consulting group has confirmed that development of these tests is seen as a high priority to safeguard Australian meat and grain exports, and that industry uptake of the tests would be high if they were simpler and faster. Testing is only the first step in minimising the risk of animal contamination with persistent compounds, but it is nonetheless an essential first step in the process. The availability of simple testing methods can induce a culture change to reinforce education of the people involved in feed grain storage on appropriate and inappropriate storage treatments. Based on prototype tests developed in this project, there is a good probability that suitable commercial kits could be developed in collaboration with a commercial partner in the next two years.

7.2 R&D Type

Estimate the R&D type expressed as a % of the total effort

Type	**R&D activity** (expressed as a %)	%
Pure Basic	Experimental or theoretical work undertaken primarily to acquire new knowledge without a specific application in view. Carried out without looking for long term economic or social benefits	
Strategic Basic	Research directed into specific broad areas in expectation of useful discoveries. Research providing the broad base knowledge necessary for the eventual solution of recognised practical problems	
Applied	Original work undertaken to acquire new knowledge with a specific application in view, to determine new methods or ways of achieving some specific and pre-determined objectives	**50**
Experimental Development	Systematic work using existing knowledge gained from research and / or practical experience for the purpose of creating new or improved materials, products, processes or services	**50**
Demonstration & Extension	Presenting the technology in way that allows a clear assessment of its technical and economic viability on a commercial scale. Extension is the broader communication of new knowledge or technologies	
Commercialisation	Commercialisation can be considered to be complementary to demonstration and relates to the investment in developing a new product or technology to the point where it is ready for release to the market	
Training & Development	Relates to the development and maintenance of the human resources relevant to the GRDC's target industries	
Total		**100%**

7.3 Flow of benefits

Specify the two 'Flow of Benefit' categories most applicable to this project (refer to the table in the guidelines)
1. Increased product quality
2.Human health

Complete 7.3.1 and 7.3.2 below to quantify the flow of benefits both to date (within project) and forecasted (post project):

7.3.1 Realised flow of benefits

a) Estimate the per unit economic impact of the project to date (eg. $/ha)	$
Nil	
b) Estimate the scale of the system to which the impact has applied to date (eg. ha, tonnes)	
Nil	
*c) Estimate the level of adoption to date (%)**	
Nil. Feed-grains industry currently relies on composite/targetted sampling for gas chromatographic analysis typically costing $200 for organochlorines (OCs), organophosphates (OPs) and synthetic	

pyrethroids (SPs) .	
Estimate the annual benefit to date (= a x b x c*)	$

**remember to convert the % figure to a decimal when calculating*

7.3.2 Forecasted flow of benefits

a. Estimate the maximum per unit economic impact of the project (eg. $/ha)	$25/30 tonne truck load
Justification Mr Ian Black (SARDI) conducted an independent economic assessment of the use of rapid tests for chemical contaminants of grains at sites of stockfeed manufacture and/or grain receival. Such an analysis involves several assumptions, because contaminant testing is essentially a form of insurance. The main risk associated with persistent agrochemicals is the appearance of detectable residues in meat or milk. His analysis assumed that 70 % of feedstuff costs in Australia are grain, and that average annual costs of feeds are $ 1300 million. A contaminant incident probability analysis indicated that the break-even investment cost for contaminants tests for feed grains in Australia was $ 13-16 million per year. If tests were made compulsory within the feeds industry, the industry could pay up to $75 for a 30 tonne truckload while for non-compulsory testing, cost of up to $50 per truckload could be borne. It is expected that a single test per truckload would usually be performed. We have set a target price of up to $ 25 for a test for a panel of contaminants, well under the maximum acceptable cost from both the economic analysis and industry survey.	
b. Estimate the maximum scale of the system to which the impact will apply (eg. ha, tonnes)	8.2 M tonnes
Justification Use in animal feeds is a growing end-use of Australian grain, accounting for utilisation of between 1/4 and 1/3 of the Australian grain crop. The main grain end-uses of different cereals are: - Wheat - almost 2 million tonnes - approximately 10 % of Australian wheat production (i.e. 50 % of the domestic usage) is for feed; - Barley - between 2-3.5 million tonnes - 60-70 % of Australian production is for feed, on average half of this is exported; - Maize - 50 -70 % of Australian production of 0.2-0.3 MT is used for feed; - 90 - 98 % of Australian production oat production of 1.5 MT, sorghum production of 0.6-1.6 MT and triticale production of 0.2 - 0.3 MT is used for feed. About one-third of the sorghum used is exported. - Feed uses are important for the non-cereal crops. Virtually all of the 1-1.5 MT of lupins are used for feed; on average half of this is exported. The majority of the 0.4 MT of field peas grown annually are also used for animal feed. In addition, oilseed meals are important feedstuffs.	
*c. Estimate the maximum level of adoption (%)**	75%
Justification Because major industry participants have been made aware of the planned development of the new tests during the BECAN consultancy and survey, and because the project involves a significant industry technology transfer component, we expect uptake to be rapid and significant. Uptake would start in November 2001, peak in July 2003 with 75 % industry uptake (although the possibility of compulsory testing has been raise by the industry). The technology will probably remain appropriate for about a further 12 years, tapering to 25 % by 2015.	
Estimate the maximum expected annual benefit (= a x b x c*)	$5,125,000
Estimate the year of initial adoption	**November, 2002**
Estimate the year of maximum adoption	**2005**

**remember to convert the % figure to a decimal when calculating*

8. Risk assessment

After considering the relevant table in the guidelines, comment on the risk that the flow of benefits to the Australian grains industry will not be realised or reach their maximum because of difficulties associated with adoption or

commercialisation of the project outputs

We consider that there will be a high risk that a flow of benefits will not occur unless post-project development with a commercial partner is now carried out. The feedback from our field demonstration of immunoassay tests indicated that current formats were not suitable for direct use, and they need an easier-to-use format such as ICT on strips. More funding is needed to modify the current format to such ICT format.

9. Certification

Project Supervisor's signature _____

Name (in capitals) IVAN R. KENNEDY Date_____

Research Organisation Signature _____

Name and Title of authorised signatory (in capitals) _____ Date_____

10. Government Time Box Initiative

If your organisation employs less than 20 people provide an estimate of the time taken to complete this form. Include the time spent by all employees reading the instructions, working on the question and obtaining relevant information.	Hours Minutes

SUPPLEMENTARY REPORT FOR GRDC PROJECT US294

Ivan R. Kennedy
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University of Sydney

The Final Report of project US294 gave details of the development of quality assurance for chemical contaminants in animal feeds. This work involved both development of new tests and modifications of existing tests for this purpose, including assessment of matrix effects and adaptation to reduce the time required for immunoassays. This Supplementary Report provides answers to a request for more details of the tests and of matters related to intellectual property and commercialisation from the GRDC (ref. Kirtsen Pietzner, 29/1/02).

Description of tests developed and marginal benefits

GRDC project US294 developed new grainstests for three classes of pesticides (methoprene, carbaryl, diflubenzuron) and added significant value to existing tests for pyrethroids, organochlorines (DDT and cyclodienes) and OPs (cholinesterase), by:

- modification for rapidity (strip-well format)
- production of more immunoreagents (pyrethroid and OC assays - haptens were resynthesised and enzyme conjugates were produced)
- optimisation of all immunoassays (diluents, overcoming matrix effects, etc.)

Apart from the pyrethroid tests, which require a pre-isomerisation stage in NaOH), all other tests can be finished in less than 20 minutes in strip-wells.

Diflubenzuron/Carbaryl/Methoprene Kit

The current strip-well test can be used to test specifically for **carbaryl and methoprene** at MRL levels in feed grains. The sensitive diflubenzuron test can also detect other benzoylphenylures at the following LODs.

Compound	Limit of Detection (ppb) in wells	ppm in grain sample (after 1/500 dilution)
Diflubenzuron[1]	**3**	**1.5**
Teflubenzuron	**4**	**2**
Chlorfluazuron	**15**	**7.5**
Lufenuron	**30**	**15**
Flufenoxuron[2]	**300**	**150**

1. MRL for diflubenzuron is 2 ppm.
2. A more sensitive, specific antibody is available.

Pyrethroids Kit

The current strip test can be used to test cypermethrin and deltamethrin at MRL levels in feed grains. Pyrethroid tests still take 45 minutes to be finished since 30-min isomerisation of pyrethroid is needed before measurement.

Compound	ppm in grain sample (after 1/500 dilution)	MRL (ppm)
Deltamethrin	**2**	**2**
Cypermethrin	**5**	**5**
Bifenthrin	**10**	**2**

Organochlorines Kits

Compound	ppm in grain sample (after 1/500 dilution)	MRL (ppm)
Endosulfan	**3**	**3**
DDT	**2**	**0.1**

Dichlorvos Cholinesterase Kit

This is a modified version of the kit available as an EnviroLogix product from ELISA SYSTEMS in Brisbane (Tel: Freecall 1800 635 472).

The current test aims to detect dichlorvos at MRL level (2 ppm) in feedgrains. However, it can also detect other OP compounds if using provided grain control sample spiked at MRL or other levels.

Compound	ppm in grain sample	MRL (ppm)
Dichlorvos	**0.5**	**2**
Chlorpyrifos-methyl*	**0.5**	**10**
Fenitrothion*	**15**	**10**
Pirimiphos-methyl*	**1**	**10**
Carbaryl	**30**	**5**

* Different protocol applies.

Intellectual Property and Commercial Issues for Practical Outcomes

From US294, University of Sydney and CSIRO own immunoreagents for carbaryl, diflubenzuron and methoprene.

The original immunoassays for cyclodiene and the DDT family organochlorines and synthetic pyrethroids have been commercially licensed by CSIRO to EnviroLogix Inc. However, an ACIAR project (PHT/1996/004) in our laboratory has also developed simple tests for organochlorines, providing an independent source of immunoreagents for potential commercial development of these OCs (DDT and cyclodiene). Those for pyrethroids are available in CSIRO Plant Industry (Dr Kevin Gale) subject to licensing to EnviroLogix, with whom suitable arrangements can be made, taking into account a written agreement set up between CSIRO and the University of Sydney as part of US294. An explanation is being sought by the University of Sydney's Business Liaison Office as to whether EnviroLogix still has the full right of control of the pyrethroid test in view of its withdrawal from development of the tests in US294.

New reagents for pyrethroids can also be prepared in future work, using methods in the public papers, which would be unencombered.

However, any issues related to the potential for commercialisation and possible returns from further investment by GRDC will be clarified when the current negotiations regarding IP between the University of Sydney, CSIRO, C-Qentec are completed. In our assessment there are good prospects that it will be possible to develop all the current tests for commercialisation by C-Qentec if technically possible. However, some sublicensing arrangement may be required for early use of a pyrethroid test.