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Survey of Glyphosate, & its degradation product AMPA, Residues in Wheat

New Zealand Food Safety Authority

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Acknowledgement

- This report is for NZFSA at its initiative and is courtesy of wheat suppliers for sampling availability and for field application information (confidential to NZFSA for research only). AgriQuality Ltd sampling and analysis services were grateful to be able to assist. The report appears to provide valuable information for assurances and progress.

Summary

The NZFSA undertook to investigate glyphosate and its residues in total NZ domestic and imported wheat in the sampling period within the 2003/4 year. This was in response to a high level of interest in glyphosate expressed at the NZFSA Consumer's Forum in July 2003. Recent publications had reported use for desiccation and use of "Round Up Ready" crops with increased glyphosate application from 11.3 to 37.5 million tons in Midwestern USA states from 1997 to 2000.

Residue results

Samples (80) were tested for glyphosate and its degradation product AMPA. The distribution of samples was according to supply tonnages from product available at sampling (2-04-04 to 07-05-04) between:

1. Imports to NZ from consignments available at sampling (got 40 result sets compared with 32 targeted)
2. NZ domestic production areas pictured (got 40 result sets from 48 targeted).

Residues vs LOQs and LODs

Results were negative compared with sensitive Limits of Quantitation and Detection (LOQ 0.05 ppm, LOD 0.02) except for a small steady increase in residues found for source locations listed: Australia (nil residues), NZ north, NZ south (mostly nil residues), and Canada (all results positive for residues).

Residues vs Defaults and MRLs

Results were negative with NZ defaults (default 0.1 ppm) with a similar small increase but well below MRLs (5ppm for wheat):

1. 0 Residues from 34 Australian samples exceeded defaults.
2. 4 Residues from 40 NZ domestic samples exceeded defaults (probably increased from north to south).
3. 5 Residues from 6 Canadian samples exceeded NZ defaults (but did not exceed the MRLs which apply to imports).
4. 0 Residues from 80 domestic and import samples exceeded Codex Maximum Residue Levels (MRLs) applied to NZ imports.
5. Note that the MRL is the minimum for good practice and with an additional safety margin over the ADI. The NZ "default" of 0.1 ppm applies to domestic production in absence of an NZ MRL.

Use survey:

NZ treatment application was nil/low/or not-declared. There was one instance each of (1) higher residues from a low treatment shortly before harvest and (2) lower residues from high treatment mostly degraded.

Conclusions:

1. Residue levels, and their variations, were low compared with local limits and especially compared with MRLs (with a further safety margin for diet variation against the Allowable Daily Intake ADI). Levels therefore appear to be limited by other controls eg economic controls (as supported by literature).
2. The NZ pattern may suggest there are situations where there is perceived need for treatment possibly especially in the south. The committee might consider progress to include investigation of domestic treatment needs (including local/southern needs), and improved technical information (including clarification of risk factors inc "defaults", MRL, ADI, and diet). Confirmation of progress may need targeted low level sampling and inclusion of investigation for other supply countries as samples become available.

Background

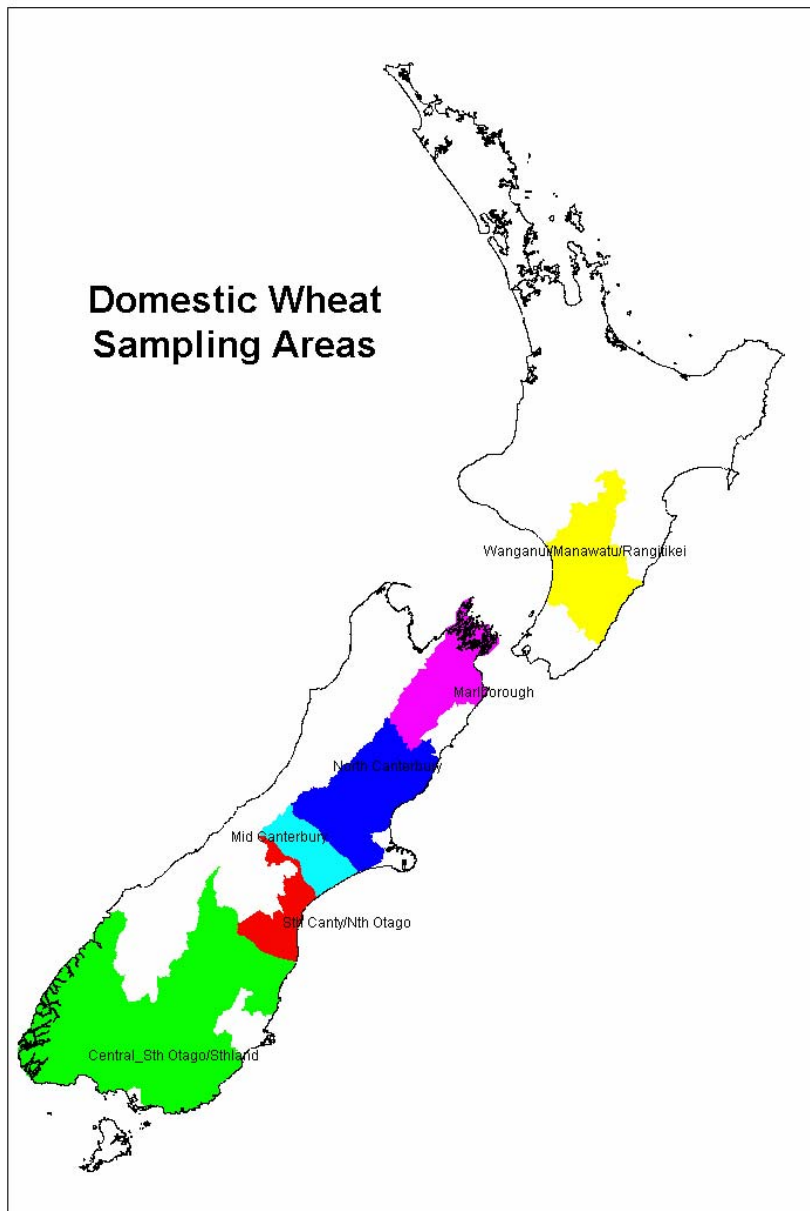
Occurrence : Glyphosate, N(phosphonomethyl)glycine has a metabolite aminomethylphosphonic acid, AMPA. It may be registered for many uses in New Zealand, including use on cereals as a herbicide and pre-harvest desiccant.

Limits

MRLs & NZ defaults ppm	Flour	Wheat	Bran
NZ defaults for domestic production	0.1	0.1	0.1
FAO/WHO Codex for imports ADI 0.3 mg/kg	0.5	5	20
Australia ANZFA		5	20
USA 40 CFR		5	20 (wheat milling fractions)

The MRL appears has a fair safety margin for diet variation against the Allowable Daily Intake ADI but this probably deserves clarification of risk factors inc “defaults”, MRL, ADI, and diet.

Map of NZ production areas sampled



Strategy of sampling and use-survey (see NZ map of production areas - previous page).

Domestic / Import Sampling Split

It was understood that approximately 300,000 tonnes of wheat was grown domestically, and 200,000 tonnes was imported. Based on this tonnage, a linear indicative sampling number split between domestic wheat samples and imported wheat samples was used for this sampling survey:

Domestic Wheat: 48 from total 80 samples (60% of the combined tonnage).

Imported Wheat: 32 from total 80 samples (40% of the combined tonnage).

Domestic Wheat (see NZ map of producing areas - previous page).

The United Wheat Growers had supplied AgriQuality with a copy listing their Growers. This had identified approximately 1200 growers throughout New Zealand distributed on a regional basis. Wheat farming methods did not vary significantly between growers or regions and a random selection of growers based on 48 samples was therefore selected for sampling as follows:

Regions	No. of Growers	% of Total	Target No of Samples	No of test sets got
Wanganui / Manawatu / Rangitikei	78	6	3	3
Marlborough	24	2	1	0
North Canterbury	409	34	16	16
Mid Canterbury	421	35	17	11
South Canterbury / North Otago	180	15	7	8
Central Otago /South Otago / Southland	107	9	4	2
Domestic total				40 of 48
Australia				34
Canada				6
Import total				40 of 32
TOTAL	1219	100	48	80

Samples taken were a fair match to the target especially given the brief sampling time available.

Results

Detailed results are in the results appendices without identifiers (which are held on file available to NZFSA for research purposes).

Results are summarised in the summary and table below and shown in graphs following as listed:

1. Result exceptions from “Residues-Not-Detected” or “Not-declared-used” (table below):
2. Sum Glyphosate + AMPA ppm (MRL 5, default 0.1, graph follows): which shows an increase from:
 - a. Australia (average residue 0ppm reflecting non-use)
 - b. to NZ north to NZ south (average 0.05 ppm reflecting mostly non-use and possibly perceived need for use especially in the south)
 - c. to Canada (average 0.28 and results reflect common use).
3. Sum Glyphosate + AMPA vs Usage (MRL 5, default 0.1 ppm, graph follows): which shows (1) low treatment very shortly before harvest giving high residues compared to (2) high earlier pre-harvest treatment subsequently partly degraded.
4. Glyphosate and AMPA proportions ppm vs Source (MRL 5, default 0.1): which shows glyphosate mostly converted to AMPA from higher earlier pre-harvest treatment mostly degraded (compare to previous graph).

Result exceptions from “Residues–Not-Detected” or “Not-Declared-Used”.				
Source	Treatment	Glyphosate	AMPA	Duplicate
Nth Canty	3L Roundup after clover	Not detected	Not detected	
Nth Canty	April 03 2L prior to sowing	Not detected	Not detected	
Nth Canty	5L prior to harvest	0.03	0.22	
Nth Canty	No	0.05	Not detected	
Mid Canty	Not Stated	0.22	Not detected	
Sth Canty	Yes	0.09	0.03	
Sth Canty	No	"0.03" <LOQ	Not detected	"0.03" <LOQ & ND
Sth Canty	Yes, 1L applied 4 days prior to harvest	1.2	Not detected	
Southland	Yes	Not detected	Not detected	
Canada	Not Stated	0.77	0.03	
Canada	Not Stated	0.48	0	
Canada	Not Stated	0.13	0	
Canada	Not Stated	0.03	0	
Canada	Not Stated	0.11	0	
Canada	Not Stated	0.11	0	

Use survey:

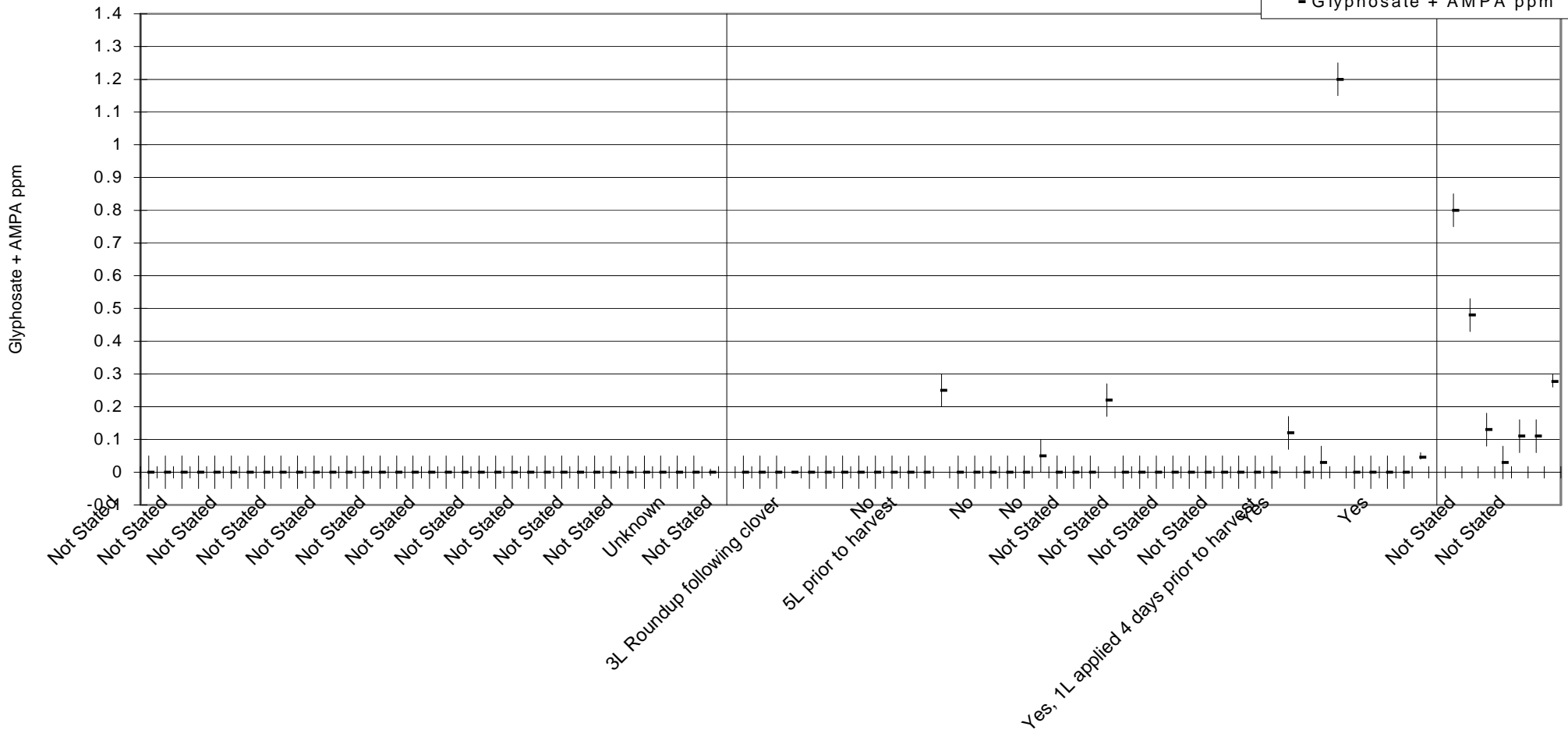
NZ treatment application was nil/low/or not-declared. There was one instance each of (1) higher residues from a low treatment shortly before harvest and (2) lower residues from high treatment mostly degraded.

Conclusions (see summary):

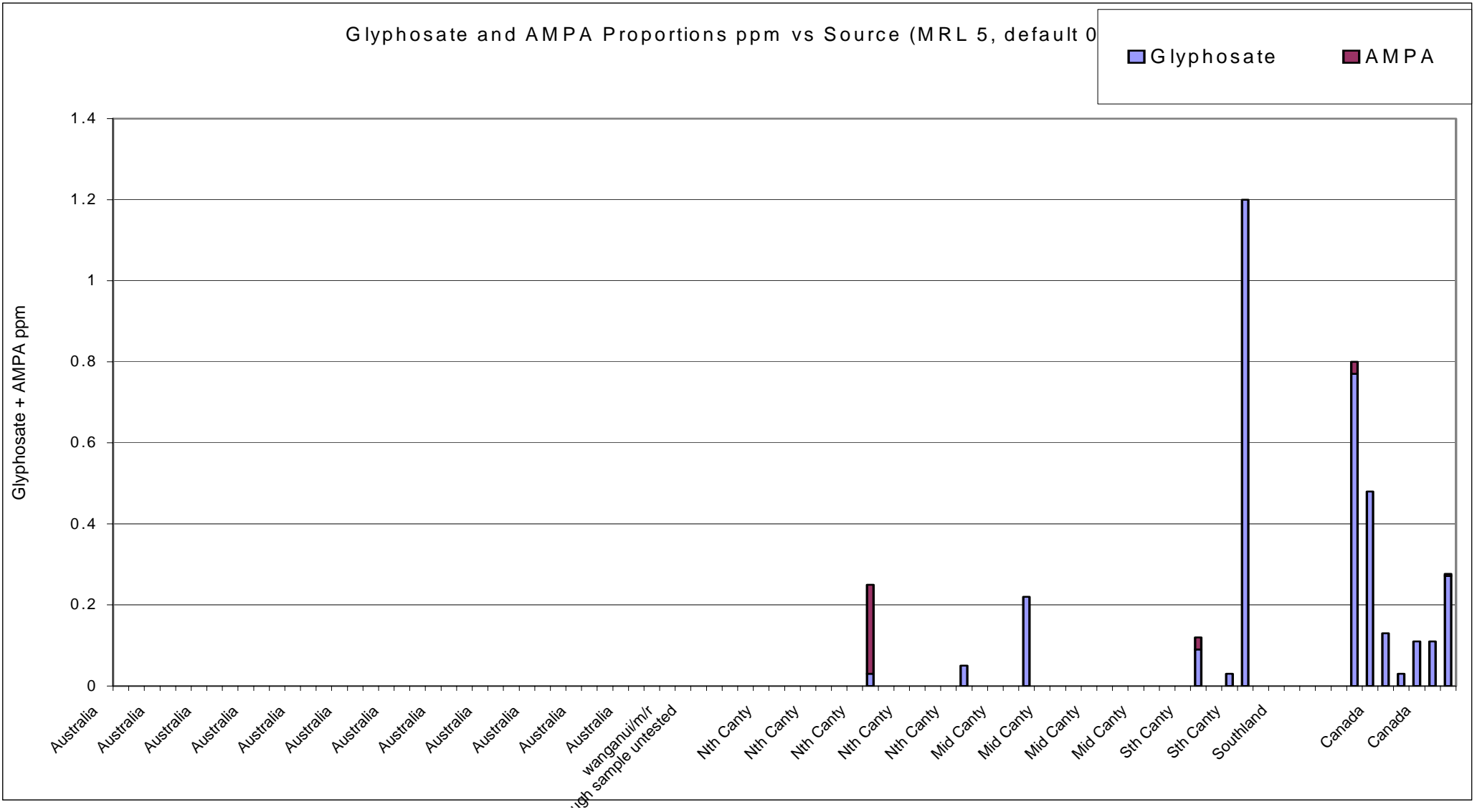
Residue levels, and their variations, were low compared with local limits and especially with MRLs (with a further safety margin for diet variation against the Allowable Daily Intake ADI). Levels may be limited by other controls eg economic controls (as supported by literature).

Sum Glyphosate + AMPA vs Usage (MRL 5, default 0.1 ppm)

high
low
- Glyphosate + AMPA ppm



Glyphosate and AMPA Proportions ppm vs Source (MRL 5, default 0)



Confidence and Probabilities for results

Measurement uncertainty has been determined (Limit of detection LOD, Limit of quantitation, and Coefficient of variation of LOQ) and is too small to influence interpretation of the results as is apparent from this and other results tables.

	AMPA	Glyphosate
LOQ	0.05	0.05
LOD	0.02	0.02
CV of LOQ	12%	9%

Probabilities from the sampling plan (consistent with the findings – which can change from time to time, refer cumulative Poisson and Codex tables CX/MAS 98/4 draft).

Sample set	Exception over limits	Limits ppm here	Average % with 95% probability
80 domestic & imports.	0	Codex MRL of 5	<3.7% exceed MRLwWith 95% probability
40 NZ domestic	4	NZ default of 0.1	<22% exceed default with 95% probability

For NZ comparison 5 from 6 Canadian samples exceed defaults (but all results comply with MRLs which apply to Canada).

- Residue levels found and their variations were low compared with limits Codex MRL 5 and unlikely to exceed them with usage in the study. Levels were mostly low compared with the NZ default 0.1.

Aust avg	0
Aust std dev	0
NZ avg	0.05
NZ std dev	0.19
Canada avg	0.28
std dev	0.30
total avg	0.04
total std dev	0.17

Methodology – sampling

Sampling assurances

- The system made provision for independence and farmer notification at the time of sampling but not earlier.
- Staff drawing seed samples were all MAF Biosecurity Accredited Phytosanitary inspectors who have been trained to source samples using ISTA methods. Many were MAF BA Accredited seed samplers, especially in the main wheat growing areas of Canterbury and Manawatu.
- All sampling documentation, instructions and equipment were coordinated through AgriQuality's Seed Certification business (see Appendices I and II). Each sample was placed into an approved Seed Certification manila seed sample packet, together with its seed sample information form and couriered to the Gracefield Laboratory.
- Each sample was identified with a unique reference number. This number was to allow for trace back purposes should further information be required or issues be identified.

Sampling of Domestic Wheat

- Growers stored seed lines in individual silos on farm. Paddocks of the same variety of seed may have been already mixed, but separate varieties are segregated.
- One sample was collected on-farm, post-harvest, for each selected grower.

Sampling of Imported Wheat

- Imported samples were collected when the ship was unloading, or at the transitional facility where seed was stored following unloading, or at the processor's site awaiting processing.
- Where an imported seed-lot exceeded 40 tonne, multiple samples were collected as per the sampling frequency identified in Appendix II.

Laboratory Sample Size

- A minimum sample of 100g was submitted to the laboratory for testing.

Transport

- All samples were transported to the laboratory using recognised national couriers. All samples were accompanied by relevant chain of custody documentation.

Seed sampling frequency was by standard methods (table ex ISTA meeting 1998)

For sacks (i.e. containers up to 100 kg capacity)

Number of containers	Sampling frequency
1-4	3 primary samples from each container
5-8	2 primary samples from each container
9-15	1 primary samples from each container
16-30	15 primary samples total
31-39	20 primary samples total
60 or more	30 primary samples total

For bulk bins (i.e. containers greater than 100 kg capacity)

Size of lot	Sampling frequency
Up to 500 kg	At least 5 primary samples
501-3000 kg	1 primary sample for each 300kg, but not less than 5
3001-20000 kg	1 primary sample for each 500kg, but not less than 10
20001 kg & above	1 primary sample for each 700kg, but not less than 40

In all cases, when sampling a lot of up to 15 containers, for those containers selected for sampling, the same number of primary samples shall be taken from each container.

Sampling method

- Sampling officers used 2 types of seed triers appropriate to "sacks" or "bulk".
- The "sack" trier was a small metal tube with a pointed end and a hole set back from it for the seed to fall into. The trier is stuck into the sack and slowly withdrawn as the seed falls into the sample container. A primary sample is one insertion and withdrawal of the trier.
- The "bulk" trier was a larger metal tube, approximately one metre long, with a pointed end and holes along its length. This is inserted in the seed lot then withdrawn. This is one primary sample.

Sample information form collected the following information:

Glyphosate Residue Testing Contract 2004

- Imported or Domestic
- Country / Region of Origin:
- Grower / Importer:
- Address:
- Import / Harvest date
- Sampling Date:
- Crop Site Reference: (eg Region of Production, paddock name/s, transitional facility address, etc)
- Storage ID (eg silo number)
- Variety (if known)
- Estimated Total Tonnage of Line
- Was Glyphosate used on crop site pre or post drilling (Domestic samples only - record rate if known)
- Send sample to:
- AgriQuality Limited, 1B Bell Road, PO Box 31242, Gracefield, Wellington

Methodology – analysis

Measurement uncertainty was determined (Limit of detection LOD, Limit of quantitation, and Coefficient of variation of LOQ) and is too small to influence interpretation of the results.

	AMPA	Glyphosate
LOQ	0.05	0.05
LOD	0.02	0.02
CV of LOQ	12%	9%

Method for sample reception testing and reporting

- On receipt, all samples were assessed for compliance against acceptance criteria, and logged into the laboratories information management system. The submission data for **all** samples, whether or not they meet acceptance criteria for testing, were loaded into the laboratory information system 'AgriLab'. Each sample received a unique laboratory sample number that it carried through all handling, sample preparation, testing, subcontracting, and storage procedures to allow tracking of the sample at all times, regardless of its suitability for testing.
- Samples were analysed in accordance with AOAC methodology – "Determination of Glyphosate and Aminomethylphosphonic Acid in Crops by Capillary Gas Chromatography with Mass-Selective Detection: Collaborative Study". Journal of AOAC International, Vol 84, No. 3, 2001 **823**. The method is based on extraction and derivitisation prior to analysis.
- The routine method LOQ for Glyphosate residues was 0.1ppm, coefficient of variation 20%, with analyte recovery in the range 60% to 120%. Full validation of the methodology specific to wheat samples complied with the NZFSA protocol (protocol and data appended).
- Quality Measures have been described in the original AgriQuality proposal (refer Appendix III Laboratory Quality Control) to provide services for this survey.
- The NZFSA Technical Liaison were advised on confirmation of all positive test results, and the information necessary for trace back, by phone / fax / email.

Laboratory test validation (result detail appended)

Procedure satisfied the minimum procedural requirements for validating glyphosate residue test method on wheat per results submitted to NZFSA (pre-trial results as previously submitted are in the NZFSA appendix- & NZFSA made no statement regarding validity or application outside the intended use for a survey to determine Glyphosate residue in wheat).

1. The laboratory must acquire a sufficient sample of wheat free from glyphosate residues to test for the LOQ (limit of reliable quantitation).
2. LOQ/variability: the lab must test 3 spiked samples at the LOQ for precision. For this study NZFSA deem the provisional LOQ to be 0.1 mg/kg and if this is refined from the analytical results to a slightly lower values (eg 0.05 mg/kg) this lower value need not be confirmed by laboratory testing. The SD of the mean and CV is to be reported.
3. Recovery: the lab to test 2 samples at 2 mg/kg spike, 2 samples at 5 mg/kg and 2 samples at 10 mg/kg spike glyphosate. Each value reported in the validation separately. The mean recovery and CV can be calculated if AQ wish to do so.
4. The linear regression of the 3 mean recoveries together with the 95 % error band of the line to be reported to NZFSA (use a forced and unforced zero intercept).
5. The linear regression of the 2, 5 and 10 mg/kg results plus those from the 0.1 LOQ analyses to be reported (use forced and unforced zero intercept).
6. Each separate suite (nos 2 and 3 above) should use 1 blank wheat sample only, ie no 2 is 4 samples in total and no 3 is 7 samples in total.

Methodology – analysis continued

Laboratory Quality Control

ISO compliance: the Wellington Laboratory's Residues Team's (Formally the National Chemical Residues Laboratory) quality control system is in compliance with ISO17025, OECD GLP and EU acceptance criteria for confirmations. This includes Standard Operation Procedures, and requirements for analytical method quality control and monitoring.

The Standard Operating Procedures describe protocols for:

- The validity and confidence in results generated by the Wellington Laboratory.
- The content and format of bench procedures for analytical methods. The protocol for identification, issue, control and review of bench procedures.
- The process for validating an analytical method to ensure that the method is fit for purpose.

Steps specific to project: to meet the specified quality assurance requirements for this proposal, every analytical assay batch performed included the following for the control of analytical method quality:

- External standards.
- Blank extracts every 10th sample analysed.
- Recoveries (spiked blank samples), at least 1 per batch.
- Blind spikes at high and low levels at least 1 every 10 samples.
- Duplicate samples at least every 20 samples.
- Critical control points are recorded e.g. percent recovery.
- Internal standards are used when available and appropriate.
- Certified Analytical standards are used for all assays.
- Intra laboratory checks – it is NCRL's practice to conduct intra laboratory checks on each assay on a monthly basis.
- Inter laboratory checks – the laboratory participates in the NARL and NRS proficiency-testing programme (ILCSS) for all relevant assays conducted when available.

The analytical variance

- Variance of methods used at the Wellington Laboratory (Residues or National Chemical Residues Laboratory's) were continuously monitored on an ongoing basis.
- All routine assays performed had monitored parameters (control samples, mean and CV of internal standards, % recovery). All % recovery control points were recorded on control charts.

Batch acceptance criteria

These included that the monitored parameter is within ± 2 standard deviations of the mean (calculated from validation and previous batches).

Anomalous results and repeat work.

Any anomalous results were investigated prior to reporting. All manual calculations and transcriptions were re-checked, the quality control data examined, and if no reason could be found, the sample was re-analysed if possible. If the apparently anomalous result was confirmed, it was reported with a comment. Normal practice is to retain samples or sub-samples for one month after reporting.

Laboratory worksheets

These were available for inspection in the company of AgriQuality staff, but not to retain. Results of analysed blanks, duplicates, spikes and standard solutions will be made available on request.

References:

- Codex Standard 193, 1995, General standard for contaminants and toxins in foods, 1, 1997, 1, 2001.
- Codex CAC, IGL 16, 1993 Guidelines for establishment of a regulatory programme for control of veterinary residues in foods.
- Joint FAO/WHO Food Standards Programme, Codex Committee on methods of analysis and sampling, Methods of sampling Proposed draft guidelines on sampling & Draft revised recommended method of sampling for the determination of pesticide residues for compliance with MRLs.
- Cumulative Poisson – Eton stat & math tables.
- Codex website pesticide MRLs.
- USA FDA 40 CFR 180.364 MRLs.
- New Zealand (Maximum Residue Limits of Agricultural Compounds) Food Standards 2004.
- Australia and New Zealand Food Standards Code std 1.4.2 Maximum Residue Levels (Australia only).
- US Department of the Interior, US Geological Survey: webmaster_co@usgs.gov
- Crop desiccation with Glyphosate.. Barrie Manikel director Focus on inputs, Morris, Manitoba.

Definitions/glossary

- Glyphosate is N(phosphonomethyl)glycine has metabolite AMPA aminomethylphosphonic acid.
- AMPA means aminomethylphosphonic acid.
- LIM means Laboratory Information Management (System).
- LOQ means Limit of Quantitation.
- MRL means Maximum Residue Limits of Agricultural Compounds) Food Standards 2004.
- NZFSA means the New Zealand Food Safety Authority

