From: Chang, Eugene

From: Sack, Chris A
Sent: Tuesday, February 21, 2017 8:38 AM
To: Mercer, Gregory E; Thompson, Richard L.; Chang, Eugene; Cooke, William
Subject: RE: PE vs no PE

Given the choice between DCM and PE I would definitely choose the DCM for convenience. I think if we want it as an option we will need to demonstrate equivalence during the collaboration. That means more analyses, unless we assign 3 labs to use DCM and three to use PE. Not sure how the CMVS would view that?

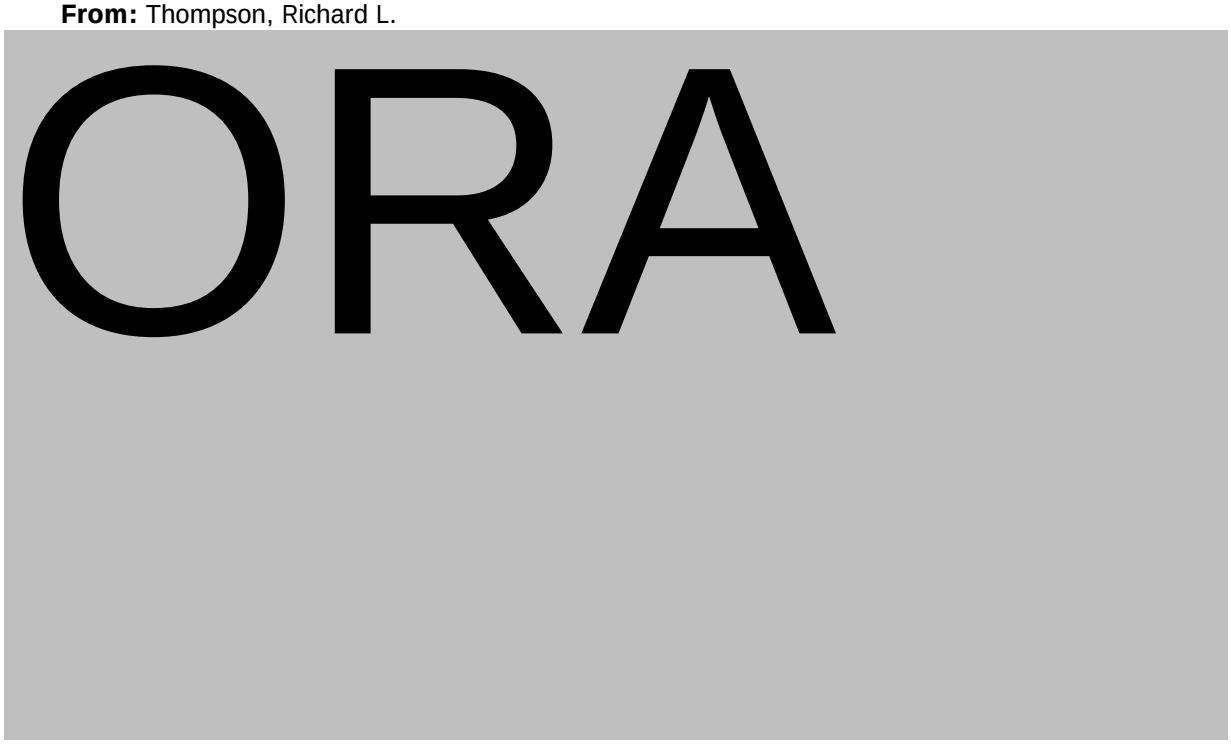
Also, what do you think about conducting the collab using the PE/DCM cleanup for all samples? If we do that we might run into some trouble in the future if we want the option to not use the cleanup. Not sure it matters all that much but I am sitting on my canola, and I don't have to do the extra work of the cleanup. What do you think?

Chris

Ph: 240-402-2464

From: Mercer, Gregory E

From: Thompson, Richard L.



From: Mercer, Gregory E

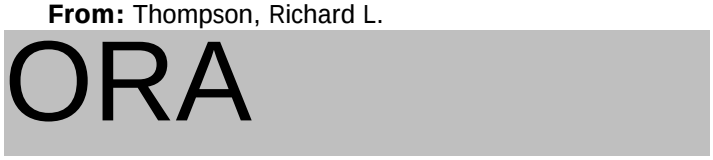
From: Sack, Chris A
Sent: Tuesday, February 21, 2017 6:52 AM
To: Thompson, Richard L.; Chang, Eugene; Cooke, William
Cc: Mercer, Gregory E
Subject: RE: PE vs no PE

And your recoveries look good. I agree with you about using the cleanup on all samples. You bring up another issue; i.e. the DCM vs PE. I know that will be an issue the QA folks will jump on unless we have some data demonstrating equivalence. I thought I remember somebody saying the PE cleanup was better. DCM is obviously easier to use. I know the west coast folks will not purchase, let alone use, DCM unless absolutely required.

Chris

Ph: 240-402-2464

From: Thompson, Richard L.



From: Sack, Chris A
Sent: Tuesday, February 21, 2017 8:18 AM
To: Thompson, Richard L.; Chang, Eugene; Cooke, William
Cc: Mercer, Gregory E
Subject: PE vs no PE

Hi Eugene, Richard, and Bill,

In the method I have indicated the PE cleanup is optional for fatty or dirty matrices. I forgot to include instructions in the collab protocol. What do you guys think? I was assuming everyone would use the PE cleanup for the avocado. Should I include analyses with and without PE cleanup for corn and carrot? I don't want some QA guy questioning the option down the road. If it was up to me I would add the PE cleanup to all analyses for the sake of simplicity and the extra cleanup probably wouldn't hurt recoveries of such polar analytes.

What do you think?

Chris

From: Sack, Chris A
To: Mercer, Gregory E
Subject: RE: Avocado with PE, 2 options
Date: Wednesday, February 22, 2017 6:48:00 AM

That is kind what I was thinking. We might need more than one lab conduct the test. Since Level three validation requires 3 labs I was thinking 3 labs. Would Seattle participate? I am pretty sure I can get ARL and PSW also. Actually, I bet everybody will agree once we mention it. It would only be two more analyses. Would you suggest corn or carrots?

Chris

Ph: 240-402-2464

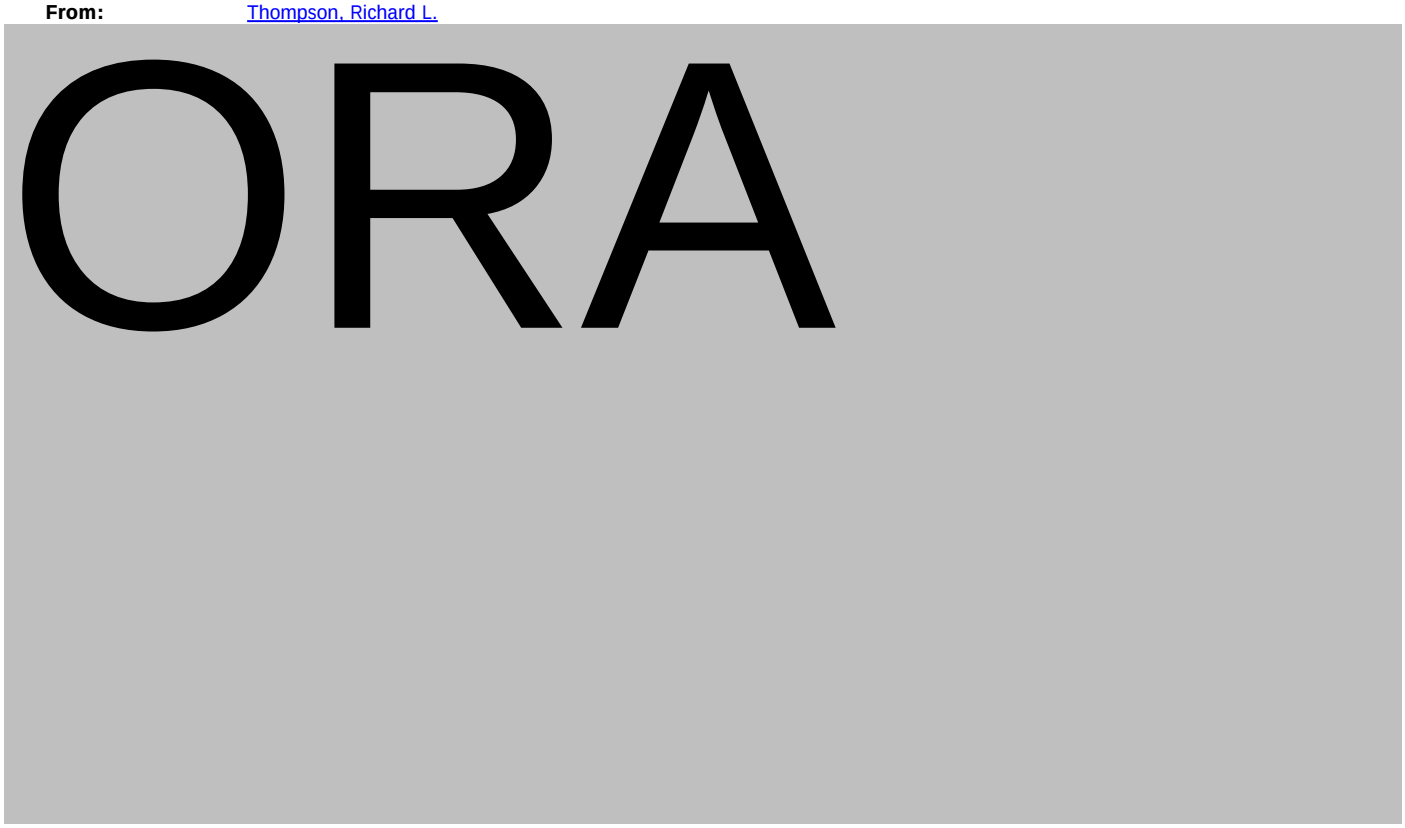
From: Mercer, Gregory E

From: Mercer, Gregory E

From: Chang, Eugene

ORA

From: Thompson, Richard L.



From: Sack, Chris A
Sent: Monday, February 20, 2017 12:16 PM
To: Chamkasem, Narong; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Noonan, Gregory; Thompson, Richard L.; Vonderbrink, John; Wong, Jon
Cc: Cassias, Irene; Eide, David J; Katsoudas, Eugenia; MacMahon, Shaun; Sack, Chris A; Podhorniak, Lynda
Subject: Glyphosate collaboration

Hi Everyone,

Bill Cooke did some work with N-acetyl glyphosate on the 6500 and found two new transitions that work better than those in the method.

Q1	Q2	RT	Transition	DP	EP	CE	CXP
210	150	4.4	N-acetyl glyphosate 1	-20	-11	-20	-13
210	63	4.4	N-acetyl glyphosate 2	-20	-11	-40	-13
210	168	4.4	N-acetyl glyphosate 3	-20	-11	-18	-13

The data and chromatograms he provided (see attached file "SEA N-acetyl 2 2-17-17.docx") clearly demonstrate the advantages of changes to the LC-MS/MS parameters. I have inserted these changes in the final method and collaboration protocol that are attached. Note I highlighted the changes in red. Note also, that I changed the transition names in the calibration method for the collab protocol – those changes are in red also. I would like everyone to try these parameters and verify they work for your instrument. Please note the DP voltage for the 5500 might be optimized at much larger levels.

Bill also analyzed some spikes using various IS calibrants for AMPA and N-acetyl glyphosate. The results tabulated below clearly indicate the benefit of using the glyphosate-13C IS for the calibration N-acetyl glyphosate. For AMPA Bill compared all three available IS isotopes. Obviously, the AMPA isotope works best, but we have already decided we will not be quantitating AMPA. The glyphosate IS appears to work satisfactorily to compensate the sample volume differences between matrices. I updated the collab protocol to use glyphosate-13C as an IS for glyphosate, AMPA, and N-acetyl glyphosate and glufosinate-D3 for glufosinate. These changes are in red also.

	AMPA Spike 200				N-acetyl glyphosate Spike 200	
IS	**AMPA**	**Glyphosate**	**Glufosinate**	**None**	**Glyphosate**	**None**
Avocado	96	22	52	17	84	65
Carrot	81	20	29	16	92	73
Corn	106	30	32	26	100	90

When I was with Bill last week, I asked him to provide me a results file formatted as directed in the collab protocol. He provided a screen shot "SEA Layout 2-17-17.png" – see attached. In his example Bill has provided all the data fields listed in the protocol along with a few extras, including Height, Ion Ratio, Accuracy, Mass Info and Area Ratio. This format is fine with me. As long as the transition masses are correct in the transition name, the Mass Info data is redundant. The other extra fields could prove useful but are not necessary.

Collab protocol	**SEA example**
Index	Index
Sample Name	Sample Name
Sample Type	Sample Type
Dilution Factor	Dilution Factor
Peak Name (Transition Name)	Component Name
Peak Area	Area
IS Peak Area	IS Area
RT	Retention Time
Concentration (Spk level or Std conc)	Actual Concentration
Calc concentration	Calculated concentration

Some notes and observations:

- All records (rows) must have both an analyte response and an IS response. This is critical for data processing.
- The Component Name (Transition name) must be identical to those I provided in the collab protocol. Note that the new transitions for N-acetyl glyphosate are based upon the outdated MS/MS parameters and must be changed (don't forget to update those Bill). This is also critical for data processing.
- Excepting the incurred soy sample that is diluted 1:10, the dilution factor should always be 1 because the calibration is set based upon spike levels. In the case of the incurred soy sample the dilution factor would be 10.
- Please use the sample descriptions provided in the collab protocol. If you want to add replicate identifiers that would be OK. For example the firs calibration standard listed in the protocol is "10 ng/ml calibration std in solvent". Since it is the first of multiple replicates you can number it 1 and subsequent injections of the same standard sequentially. If you follow the injection protocol as written, you should have 10 ng solvent

standards 1 thru 5.

- The data should be in XLSX format. If you can add the chroms in the report as shown in Bill's example, that would be OK, but not necessary. If I see an anomaly in the data, e.g. replicate recoveries do not match, then we will need to re-examine the integrations to ensure that something was not amiss.
- Before you submit the data, please review it closely. The responses for all IS of glyphosate-13C should be very nearly identical. The same applies to the response for the glufosinate IS glufosinate-D3. Any variations in the IS responses indicate a critical failure of the process and might require re-analysis.

Before you start the collab let everyone know how the revised MS/MS parmeters work for you. If you are using the 5500, let everyone know the optimized DP voltage you use.

Thanks everyone,

Chris

From:	Sack, Chris A
To:	Chamkasem, Narong; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Noonan, Gregory; Thompson, Richard L.; Vonderbrink, John; Wong, Jon
Subject:	Glyphosate level in N-acetyl glyphosate standard
Date:	Wednesday, February 22, 2017 11:37:00 AM

Hi Everyone,

Several of you have noticed their N-acetyl glyphosate standard has a trace amount of glyphosate impurity. Richard assayed his N-acetyl glyphosate standard and found 1.5 % glyphosate. Before you start the collab, I need everyone to do assay their N-acetyl glyphosate standard for glyphosate and let me know the level you find. For the collaboration most of the results we will be evaluating are based upon recoveries which are not affected by the low level glyphosate contaminate in the N-acetyl glyphosate. So, stick with the collab protocol to prepare the standard for recovery studies.

The contaminate will affect the analysis of incurred residues in two proficiency samples (corn and soy) we are analyzing. At 1.5 % the effect is negligible. However, if the glyphosate level in the N-acetyl glyphosate is up around (b) (5) the incurred residue levels will drop noticeably. In that case we might need to prepare a separate glyphosate standard with no N-acetyl glyphosate for the analysis of incurred residues.

Let me know what the glyphosate level you find in the N-acetyl glyphosate standard.

Thanks,

Chris

From: Sack, Chris A
To: Chang, Eugene; Mercer, Gregory E; Thompson, Richard L.; Cooke, William
Subject: RE: Avocado with PE, 2 options
Date: Wednesday, February 22, 2017 11:54:00 AM

Thanks Eugene for looking at DCM. To be clear, we will collaborate the current method. If you want to pursue other solvent mixes later, I wish you the best. But we really needed to have the collab finished by now. Couple questions for you.

Did you resolve your issues with N-acetyl glyphosate? Were you able to demonstrate instrument efficiency for N-acetyl? What about method proficiency?

How soon can you begin the collab?

Chris

Ph: 240-402-2464

From: Chang, Eugene

ORA

From: Mercer, Gregory E

ORA

From: Chang, Eugene

From: Sack, Chris A
To: Thompson, Richard L.; Chang, Eugene; Mercer, Gregory E; Cooke, William
Cc: Chamkasem, Narong; Masse, Claude; Vonderbrink, John; Noonan, Gregory
Subject: RE: Avocado with PE, 2 options
Date: Wednesday, February 22, 2017 2:15:00 PM

Wow. Good job Richard.

In our collab I built in a study to demonstrate equivalence between using and not using the PE clean option with the non fatty samples. It cost each lab an additional 6 samples for the collab. How dedicated is everyone to demonstrating that DCM and PE are essentially equivalent? PSW and PNW have expressed serious concern about the health effects of DCM exposure. If I can get 3 labs to agree to demonstrate that cleanup using DCM is equivalent to using PE I think we can allow the labs to use them interchangeably. Of course, as it stands now, we will be using (b) (5)
(b) (5)

Thanks

Chris

Ph: 240-402-2464

From: Thompson, Richard L.

From:	Sack, Chris A
To:	Chamkasem, Narong; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Noonan, Gregory; Thompson, Richard L.; Vonderbrink, John; Wong, Jon
Subject:	PE vs DCM cleanup
Date:	Friday, February 24, 2017 11:35:00 AM
Attachments:	Glyphosate method Collab Final.docx Collab-Glyphosate Final.xlsx

Hi Everyone,

Feedback re the need to use cleanup for nonfat items indicates it is unnecessary, so we will not be conducting any analyses using the cleanup step with carrots or corn. In the attached collab protocol I removed the extra cleanup study.

That brings us back to the use of petroleum ether (PE) or dichloromethane (DCM) as a cleanup solvent. Monsanto and Narong both use DCM in their methods and I believe that KAN and ARL is using DCM. In his modification Eugene showed that PE was equivalent in effectiveness and recovery to DCM. Since they are equivalent, I think (b) (5) (b) (5) CFSAN and EPA can choose either when they come on line. PNW and PSW want to use PE; and SRL, KAN and ARL have been using DCM. That just leaves NRL to use PE. How does that sound to everyone?

In the attached method I corrected a dilution error for reagent no. 15.

Thanks and have a nice weekend,

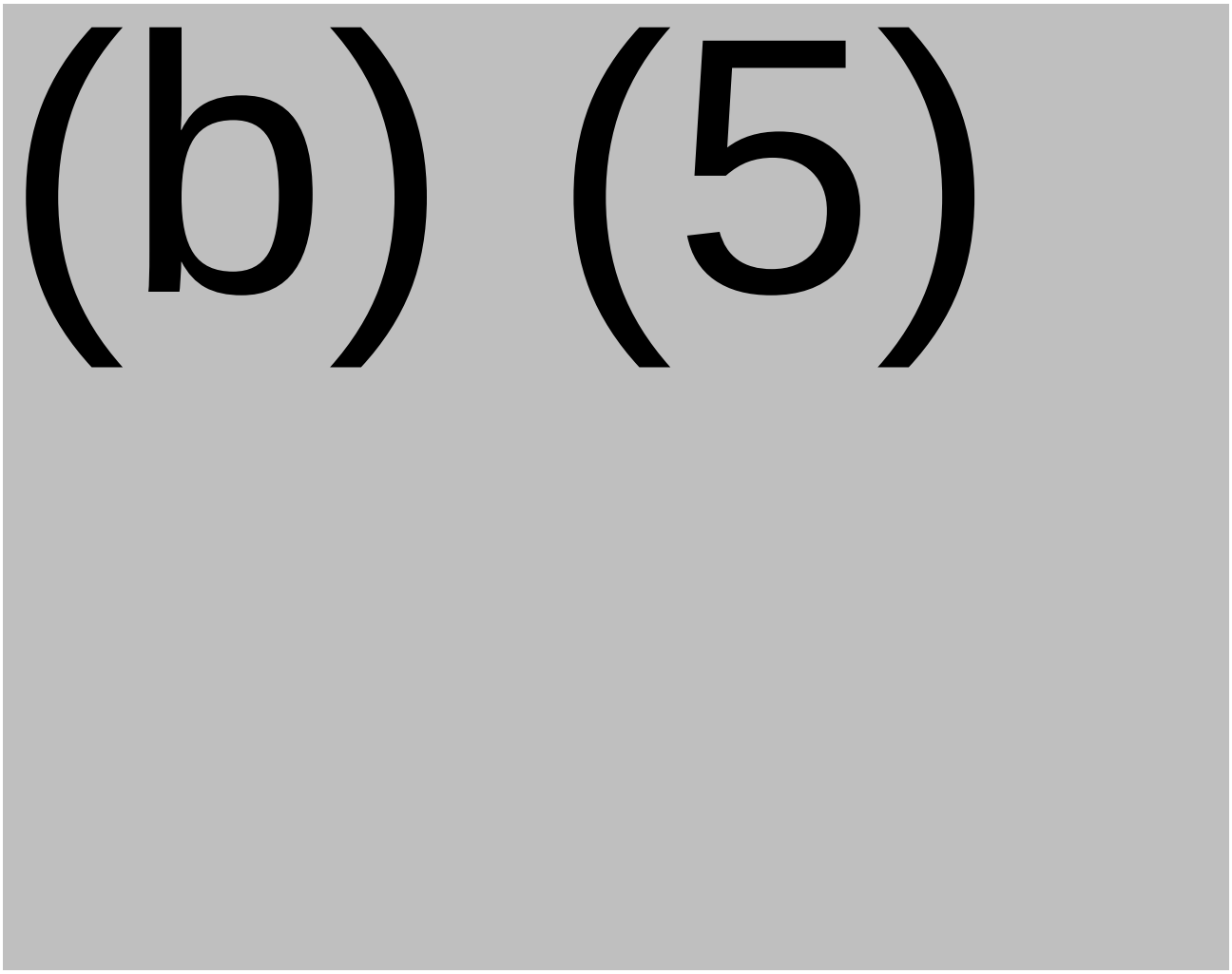
Chris

From: Cooke, William

From: Chang, Eugene

From: Sack, Chris A
Sent: Tuesday, February 28, 2017 10:17 AM
To: Chamkasem, Narong; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Noonan, Gregory; Thompson, Richard L.; Vonderbrink, John; Wong, Jon; Cassias, Irene; Eide, David J; Katsoudas, Eugenia; MacMahon, Shaun; Sack, Chris A
Subject: Draft minutes for PesTAG PMC meeting Feb 28, 2017

**********Draft**********
Please give this a look and let me know if you see anything that needs to be corrected.
Thanks,
Chris
****



(b) (5)

(b) (5)

Good luck with the collaboration,

Chris

From: Chang, Eugene

From: Sack, Chris A
Sent: Thursday, March 02, 2017 8:18 AM
To: Chang, Eugene
Cc: Chamkasem, Narong; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Noonan, Gregory; Parker, Christine; Thompson, Richard L.; Vonderbrink, John; Wong, Jon
Subject: RE: New MDL and LOQ data

Hi Eugene,

Data looks great.

Re the quantitation limit, we need to keep things in perspective. When talking about limits it is important to keep in mind we are talking about limits. By definition limits are **ESTIMATES** subject to considerable variation and fluctuation. So, it is really meaningless for us to dwell on exactness when discussing them. Your data actually exemplies this point. For glyphosate in carrot you came up with a quantitation limit of 7.11 by using 3*MDL and 7.54 when using 10xSD. For residue work, an MU of ± 50 % for residues below 10 ppb is absolutely acceptable. In our case at 8 ppb we would be happy with values ranging from 4-12 ppb; that is a difference of 8 ppb. Your estimates of the quantitation limit are only 0.43 ppb (7.54-7.11) different. Kinda puts the limits discussion into perspective. Add the fact that we designate every residue level below 10 ppb as Trace and do not act on them. We really need perspective on this issue. Nonetheless, we should

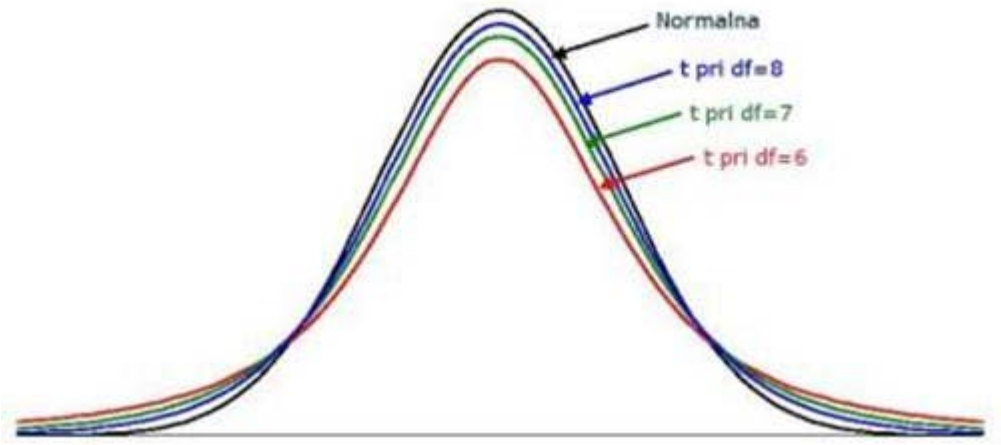
at least be on the same page. In the FDA pesticide program we generally agree on the definitions below.

Limit of Detection (LOD): 3 x background, or SN = 3, I use this term for instruments only
Method Detection Limit (MDL): 3 x SD of replicate analyses at a level near the MDL, I use this term for full methods only
Limit of Quantitation (Lq): 10 x instrumental background, the pesticide program uses this term for the instruments only
Limit of Quantitation (LOQ): 10 x SD of replicates at a level near the MDL, we use this term for full methods only

The scientific community generally agrees that the LOD for an analysis is that level at which a signal can be distinguished from background response with 99 % confidence. Statistically, that is between 2-3 times (~2.4) the SD of the background, or as we say a SN = 3. For an instrument the noise can be measured directly, for a method we use the SD of replicate analyses to measure the background.

Just doing the math, the LOQ is 3.3 x the MDL (10/3). I apologize if I have not made that clear in our communications. So, "they" are correct. Of course, I should mention that there is consensus amongst the sci community re LOD and MDL, the same is not true for quantitation limits. Although 10 * SD is accepted generally, I could not find a justification for that statistically. Many have chosen other methods to defined the LOQ based upon acceptable confidence levels. The SANCO document for pesticides simply defines it as the lowest level of "acceptable accuracy".

There is some confusion around understanding the normal distribution for standard deviation. It is important to know that the distribution used for standard deviation assumes an infinite data set. As the data set shrinks the distribution broadens – see below. In a normal distribution an SD of 2.4 includes ~99 % of the possible values using a one-sided distribution, however, in smaller distribution the SD for 99 % of the population increases. That is why we multiply the SD of 7 replicates by 3.14 instead of 3 (nobody uses the multiplier 2.4).



I apologize if I have been unclear in my communications. I am looking forward to working with the glyphosate data.

Chris

Ph: 240-402-2464

From: Chang, Eugene

From: Islam, Mohammed R

From: Sack, Chris A
Sent: Friday, March 03, 2017 11:39 AM
To: Shireen, Kaniz F
Cc: Islam, Mohammed R; Robin, Lauren P
Subject: RE: herbicide assignment

Hi Kaniz,

We are about ready to resume the acid herbicide assignment. Before we do that we need to amend the assignment. Previously, samples were shipped to SRL for glyphosate analysis and to KAN for acid herbicide analysis. As of FY-17 ORA-ORS shut down the pesticide program at SRL, so the glyphosate samples need to be re-assigned to other labs. Just this week, ORA-ORS has decided that the glyphosate samples will be split between 3 different ORA laboratories that have demonstrated proficiency with the revised method: PNW (Seattle), PSW (LA), and ARL (Arkansas). Samples will be shipped to KAN for acid herbicide analysis, however the glyphosate samples will need to be re-directed from SRL to one of these three labs. Logistically, this is a little bit tricky.

Moh and I have been discussing how to address this issue and we decided that we would modify the current lab assignments for routine pesticide analysis. I am waiting for Moh to provide that table to me so I can incorporate it into the assignment. I need you to send me an editable copy of the original assignment so I can work with Moh to modify.

Thanks,

Chris

Ph: 240-402-2464

From: Shireen, Kaniz F
Sent: Thursday, March 02, 2017 1:45 PM

To: Sack, Chris A
Subject: herbicide assignment

Hi Chris:
Is lab ready to analyze the herbicide samples yet? When would we reissue this assignment?
Just wondering ☺

Thanks,

Kaniz F. Shireen, MS
Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Compliance
U.S. Food and Drug Administration
Tel: 240-402-2775
Kaniz.Shireen@fda.hhs.gov

FDA U.S. FOOD & DRUG ADMINISTRATION

From: Islam, Mohammed R

From: Islam, Mohammed R

From: Islam, Mohammed R

From: McLaughlin, Michael A

From: Islam, Mohammed R

From: Islam, Mohammed R

From: Sack, Chris A
Sent: Tuesday, February 14, 2017 4:23 PM
To: Chamkasem, Narong; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Noonan, Gregory; Thompson, Richard L.; Vonderbrink, John; Wong, Jon
Subject: Update of instrument and method proficiency

Hi Everyone,

Just want to give you a quick update of our progress with proficiency demonstration at each lab. I have received full instrument proficiency from 3 labs: ARL, PNW, and KAN. I have received partial instrument proficiency from NRL. Except for slightly elevated Lq for glufosinate at PNW, the instrument proficiency data indicates the LCMS method works exceptionally.

	ARL	PNW	NRL	KAN
	Glyphosate			
Lq (ng/ml)	0.2	0.4	0.2	0.5
Accuracy	100.3	98.4		100.3
Precision	6.3	2.8		1.2
Linearity	0.9970	0.9999		0.9999
	Glufosinate			
Lq (ng/ml)	0.3	4	0.1	0.6
Accuracy	99.8	96.2		100.2
Precision	1.9	0.7		0.6
Linearity	0.9999	0.9999		0.99999
	AMPA			
Lq (ng/ml)	0.2	2	0.3	0.3
Accuracy	100.5	96.4		100.2
Precision	11.9	3.3		1.6
Linearity	0.9985	0.9999		0.9999
	N acetylglphosate			
Lq (ng/ml)		6	0.3	
Accuracy		97.2		

From: Robin, Lauren P
To: Sack, Chris A
Subject: FW: Benzopyrene in coconut o l
Date: Friday, March 03, 2017 9:42:54 AM

Chris,

Mike McLaughlin provided the update below re glyphosate/herbicide analysis Can you please provide an update as well? If we are ready to start up again, I think you would work with Kaniz and Page to restart the assignment

Lauren

From: McLaughlin, Michael A

From: McLaughlin, Michael A

From: Morris, Cynthia


ORA

From: Chang, Eugene

From: Chang, Eugene

From: Sack, Chris A
Sent: Tuesday, March 28, 2017 10:47 AM
To: Chang, Eugene; Islam, Mohammed R
Subject: RE: CARTS project for glyphosate

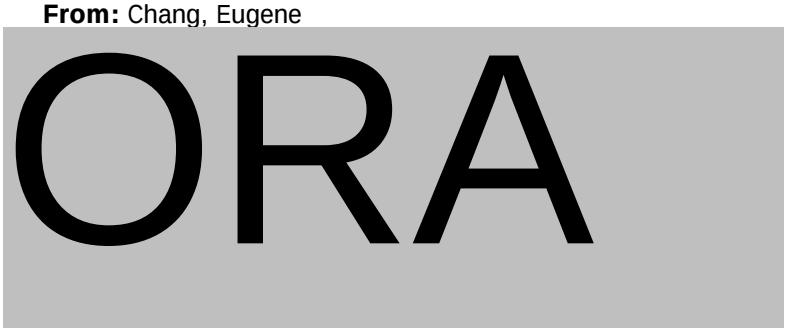
Hi Eugene,

Looks OK to me, however I am concerned about Milestone 3. "Commodity tests will expand the method from three representative food matrices to more than 15 matrices." This suggests the (b) (5) The whole reason we chose the three matrices (carrot, corn, and avocado) was because they represent the totality of matrix types we analyze in pesticides (high moisture, low moisture, and fatty). Now you are suggesting (b) (5) (b) (5) So where will the "expansion" end. What if we are analyzing a matrix different than the 15 you validate? Will we need to do matrix expansion? Where does this end. ORA-ORS and the national QA manager are already visiting each pesticide lab and requiring matrix extension validations. BTW, these are all local validations that do not advance the national pesticide program, but rather solidify the compartmentalization of the program at the lab level. In the pesticide program we analyze between 700-1000 different matrices per year. Maybe we should just shut down the program until we have completed matrix extension validation of all the matrices in the universe. What I am attempting with the glyphosate collaboration is to have a (b) (5)
The way you wrote milestone 3 indicates otherwise. Any chance you can fix this before it goes too far?

Chris

Ph: 240-402-2464

From: Chang, Eugene



ORA

From:	Sack, Chris A
To:	Chamkasem, Narong; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Noonan, Gregory; Parker, Christine; Thompson, Richard L.; Vonderbrink, John; Wong, Jon; Cassias, Irene; Eide, David J; Katsoudas, Eugenia; MacMahon, Shaun; Sack, Chris A
Subject:	Collaboration Report
Date:	Friday, March 31, 2017 1:24:08 PM
Attachments:	Glyphosate MLV Rpt.docx

Hi everyone,

Attached is my first draft for the glyphosate collaboration. Please review and send me your corrections and thoughts by early next week. I would appreciate it if you could expedite your review as the report needs to be submitted last week.

Thanks and have most wonderful weekend,

Chris

From: Thompson, Richard L.

From: Masse, Claude

[redacted]

From: Sack, Chris A
Sent: Monday, April 03, 2017 11:58 AM
To: Masse, Claude
Subject: RE:

Hi Claude,

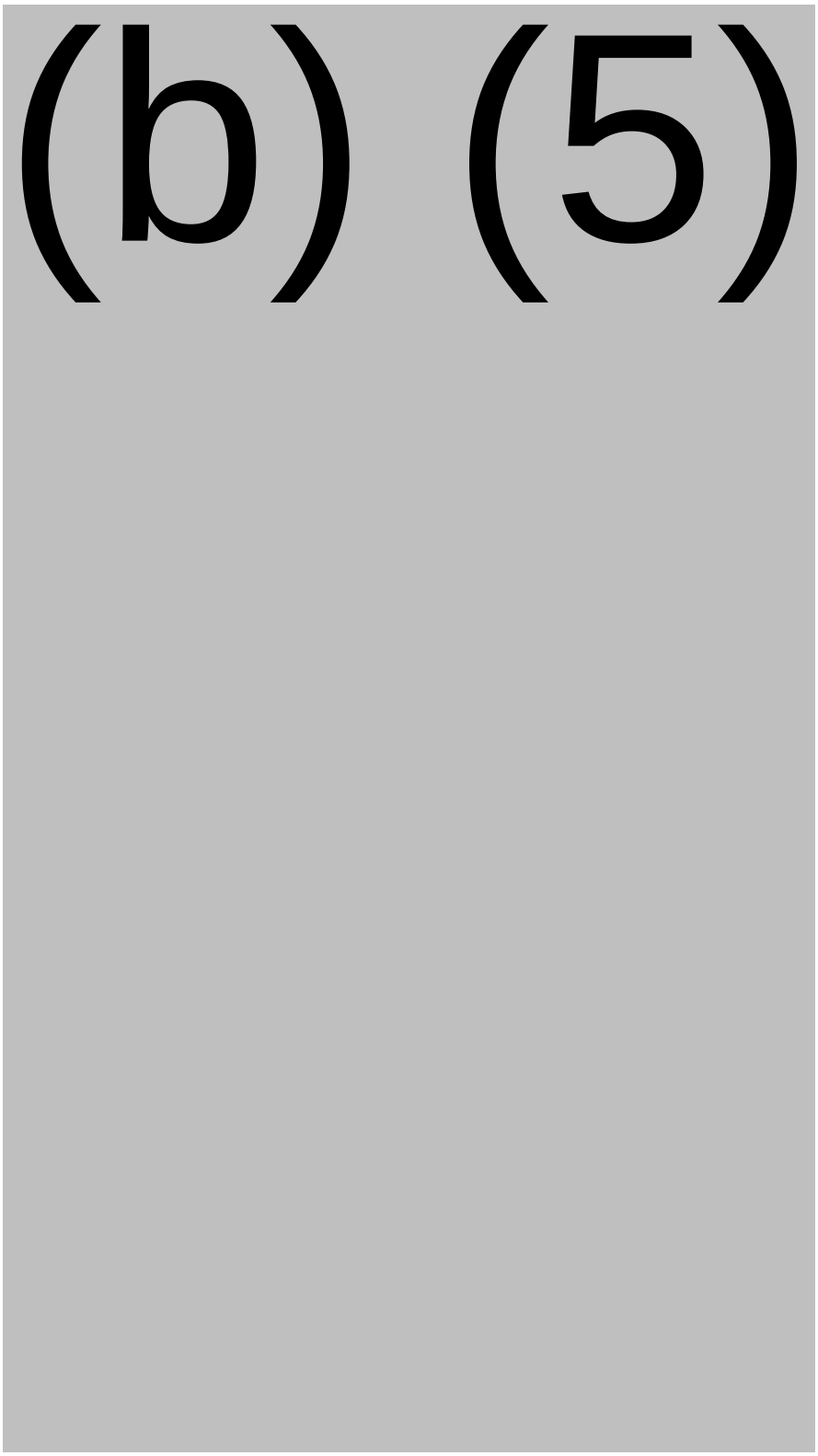
I finally got around to uploading your data this AM and I cannot make head or tails of it. Using the average ratios of the glyphosate/glyphosate IS responses recoveries for glyphosate range from 70 to over 800 – see below. ????? Both the glyphosate and glufosinate IS responses vary signgificantly. I am not sure what you are doing wrong.

[redacted]

(b) (5)

Both the glyphosate and glufosinate IS responses vary significantly. See areas of glyphosate IS below.

(b) (5)



Chris

Ph: 240-402-2464

From: Masse, Claude
Sent: Sunday, March 26, 2017 1:01 PM
To: Sack, Chris A
Subject:

Chris,
Here is another collab data.

Claude

From:	Sack, Chris A
To:	Chamkasem, Narong; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Noonan, Gregory; Parker, Christine; Thompson, Richard L.; Vonderbrink, John; Wong, Jon; Cassias, Irene; Eide, David J; Katsoudas, Eugenia; MacMahon, Shaun; Sack, Chris A
Subject:	Glyphosate collaboration report
Date:	Tuesday, April 04, 2017 2:12:40 PM
Attachments:	Glyphosate MLV Rpt.docx

Hi Everyone,

I updated the glyphosate collaboration report per the input I have received. I plan to submit to the CMVS tomorrow, unless I hear from you. Richard, Eugene, and Bill, take a look at the attachment for your lab and let me know if you have any changes you would make.

Thanks,

Chris

From:	Sack, Chris A
To:	Shireen, Kaniz F
Subject:	RE: Acid Herbicide assignment
Date:	Tuesday, April 04, 2017 6:07:00 AM
Attachments:	image001.png

Hi Kaniz,

Good job. I am submitting the report for the method collaboration this week. I will let you know when the labs are ready for samples.

Chris

Ph: 240-402-2464

From: Shireen, Kaniz F
Sent: Monday, April 03, 2017 8:54 AM
To: Sack, Chris A
Subject: Acid Herbicide assignment

Chris:
I've updated the assignment with lab information and import sample numbers within the Table. Please review once again and let me know, if I can reissue the assignment.

Thanks,
Kaniz F. Shireen, MS
Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Compliance
U.S. Food and Drug Administration
Tel: 240-402-2775
Kaniz.Shireen@fda.hhs.gov

FDA U.S. FOOD & DRUG ADMINISTRATION

From: Vonderbrink, John

<http://www.fda.gov/>

<https://www.facebook.com/FDA> <https://twitter.com/US_FDA>
<http://www.youtube.com/user/USFoodandDrugAdmin> <http://www flickr.com/photos/fdaphotos/>
<http://www fda.gov/AboutFDA/ContactFDA/StayInformed/RSSFeeds/default htm>

"The contents of this message are mine personally and do not necessarily reflect any position of the Government or the Food and Drug Administration."

From: Sack, Chris A
Sent: Monday, April 03, 2017 10:59 AM
To: Vonderbrink, John
Subject: RE: Update

Thanks for the update.

Chris

Ph: 240-402-2464

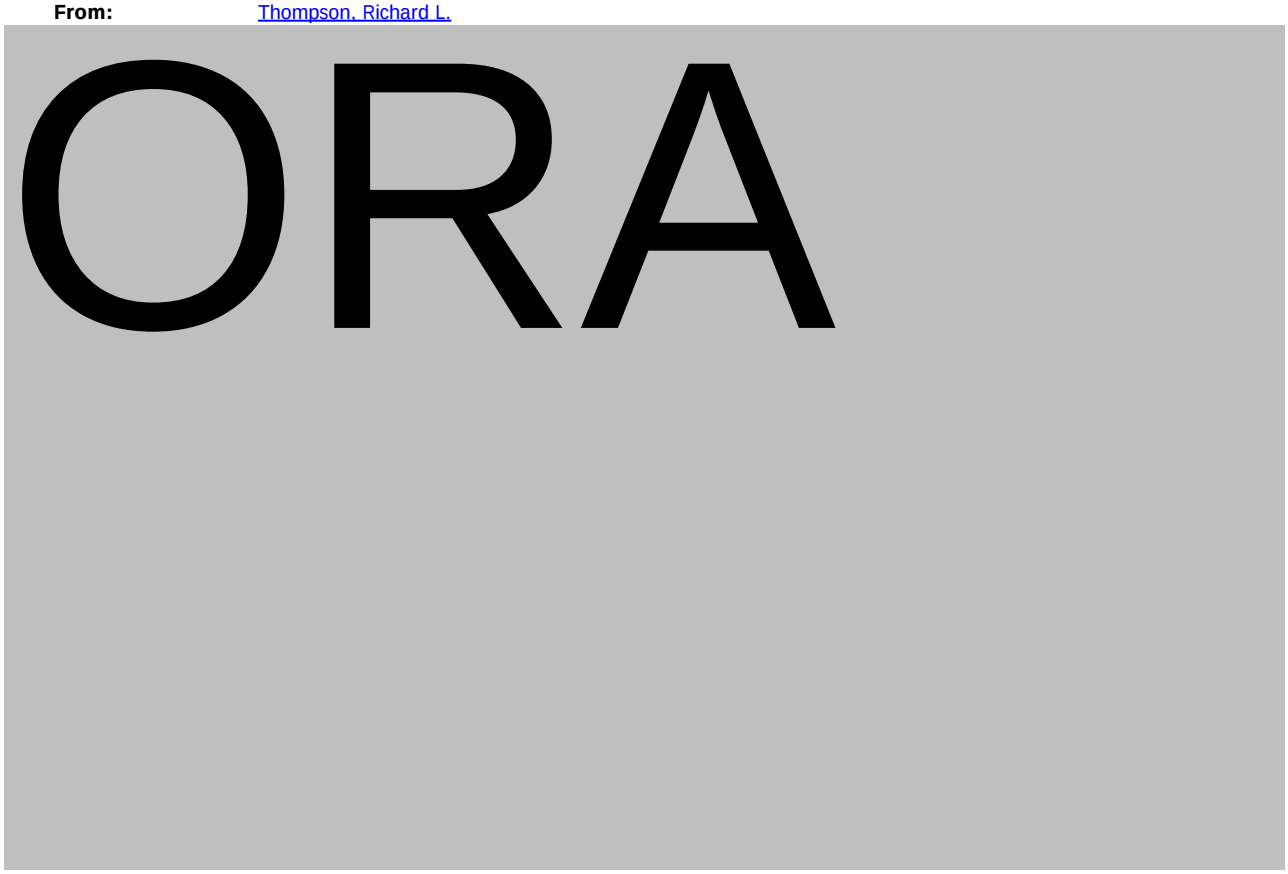
From: Vonderbrink, John

<http://www.fda.gov/>

<https://www.facebook.com/FDA> <https://twitter.com/US_FDA>
<http://www.youtube.com/user/USFoodandDrugAdmin> <http://www flickr.com/photos/fdaphotos/>
<http://www fda.gov/AboutFDA/ContactFDA/StayInformed/RSSFeeds/default htm>

"The contents of this message are mine personally and do not necessarily reflect any position of the Government or the Food and Drug Administration."

From: Thompson, Richard L.



From:	MacMahon, Shaun
To:	Sack, Chris A
Cc:	Bowers, John C; Cai, Yanxuan (Tina); Chu, Pak S; Deeds, Jonathan; Eischeid, Anne; Heitkemper, Douglas T; Oakes, Gregg P.; Turnipseed, Sherri B; Callahan, John
Subject:	CMVS comments on the glyphosate MLV proposal
Date:	Wednesday, April 05, 2017 1:52:00 PM
Attachments:	Glyphosate_Response.doc

Hi Chris,

The CMVS has reviewed your multi-laboratory validation plan for the method, “Determination of Glyphosate and Glufosinate Residues in Food.” The enclosed report summarizes the findings of the subcommittee and includes a number of comments and suggestions which need to be addressed before the MLV proposal can be approved. Please let me know if you have any questions or if there is anything you’d like to discuss.

Kind regards,
Shaun

Shaun MacMahon, PhD
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Shaun.MacMahon@fda.hhs.gov

From: Sack, Chris A
To: Chang, Eugene; Chamkasem, Narong; Mercer, Gregory E; Thompson, Richard L.
Subject: CMVS questions
Date: Thursday, April 06, 2017 7:55:00 AM
Attachments: Glyphosate method Collab Final.docx

Hey guys,

Just got the CMVS response to our glyphosate collaboration proposal. They have a lot a questions, most of which I can answer. There are a few which I can't without your help. I would like to respond today if possible. The question/remarks are in italics in bullets; you can respond below each. Some of my thoughts are in blue below the question. I am attaching the method for you to reference.

- *Does the method employ a divert valve? Instructions are not provided.*

(b) (5)

- *Has arcing been observed during analysis? This is a common issue in negative ion methods, particularly when employing a divert valve, and can damage the electrode.*

(b) (5)

- *Why is tetrabutylammonium formate used as the buffer? It's an unusual buffer selection for MS and is likely to have significant carryover.*

(b) (5)

- *What conditions should be used for a 5500 QTRAP and which for a 6500 QTRAP? Any other details on condition differences between the platforms should be included. At a minimum, the 6500 QTRAP commonly requires a lower source temperature, higher curtain gas, and higher declustering potentials than the 5500.*

(b) (5)

- *What is the linear dynamic range of the method? At what point does response become quadratic? Saturated?*

(b) (5)

- *Some reagents are listed as "optional" e.g tetrabutyl ammonium acetate solid or solution. Apparently these reagents can be used to make mobile phase A (or not)? What is the effect of making the mobile phase three different ways? It seems better to have all laboratories prepare the reagents in a consistent manner.*

(b) (5)

- *Stability (and storage conditions) of standards should be specified.*

(b) (5)

- *The SPE clean-up step should also be described in more detail. How is the SPE conditioned? Is there a wash or elution step or is this just a "pass-through" procedure?*

(b) (5)

- *What type of filters should be used? Nylon or PTFE, etc.? This can be critical for some analytes.*

(b) (5)

- *The analytical column listed in section D is a 4.6 mm (see concerns above), but in section F, there is the option of using a 2.0 mm LC column (more common). Is the guard column recommended for both? Again, it seems better to provide consistent instruction for the laboratories.*

(b) (5)

- *Section D.2, what was the type of rotor used? Does the centrifuge require temperature controlling capability?*

(b) (5)

- Section E.4, what is the centrifugation g-force?

(b) (5)

May the g-force be with you,

Chris

From: Cooke, William

From: Sack, Chris A
Sent: Thursday, April 06, 2017 7:18 AM
To: Mercer, Gregory E; Cooke, William
Subject: System Suitability LOQ

Hi Bill and Greg,

PNW failed the SS LOQ for glufosinate. We need <= 2 for 10 ppb limit. Any way you can rectify that? Take a look at the summary.

	ARL	PNW	NRL	KAN	SRL	PSW	Avg
	Glyphosate						
Lq (ng/ml)	0.2	0.4	0.2	0.5	0.2	0.3	0.3
Accuracy	100.3	98.4	101.4	100.3	99.3	99.1	99.9
Precision	6.3	2.8	1.6	1.2	0.5	1.4	2.5
Linearity	0.9940	0.9998	0.9999	0.9999	0.9997	0.9997	0.9987
	Glufosinate						
Lq (ng/ml)	0.3	4	0.1	0.6	1.5	0.3	1.3
Accuracy	99.8	96.2	101.4	100.2	98.9	99.8	99.3
Precision	1.9	0.7	4.7	0.6	1.0	2.3	1.8
Linearity	0.9998	0.9999	0.9996	0.99999	0.9995	0.9996	0.9998
	AMPA						
Lq (ng/ml)	0.2	2	0.3	0.3	0.3	0.5	0.62
Accuracy	100.5	96.4	105.1	100.2	98.8	97.7	100.2
Precision	11.9	3.3	2.2	1.6	1.0	2.1	4.0

Linearity	0.9976	0.9999	0.9988	0.9999	0.9991	0.9998	0.9991
	N acetylglphosate						
Lq (ng/ml)		6	0.3	7		0.2	4.4
Accuracy		97.2	102.1	99.3		102.3	99.5
Precision		6.7	5.5	4.6		1.16	5.6
Linearity		0.9998	0.9999	0.9997		0.9998	0.9998

From:	Sack, Chris A
To:	Chamkasem, Narong; Chang, Eugene; Cooke, William; Masse, Claude; Mercer, Gregory E; Thompson, Richard L.; Vonderbrink, John
Subject:	Deviants
Date:	Thursday, April 06, 2017 2:08:00 PM

Men,

I am working on these questions. When I am finished I will forward my response to all of you for a quick review. I just came across a question I did not bother you with initially but now I see I need **ALL OF YOU** to answer for me.

(b) (5)

Forget about the spreadsheet; I will handle that. What I need to know from you is if you DEVIATED from the method or protocol; and if so What was your deviation? A simple NO is the right answer; any YES men out there will be disinvited from the club and I will see if we can find some melamine samples for you; maybe some PAHs to boot.

Also, since we have few options in our method I would like you to tell me which you are using. For example, which HPLC column, with or without the guard column (it looks like some of you have already answered that). Also, tell me if you used Pet Ether or DCM – for the avocado samples.

Thanks,

Chris

From: Sack, Chris A
To: Masse, Claude; Viner, Marianna
Cc: Islam, Mohammed R
Subject: Glyphosate data
Date: Tuesday, April 11, 2017 9:04:00 AM

Hi Claude,

I have reviewed all three sets of collaboration data you provided and found none of them acceptable. In two the IS responses were extremely erratic indicating incorrect preparation. In the last set I received the IS responses were better but whole the responses of many of the standards were zero, even glyphosate at 500 ng/ml. ??? Please don't send me any more collab analyses until you can provide me a simple small set of data demonstrating the method is working in your lab. For example, I would like to see some standards and matrix matched standards that agree.

Chris

From:	Sack, Chris A
To:	MacMahon, Shaun
Cc:	Bowers, John C; Cai, Yanxuan (Tina); Chu, Pak S; Deeds, Jonathan; Eischeid, Anne; Heitkemper, Douglas T; Oakes, Gregg P.; Turnipseed, Sherri B; Callahan, John; McLaughlin, Michael A; Islam, Mohammed R
Subject:	RE: CMVS comments on the glyphosate MLV proposal
Date:	Tuesday, April 11, 2017 11:14:00 AM
Attachments:	Glyphosate MLV Rpt.docx CMVS Review Glyphosate MLV Proposal - PesTAG Reply.docx

Hi Shawn,

The PesTAG answers to your questions about the glyphosate MLV proposal are provided in the attached response. Changes to the procedure are included in Attachment B of the attached Glyphosate MLV report. The glyphosate MLV report is a preliminary report prepared from the data provided from PSW for the single laboratory validation (SLV), and collaboration data from three laboratories (PSW, PNW, and ARL). A subsequent MLV report will be submitted when all participating laboratories have submitted their collaboration data.

The MLV report demonstrates the method is suitable for the purpose of quantitative determination for residues of glyphosate, glufosinate and N-acetylglyphosate and semi quantitative determination of AMPA residues in the three primary matrix types analyzed in the FDA pesticide program, i.e., high moisture, low moisture, and high fat items. The collaboration meets all the requirements of a level three multi-laboratory validation as per the "Guidelines for Validation of Chemical Methods for the FDA FVM Program, 2nd Edition.

As you know it is imperative that we expedite the review and approval of glyphosate method for immediate implementation in the three laboratories that have successfully completed the collaboration, i.e., PSW, PNW, and ARL. Original projections of restarting the glyphosate assignment by the end of January proved to be optimistic. With the completion of this initial phase of the collaboration, our hope is to restart the glyphosate assignment in April. You can address any further questions to me.

Thank you,

Chris Sack

Residue Expert

Office of Food Safety

Center for Food Safety and Applied Nutrition

US Food and Drug Administration

Phone: 240-402-2464

From: MacMahon, Shaun
Sent: Wednesday, April 05, 2017 1:52 PM

From: Cooke, William

From: Chang, Eugene

From: Sack, Chris A
Sent: Friday, April 07, 2017 5:56 AM
To: Chamkasem, Narong; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Thompson, Richard L.; Vonderbrink, John; Wong, Jon
Subject: RE: Respond to CMVS evaluation of proposed glyphosate method collaboration

I guess I could have called it glyphosate and its common degradants but that seemed unwieldy. We might drop (b) (5) since we don't really need it. We do need to monitor N-

acetylglyphosate because it is in the tolerance expression.

Do you have any other comments/corrections for the response I sent out late yesterday?

Chris

Ph: 240-402-2464

From: Chamkasem, Narong

From: Sack, Chris A
Sent: Thursday, April 06, 2017 6:54 PM
To: Chamkasem, Narong; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Thompson, Richard L.; Vonderbrink, John; Wong, Jon
Subject: Respond to CMVS evaluation of proposed glyphosate method collaboration

Hi Everyone,

CMVS reviewed our glyphosate method SOP and collaboration protocol and they have provided us with some questions and observations. Please review the attached response and send me your comments and corrections ASAP. Some of our responses require modification of the method SOP we submitted so I am attaching the revised method. I highlighted all the changes I made in the method (I hope I got them all anyway).

Thanks,

Chris

From: Sack, Chris A
To: Cassias, Irene; Islam, Mohammed R; McLaughlin, Michael A
Subject: FW: Implementation of the glyphoste method in your lab
Date: Wednesday, April 12, 2017 10:08:00 AM
Attachments: Glyphosate method postCollab.docx
Glyphosate MLV Rpt.docx

Hi Moh,

Just to be clear, the chair of the CMVS and I agree that all three labs (PSW, PNW and ARL) have completed all necessary validation and method verification for implementation of the glyphosate method. This is particularly true for PSW. While PNW and ARL might need to conduct some limit testing for method verification (I had intended that the system suitability testing would cover the limit testing for method verification), PSW has completed all the method validation and verification requirements because they did the SLV. Reports for both the SLV and PNW's contribution to the MLV are included as attachments in the MLV report.

ORA-ORS needs to help facilitate getting the glyphosate assignment restarted.

Thanks,

Chris

Ph: 240-402-2464

From: Sack, Chris A
Sent: Tuesday, April 11, 2017 12:09 PM
To: Gonzales, Steven A.; Cassias, Irene; Mabry-Smith, Ronald C
Cc: McLaughlin, Michael A; Islam, Mohammed R
Subject: Implementation of the glyphoste method in your lab

Hi,

Now that your lab has completed the collaboration of the glyphosate method and I have submitted an initial report (attached), I am writing you to ask what we need to do the begin the analysis of glyphosate in your laboratory. In our last meeting we agreed we needed an SOP. I am attaching the method that I submitted to the CMVS in the MLV report. In response to some questions from the CMVS review of the glyphosate MLV proposal I had to make a few minor modifications to the method, mostly for clarification. Those changes are highlighted in the attached method. I believe your laboratories have begun preparation of the SOP. We also discussed method verification requirements. According to the chair of the CMVS participation in the collaboration is a full demonstration of method verification. However, I know that may not be the opinion of some local laboratory directors and QA managers. In the attached MLV report I provided separated attachments summarizing the contributions of each laboratory. Let me know what I can do to help you with getting the method verified for use in your laboratory.

Please understand that I AM TALKING ABOUT IMMEDIATE IMPLEMENTATION. According to the chair of the CMVS ORA has implemented methods for which the CMVS has not finished review of the collaboration report. The glyphosate assignment is waiting upon us to implement the method in the laboratories, so let's get this puppy rolling. Feel free to forward this to anyone necessary to get the process moving.

From:	MacMahon, Shaun
To:	Sack, Chris A
Cc:	Callahan, John; Noonan, Gregory
Subject:	Glyphosate MLV
Date:	Thursday, April 13, 2017 8:27:03 AM

Hi Chris,

I just spoke with John Callahan, the chair of the CRCG, about the approval of the glyphosate MLV and wanted to give you an update. All of this is tentative at this point and will be formally discussed and decided at next Thursday's CRCG meeting. Given the possibility that the data from the additional labs could impact the validation, as well as the possibility that the MLV could (b) (5) (b) (5) the formal approval of the MLV is going to await the submission of data from all the participating labs. In addition, given the likely widespread, long term nature of the method, and the fact that it is of high public visibility and could be (b) (5) (b) (5) would definitely be appropriate and is well worth pursuing.

That said, the CMVS could provide a preliminary review of the MLV report and provide feedback. This will likely help expedite the final approval once all the data is submitted. In addition, given the immediate need for this method, any labs that have successfully completed the MLV should be able to begin running regulatory samples, without waiting on the formal approval of the entire MLV. The successful completion of the MLV can also serve as a method verification at a local level. Implementing a method prior to the MLV approval is not done typically, but it is worthwhile in this case in order to get this method up and running on regulatory samples ASAP.

John, please feel free to correct anything I wrote that doesn't accurately represent our discussion. And Chris, please let us know if you have any questions.

Shaun

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Shaun.MacMahon@fda.hhs.gov

From: Vonderbrink, John

From: Sack, Chris A
Sent: Wednesday, April 19, 2017 9:54 AM
To: Vonderbrink, John
Subject: RE: Mat Std

Hi John,

Glad to hear I will be seeing you in NYK. I have a call tomorrow AM at 9. I can talk before that, or after 10. Just give me a time and I will be waiting for your call.

Talk soon,

Chris

Ph: 240-402-2464

From: Vonderbrink, John

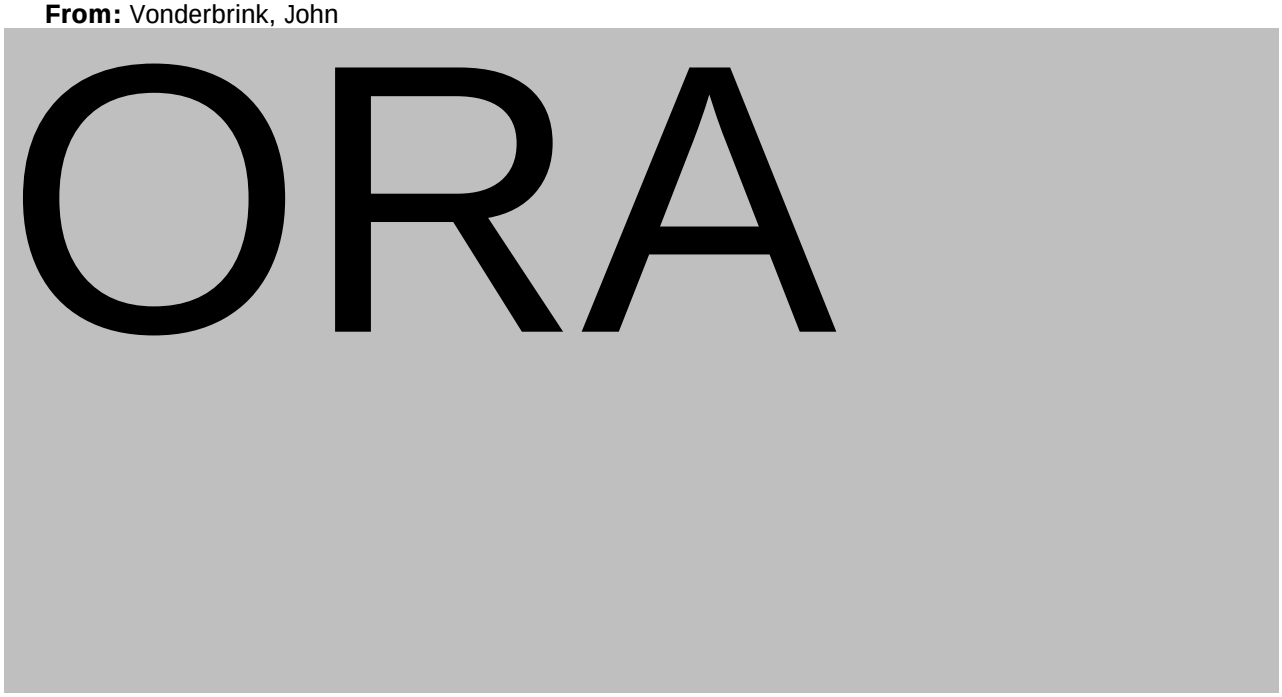
From: Sack, Chris A
Sent: Wednesday, April 19, 2017 8:43 AM
To: Vonderbrink, John
Subject: RE: Mat Std

Thanks John. Data looks much better. Looking at your Soy results and see that you had the highest results initially, just over 5000; and now your results are the lowest at 3870 ppb. If I average those two values (b) (5)

Chris

Ph: 240-402-2464

From: Vonderbrink, John



From: Sack, Chris A
Sent: Tuesday, April 18, 2017 12:58 PM
To: Vonderbrink, John
Subject: RE: Mat Std

Hey,

Did you fall off the face of the earth? Any news on the soy? N-acetyl LOQ?

Chris

Ph: 240-402-2464

From: Vonderbrink, John

From: Thomas Cruse, Kim

From: Sack, Chris A
Sent: Friday, April 21, 2017 11:25 AM
To: McLaughlin, Michael A; Cassias, Irene; Chamkasem, Narong; Eide, David J; Islam, Mohammed R; Katsoudas, Eugenia; Liang, Charlotte; MacMahon, Shaun; Mercer, Gregory E; Noonan, Gregory; Sack, Chris A; Thompson, Richard L.; Wong, Jon
Cc: Humphries, Susan; Knox, Valerie; Kwan, Thao T.
Subject: FW: Glyphosate MLV proposal

Hi everyone,

The CMVS has reviewed the preliminary MLV report for the glyphosate collaboration and deemed that each participating laboratory has met the requirements of a Level II SLV and agreed "With the concurrence of local QA management, Level II SLV data is sufficient for using the method for regulatory samples." Suggested changes to the procedure were provided to the CMVS and have already been incorporated into the final procedure and SOP as Richard indicated in our meeting earlier this week. Suggested changes to the MLV report will be incorporated into the final report when all laboratories have participated in the collaboration. Please let me know if you need anything else to implement the glyphosate method in your lab.

Thanks and have a wonderful weekend,

Chris

Ph: 240-402-2464

From: MacMahon, Shaun
Sent: Friday, April 21, 2017 12:34 PM
To: Sack, Chris A
Cc: Bowers, John C; Cai, Yanxuan (Tina); Chu, Pak S; Deeds, Jonathan; Eischeid, Anne; Heitkemper, Douglas T; Oakes, Gregg P.; Turnipseed, Sherri B; Callahan, John; Noonan, Gregory
Subject: Glyphosate MLV proposal

Chris,

On behalf of the CMVS, thank you and the Pesticides TAG for providing thorough, point by point responses to all of our comments. The MLV proposal for the method "Determination of Glyphosate and Glufosinate Residues in Food" is approved by the CMVS. There are a few areas requiring minor clarification. The first is please ensure these changes you suggested in your response are incorporated in the method SOP. As you mentioned, the AMPA is going to be included in the validation but not be used for routine monitoring. We suggest (b) (5) (b) (5) In addition, for future MLV's, the CMVS strongly suggests (b) (5) in the future. While it's clearly precautionary, the effort is minimal. In addition, if down the line submission to AOAC as an Official Method is considered, they will not accept results of an MLV that did not employ blinded/randomized samples.

While the full CMVS review of the MLV report will wait until all labs have reported, the format looks good. We would suggest clarifying in the report which labs (b) (5) (b) (5) to make it easier to confirm equivalence. In addition, the use of R2 of true (spike) levels versus observations is not an ideal measure of performance (accuracy) because it depends on various factors like the range of spike levels. If you have questions on statistics related to the MLV report, I would encourage you to contact John Bowers, who is the Stats lead on the CMVS.

Regarding implementation of the method, each lab that submitted results has completed a Level II SLV (3 spiking concentrations in 3 matrices, analyzed in duplicate, along with a control, matrix) according to the OFVM guidelines. With the concurrence of local QA management, Level II SLV data is sufficient for using the method for regulatory samples. Typically implementation is held until the MLV report is approved, but given the understandable desire to begin using this method ASAP, the CRCG and the CMVS support moving forward with regulatory testing at ARL, PRL-SW, PRL-NW, based on their Level II SLV's.

As always, happy to discuss any of this further.

Kind regards,
Shaun

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From: Sack, Chris A
To: Robin, Lauren P
Subject: RE: update
Date: Tuesday, April 25, 2017 1:43:00 PM
Attachments: image001.png

Hi Lauren,

I submitted the preliminary (partial) MLV report to the CMVS for review and approval on April 11. Last Friday they sent me notice that they approved the method for use in the three laboratories that completed the collaboration (ARL, PSW, and PNW), the same three laboratories assigned to the glyphosate assignment. I forwarded that approval notice to the lab managers and QSMs of those three labs and let them know that they can implement in their laboratories. They have begun that process. ARL provided a national SOP for the method. In the MLV report I provided an attachment for each lab summarizing their contribution to the collaboration. I was hoping those lab reports would be sufficient documentation of method verification and validation at the local level, but I guess it wasn't enough. So, each lab is preparing a method validation and verification report to meet their local requirements. In the case of ARL and PNW they had to do some additional work to demonstrate they are able to meet minimum sensitivity requirements.

That's where we are at. When the PesTAG met last week with the local QSMs we found they had been given some misinformation at which they were alarmed. I am pretty sure I was able to work through that with them. Another thing that was interesting, they each indicated they were in the dark. That really surprised me because I have repeatedly told the pesticide people what we are doing and pleaded with them to do whatever it takes to expedite this process. I pleaded with Moh and Mike at ORA to move the process along.

Don't ask me how long it will take the labs to work through their process. I will keep you informed as I hear back from them. Kaniz contacts me about once a week to see if she can start the assignment.

Chris

Ph: 240-402-2464

From: Robin, Lauren P
Sent: Tuesday, April 25, 2017 11:10 AM
To: Sack, Chris A
Subject: update

Hi Chris

After your PESTAG meeting tomorrow, can you please provide me with a brief update of the glyphosate assignment restart status?

Thanks
Lauren

Lauren Posnick Robin, Sc.D.
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From: Sack, Chris A
To: MacMahon, Shaun
Subject: FW: Glyphosate MLV proposal
Date: Wednesday, April 26, 2017 12:41:00 PM
Attachments: Glyphosate MLV Rpt 4-24-17.docx

Hi Shaun,

The PesTAG met with the QSMs and QSSs from the three labs that have participated in the collaboration to date (PSW, PNW, and ARL). I included a copy of the MLV report in the minutes. The QSMs found some errors in the MLV report that I corrected and changed in the attached report. You can see their observations in the email thread below. One of their concerns was the calculation of the MU; i.e. they wanted the K multiplier using the Student t distribution added included in the calculation of the MU. I made those corrections throughout the report. The MUs for the summary of all laboratories were only very slightly affected by the multiplier; for the individual lab reports where the degrees of freedom for each test was only 5 the new MUs were somewhat higher but none were out of the specification of 30 %.

The QSMs also found an error where I inadvertently entered the PNW results for N-acetylglyphosate for PSW. It was a case of the alphabet soup in the brain of a 60 year old. Fortunately, the switch did not affect the overall results. I did find another cut-n-paste error for the average recovery of the N-acetylglyphosate for ARL. This was corrected also. None of these changes would affect the validity of the method. In the attached report all the changes have been highlighted.

My apologies for the additional work and mistakes. I sure wish I had included the QSMs in the review process for the original report. I won't make this mistake twice. Let me know if you need to cuss or discuss.

Thanks,

Chris

Ph: 240-402-2464

From: Sack, Chris A
Sent: Tuesday, April 25, 2017 11:31 AM
To: Humphries, Susan
Cc: Knox, Valerie; Kwan, Thao T.; Kontas, Cassandra
Subject: RE: Glyphosate MLV proposal

Thanks Susan, Thao, Valerie and Cassandra for your excellent review.

Re the method, none of the changes suggested by the CMVS affected the actual procedure. They were really more for clarity. I am attaching the method I sent out to the labs on April 11 when I submitted the MLV report. The changes we made in response to the CMVS are highlighted. These changes have been incorporated into the national SOP prepared by ARL. On the phone call we agreed to use a common SOP for this method. Given the parochial nature of ORA, I have my doubts this is possible but at least we will start with a common procedure.

Re your other questions:

1) *It appears MU values were calculated with a coverage factor of K=2 instead of K based on the desired confidence level (usually 95%) and degrees of freedom. K=2 is acceptable when there are more samples. The true uncertainties are larger, because the number of samples is small.*

   For the individual lab reports however the K value for 5 degrees of freedom is (b) (5) and for the overall report the K value for 17 degrees of freedom is (b) (5) I have applied those factors to the attached revision of the MLV report.

2) *Values for n-acetylglyphosate are identical in all of the following tables, which would seem highly unlikely:*
   - *Main report, Table 1, Northwest data*
   - *Att. C, Table 1, Southwest data*
   - *Att. F, Table F1, Southwest data*
   - *Att. G, Table G1, Northwest data*

   Thanks for catching this little mistake. When I recalculated the data for N-acetylglyphosate using external standard calibration I believe I confused "PNW" and "PSW", should have stuck to SEA and LA. I have corrected in the attached report.

   In addition, check ARL's data for the same compound. It does not match between their table H1 and the summary table. I have not yet had time to look at my group's raw data so am not able to say what is correct. Please let me know if I am misreading the report with respect to the various tables.

   You are correct. In the main Table 1, the ARL average recovery of N-acetylglyphosate was incorrectly copied into the main table. This affected the overall average and stats for all labs. These are corrected in the attached report.

I found one additional mistake in Table 1; i.e. the incorrect overall RSDs were used for the calculation of the MU. Not sure how that happened. This has also been corrected. Fortunately, the MUs are all still excellent.

Theoretically, the entire PesTAG reviewed this report before I submitted it. I really appreciate your excellent critique and wish I had sent it to you before I submitted to the CMVS. Take a look at the attached doc and let me know if you have any further observations. I highlighted the changes I made. I will re-submit to the CMVS when I hear back from you. None of these changes actually affect the validity of the method validation.

Have a really great day,

Chris

Ph: 240-402-2464

From: Mercer, Gregory E

From: David Kennedy [mailto:DavidK@phenomenex.com]
Sent: Wednesday, April 26, 2017 2:28 PM
To: Mercer, Gregory E

From: David Kennedy
To: Mercer, Gregory E
Cc: Sack, Chris A
Subject: Re: FDA Glyphosate MLV
Date: Wednesday, April 26, 2017 6:45:44 PM

Thanks Greg!

Hey Chris, is there anything you can share on the glyphosate method MLV? I'm just interested in the basics.

Best regards,

Dave Kennedy

David C. Kennedy, PhD
Business Development Manager
Phenomenex
Torrance, CA

From: Mercer, Gregory E <Greg.Mercer@fda.hhs.gov>

From: David Kennedy [mailto:DavidK@phenomenex.com]
Sent: Wednesday, April 26, 2017 2:28 PM
To: Mercer, Gregory E

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From: Sack, Chris A
To: Humphries, Susan
Cc: Kontas, Cassandra; Knox, Valerie; Kwan, Thao T.
Subject: RE: Glyphosate LOQ study
Date: Thursday, April 27, 2017 1:48:00 PM
Attachments: image001.png

Thanks Susan,

Just one thought re the calculation of the LOQ. I use 10 x SD for all LOQ calculations. That is how I calculated the LOQ in the SLV report (Attach C of the MLV report). You mention 95 % confidence level (3.14 x SD at 6 DF) which is the MDL. In pesticides we do not use the MDL as a limit parameter. Also, note on the method we are using (b) (5) for the primary pesticides glyphosate and glufosinate, and external standard calibration for the N-acetylglyphosate. If you choose the calculate the LOQ for AMPA, we are calculating residue levels using the glyphosate (b) (5) as an internal standard.

Have a wonderful day and weekend,

Chris

Ph: 240-402-2464

From: Humphries, Susan

From: Cooke, William

<http://www.fda.gov/>

From: Cassias, Irene

From: Robin, Lauren P
To: McLaughlin, Michael A
Cc: Sack, Chris A
Subject: FW: update
Date: Friday, April 28, 2017 2:03:48 PM
Attachments: image001.png

Hi Mike

Do you have an estimate of how long it will take for the ORA labs to be ready for the herbicide assignment to restart?

Thanks
Lauren

Lauren Posnick Robin, Sc.D.
Chief, Plant Products Branch
DPPB/OFS/CFSAN
U.S. Food and Drug Administration
HFS-317
5001 Campus Drive
College Park, MD 20740
240-402-1639
lauren.robin@fda.hhs.gov

From:	Shireen, Kaniz F
To:	Sack, Chris A
Subject:	RE: Glyphosate assignment
Date:	Tuesday, May 16, 2017 2:00:36 PM
Attachments:	Acid Herbicide Assignment FY17.docx image002.png image008.png

Chris:
I have updated the assignment per your note below.
Please let me know, if I can issue the assignment soon.

Thanks, Kaniz

From: Sack, Chris A
Sent: Friday, May 12, 2017 4:47 PM
To: Shireen, Kaniz F
Subject: FW: Glyphosate assignment

Hi Kaniz,

One of the labs (Arkansas Regional Laboratory) we were planning to send glyphosate samples was not able to meet the method specifications so we are dropping them from the assignment. The other two labs (PNW and PSW) are ready to receive samples. So, we need to amend the assignment to remove ARL as a servicing laboratory for glyphosate. Let me know if you would like me to make the changes to the assignment. I am not sure if I have the final version, so if you would like me to make the changes send me your latest version. Otherwise, you can make the changes and send to me for review. I am out of the office Mon-Wed next week and I will have very limited access to the internet. I will make it a point to access at least daily. I will work on it this weekend if you send me something before Monday.

Have a wonderful weekend,

Chris

Ph: 240-402-2464

From: Islam, Mohammed R

From: McLaughlin, Michael A

From: Islam, Mohammed R

From: Sack, Chris A
Sent: Thursday, May 11, 2017 2:13 PM
To: Islam, Mohammed R
Subject: FW: Glyphosate LOQ and MLV data

Hi Moh,

PNW and PSW were both able to achieve the 10 ppb LOQ for all analytes. I am OK with using just those two labs for the glyphosate assignment. ARL will need to do some more work before I agree for them to analyze the glyphosate samples. Let me know so I can modify the assignment, if necessary.

Thanks,

Chris

Ph: 240-402-2464

From: Sack, Chris A
Sent: Thursday, May 11, 2017 1:09 PM
To: Cooke, William; Humphries, Susan; Kontas, Cassandra
Cc: Mabry-Smith, Ronald C; Chow, Peter C; Islam, Mohammed R
Subject: RE: Glyphosate LOQ and MLV data

FYI. For the MDL we have been using the 40 CFR 136 calculation, i.e., for seven reps the MDL is the SD x 3.14 which is the one-tail student T value DF = 6 at 99 % confidence. For the LOQ we are using 10 x SD. The 95 % confidence interval is not used for detection limits because you allow a false positive rate of 5 %. It is essentially 3 x SN where the SD becomes the noise and the Student T multiplier corrects for the broader distribution of smaller sample sets. Let me know if you disagree.

Otherwise the data looks incredible. Good work.

Chris

Ph: 240-402-2464

From: Cooke, William

From: Sack, Chris A
To: Masse, Claude
Subject: RE:
Date: Tuesday, June 20, 2017 1:32:00 PM

I don't see a 100 ng/ml mat std for corn. It looks like you might have prepared a 100 ng/ml mat std at 50 ng/ml.

Chris

Ph: 240-402-2464

From: Masse, Claude

From: Chang, Eugene

From: Sack, Chris A
Sent: Wednesday, June 21, 2017 8:30 AM
To: Chang, Eugene
Cc: Cooke, William; Cassias, Irene
Subject: RE: Progress for Egg Extraction

Hi Eugene,

I am not sure I understand the issues with eggs. Why did SRL analyze over 100 egg samples with no problem, however we are unable to analyze them using the modified and "improved" method? We really only changed the LC method, Right? Do we need to convene a call to discuss egg issues?

Chris

Ph: 240-402-2464

From: Chang, Eugene

ORA

From:	Sack, Chris A
To:	Vonderbrink, John
Subject:	Collabdata
Date:	Thursday, June 22, 2017 8:40:00 AM
Attachments:	KAN500IntQuestion.xlsx

Hi John,

I am reviewing the collab data for the final report and I have a question. Kan gly results for carrots failed the linearity spec of 0.99 by just a hair. I looked at each data and I see that the area of the internal standard for the second analysis of the 500 ppb spike for carrots is substantially higher (1306604) than that of the other spike (1138554) and the corresponding mat standards (1100448 and 1246199). I attached the 500 ppb data for your to look at. I used red and blue font to accent the integrations to which I refer. Can you look at the gly peak of the IS for the second carrot spike and let me know if it is correctly integrated? If not, send me the corrected integration area. Let me know what you find in any case.

Thanks,

Chris

From:	Sack, Chris A
To:	Wong, Jon
Cc:	Wittenberg, James
Subject:	RE: MLV Raw Data
Date:	Friday, June 23, 2017 10:30:00 AM
Attachments:	Glyphosate MLV Rpt - CFSAN Att only.docx

Hi Jon and Jim,

Your data looks great. Take a look at the CFSAN Att I plan to include with the MLV report.

Have a wonderful weekend,

Chris

Ph: 240-402-2464

From: Wong, Jon
Sent: Friday, June 16, 2017 12:41 PM
To: Sack, Chris A
Cc: Wittenberg, James
Subject: FW: MLV Raw Data

Hi Chris,

Here is the data for the glyphosate work. Our N-acetyl glyphosate results are much better this time with the smaller ID column. We also used the four labeled IS for each of the four compounds.

Have a great weekend.

Best regards,

Jon

From: Wittenberg, James
Sent: Friday, June 16, 2017 1:31 PM
To: Wong, Jon
Subject: MLV Raw Data

Jon,

Attached is the raw data requested by Chris. The first tab is the data corrected using Glyphosate IS. The second tab is the data corrected using all four native compound-correlated internal standards. Please take a look and let me know if you need anything else from me.

Thanks,
Jim

From: Shireen, Kaniz F
To: Sack, Chris A
Subject: RE: Glyphosate assignment
Date: Tuesday, June 27, 2017 11:33:37 AM

Hi Chris:
I requested ORA contact to hold off milk and egg sample collection until July.

Thanks, Kaniz

From: Sack, Chris A
Sent: Tuesday, June 27, 2017 11:39 AM
To: Shireen, Kaniz F
Subject: Glyphosate assignment

Hi Kaniz,

I know you issued the glyphosate assignment. Did you put a hold on the milk and egg collection?

Thanks,

Chris

From: Cromer, Michele

From: Sack, Chris A
To: Cromer, Michele
Subject: RE: Acid herbicides
Date: Tuesday, June 27, 2017 10:36:00 AM

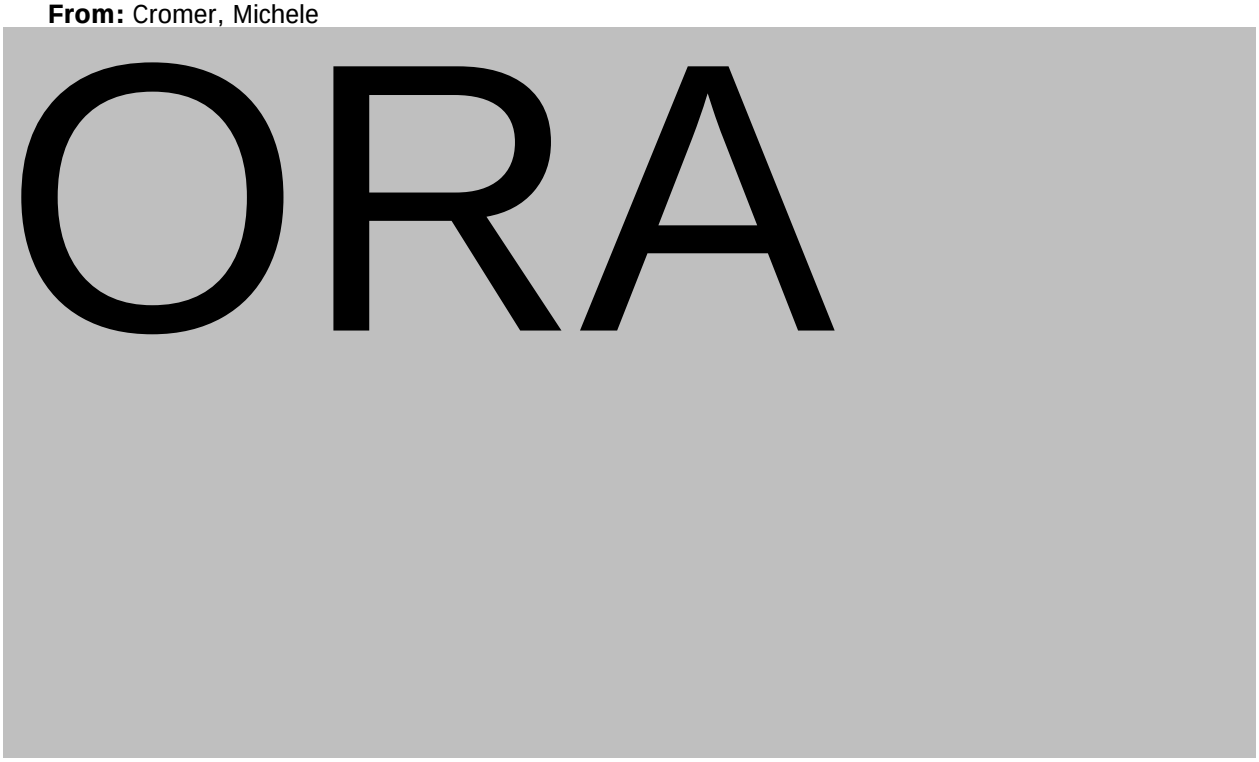
Hi Michele,

I pleaded with the LA lab not to forward couscous because it is not what we want for the AcH assignment. You can either reject the couscous or analyze as a normal pesticide sample. Farro is OK for AcH, assuming it is a whole grain and not processed.

Chris

Ph: 240-402-2464

From: Cromer, Michele



F om [illegible]
To [illegible]
Subject YI
Date Thu uday July 13 2017 7 18 46 AM
Attachmen s image011.png

Bl g h C y & l P Bl g y h R g Bl g T l D R

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Does the World's Top Weed Killer Cause Cancer? Trump's EPA Will Decide

Roundup has evolutionized fa ming. Now, human health and Baye s $66 billion deal fo Monsanto depend on an honest app aisal of its safety.

Spraying a mix of Roundup and another product on soy fields in Wisconsin.

Photog aphe Jesse Chehak fo Bloombe g Businessweek

By
Pete Waldman
,
Lydia Mulvany
,
Tiffany Stecke
, and
Joel Rosenblatt
July 13, 2017, 4 30 AM EDT

Every year, farmers spray, on average, almost a pound of the herbicide glyphosate on every acre of cropland in the U.S., and nearly half a pound on every acre of cropland worldwide. Glyphosate is the active ingredient in Roundup, a huge source of income for its manufacturer, Monsanto Co., and the foundation for its epochal foray into genetically modified organisms. If you know nothing else about GMOs and Monsanto, know this: The St. Louis-based company reengineered the DNA of corn, soybeans, and other crops for the primary purpose of making them resistant to Roundup.

Farmers spray the chemical on crops grown from Monsanto's Roundup Ready seeds. The weeds die, harvests expand, and expensive, laborious tillage is no longer necessary. Large-scale agriculture is built on this model, and not only in the U.S., which is why Bayer AG, the German drug and chemical company, agreed in September to buy Monsanto for $66 billion, pending regulatory approvals. Other than government antitrust objections, about the only thing that could mess up the purchase would be for the U.S. Environmental Protection Agency to reverse its position on the active ingredient of Roundup, glyphosate.

Last December, the EPA convened a panel of outside scientists to peer-review the agency's long-standing conclusion that glyphosate is unlikely to cause cancer. The peer reviewers, a mix of academics, federal scientists, and chemical industry consultants, gathered at an EPA conference center in Arlington, Va. From the agency's point of view, this was something of a formality. Federal law requires an EPA health-effects review for every pesticide at least once every 15 years, and glyphosate has enjoyed a clean bill of health since 1991, when the agency cleared the way for Monsanto's GMO breakout by classifying the herbicide as noncarcinogenic to humans.

Its use in global agriculture has soared almost fifteenfold since Monsanto introduced Roundup Ready seeds in 1996. As a result, traces of glyphosate have been detected in cookies, crackers, chips, breakfast cereals, and honey, and in human urine and breast milk. Monsanto says it's nothing to worry about. "Glyphosate is about half as toxic as table salt and more than 10 times less toxic than caffeine," the company says on one of its websites. More than 1,000 farmers and other agricultural workers stricken with non-Hodgkin lymphoma disagree. They're suing Monsanto in state and federal courts across the country, claiming Roundup caused their cancer. Monsanto is vigorously contesting the claims. "There's never been a more studied herbicide in the history of farming," says Scott Partridge, Monsanto's vice president for strategy. "In more than 700 studies, not one has associated cancer with the use of glyphosate. And in the more than 160 countries that have registered glyphosate for use, not a single government agency has found glyphosate is a carcinogen."

December's Scientific Advisory Panel meeting followed the typical script for a federal peer review, with some twists. Officials from the EPA's Office of Pesticide Programs opened the public hearing by laying out 45 years of study data and describing why, in the agency's view, they indicate that glyphosate is an unlikely human carcinogen at current exposure levels. The agency's conclusion, elucidated in a 227-page issue paper released the previous September, reflects the "weight of evidence," Jack Housenger, then chief of the pesticide office (he retired this year), told the panel. "Now it's your turn to tell us what you think of our analysis," he said, "and hopefully put the subject to bed so we can move on."

Roundup wait ng fo dilution and application in Wisconsin.
Photog aphe Jesse Chehak fo Bloombe g Businessweek

Far from settling the matter, eight of the 15 experts expressed significant concerns about the EPA's benign view of glyphosate, and three more expressed concerns about the data. Their skepticism also raised, again, questions about the independence of the Office of Pesticide Programs, which has the final say on permitting pesticides. The office relies on pesticide manufacturers for the data it uses in making health decisions—and got almost 30 percent of its operating budget from the industry last year.

The EPA paper had a whack-a-mole quality to it. Throughout, the authors included data sets suggesting that glyphosate could cause cancer, only to knock them down. On epidemiology studies, for example, they said farmers' recollection of their own glyphosate exposure was biased and unreliable. On meta-analyses pooling human data from multiple studies to identify trends, the EPA assessors shaved decimal points from the results, which made it possible for them to shrug off data showing exposed farmers had an elevated risk of cancer.

Many of the reasons cited in the paper contradicted the agency's own carcinogenicity guidelines, multiple panelists pointed out. "Every time there's something positive there, you said there's something wrong with the study," Eric Johnson, an epidemiologist at the University of Arkansas for Medical Sciences, scolded EPA officials at the meeting. Lianne Sheppard, a biostatistician at the University of Washington at Seattle, says there was "consensus" on the panel that the agency hadn't followed its own standards. "The available evidence did not fit with the conclusions drawn in the issue paper, particularly when put in the context of the guidelines," she says.

The EPA's report on the peer review, posted on March 16, raises obfuscation to a high bureaucratic art. While spelling out the panel's criticisms, the report gives no indication which, or how many, reviewers felt strongly about which particular problems. Instead, it uses the phrase "some panel members" 76 times—as in "some panel members noted," "some panel members emphasized," "some panel members suggested." The imprecision obscures that the majority of peer reviewers expressed doubts about the EPA's methods or conclusions. Under the law, the agency must consider the panel's input in its final evaluation of glyphosate, scheduled for completion later this year. By enshrining the reviewers' comments in such vague terms, however, the EPA can more easily ignore them.

"I asked for a vote on the main issues to make our guidance clear, but this committee apparently never does that," says Emanuela Taioli, an epidemiologist at Icahn School of Medicine at Mount Sinai in New York and one of the panelists who disagreed with the EPA's interpretation of the evidence. "Not giving a tally leaves them more flexibility to interpret our advice."

Monsanto, a company that has genetically altered vast swaths of cropland to vanquish farm pests, has lately been struggling to control an invasive species on its own turf: scientific doubt. It's an exotic. Ever since President Bill Clinton awarded four Monsanto gene scientists the National Medal of Technology and Innovation in 1998, the company's ag-biotech enterprise has been the closest thing America has to a Japanese-style strategic industry. From Clinton to Bush to Obama, successive administrations mobilized federal agencies and embassies around the world to promote GMOs, often against the vehement opposition of environmenta ists and food purists, particularly in Europe. Dozens of diplomatic cables released by WikiLeaks show how U.S. missions received special funding from Congress to advocate for genetically engineered products and how Monsanto prevailed on American diplomats to lobby on its behalf when problems arose. In Argentina, for example, when the ministry of defense banned the use of glyphosate on its urban farmland in 2009, the U S. embassy intervened, according to a diplomatic cable to Washington. "Post contacts within the Secretariat of Agriculture assure us that Argentina will continue to support biotechnology," said the cable, signed by Thomas Kelly III, deputy chief of mission at the time.

The first breach in Monsanto's fortress opened in 2015, when the International Agency for Research on Cancer labeled glyphosate a probable carcinogen. IARC, a France-based arm of the World Health Organization, has no regulatory power, but its carcinogenicity studies are widely cited in court cases and government health assessments worldwide. The agency's assessment, based on published, peer-reviewed research, foreshadowed many of the concerns the EPA's scientific advisory panel expressed last December. IARC acknowledged the studies were all flawed in different ways, but it concluded that their findings pointed toward cancer and couldn't be dismissed. This led California on July 7 to list glyphosate as a known carcinogen, over Monsanto's objection.

"Every one of us takes risks every day when we take our car on the road or get on an airplane or dump table salt into something we're cooking"

The question now falls to the Trump EPA and the courts. Led by Administrator Scott Pruitt, the former Oklahoma attorney general who sued the EPA more than a dozen times to stop environmental regulations, the agency has already canceled an Obama-era proposed ban on chlorpyrifos, a pesticide linked to cognitive damage in farmworkers and children. The chances that Pruitt will move against glyphosate, with all the attendant repercussions for industrial agriculture, appear slim.

The considerations are much different, however, for U.S. District Court Judge Vince Chhabria in San Francisco. The judge is presiding over multidistrict litigation composed of 310 plaintiff lawsuits against Monsanto filed by cancer victims around the country. (It will likely consolidate hundreds more suits.) Chhabria has told both sides that the question of whether Roundup can cause cancer will turn on the scientific evidence presented at trial, not on what agencies such as IARC and EPA say. In this instance, the difference between Roundup and glyphosate is crucial. The EPA focuses on the latter. The plaintiffs in the court case claim that Roundup contains ingredients that exacerbate the effects of glyphosate alone.

Chhabria has allowed the plaintiffs wide latitude to collect evidence on Monsanto's health-effects research over the years, which the plaintiffs hope will show the company manipulated the data. In March he unsealed dozens of Monsanto's confidential documents for the public to see. The records show internal deliberations on how to present the science on glyphosate's health impacts and manage a global public-relations campaign to assure consumers and regulators that Roundup is safe.

Containe s of Roundup that a e kept outside the cockpit of the sp aye .
Photog aphe Jesse Chehak fo Bloombe g Businessweek

Monsanto documents show the company commissioned scientists to publish papers rebutting IARC. Reminiscent of tobacco companies, it also funneled money to front groups, according to a plaintiffs' court filing. The groups, with names such as Genetic Literacy Project and American Council on Science and Health, published articles praising the EPA and attacking IARC, which they called on Congress to defund. The plaintiffs claim that Monsanto established a program called Let Nothing Go, through which it made payments to people with no apparent industry ties to post online comments defending Monsanto, its chemicals, and GMOs in news articles and Facebook posts. "That's simply false," says Monsanto's Partridge. "We don't need to do that type of stuff."

In Washington, where Monsanto has spent almost $60 million on lobbying since 2008, the company cultivates allies on both sides of the aisle and in the relevant federal agencies. It deployed five lobbyists in 2015 to trash IARC's findings at the White House, Congress, and the agencies. Monsanto employees are regular visitors to the EPA's Office of Pesticide Programs, according to logs obtained through the Freedom of Information Act.

Relations were warm, even jocular. "So Jess called me out of the blue this morning," wrote Monsanto's lead EPA liaison, Dan Jenkins, to William Heydens, the company's chief of regulatory research, in an April 2015 email released in the court case. Jess was Jess Rowland, a senior official in the EPA's pesticide office who was chairing the agency's cancer assessment of glyphosate at the time. Heydens had emailed Jenkins the day before, asking his colleague to reach out to the EPA and find out "what area they see as most problematic (e g., human epidemiology vs. animal bioassays vs. genotoxicity), or just ask if there is anything that would help them defend the situation?"

Rowland was all set, Jenkins reported back to Heydens. "We have enough to sustain our conclusions," Rowland told Jenkins on the phone, according to Jenkins's email. "I am the chair of the [cancer review]," he added, "and my folks are running this process for glyphosate."

On the same call, Jenkins wrote, Rowland said he was working to control a separate glyphosate assessment by another federal unit, the Agency for Toxic Substances and Disease Registry (ATSDR), a division of the Centers for Disease Control and Prevention. "If I can kill this, I should get a medal," Jenkins quoted Rowland as telling him.

"Wow!" Heydens wrote back to Jenkins. "That's very encouraging. Thanks for the news update."

In April 2016, as Rowland was preparing to retire after 26 years at the EPA, his assessment that glyphosate is unlikely to cause cancer leaked online—just in time for Monsanto's lawyers to cite it at an important court hearing in San Francisco. The EPA quickly characterized the report as "preliminary" and "not final," but Monsanto's lawyers still told Judge Chhabria, "the scientists have spoken." As for the ATSDR review, another internal Monsanto document reported that the CDC division had "agreed, for now, to take direction from EPA." This May, EPA Inspector General Arthur Elkins Jr. confirmed he's investigating if EPA employees colluded with Monsanto. Through his lawyer, Rowland declined to comment for this story. Monsanto's relationships with Rowland and other EPA employees were professional and proper, Partridge says.

Bayer and Wall Street are betting none of this matters. Monsanto has built the kind of virtuous circle that management experts and business school professors rave about. More sales of Roundup Ready seeds beget more use of Roundup; more herbicide use drives up demand for Monsanto's GMO seeds. The global chemical dependency could be too big to kick.

It's hard to quantify what a shift to glyphosate-free farming would look like. A study by chemical industry consultants in the U.K., where about a third of the nation's wheat fields are sprayed with the herbicide, estimated that yields of the grain would fall 12 percent if glyphosate were banned. A study last year by Andrew Kniss, an associate professor of plant sciences at the University of Wyoming, showed the yields from organic farms were roughly two-thirds of those from conventional farms for corn, wheat, soybeans, and barley, and less than half for grapes, tomatoes, bell peppers, and onions.

Kniss did another study that found planting genetically modified sugar beets saved farmers $200 an acre, equal to about 15 percent of their revenue, compared with planting non-GMO seeds. With no glyphosate, farmers would have to resort to using more-toxic chemicals for weed control, Kniss says, or revert to grueling tillage by hand. "Getting rid of glyphosate would have a major impact on farmers and their bottom lines," he says. "It's not like there's a risk-free scenario here."

Robert Fraley grew up on a farm in Hoopeston, Ill., in the 1950s and 1960s, and one of his earliest memories is of the fields turning black each November. He recalls tractors churning up a foot of dark topsoil to keep the weeds from taking over. His dad cranked up the tractor every morning by 4 a m. and plowed until Fraley got home from school and took over until midnight. In summers the boy walked the rows of beanstalks with his friends and cousins, pulling the weeds by hand. Fraley, now 64, helped develop Roundup Ready seeds and is Monsanto's chief technology officer. "Any kid my age who spent time on the farm, the first thing you realize is the greatest challenge is weeds and bugs," he says. "We freed America's farm youth."

By 1980, when Fraley joined Monsanto as a 27-year-old with a doctorate in microbiology and biochemistry, scientists were experimenting with recombinant DNA in yeast and animal cells, but no one had introduced a new gene in a plant. Fraley's team, working with a germ called agrobacterium, which normally causes blight in plants, isolated the part of the germ that binds to plant cells and can inject its DNA into plants, and eliminated the blight-producing sequence. They worked with petunia plants to fine-tune a gene insertion process, using the flower's color genetics to map the plant's chromosomes. It took more than a decade to develop commercially useful traits such as resistance to insects and glyphosate. The breakthrough finally made glyphosate, which was invented in 1970, a significant commercial power.

Even as environmentalists vilify Monsanto for its link to GMOs, its mantra internally remains stewardship and sustainability. "We get to enjoy more of our forest and wetlands and prairies because we've increased yields on the land we're already farming," Fraley says. The alternative to genetic engineering and the accompanying chemicals, he says, is plowing up an additional 30 million or 40 million acres of land to feed a hungry planet.

"Looking at the cost-benefit ratio, I'm extremely reluctant to give up glyphosate"

That probably overstates the trade-off. Land spared from cultivation is seldom set aside for conservation. And there are alternatives to Roundup Ready farming other than going organic. "It's such a Monsanto-based perspective to say food prices will spike if we use less glyphosate," says Claire Kremen, a conservation biologist at the University of California at Berkeley. "There are other methods, besides organics, that are just as productive as conventional farming and don't rely on toxic chemicals that endanger lives and harm the environment." A more realistic alternative, she says, is finding middle ground between the industrial and organic farming models. Researchers at Iowa State University, for example, have shown that rotating diverse crops in three- and four-year cycles and controlling weeds with limited herbicide spraying produce similar yields and profits to conventional farming—with only 1 percent of the water toxicity. And such alternatives are becoming necessary anyway, because weeds are developing resistance to glyphosate at an accelerating rate.

Nonetheless, a lot of farmers are deeply committed to glyphosate. "The cancer issue doesn't concern me at all," says Paul Jeschke, 64, who farms 4,000 acres of corn and soybeans with his brother-in-law and nephew in Mazon, Ill. Before he started using Roundup in the 1980s, Jeschke says, his topsoil, after constant tillage, would wash away in the rain. The quack grass would get so bad some farmers would have to put up a fence around their fields and turn the pigs loose, losing a year's harvest. "Every one of us takes risks every day when we take our car on the road or get on an airplane or dump table salt into something we're cooking," Jeschke says. "Looking at the cost-benefit ratio, I'm extremely reluctant to give up glyphosate."

The first cancer concerns came from within the EPA's Office of Pesticide Programs in 1984. This was despite the traditionally close relationships between the agency and the companies it regulates. Pesticide makers, called registrants, pay the office to review their compounds for registration. In 2016 they provided $47 million, or 28 percent, of OPP's budget. The OPP is also the only EPA branch that does its own health assessments; the agency's National Center for Environmental Assessment is in charge of evaluations for the other EPA branches. The OPP's studies are based, by law, on data provided by pesticide registrants themselves. This has fueled intra-agency criticism that some of its cancer reviews are too lax.

Soy fields t eated with Roundup.
Photog aphe Jesse Chehak fo Bloombe g Businessweek

Still, its scientists did come down hard on glyphosate early on. In Februuary 1984, EPA toxicologists sounded the alarm internally when a Monsanto-sponsored study showed that 4 of 100 mice that were fed large amounts of glyphosate developed rare kidney tumors called tubular adenomas, compared with zero of 98 mice given little or no glyphosate. Monsanto insisted glyphosate wasn't the cause, invoking an argument that would become a pillar of its—and the EPA's—defense of the chemical for the next 30 years. The mice with the tumors weren't the problem, the company said. The healthy mice were. The control group ought to have had more tumors.

An OPP statistician was having none of it. "Our viewpoint is one of protecting the public health when we see suspicious data. It is not our job to protect registrants from false positives," wrote Herbert Lacayo, who analyzed Monsanto's complaints in a memo in February 1985. A week later, OPP's toxicology branch cited the mouse tumors in a "consensus report" naming glyphosate a possible human carcinogen.

Monsanto was apoplectic. The Roundup Ready world that was coming to life inside Fraley's gene-splicing lab depended on the copious use of glyphosate. To change the EPA's mind, the company solicited 10 outside experts to examine the mice in the control group. One of the scientists, who'd been a Monsanto consultant for many years, reported to the company that he'd found cellular changes in the kidney of one mouse. Monsanto argued it was another adenoma. If so, it would render the four tumors in the exposed mice statistically insignificant. The other experts then supported the finding of the single scientist.

The EPA's toxicology branch recut new sections of all the mouse kidneys for another look. Its scientists reconfirmed there was no control adenoma. Nevertheless, in February 1986, the EPA's scientific advisory panel overruled the agency scientists, asserting that the "vast majority" of pathologists who'd looked at the control kidney in question saw a tumor. If the reviewers knew those experts were brought on by Monsanto, they didn't care. The panel reclassified glyphosate as a chemical of uncertain carcinogenicity. Five years later, another EPA panel looked at the same data and a new rat study and reclassified glyphosate again, this time as noncarcinogenic to humans.

By 1999, with Roundup Ready soybean, cotton, and corn seeds already changing global farm markets, Monsanto was facing questions about how glyphosate affected animal genes. Emails unsealed in March by Judge Chhabria show that the company hired James Parry, a prominent genetic toxicologist at Swansea University in Wales, to publicly advocate that the chemical wasn't genotoxic—that is, it didn't have a destructive effect on DNA and RNA. But after reviewing studies that Monsanto provided, Parry reached the opposite conclusion.

He wrote a report for Monsanto that said glyphosate appeared to damage genes through a biochemical process called oxidative stress—the same cancer-causing mechanism IARC identified 16 years later. He recommended Monsanto do a series of studies to find out. If glyphosate was confirmed to be genotoxic, Parry said, the company should analyze the lymphatic cells of exposed humans to check for chromosome damage.

Monsanto scientists deliberated for weeks about their consultant's unwelcome advice. The company was in a "genotox hole," wrote senior toxicologist Donna Farmer in a September 1999 email. "I am concerned about leaving Parry out there with this as the final project/his final impressions."

"Maybe you should invite Parry to St. Louis to get him more familiarized with the complete database," suggested another Monsanto toxicologist.

In an email Monsanto must surely regret, Heydens, the regulatory research chief, wrote that changing Parry's mind would be expensive and probably not worth it. "Let's step back and look at what we are really trying to achieve here," Heydens wrote to Farmer and two others. "We want to find/develop someone who is comfortable with the genetox profile of glyphosate/Roundup and who can be influential with regulators and Scientific Outreach operations when genetox issues arise. My read is that Parry is not currently such a person, and it would take quite some time and $$$/studies to get him there. We simply aren't going to do the studies Parry suggests."

Parry's report was never submitted to the EPA. (He died in 2010.) The episode points to an ongoing concern at Monsanto, which was concisely stated by Heydens in a later email: "Data generated by academics has always been a major concern for us in the defense of our products." Partridge says Heydens sometimes made "an unfortunate choice of words," but that subsequent studies by Monsanto resolved Parry's concerns.

"The agency's conclusion is seriously flawed and needs to be strongly revised"

The transcript of the EPA's scientific advisory panel meeting runs 1,300 pages. Reading the document is the only way to know that four of the six reviewers charged with evaluating the crucial epidemiological data lambasted the EPA. (The four critics are all biomedical researchers at major universities; the two who supported the EPA's evaluation are private consultants.) The agency disregarded all but one meta-analysis of the epidemiological data, because agency evaluators said the results weren't statistically valid. When several of the panelists reran the pooled data, they found the EPA was plain wrong. Not only were the meta-analyses statistically significant, but they also showed farmers exposed to glyphosate had an elevated risk ratio for non-Hodgkin lymphoma of 1.27 to 1.5, meaning they were at 27 percent to 50 percent higher risk than control groups.

"For a human epidemiologic study, an association of 1.2 or 1.3 is very meaningful and impactful," says Mount Sinai's Taioli. At the meeting, she pointed out that millions of American women no longer take estrogen after menopause, because studies found that it increased the risk of breast cancer by about 22 percent. Sheppard, the biostatistician with the University of Washington, said at the meeting that the EPA's evidence assessment was "highly imbalanced" and that the agency downplayed statistical findings in favor of other criteria. "The agency's conclusion is seriously flawed and needs to be strongly revised," she said.

Several panelists asserted that while glyphosate probably doesn't initiate cancer by causing gene mutations, it appears to promote malignancies by spurring tumor growth. Such a carcinogen is more dangerous to humans than to rodents, because people live much longer and thus accumulate more lesions susceptible to glyphosate's catalytic effect, panelist Barbara Parsons, a molecular toxicologist with the U S. Food and Drug Administration, said at the meeting. She warned that mixing a tumor promoter such as glyphosate in formulations with other chemicals that have "any genotoxic potential would be a significant public-health concern."

That argument is the crux of the plaintiffs' case in the consolidated federal suits. Their lawyers say they have evidence that Monsanto knew for years that some of he nonactive ingredients in Roundup are carcinogenic, and thus the danger of those chemicals is compounded when they're combined with glyphosate. They say that the OPP, by focusing its concerns on the active ingredient instead of on the formulated product, has let Monsanto off the hook. Partridge denies any Roundup ingredients are genotoxic and says potentially carcinogenic impurities in the product are strictly controlled.

The doubt invading Monsanto's prize product is as strong as it's ever been, even as Roundup has become instrumental in industrial agriculture. Farmers and consumers have reaped huge savings from productivity gains made possible by taming the scourge of weeds. Improbable as it seems, suppose the EPA now moves glyphosate from the category of unlikely carcinogen to suggestive or even likely. That would trigger extensive cost-benefit analyses. Then the questions get really difficult.

From:	Purnell, Standra
To:	Lee, Mabel
Cc:	Shireen, Kaniz F; Sack, Chris A
Subject:	RE: Glyphosate in honey
Date:	Friday, July 21, 2017 9:12:56 AM

Hi Mabel,

Per CFR 180.136 there is no established tolerance for glyphosate in honey however there are over established tolerances for over 140 commodities to include fruits, vegetables, nuts, fish and shellfish etc. I checked IA 99-08 , manufacturers of honey were listed on the red list for reported finding of boscalid, chlordimeform, carbendazim, or methamidophos.

CFSAN issued an assignment 16-08.pdf – to collect and analyze domestic and import samples for glyphosate and acid herbicides however honey is not listed as a commodity to collect. The assignment states "FDA has never monitored glyphosate and the acid herbicides in its regulatory pesticide program.

I have included Kaniz and Chris on this email response. Chris (he was named in the Huffington post article linked below) may be able to provide additional information regarding the pesticide glyphosate. Chris works for OFS/PPB here at the Center.

Standra

From: Lee, Mabel
Sent: Friday, July 21, 2017 9:35 AM
To: Purnell, Standra
Subject: Glyphosate in honey

Hi Standra,

I am working on a Congressional that deals with glyphosate in honey. Do you have any insights or referrals you can provide? From the article in this link, it looks like we do not have a tolerance level for glyphosate in honey. Have we taken any action on it? (I assumed no, since there is no safety concern based on the article, but I want to confirm with you.)

http://www.huffingtonpost.com/carey-gillam/fda-finds-monsantos-weed_b_12008680.html

Thank you!

Mabel

Mabel M. Lee
Consumer Safety Officer, Labeling Regulations Implementation Team

Center for Food Safety and Applied Nutrition
Office of Nutrition and Food Labeling
Food Labeling and Standards Staff

U.S. Food and Drug Administration
5001 Campus Drive
College Park, MD 20740
Tel: 240-402-2371
mabel.lee@fda.hhs.gov

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From:	Strachman-Miller, Jason
To:	Sack, Chris A; Robin, Lauren (Posnick); Christin, Charlotte - OC
Subject:	FW: NY Times story on glyphosate is out
Date:	Tuesday, July 25, 2017 5:07:37 PM
Attachments:	image001.png

Good evening,
Passing along this info on the NYT story that came out today.
Cheers, Jason

From: DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>
Date: July 25, 2017 at 5:23:38 PM EDT
To: Mettler, Erik <Erik.Mettler@fda.hhs.gov>, Mayne, Susan <Susan.Mayne@fda.hhs.gov>
Cc: Strachman-Miller, Jason <Jason.Strachman-Miller@fda.hhs.gov>, Shapinsky, David <David.Shapinsky@fda.hhs.gov>
Subject: FW: NY Times story on glyphosate is out

Wanted to let you both know that a NYT story on glyphostate is out.

This is the story - https://mobile.nytimes.com/2017/07/25/dining/ben-and-jerrys-ice-cream-herbicide-glyphosate.html?referer=https://www.google.com/

OMA has asked for correction/update on the part about testing that said we weren't sure when testing would begin.

Traces of Controversial Herbicide Are Found in Ben & Jerry's Ice Cream

By STEPHANIE STROM

July 25, 2017

A growing number of foods commonly found in kitchens across America have tested positive for glyphosate, the herbicide that is the main ingredient in the popular consumer pesticide Roundup, which is widely used in agriculture. But few brands on that list are as startling as the latest: Ben & Jerry's, the Vermont ice

cream company known for its family-friendly image and environmental advocacy.

The Organic Consumers Association announced Tuesday that it found traces of glyphosate in 10 of 11 samples of the company's ice creams — although at levels far below the ceiling set by the Environmental Protection Agency.

Rob Michalak, global director of social mission at Ben & Jerry's, said the company was working to ensure that all the ingredients in its supply chain come from sources that do not include genetically modified organisms, known as G.M.O.s. None of its plant-based ingredients, for instance, come from agenetically engineered crop like corn or soy, where glyphosate is used in production. The company is also trying to figure out a cost-effective way for the dairy farms that supply its milk to use non-G.M.O. feed.

"We're working to transition away from G.M.O., as far away as we can get," Mr. Michalak said. "But then these tests come along, and we need to better understand where the glyphosate they're finding is coming from. Maybe it's from something that's not even in our supply chain, and so we're missing it."

Consumer groups around the country, including the Organic Consumers Association, have begun raising awareness of glyphosate in food, because some studies have linked it to a variety of diseases. The International Agency for Research on Cancer, a unit of the World Health Organization,declared this year that it "probably" could cause some cancers. The agency reviewed scientific studies involving people, laboratory animals and cells to assess whether glyphosate might cause cancer.

Monsanto and other companies that make products containing glyphosate hotly dispute those studies and say there is no reason for concern. Government and other regulators tend to agree that very low levels are not harmful to humans.

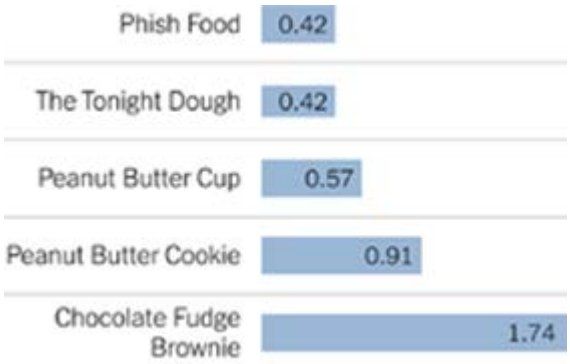
Ronnie Cummins, a founder and the international director of the Organic Consumers Association, said the amount found in Ben & Jerry's ice cream would not violate any regulations. "Not everyone agrees with the acceptable levels governments have set," Mr. Cummins said. "And, anyway, would you want to be eating this stuff at all?"

It's far from clear. Divergent findings over glyphosate's impact on health have divided governments, scientists, regulators and even the World Health Organization, with its International Agency for Research on Cancer linking it to cancer and another unit of the organization insisting on its safety.

Here is what we know:

• **The levels of glyphosate found in Ben & Jerry's ice creams are, indeed, small,according to government regulators and the scientist who did the testing.**

Among the flavors tested, Ben & Jerry's Chocolate Fudge Brownie showed the highest levels of glyphosate, with 1.74 parts per billion, and glyphosate's byproduct aminomethylphosphonic acid registering 0.91 parts per billion.



Graphic | The Test Results

Such amounts might seem negligible. John Fagan, the chief executive of the Health Research Institute Laboratories, which did the testing for the Organic Consumers Association, calculated that a 75-pound child would have to consume 145,000 eight-ounce servings a day of Ben & Jerry's Chocolate Fudge Brownie ice cream to hit the limit set by the Environmental Protection Agency, the government body charged with setting a ceiling on the amount of glyphosate allowed in food.

An adult would have to eat 290,000 servings to hit the agency's cutoff, Dr. Fagan said.

Even European regulatory limits for glyphosate consumption, which are almost six times lower than limits in the United States, find that a child would have to eat 25,000 servings a day and an adult 50,000 for the herbicide to pose a threat.

"Based on these government thresholds, the levels found in Ben & Jerry's Chocolate Fudge Brownie ice cream would seem totally irrelevant," he said.

• **But recent research suggests that the glyphosate levels still might be significant.** Inresearch published this year in the journal Nature, rats that consumed very low doses of glyphosate each day showed early signs of fatty liver disease within three months, which worsened over time.

In that study, conducted by a group of scientists at King's College London and led by Michael Antoniou, a molecular biologist, the rats consumed in a day an amount of glyphosate equivalent to a child's portion of Ben & Jerry's Chocolate Fudge Brownie ice cream, Dr. Fagan said.

Monsanto, the largest seller of products containing glyphosate, labeledthe research "bad science" and the rehashing of a study done five years earlier. Some scientists criticized the more recent study for failing to disclose the age of the rats, which could affect outcomes, and for using a breed prone to tumors.

"There were a number of criticisms of that study that were absolutely not true," said David Schubert, a professor at the Salk Institute for Biological Studies who works on neurodegenerative diseases. "But the industry does what it can to make the science very confusing to a layperson."

Dr. Schubert pointed toa study in the journal Cell Chemical Biology that came out

shortly after the one led by Dr. Antoniou, which found that when a body processes glyphosate, one of the herbicide's byproducts interfered with the body's ability to break down fatty acids. The accumulation of fatty acids is a signature of fatty liver disease.

"It basically confirms what Antoniou showed in his research," Dr. Schubert said.

• One of the consumer groups pointing at Ben & Jerry's may have a larger motive.

The Organic Consumers Association has been working with an organization called Regeneration Vermont to persuade Ben & Jerry's to go organic. Federal regulations governing organic agriculture prohibit the use of glyphosate.

To make its point, the association also had the Health Research Institute test four organic brands of vanilla ice cream — Alden's, Three Twins, Julie's and the Whole Foods Market brand 365. The lab found 0.25 to 0.5 parts per billion of glyphosate's byproduct, aminomethylphosphonic acid, in the 365 sample, but no detectable traces of glyphosate or its byproduct in the other samples.

"If they went organic, they wouldn't have this problem," said Will Allen, a founder of Regeneration Vermont and an organic farmer who has met with Ben & Jerry's executives.

Other groupstesting for glyphosate have found it in Quaker Oats, Cheerios, Ritz Crackers and Stacy's Simply Naked Pita Chips, among a range of other products. The companies behind those products have all noted that the glyphosate amounts fell well below regulatory limits.

Many of those products have few or no ingredients derived from genetically engineered crops like corn, soy and sugar beets, which are meant to withstand glyphosate. Some of those products have nonetheless tested for glyphosate registered at much higher levels than those found in Ben & Jerry's ice creams.

Both Mr. Cummins, of the Organic Consumers Association, and Mr. Michalak, of Ben & Jerry's, said the glyphosate found in Ben & Jerry's probably comes from add-ins like peanut butter and cookie dough. Such products contain ingredients like wheat, oats and peanuts that are often sprayed with the herbicide to dry them out.

• Regardless, this may be only the beginning for consumer brands, whichwill face increasing scrutiny over glyphosate.

For the past few years, consumer and environmental groups have started testing for glyphosate in food, because, while the governmentroutinely tests foods for a variety of pesticides, it does not regularly test for glyphosate.

In 2011, the Agriculture Department conducted a special test of 300 soybean samples for glyphosate and found the herbicide in 271 of them, according to Carey Gillam, the author of "Whitewash: The Story of a Weed Killer, Cancer, and the Corruption of Science," a book about glyphosate that will go on sale in October.

“Regulators have turned a blind eye toward trying to figure out what levels of glyphosate are in our food supply,” Ms. Gillam said.

The Agriculture Department did not respond to a request for comment.

The Food and Drug Administration is responsible for enforcing maximum pesticide residue levels for any foods in interstate commerce, and itissues an annual report on pesticide residue found in food — with the exception of glyphosate.

Megan McSeveney, a spokeswoman for the agency, said the methods used in its annual tests cannot detect glyphosate because of its chemical makeup and how it degrades. Available methods of testing, she added, are costly and labor intensive. In 2014, after the Government Accountability Officesharply criticized the agency for failing to test for glyphosate — and also for not disclosing that fact to the public — the Food and Drug Administration said it would cost about $5 million to start such testing.

The agency, Ms. McSeveney said, planned to test four food commodities — corn, soy, eggs and milk — although she could not say when such testing would begin.

Some food and commodity companies have decided they can’t wait on the government. The Scoular Company, which sells grains and other commodities, has begun requiring farmers who sell the company soybeans and corn to notify it before using any defoliants, including glyphosate.

“We are concerned about the general increase in chemical residues in foods,” said Greg Lickteig, a director at Scoular, “and some of our customers are concerned, too. That’s just the way it is. We now have the ability to know what’s in our food more than we ever have before.”

From: Islam, Mohammed R

From: Sack, Chris A
Sent: Wednesday, July 26, 2017 10:40 AM
To: Islam, Mohammed R; Mercer, Gregory E
Subject: RE: Ben & Jerry's Ice Creams Found To Have Trace Amounts Of Glyphosate.

Did you see those levels? Good grief.

Chris

Ph: 240-402-2464

From: Islam, Mohammed R

From:	MacMahon, Shaun
To:	Sack, Chris A; Pawar, Rahul
Subject:	FW: dairyreporter.com: Ben & Jerry's says product is safe after traces of glyphosate found in ice cream
Date:	Thursday, July 27, 2017 10:34:03 AM

FYI

Shaun MacMahon, PhD
Phone: 240-402-1998

From: Bunning, Vincent
Sent: Thursday, July 27, 2017 11:29 AM
To: Musser, Steven M; Bunning, Vincent; Diachenko, Gregory W; Callahan, John; Noonan, Gregory; Begley, Timothy H; MacMahon, Shaun
Subject: dairyreporter.com: Ben & Jerry's says product is safe after traces of glyphosate found in ice cream

Ben & Jerry's says product is safe after traces of glyphosate found in ice cream

1 comment

By Mary Ellen Shoup+Mary Ellen Shoup
27-Jul-20172017-07-27T00:00:00Z
Last updated on 27-Jul-2017 at 17:16 GMT2017-07-27T17:16:21Z
A person would have to consume 145,000 eight-ounce servings per day to reach the limit set by the US EPA, Ben & Jerry's said.
Related tags: Ben & Jerry's, Ice cream, Food safety, Pesticides, Herbicides, Glyphosate, EPA

Unilever-owned Ben & Jerry's has said that its ice cream products are safe to consume after independent lab testing by the Organic Consumers Association (OCA) found that certain ice cream samples tested positive for glyphosate.

"*While we have not yet seen the results, we can confirm all Ben & Jerry's products are safe to consume,"* Ben & Jerry's told DairyReporter.

Glyphosate-based herbicides (GBH) and their byproduct aminomethylphosphonic acid (AMPA) are found in common pesticides used worldwide on a variety of crops as well as non-crop land.

The results of the lab testing detected trace amounts of glyphosate in 10 out 11 Ben & Jerry's ice cream samples including the flavors: Peanut Butter Cup, Peanut Butter Cookie, Vanilla (two samples), Phish Food, The Tonight Dough, Half Baked, Chocolate Fudge Brownie, Americone Dream and Chocolate Chip Cookie Dough.

Chocolate Fudge Brownie registered the highest level of GBH at 1.74 parts per billion and AMPA at 0.91 parts per billion. Cherry Garcia was the only flavor to test negative for the herbicide.

"Even if the reported results are accurate, as the laboratory that conducted the test stated, a person would have to consume 145,000 eight-ounce servings PER DAY to reach the limit set by the US Environmental Protection Agency (EPA)," Ben & Jerry's said.

OCA founder Ronnie Cummins and Ben & Jerry's global director of social mission, Rob Michalak, told *The New York Times* that the detected glyphosate in its ice cream probably comes from *"add-ins"* like peanut butter or cookie dough or ingredients such as wheat, oats, and peanuts, which are sprayed by the herbicide.

What are safe levels of glyphosate?

Glyphosate residues are routinely detected in food products and the acceptable daily intake of the herbicide is 1.75mg/kg per day in the US, according to a report published in January 2017 in the scientific journal Nature.

Toxicity studies have shown that glyphosate may provoke toxic effects on liver and kidney functions. However, *"it should be noted that most results from these GBH toxicity studies were obtained at doses far greater than general human population exposure,"* scientists added.

OCA argued that there is no *"safe"* level of glyphosate suggested by regulatory agencies and has called for Ben & Jerry's to begin an immediate transition to using only organic ingredients, including milk.

The group has also urged natural and organic food stores to drop the Ben & Jerry's brand unless the company commits to transitioning to organic.

Ben & Jerry's says product is safe after traces of glyphosate found in ice cream

By Mary Ellen Shoup+Mary Ellen Shoup, 27-Jul-2017

Unilever-owned Ben & Jerry's has said that its ice cream products are safe to consume after independent lab testing by the Organic Consumers Association (OCA) found that certain ice cream samples tested positive for glyphosate.

http://www.dairyreporter.com/Regulation-Safety/Ben-Jerry-s-says-traces-of-glyphosate-in-ice-cream-are-negligible

RELATED TOPICS:

Dairy Health Check, Regulation & Safety, Ice Cream, Unilever

From:	Sack, Chris A
To:	Chang, Eugene; Islam, Mohammed R; Mercer, Gregory E; Thompson, Richard L.; Vonderbrink, John; Wong, Jon
Subject:	Glyphosate MLV Report
Date:	Wednesday, July 05, 2017 10:44:00 AM
Attachments:	Glyphosate MLV Rpt.docx

Hi everyone,

Take a look at the attached final MLV report for the glyphosate collaboration. The report includes a summary of results for all labs (pp 1-5) and a summary for each participating laboratory – see attachments E-I. I need everyone to look over the summary and the individual lab report for which you contributed. Please send me your comments and corrections by next Wednesday (July 12). I have turned on change tracking in the attached doc, so feel free to make changes to it and send it back to me.

I hope to submit the final report by the end of next week.

Thanks,

Chris

From:	Sack, Chris A
To:	MacMahon, Shaun
Cc:	Cassias, Irene; Eide, David J; Islam, Mohammed R; Katsoudas, Eugenia; Liang, Charlotte; Mercer, Gregory E; Noonan, Gregory; Sack, Chris A; Thompson, Richard L.; Wong, Jon
Subject:	Glyphosate method collaboration report
Date:	Friday, July 21, 2017 8:34:21 AM
Attachments:	Glyphosate MLV Rpt.docx

Hi Shaun,

The multi-laboratory validation report for the glyphosate collaboration is attached for CMVS/CRCG review and approval. Please note that five laboratories participated in the collaboration, therefore the procedure has been successfully validated at Level III. The procedure is currently in use at selected laboratories for the acid herbicide/glyphosate assignment. The single laboratory validation (SLV) was conducted at PSFFL; you can contact them if you would like to see the SLV report.

Let me know if you need anything further or have any questions.

Have a wonderful weekend,

Chris

From:	Shireen, Kaniz F
To:	Sack, Chris A
Subject:	barley samples for herbicides
Date:	Tuesday, July 25, 2017 9:32:11 AM
Attachments:	image001.png

Chris: Good Morning.
Can Divisions collect roasted single ingredient and malt barley for subject analysis?

Thanks,

Kaniz F. Shireen, MS
Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Compliance
U.S. Food and Drug Administration
Tel: 240-402-2775
Kaniz.Shireen@fda.hhs.gov

FDA U.S. FOOD & DRUG ADMINISTRATION

From:	MacMahon, Shaun
To:	Sack, Chris A
Subject:	NRL"s MLV data
Date:	Monday, July 31, 2017 9:11:55 AM

Hey Chris,

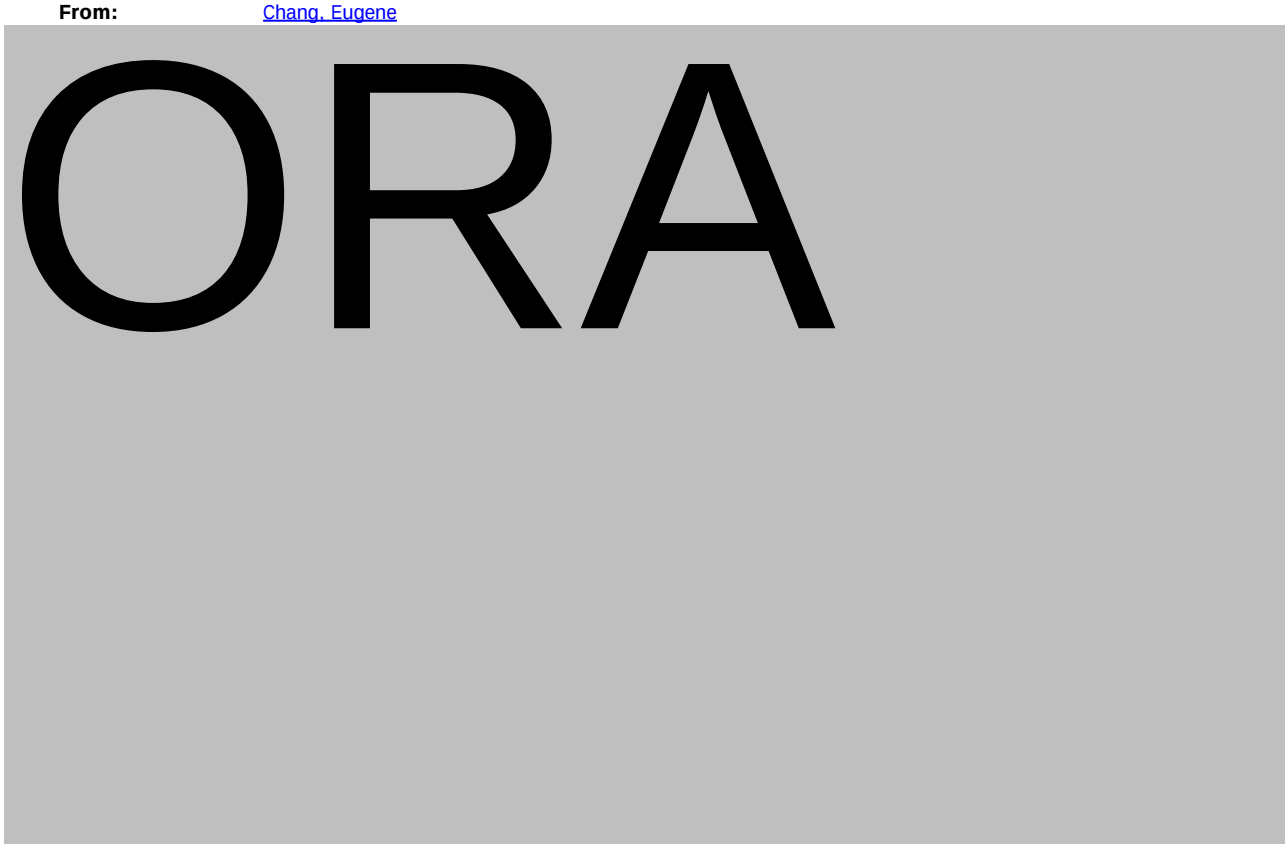
The CMVS doesn't meet until 1 week from tomorrow, but I noticed that results from NRL were not included in the MLV report. Was there an issue with their data and, if so, was a root cause determined for what went wrong? Will they be one of the servicing labs for glyphosate? It's not uncommon for a lab's results to be excluded, just wanted to see if there was any more detail on it. I know these questions are going to come up so any information you could provide would be very helpful.

Thanks,
Shaun

Shaun MacMahon, PhD
Branch Chief, Chemical Contaminants Branch
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
5001 Campus Drive
College Park, MD 20740

Phone: 240-402-1998
Blackberry: 240-731-9797
Fax: 301-436-2634
Shaun.MacMahon@fda.hhs.gov

From: Chang, Eugene



From: Mercer, Gregory E

From: Mercer, Gregory E

From: Chang, Eugene

From:	Wirtz, Mark S
To:	Sack, Chris A; Wong, Jon; Councell, Terry
Subject:	FW: glyphosate ... FYI
Date:	Thursday, August 17, 2017 10:33:59 AM

From: Dennis, Sherri
Sent: Thursday, August 17, 2017 11:33 AM
To: Wirtz, Mark S
Subject: FW: glyphosate ... FYI

From: Das, Sharmi
Sent: Thursday, August 17, 2017 11:26 AM
To: Dietz, Jason; Vierk, Katherine; Dennis, Sherri; Taubenheim, Ann; Choiniere, Conrad
Subject: glyphosate ... FYI

MONSANTO STRIKES BACK ON GLYPHOSATE: Monsanto is pointing to a deposition in the ongoing U.S. class action suit brought by farmers who say their cancer is linked to glyphosate that reveals the International Agency for Research on Cancer unfairly disregarded two important pieces of research from Germany that suggested the herbicide was safe. IARC's controversial review of the herbicide found it was a probable carcinogen. The deposition of Charles William Jameson, a scientist who specialized in animal studies at IARC and was a member of IARC's review panel for the herbicide, was part of hundreds of previously undisclosed documents from the high-profile court case in San Francisco that Monsanto provided exclusively to our colleague Simon Marks at POLITICO Europe.
Jameson said in the deposition that he was not provided with research from two German scientists in time to include it in the review - key data, he said, that would have contributed more deeply to IARC's assessment. The studies found glyphosate does not pose cancer risks. Simon's reporting, supported by interviews with numerous scientific experts, follows a report by Reuters in June that presented evidence that Aaron Blair, a U.S. researcher who led the IARC panel for glyphosate, didn't disclose to panel members unpublished research that he was involved in that found no evidence of a cancer link to glyphosate exposure.
Digging through the dirt: The POLITICO story is the latest revelation to come out of the federal court case as both sides use documents contained in the suit to make their case publicly. While Monsanto continues to push that key data was left out of the IARC assessment, opponents of the chemical are pointing to memos and emails that show the company pressured EPA and independent scientists to back the herbicide.
Timing is everything: The courtroom fight in San Francisco is happening as both the U.S. and EU regulators decide whether to keep the chemical - the most widely used herbicide in the world - available for farmers. The EU has to make a decision by the end of the year, while the EPA could release a proposal at any time - many expected it to be out last spring. While EPA and EU regulators have already said the chemical is safe in current uses, Monsanto seems to be taking no risks in its effort to undermine the IARC report. Later this year, Judge Vince Chhabria of U.S. District Court for the Northern District of California will issue an unusual verdict on whether decades of scientific evidence support a direct link between glyphosate and cancer. A hearing on the case is scheduled for next week. Pros, Simon's full story is here.

From: Cromer, Michele

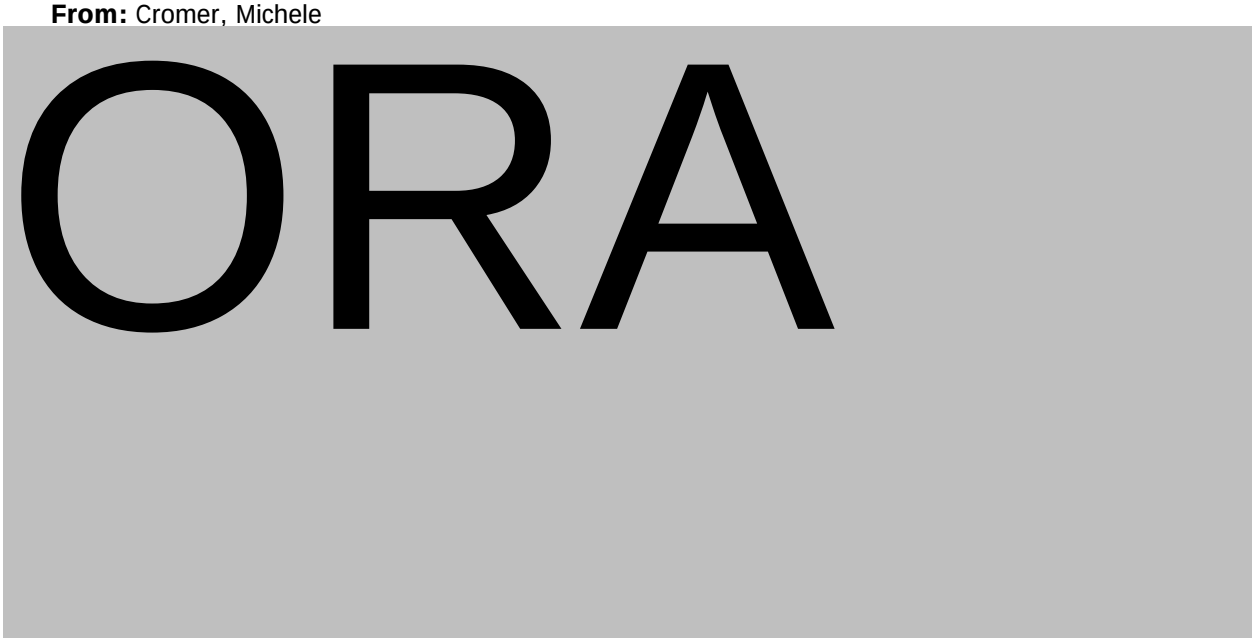
From: Sack, Chris A
To: Cromer, Michele
Subject: RE: Question
Date: Wednesday, August 02, 2017 11:58:00 AM

I am OK with white rice. Any grain with minimal processing.

Chris

Ph: 240-402-2464

From: Cromer, Michele

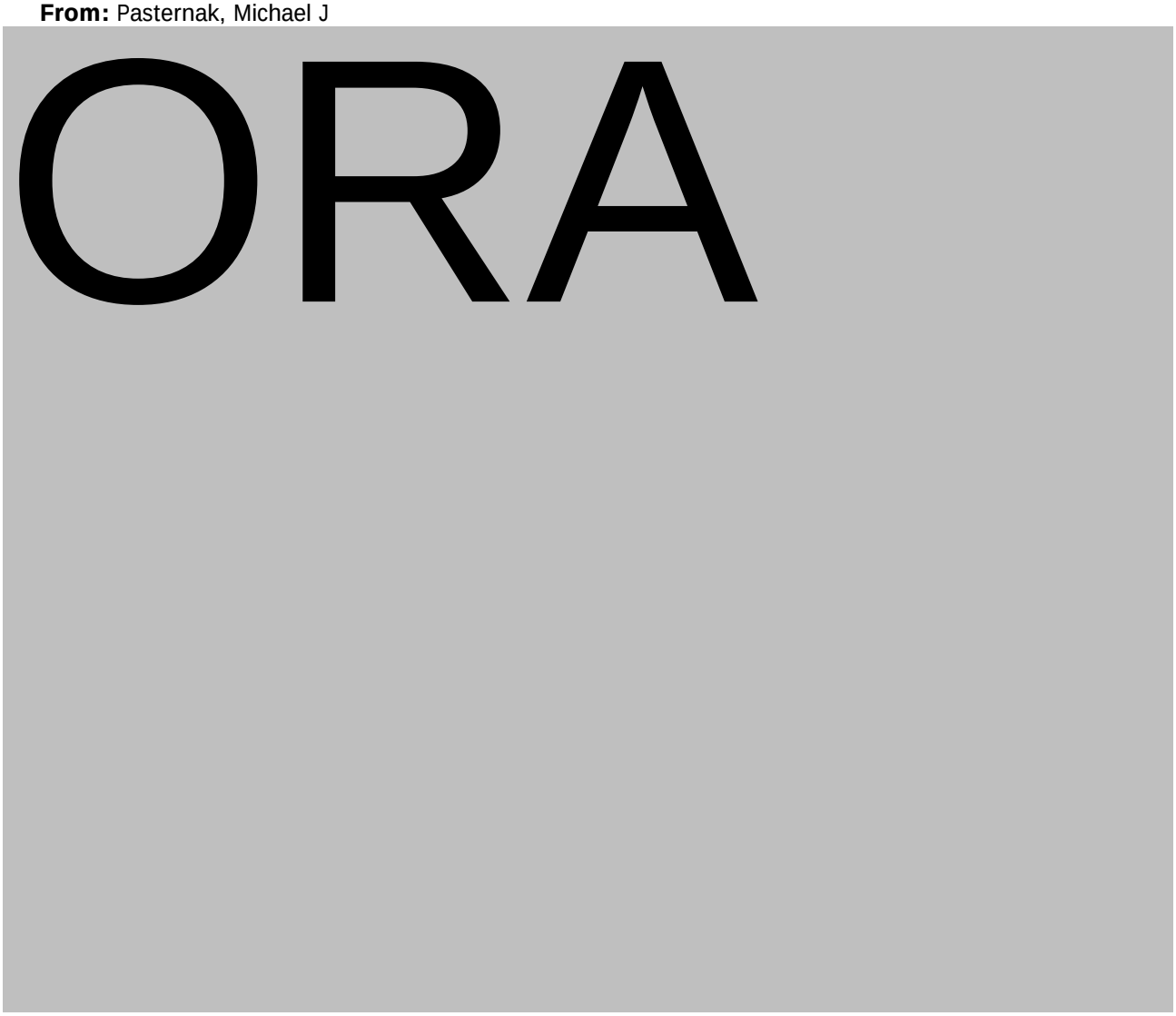


From: Shireen, Kaniz F
To: Sack, Chris A
Cc: Pasternak, Michael J; Jones, April (CDC); Vora, Rina (Patel)
Subject: FW: Domestic Sample Collection question for DFPG 16-08
Date: Thursday, August 10, 2017 1:14:45 PM

Hi Chris,
Please advise.

Thanks, Kaniz

From: Pasternak, Michael J



From: Vonderbrink, John

From: Mabry-Smith, Ronald C

ORA

From: Sack, Chris A
Sent: Tuesday, August 15, 2017 2:19 PM
To: Mabry-Smith, Ronald C; Lane, Shannon; Chang, Eugene
Subject: Glyphosate samples

Hey guys,

Could you give me a ballpark on the number of samples you have received for each of the 4 commodities in the assignment? I need to provide a report to my boss early tomorrow AM.

Thanks,

Chris

From:	Sack, Chris A
To:	Vonderbrink, John
Cc:	Cromer, Michele; Ross, Mark S; Adams, Neal L
Subject:	RE: Peanuts for AcH
Date:	Tuesday, August 15, 2017 2:19:00 PM
Attachments:	image001.png

Hi John,

Good to see you in NYK. Give that peanut sample a LC 1 and close it out. In the future, you can assume that 4-CPA is a degradant of 2,4-D. If you find it in a sample without 2,4-D at a significant level, say >100 ppb, then alert me.

Thanks for asking,

Chris

Ph: 240-402-2464

From: Vonderbrink, John

From:	MacMahon, Shaun
To:	Sack, Chris A; Mercer, Gregory E
Cc:	Callahan, John; Noonan, Gregory; Bowers, John C; Cai, Yanxuan (Tina); Chu, Pak S; Deeds, Jonathan; Eischeid, Anne; Heitkemper, Douglas T; Krynitsky, Alexander; Linder, Sean; Oakes, Gregg P.
Subject:	CMVS review of Glyphosate MLV report
Date:	Friday, August 18, 2017 7:11:16 AM
Attachments:	Glyphosate MLV Response.doc Glyphosate MLV Rpt.docx

---

Chris/Greg,

The CMVS has reviewed your submission of a multi-laboratory validation report for the method, “Determination of Glyphosate and Glufosinate Residues in Food.” While the results are very encouraging, the enclosed report summarizes the findings of the subcommittee and includes comments and suggestions which need to be addressed before the study can be approved as a Level III Multi-Laboratory Validation. As always, I’m happy to discuss or clarify any of the questions raised by the Committee.

Shaun

Shaun MacMahon, PhD
Branch Chief, Chemical Contaminants Branch
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
5001 Campus Drive
College Park, MD 20740

Phone: 240-402-1998
Blackberry: 240-731-9797
Fax: 301-436-2634
Shaun.MacMahon@fda.hhs.gov

From:	Shireen, Kaniz F
To:	Sack, Chris A
Subject:	FW: ACTION ITEM: AMENDED: Collection of Selected Domestic and Imported Foods for Herbicides Analysis
Date:	Friday, August 18, 2017 8:48:23 AM
Attachments:	image001.png Acid Herbicide Assignment FY17.docx
Importance:	High

FYI

From: Bass, Glenn
Sent: Friday, August 18, 2017 9:45 AM
To: ORA HAF EAST Div DIBs; ORA HAF WEST Div DIBs
Cc: Pittman, Eric; Vora, Rina (Patel); Shireen, Kaniz F; Shelborne, Paige
Subject: ACTION ITEM: AMENDED: Collection of Selected Domestic and Imported Foods for Herbicides Analysis
Importance: High

Good Morning All,

See revised instructions from CFSAN/OC below.

Thanks

Glenn T. Bass, MS.
Human and Animal Food, Program Deputy Director-West
240-402-4894
White Oak, Building 31
Room 2530

"Dedicated to Promoting and Protecting Public Health by Assuring Safe and Effective FDA Regulated Products"

From: Shireen, Kaniz F
Sent: Friday, August 18, 2017 8:46 AM
To: Bass, Glenn
Cc: Vora, Rina (Patel); Pasternak, Michael J; Shelborne, Paige
Subject: FW: AMENDED: Collection of Selected Domestic and Imported Foods for Herbicides Analysis

Hi Glenn:
This is just a friendly reminder that the attached assignment will end September 30, 2017.

(b) (5)

We would like for labs to report results in FACTS by the end of September.

If you have questions, please let me know.
Thank you.

Kaniz

From: Shireen, Kaniz F
Sent: Thursday, May 18, 2017 2:58 PM
To: Price, Derek C; Wilkinson, Kelli; Clarida, Thomas D; Williams, Toniette K; Daugherty, Karen C; Shambaugh, Shari J; Harris, Mark; Hernandez, Ramon; Ramos, Edwin; Bromley Jr, Gerald D; Almogela, Darlene B; vanTwuyver, Chris; Holmquist, Lori; Althar, Lisa M; Below, Stacy M; ORA KAN Lab; ORA PAR Lab Directors
Cc: Zambrana, Ingrid; Weissinger, William; Barber, Steven; Garcia, Edmundo; Mitchell, LaTonya M; Torres Irizarry, Maridalia; Bigham, Cheryl A; Dutcher, Michael; Pace, Ronald; Burbach, Miriam R; Cato, Todd W; Beru, Nega; Robin, Lauren (Posnick); Sack, Chris A; Vora, Rina (Patel); Rudnitsky, Samuel; Pasternak, Michael J; Preciados, Mark V.; Islam, Mohammed R; CFSAN-OC
Subject: AMENDED: Collection of Selected Domestic and Imported Foods for Herbicides Analysis

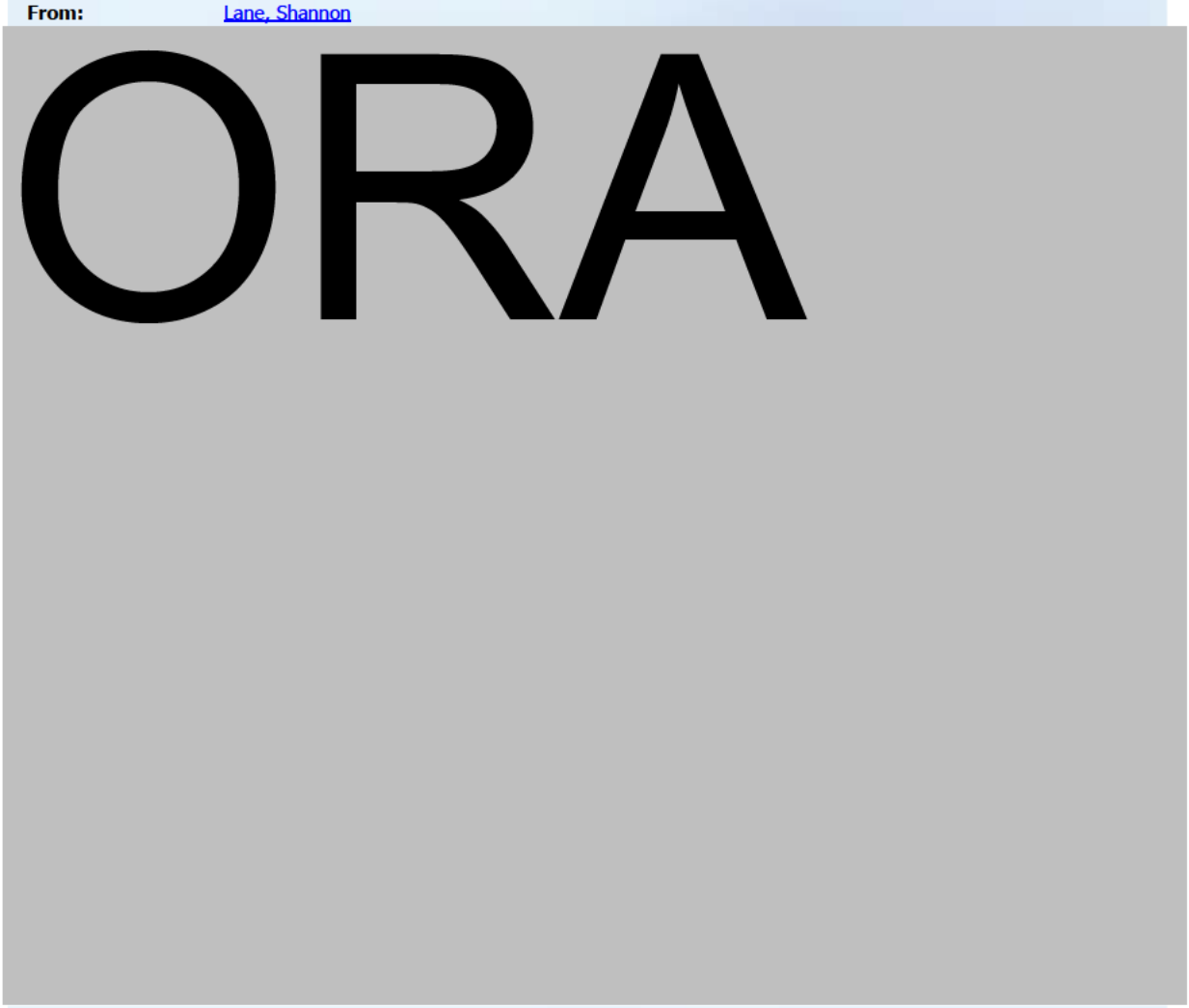
(b) (5)

Thank you.
Kaniz F. Shireen, MS
Consumer Safety Officer

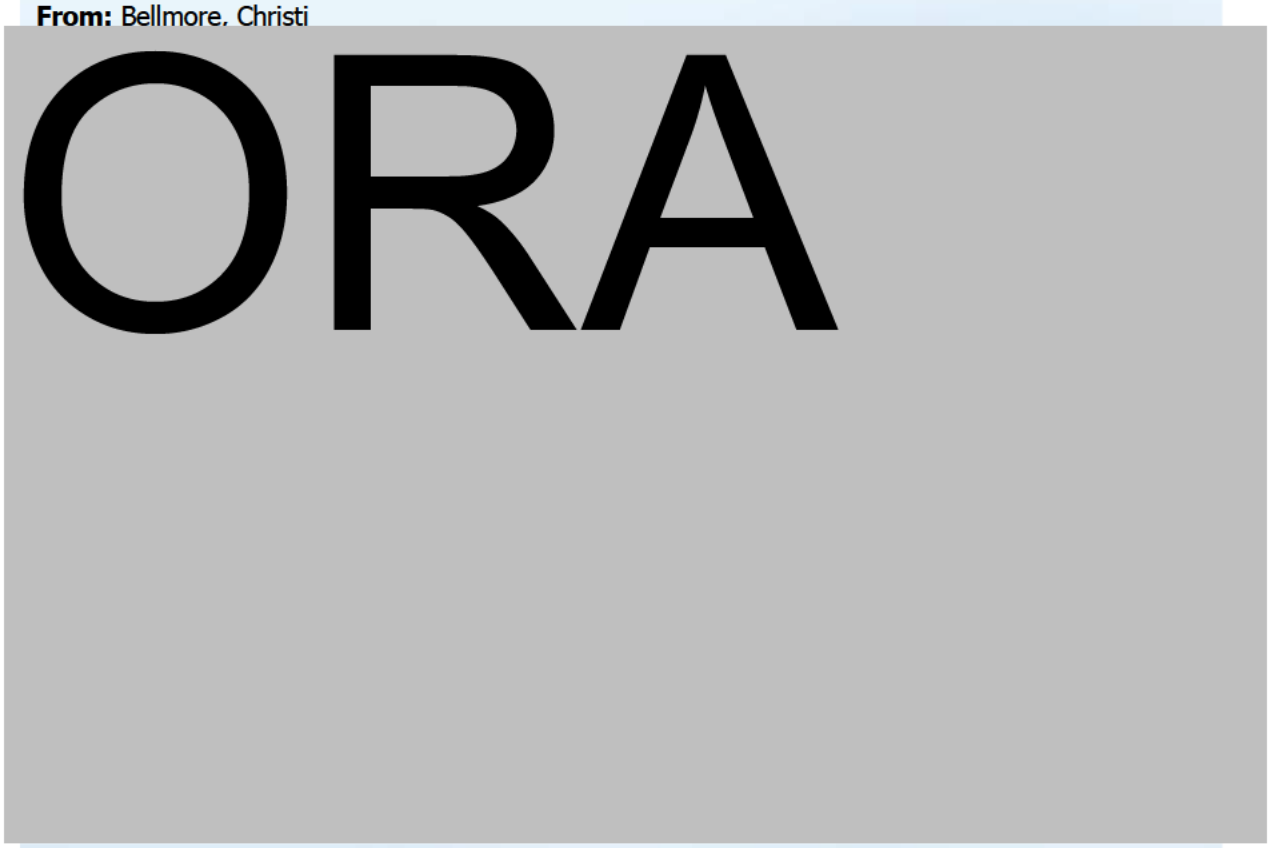
Center for Food Safety and Applied Nutrition
Office of Compliance
U.S. Food and Drug Administration
Tel: 240-402-2775
Kaniz.Shireen@fda.hhs.gov

FDA U.S. FOOD & DRUG ADMINISTRATION

From: Lane, Shannon



From: Bellmore, Christi

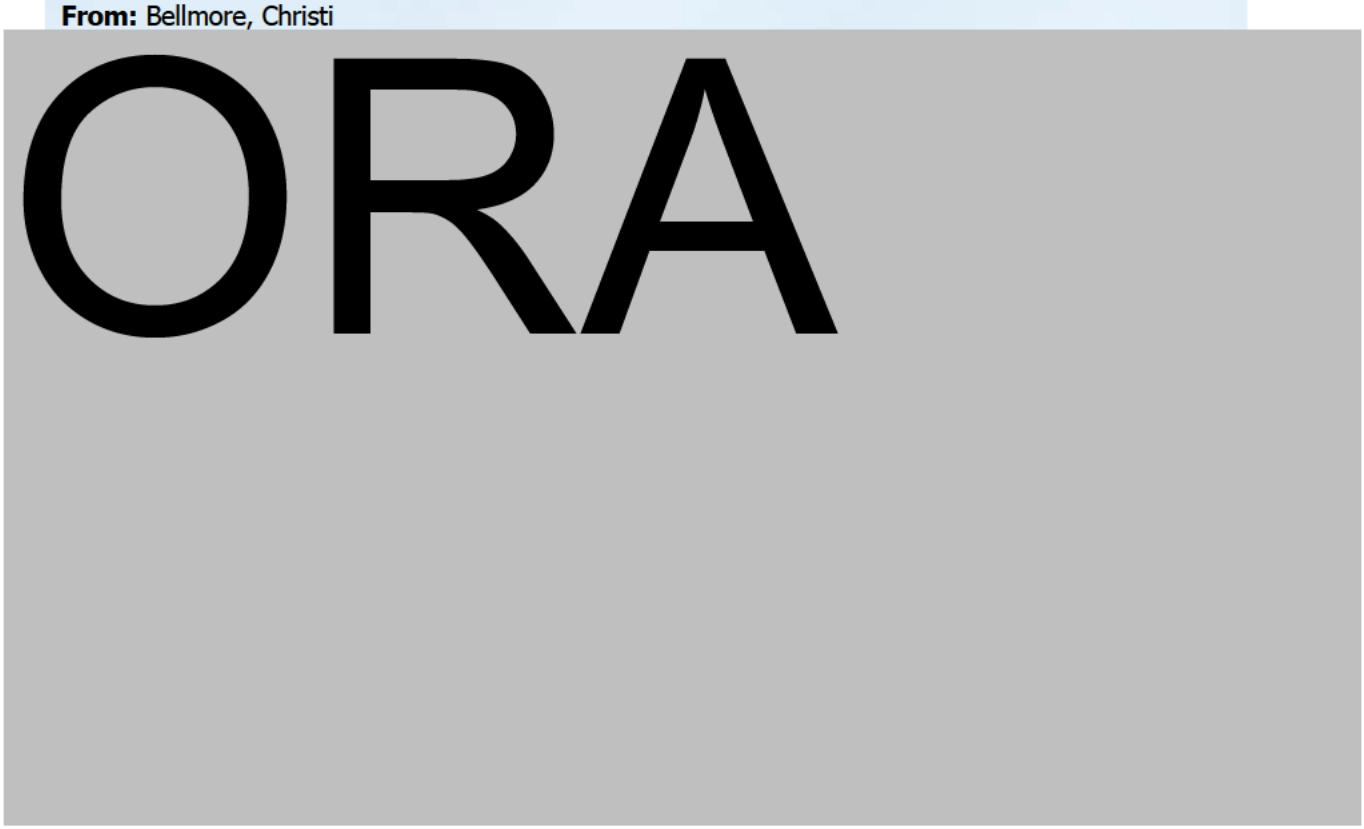


From: Islam, Mohammed R

From: Eide, David J

From: Dietzel, Rachel E

From: Bellmore, Christi



ORA

From: Sack, Chris A
To: Lane, Shannon; Islam, Mohammed R
Cc: Cassias, Irene
Subject: RE: Glyphosate and Acid Herbicides Assignment #11750792, DFPG # 16-08
Date: Saturday, August 26, 2017 5:48:00 AM

Dried corn on the cob is acceptable (b) (5) Looking for grain corn.

Chris Sack
Residue Expert
Office of Food Safety
Center for Food Safety and Applied Nutrition
US Food and Drug Administration

Phone: 240-402-2464

From: Lane, Shannon

From: Bellmore, Christi

ORA

From:	Shireen, Kaniz F
To:	Sack, Chris A; Robin, Lauren (Posnick)
Subject:	Corn sample collection
Date:	Monday, August 28, 2017 2:36:21 PM
Attachments:	image001.png

Hi Chris and Lauren:
I received call from the investigator who wants to know
-can he collect corn sample before and/or after milling .
-can he collect dried crushed corn?

Please let me know so that I can provide instructions.
Thanks,

Kaniz F. Shireen, MS
Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Compliance
U.S. Food and Drug Administration
Tel: 240-402-2775
Kaniz.Shireen@fda.hhs.gov

FDA U.S. FOOD & DRUG ADMINISTRATION

From: Shireen, Kaniz F
To: Sack, Chris A; Robin, Lauren (Posnick)
Subject: FW: Corn samples collected under DFPG #16-08 at PSFFL
Date: Wednesday, August 30, 2017 3:37:57 PM

Please advise.

Thanks, Kaniz

From: Tuntevski, Danny

From: Lane, Shannon

From:	Sack, Chris A
To:	Lane, Shannon
Subject:	RE: Problem
Date:	Thursday, August 31, 2017 5:38:00 AM

Hi Shannon,

Samples that do not meet the requirements of the assignment can be analyzed for the normal pesticides MRMs if they are appropriate for the pesticide program. Fresh corn would be OK for routine pesticide screening, but not the AcH assignment.

Chris

Ph: 240-402-2464

From: Lane, Shannon

FDA Glyphosate Method

A. Reagents and Supplies

1. Acetonitrile, HPLC grade
2. Petroleum ether
3. Methylene chloride
4. Water, HPLC grade
5. Formic acid, 98% solution
6. Acetic acid
7. Ammonium formate
8. Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA)
9. Tetrabutylammonium hydroxide (TBAOH) titrant, 0.4 M in Water, HPLC Grade, ACROS Organics
10. Tetrabutylammonium acetate (TBuAA), Aldrich No. 335991-10G (optional)
11. Tetrabutylammonium acetate 1 M (TBuAA), Aldrich No. 401803 – 50 ML (optional)
12. 50-mL plastic centrifuge tubes
13. Waters Oasis HLB SPE, 60 mg, 3cc, 30 µm
14. Extraction solvent (50 mM acetic acid/10 mM Na_2EDTA): mix 572 µL acetic acid and 0.74 g Na_2EDTA in 200-mL of purified water.
15. Mobile phase A (4 mM tetrabutylammonium formate)
    a. Add 10.0 ml of 0.4 M TBAOH to ~900 mL HPLC water, and adjust the pH to 2.8±0.05 using formic acid (~ 3 ml). OR
    b. Add 1.20 g TBA acetate in 1 L HPLC water; and adjust the pH to 2.8±0.05 using formic acid (~2 mL).

B. Standard Reference Materials

1. Glyphosate
2. Glufosinate
3. AMPA
4. Glyphosate-^{13}C
5. Glufosinate-D^3
6. N-acetyl-glyphosate, available from Toronto Research Chemicals (TRC No A178245), or Santa Cruz BioTech (SCBT No. sc-479500)

C. Standard Solutions

(b) (5)

D. Equipment and Instrumentation

1. Genogrinder

2. Centrifuge
3. Pipettes
4. LC-MS/MS
   a. Shimadzu HPLC system: two LC-20AD pumps, Sil-20AC autosampler, CTO-20AC column oven
      NOTE: Replace all metal LC tubing with PEEK tubing between the autosampler and injection valve because glyphosate can be retained on metal surfaces.
   b. AB 6500 Q-TRAP mass spectrometer
   c. HPLC column: Phenomenex Luna C8(2), 100 Å, 5 µm, 150 x 4.6 mm, Phenomenex 00F-4249-E0
   d. HPLC guard column: Phenomenex guard column KrudKatcher P/N AFO-8497

E. **Extraction Procedure**

1. 5 g sample + 25 ml extraction solvent
   2g sample plus 10 ml extraction solvent for dry products
2. Add 10 ml PE or MeCl to fatty matrices
3. Spike with isotopes @ 200 ng/g (could be included in the extraction solvent)
4. Shake @ 1000 for 10 min
5. Centrifuge at ≥ 3000 rpm for 5 min
6. Filter aqueous extract thru HLB SPE cartridge
7. Filter for injection (could be included with SPE step)
8. Sample concentration: 0.2 g/ml

F. **LC-MS/MS method**

LC Parameters		**Gradient**	
Column:	Phenomenex Luna C8(2), 150 x 4.6 mm, 5 µm, with Phenomenex KrudKatcher guard column	**Time**	**MPB**
MP A:	4 mM tetrabutlyammonium formate + 0.1 % formic acid in water (pH 2.8±0.05)	0.00	5
MP B:	MeCN	1.00	5
Flow:	0.6 mL/min (4.6 mm column)	5.00	95
Inj Vol:	10 µL	6.50	95
Temp	45 °C	6.60	5
		10.00	5

MS/MS Parameters

Q1	Q3	RT	Transition	DP	EP	CE	CXP
110	63	2.5	AMPA 1	-40	-11	-30	-9
110	79	2.5	AMPA 2	-40	-11	-34	-9
112	63	2.5	AMPA IS	-60	-11	-26	-9

FDA Glyphosate Method

180	63	4.0	Glufosinate 1	-60	-11	-66	-9
180	95	4.0	Glufosinate 2	-40	-11	-24	-5
180	85	4.0	Glufosinate 3	-60	-11	-25	-9
183	63	4.0	Glufosinate IS	-60	-11	-40	-9
168	63	5.0	Glyphosate 1	-30	-11	-28	-9
168	79	5.0	Glyphosate 2	-30	-11	-56	-9
168	150	5.0	Glyphosate 3	-30	-11	-16	-9
171	63	5.0	Glyphosate IS	-30	-11	-28	-9
210	63	6.0	N-acetyl glyphosate 1	-85	-11	-40	-13
210	124	6.0	N-acetyl glyphosate 2	-85	-11	-17	-13
210	79	6.0	N-acetyl glyphosate 3	-85	-11	-50	-13

MS Parameters

CUR	25
CAD	MEDIUM
IS	-4000
GAS 1	65
GAS 2	65
TEM	450-650 ˚C
Q1	UNIT
Q3	UNIT

2. Centrifuge
3. Pipettes
4. LC-MS/MS
   a. Shimadzu HPLC system: two LC-20AD pumps, Sil-20AC autosampler, CTO-20AC column oven
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   b. AB 6500 Q-TRAP mass spectrometer
   c. HPLC column: Phenomenex Luna C8(2), 100 Å, 5 µm, 150 x 4.6 mm, Phenomenex 00F-4249-E0
   d. HPLC guard column: Phenomenex guard column KrudKatcher P/N AFO-8497

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1. 5 g sample + 25 ml extraction solvent
   2g sample plus 10 ml extraction solvent for dry products
2. Add 10 ml PE or MeCl to fatty matrices
3. Spike with isotopes @ 200 ng/g (= 40 ng/ml in the extraction solvent)
4. Shake @ 1000 for 10 min
5. Centrifuge at ≥ 3000 rpm for 5 min
6. Filter aqueous extract thru HLB SPE cartridge
7. Filter for injection (could be included with SPE step)
8. Sample concentration: 0.2 g/ml

F. **LC-MS/MS method**

LC Parameters		**Gradient**	
Column:	Phenomenex Luna C8(2), 150 x 4.6 mm, 5 µm, with Phenomenex KrudKatcher guard column	**Time**	**MPB**
MP A:	4 mM tetrabutlyammonium formate + 0.1 % formic acid in water (pH 2.8±0.05)	0.00	5
MP B:	MeCN	1.00	5
Flow:	0.6 mL/min (4.6 mm column)	5.00	95
Inj Vol:	10 µL	6.50	95
Temp	45 °C	6.60	5
		10.00	5

MS/MS Parameters

Q1	Q3	RT	Transition	DP	EP	CE	CXP
110	63	2.5	AMPA 1	-40	-11	-30	-9
110	79	2.5	AMPA 2	-40	-11	-34	-9
112	63	2.5	AMPA IS	-60	-11	-26	-9

FDA Glyphosate Method

A. Reagents and Supplies

1. Acetonitrile, HPLC grade
2. Petroleum ether
3. Methylene chloride
4. Water, HPLC grade
5. Formic acid, 98% solution
6. Acetic acid
7. Ammonium formate
8. Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA)
9. Tetrabutylammonium hydroxide (TBAOH) titrant, 0.4 M in Water, HPLC Grade, ACROS Organics
10. Tetrabutylammonium acetate (TBuAA), Aldrich No. 335991-10G (optional)
11. Tetrabutylammonium acetate 1 M (TBuAA), Aldrich No. 401803 – 50 ML (optional)
12. 50-mL plastic centrifuge tubes
13. Waters Oasis HLB SPE, 60 mg, 3cc, 30 µm
14. Extraction solvent (50 mM acetic acid/10 mM Na_2EDTA): mix 572 µL acetic acid and 0.74 g Na_2EDTA in 200-mL of purified water.
15. Mobile phase A (4 mM tetrabutylammonium formate)
    a. Add 10.0 ml of 0.4 M TBAOH to ~900 mL HPLC water, and adjust the pH to 2.8±0.05 using formic acid (~ 3 ml). OR
    b. Add 1.20 g TBA acetate in 1 L HPLC water; and adjust the pH to 2.8±0.05 using formic acid (~2 mL).

B. Standard Reference Materials

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2. Glufosinate
3. AMPA
4. Glyphosate-^{13}C
5. Glufosinate-D^3
6. N-acetyl-glyphosate, available from Toronto Research Chemicals (TRC No A178245), or Santa Cruz BioTech (SCBT No. sc-479500)

C. Standard Solutions

(b) (5)

D. Equipment and Instrumentation

1. Genogrinder
2. Centrifuge
3. Pipettes
4. LC-MS/MS
    a. Shimadzu HPLC system: two LC-20AD pumps, Sil-20AC autosampler, CTO-20AC column oven
    NOTE: Replace all metal LC tubing with PEEK tubing between the autosampler and injection valve because glyphosate can be retained on metal surfaces.
    b. AB 6500 Q-TRAP mass spectrometer

c. HPLC column: Phenomenex Luna C8(2), 100 Å, 5 µm, 150 x 4.6 mm, Phenomenex 00F-4249-E0
d. HPLC guard column: Phenomenex guard column KrudKatcher P/N AFO-8497

E. **Extraction Procedure**

1. 5 g sample + 25 ml extraction solvent
   2 g sample plus 10 ml extraction solvent for dry products
2. Add 10 ml PE or MeCl as needed for fatty or dirty matrices
3. Spike with isotopes @ 200 ng/g (could be included in the extraction solvent)
4. Shake @ 1000 for 10 min
5. Centrifuge at ≥ 3000 rpm for 5 min
6. Filter aqueous extract thru HLB SPE cartridge
7. Filter for injection (could be included with SPE step)
8. Sample concentration: 0.2 g/ml

F. **LC-MS/MS method**

LC Parameters		**Gradient**	
Column:	Phenomenex Luna C8(2), 150 x 4.6 mm, 5 µm OR Phenomenex Luna C8, 150 x 2 mm, 5 µm, with Phenomenex KrudKatcher guard column	**Time**	**MPB**
MP A:	4 mM tetrabutlyammonium formate + 0.1 % formic acid in water (pH 2.8±0.05)	0.00	5
MP B:	MeCN	1.00	5
Flow:	0.6 mL/min (4.6 mm column)	5.00	95
	0.3 mL/min (2.0 mm column)	7.00	95
Inj Vol:	10 µL	8.00	5
Temp	45 °C	14.00	5

MS/MS Parameters

Q1	Q3	RT	Transition	DP	EP	CE	CXP
110	63	2.5	AMPA 1	-40	-11	-30	-9
110	79	2.5	AMPA 2	-40	-11	-34	-9
112	63	2.5	AMPA IS	-60	-11	-26	-9
180	63	4.0	Glufosinate 1	-60	-11	-66	-9
180	95	4.0	Glufosinate 2	-40	-11	-24	-5
180	85	4.0	Glufosinate 3	-60	-11	-25	-9
183	63	4.0	Glufosinate IS	-60	-11	-40	-9
168	63	5.0	Glyphosate 1	-30	-11	-28	-9
168	79	5.0	Glyphosate 2	-30	-11	-56	-9

FDA Glyphosate Method

168	150	5.0	Glyphosate 3	-30	-11	-16	-9
171	63	5.0	Glyphosate IS	-30	-11	-28	-9
210	63	6.0	N-acetyl glyphosate 1	-85	-11	-40	-13
210	124	6.0	N-acetyl glyphosate 2	-85	-11	-17	-13
210	79	6.0	N-acetyl glyphosate 3	-85	-11	-50	-13

MS Parameters

CUR	25
CAD	MEDIUM
IS	-4000
GAS 1	65
GAS 2	65
TEM	450-650 ˚C
Q1	UNIT
Q3	UNIT

FDA Glyphosate Method

A. Reagents and Supplies

1. Acetonitrile, HPLC grade
2. Petroleum ether
3. Methylene chloride
4. Water, HPLC grade
5. Formic acid, 98% solution
6. Acetic acid
7. Ammonium formate
8. Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA)
9. Tetrabutylammonium hydroxide (TBAOH) titrant, 0.4 M in Water, HPLC Grade, ACROS Organics
10. Tetrabutylammonium acetate (TBuAA), Aldrich No. 335991-10G (optional)
11. Tetrabutylammonium acetate 1 M (TBuAA 1M), Aldrich No. 401803 – 50 ML (optional)
12. 50-mL plastic centrifuge tubes
13. Waters Oasis HLB SPE, 60 mg, 3cc, 30 µm
14. Extraction solvent (50 mM acetic acid/10 mM Na_2EDTA): mix 572 µL acetic acid and 0.74 g Na_2EDTA in 200-mL of purified water.
15. Mobile phase A (4 mM tetrabutylammonium formate)
    a. Add 10.0 ml of 0.4 M TBAOH to ~900 mL HPLC water, and adjust the pH to 2.8±0.05 using formic acid (~ 3 ml). OR
    b. Add 1.20 g TBA acetate in 1 L HPLC water; and adjust the pH to 2.8±0.05 using formic acid (~2 mL). OR
    c. 4 ml 1M TBuAA in 1 L HPLC water; and adjust the pH to 2.8±0.05 using formic acid (~2 mL).

B. Standard Reference Materials

1. Glyphosate
2. Glufosinate
3. AMPA
4. Glyphosate-^{13}C
5. Glufosinate-D^3
6. N-acetyl-glyphosate, available from Toronto Research Chemicals (TRC No A178245), or Santa Cruz BioTech (SCBT No. sc-479500)

C. Standard Solutions

1. General instructions
    a. Unless otherwise indicated prepare standards in DI water
    b. Store standard solutions in plastic containers because glass can leach standard reference material from solution. Use of glass volumetric flasks for standard preparation is OK if solution is removed from the glassware after preparation.
2. Stock standards 1 mg/ml (includes all native and isotopic standards listed in Section B)
3. Isotopic working solutions
    a. 20 µg/ml – Combine and dilute 1 mg/ml stock isotopic standards 50:1

   b. IS Fortified Extraction solvent containing isotopic standards @ 50 ng/ml – Dilute 20 µg/ml mixed isotopic standard 400:1 using extraction solvent, e.g. 2.5 ml (Iso 20 µg/ml) to 1000 ml extraction solvent
4. Intermediate mixed standards
   a. 50 µg/ml mixed native standard – Combine and dilute native 1 mg/ml stock standards 20:1
   b. 5.0 µg/ml mixed native standard– Dilute 50 µg/ml mixed standard 10:1
5. LC-MS/MS calibration standard 50 ng/ml – Dilute 5.0 µg/ml mixed native standard 100:1 using Iso fortified extraction solvent.

D. **Equipment and Instrumentation**
1. Genogrinder
2. Centrifuge
3. Pipettes
4. LC-MS/MS
   a. Shimadzu HPLC system: two LC-20AD pumps, Sil-20AC autosampler, CTO-20AC column oven
   NOTE: Replace all metal LC tubing with PEEK tubing between the autosampler and injection valve because glyphosate can be retained on metal surfaces.
   b. AB 6500 Q-TRAP mass spectrometer
   c. HPLC column: Phenomenex Luna C8(2), 100 Å, 5 µm, 150 x 4.6 mm, Phenomenex 00F-4249-E0
   d. HPLC guard column: Phenomenex guard column KrudKatcher P/N AFO-8497

E. **Extraction Procedure**
1. 5 g sample + 25 ml extraction solvent containing 100 ng/ml isotopes
   2 g sample plus 10 ml extraction solvent for dry products
2. Add 10 ml PE or MeCl as needed for fatty or dirty matrices
3. Shake @ 1000 for 10 min
4. Centrifuge at ≥ 3000 rpm for 5 min
5. Filter aqueous extract thru HLB SPE cartridge
6. Filter for injection (could be included with SPE step)
7. Sample concentration: 0.2 g/ml

F. **LC-MS/MS method**

	LC Parameters	**Gradient**	
Column:	Phenomenex Luna C8(2), 150 x 4.6 mm, 5 µm OR Phenomenex Luna C8, 150 x 2 mm, 5 µm, with Phenomenex KrudKatcher guard column	**Time**	**MPB**
MP A:	4 mM tetrabutlyammonium formate + 0.1 % formic acid in water (pH 2.8±0.05)	0.00	5
MP B:	MeCN	1.00	5
Flow:	0.6 mL/min (4.6 mm column)	5.00	95
	0.3 mL/min (2.0 mm column)	7.00	95

FDA Glyphosate Method

Inj Vol:	10 µL	8.00	5
Temp	45 ˚C	14.00	5

MS/MS Parameters

Q1	Q3	RT	Transition	DP	EP	CE	CXP
110	63	2.5	AMPA 1	-40	-11	-30	-9
110	79	2.5	AMPA 2	-40	-11	-34	-9
112	63	2.5	AMPA IS	-60	-11	-26	-9
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180	85	4.0	Glufosinate 3	-60	-11	-25	-9
183	63	4.0	Glufosinate IS	-60	-11	-40	-9
168	63	5.0	Glyphosate 1	-30	-11	-28	-9
168	79	5.0	Glyphosate 2	-30	-11	-56	-9
168	150	5.0	Glyphosate 3	-30	-11	-16	-9
171	63	5.0	Glyphosate IS	-30	-11	-28	-9
210	63	6.0	N-acetyl glyphosate 1	-85	-11	-40	-13
210	124	6.0	N-acetyl glyphosate 2	-85	-11	-17	-13
210	79	6.0	N-acetyl glyphosate 3	-85	-11	-50	-13

MS Parameters

CUR	25
CAD	MEDIUM
IS	-4000
GAS 1	65
GAS 2	65
TEM	450-650 ˚C
Q1	UNIT
Q3	UNIT

FDA Glyphosate Method

A. Reagents and Supplies

1. Acetonitrile, HPLC grade
2. Petroleum ether
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7. Ammonium formate
8. Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA)
9. Tetrabutylammonium hydroxide (TBAOH) titrant, 0.4 M in Water, HPLC Grade, ACROS Organics
10. Tetrabutylammonium acetate (TBuAA), Aldrich No. 335991-10G (optional)
11. Tetrabutylammonium acetate 1 M (TBuAA 1M), Aldrich No. 401803 – 50 ML (optional)
12. 50-mL plastic centrifuge tubes
13. Waters Oasis HLB SPE, 60 mg, 3cc, 30 µm
14. Extraction solvent (50 mM acetic acid/10 mM Na_2EDTA): mix 572 µL acetic acid and 0.74 g Na_2EDTA in 200-mL of purified water.
15. 50 ng/ml IS fortified extraction solvent: dilute IS 20 µg/ml mixed isotope internal standard, prepared in step C.2.a, 400:1 using extraction solvent, prepared in step A.14, e.g. 2.5 ml (IS 20 µg/ml) to 1000 ml extraction solvent
16. Mobile phase A (4 mM tetrabutylammonium formate)
    a. Add 10.0 ml of 0.4 M TBAOH to ~900 mL HPLC water, and adjust the pH to 2.8±0.05 using formic acid (~ 3 ml). OR
    b. Add 1.20 g TBA acetate in 1 L HPLC water; and adjust the pH to 2.8±0.05 using formic acid (~2 mL). OR
    c. 4 ml 1M TBuAA in 1 L HPLC water; and adjust the pH to 2.8±0.05 using formic acid (~2 mL).

B. Standard Reference Materials

1. Glyphosate
2. Glufosinate
3. AMPA
4. Glyphosate-^{13}C
5. Glufosinate-D^3
6. N-acetyl-glyphosate, available from EPA and Toronto Research Chemicals (TRC No A178245)

C. Standard Solutions

1. General instructions
    a. Unless otherwise indicated prepare standards in DI water
    b. Store standard solutions in plastic containers because glass can leach standard reference material from solution. Use of glass volumetric flasks for standard preparation is OK if solution is removed from the glassware after preparation.
    c. Do not store standards prepared with water or aqueous media in the freezer.
2. Stock standards 1 mg/ml

a. Includes all native and isotopic standards listed in Section B
b. Prepare individual stock standard for each

3. Isotopic working solutions
   a. IS 20 µg/ml mixed isotope internal standard
      i) Combine isotopes Glyphosate-^{13}C and Glufosinate-D^3
      ii) Dilute 1 mg/ml stock isotope internal standards, prepared in step C.2, 50:1
4. Intermediate mixed standards
   a. 50 µg/ml mixed native standard
      i) Combine native 1 mg/ml stock standards, prepared in step C.2
      ii) Include glyphosate, glufosinate, AMPA, and N-acetyl-glyphosate
      iii) Dilute 20:1
   b. 5.0 µg/ml mixed native standard
      i) Dilute 50 µg/ml mixed standard, prepared in step C.4.a, 10:1
   c. 1.0 µg/ml mixed native standard
      i) Dilute50 µg/ml mixed standard, prepared in step C.4.a, 50:1
5. LC-MS/MS calibration standard 50 ng/ml
   a. Dilute 5.0 µg/ml mixed native standard, prepared in step C.4.b, 100:1, using 50 ng/ml IS fortified extraction solvent

D. **Equipment and Instrumentation**

1. Genogrinder
2. Centrifuge
3. Pipettes
4. LC-MS/MS
   a. Shimadzu HPLC system: two LC-20AD pumps, Sil-20AC autosampler, CTO-20AC column oven
      NOTE: Replace all metal LC tubing with PEEK tubing between the autosampler and injection valve because glyphosate can be retained on metal surfaces.
   b. AB 6500 Q-TRAP mass spectrometer
   c. HPLC column: Phenomenex Luna C8(2), 100 Å, 5 µm, 150 x 4.6 mm, Phenomenex 00F-4249-E0
   d. HPLC guard column: Phenomenex guard column KrudKatcher P/N AFO-8497

E. **Extraction Procedure**

1. 5 g sample + 25 ml 50 ng/ml IS fortified extraction solvent prepared in step A.15
   For dry products containing less than 50 % moisture: 2 g sample plus 10 ml 50 ng/ml IS fortified extraction solvent prepared in step A.15 for dry products
2. Add 10 ml PE or MeCl as needed for fatty or dirty matrices
3. Shake @ 1000 for 10 min
4. Centrifuge at ≥ 3000 rpm for 5 min
5. Filter aqueous extract thru HLB SPE cartridge
6. Filter for injection (could be included with SPE step)
7. Sample concentration: 0.2 g/ml

F. **LC-MS/MS method**

FDA Glyphosate Method

LC Parameters		Gradient	
Column:	Phenomenex Luna C8(2), 150 x 4.6 mm, 5 µm OR Phenomenex Luna C8, 150 x 2 mm, 5 µm, with Phenomenex KrudKatcher guard column	**Time**	**MPB**
MP A:	4 mM tetrabutlyammonium formate + 0.1 % formic acid in water (pH 2.8±0.05)	0.00	5
MP B:	MeCN	1.00	5
Flow:	0.45 mL/min (4.6 mm column)	5.00	90
	0.3 mL/min (2.0 mm column)	7.00	90
Inj Vol:	10 µL	8.00	5
Temp	40 ˚C	14.00	5

MS/MS Parameters

Q1	Q3	RT	Transition	DP	EP	CE	CXP
110	63	2.5	AMPA 1	-40	-11	-30	-9
110	79	2.5	AMPA 2	-40	-11	-34	-9
112	63	2.5	AMPA IS	-60	-11	-26	-9
180	63	4.0	Glufosinate 1	-60	-11	-66	-9
180	95	4.0	Glufosinate 2	-40	-11	-19	-5
180	85	4.0	Glufosinate 3	-60	-11	-25	-9
183	63	4.0	Glufosinate IS	-60	-11	-40	-9
168	63	5.0	Glyphosate 1	-30	-11	-28	-9
168	79	5.0	Glyphosate 2	-30	-11	-56	-9
168	150	5.0	Glyphosate 3	-30	-11	-16	-9
171	63	5.0	Glyphosate IS	-30	-11	-28	-9
210	63	6.0	N-acetyl glyphosate 1	-85	-11	-40	-13
210	124	6.0	N-acetyl glyphosate 2	-85	-11	-17	-13
210	79	6.0	N-acetyl glyphosate 3	-85	-11	-50	-13

MS Parameters

CUR	25
CAD	MEDIUM
IS	-4000
GAS 1	65
GAS 2	65
TEM	450-650 ˚C

FDA Glyphosate Method

Q1 UNIT

Q3 UNIT

FDA Glyphosate Method

A. Reagents and Supplies

1. Acetonitrile, HPLC grade
2. Petroleum ether
3. Methylene chloride
4. Water, HPLC grade
5. Formic acid, 98% solution
6. Acetic acid
7. Ammonium formate
8. Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA)
9. Tetrabutylammonium hydroxide (TBAOH) titrant, 0.4 M in Water, HPLC Grade, ACROS Organics
10. Tetrabutylammonium acetate (TBuAA), Aldrich No. 335991-10G (optional)
11. Tetrabutylammonium acetate 1 M (TBuAA 1M), Aldrich No. 401803 – 50 ML (optional)
12. 50-mL plastic centrifuge tubes
13. Waters Oasis HLB SPE, 60 mg, 3cc, 30 µm
14. Extraction solvent (50 mM acetic acid/10 mM Na_2EDTA): mix 572 µL acetic acid and 0.74 g Na_2EDTA in 200-mL of purified water.
15. 50 ng/ml IS fortified extraction solvent: dilute IS 20 µg/ml mixed isotope internal standard, prepared in step C.2.a, 400:1 using extraction solvent, prepared in step A.14, e.g. 2.5 ml (IS 20 µg/ml) to 1000 ml extraction solvent
16. Mobile phase A (4 mM tetrabutylammonium formate)
    a. Add 10.0 ml of 0.4 M TBAOH to ~900 mL HPLC water, and adjust the pH to 2.8±0.05 using formic acid (~ 3 ml). OR
    b. Add 1.20 g TBA acetate in 1 L HPLC water; and adjust the pH to 2.8±0.05 using formic acid (~2 mL). OR
    c. 4 ml 1M TBuAA in 1 L HPLC water; and adjust the pH to 2.8±0.05 using formic acid (~2 mL).

B. Standard Reference Materials

1. Glyphosate
2. Glufosinate
3. AMPA
4. N-acetyl-glyphosate, available from EPA and Toronto Research Chemicals (TRC No A178245)
5. Glyphosate-^{13}C
6. Glufosinate-D^3

C. Standard Solutions

1. General instructions
    a. Unless otherwise indicated prepare standards in DI water
    b. Store standard solutions in plastic containers because glass can leach standard reference material from solution. Use of glass volumetric flasks for standard preparation is OK if solution is removed from the glassware after preparation.
    c. Do not store standards prepared with water or aqueous media in the freezer.
2. Stock standards 1 mg/ml

   a. Includes all native and isotopic standards listed in Section B
   b. Prepare individual stock standard for each compound
3. Isotopic working solutions
   a. IS 20 µg/ml mixed isotope internal standard
      i) Combine isotopes Glyphosate-^{13}C and Glufosinate-D^3 (step B.5 & 6)
      ii) Dilute 1 mg/ml stock isotope internal standards, prepared in step C.2, 50:1
4. Intermediate mixed standards
   a. 50 µg/ml mixed native standard
      i) Combine native 1 mg/ml stock standards, prepared in step C.2
      ii) Include glyphosate, glufosinate, AMPA, and N-acetyl-glyphosate (Step B.1-4)
      iii) Dilute 20:1
   b. 5.0 µg/ml mixed native standard
      i) Dilute 50 µg/ml mixed standard, prepared in step C.4.a, 10:1
   c. 1.0 µg/ml mixed native standard
      i) Dilute50 µg/ml mixed standard, prepared in step C.4.a, 50:1
5. LC-MS/MS calibration standard 50 ng/ml
   a. Dilute 5.0 µg/ml mixed native standard, prepared in step C.4.b, 100:1, using 50 ng/ml IS fortified extraction solvent

D. **Equipment and Instrumentation**
1. Genogrinder
2. Centrifuge
3. Pipettes
4. LC-MS/MS
   a. Shimadzu HPLC system: two LC-20AD pumps, Sil-20AC autosampler, CTO-20AC column oven
      NOTE: Replace all metal LC tubing with PEEK tubing between the autosampler and injection valve because glyphosate can be retained on metal surfaces.
   b. AB 6500 Q-TRAP mass spectrometer
   c. HPLC column: Phenomenex Luna C8(2), 100 Å, 5 µm, 150 x 4.6 mm, Phenomenex 00F-4249-E0
   d. HPLC guard column: Phenomenex guard column KrudKatcher P/N AFO-8497

E. **Extraction Procedure**
1. 5 g sample + 25 ml 50 ng/ml IS fortified extraction solvent prepared in step A.15
   For dry products containing less than 50 % moisture: 2 g sample plus 10 ml 50 ng/ml IS fortified extraction solvent prepared in step A.15 for dry products
2. Add 10 ml PE or MeCl as needed for fatty or dirty matrices
3. Shake @ 1000 for 10 min
4. Centrifuge at ≥ 3000 rpm for 5 min
5. Filter aqueous extract thru HLB SPE cartridge
6. Filter for injection (could be included with SPE step)
7. Sample concentration: 0.2 g/ml

F. **LC-MS/MS method**

FDA Glyphosate Method

A. Reagents and Supplies

1. Acetonitrile, HPLC grade
2. Petroleum ether
3. Methylene chloride
4. Water, HPLC grade
5. Formic acid, 98% solution
6. Acetic acid
7. Ammonium formate
8. Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA)
9. Tetrabutylammonium hydroxide (TBAOH) titrant, 0.4 M in Water, HPLC Grade, ACROS Organics
10. Tetrabutylammonium acetate (TBuAA), Aldrich No. 335991-10G (optional)
11. Tetrabutylammonium acetate 1 M (TBuAA 1M), Aldrich No. 401803 – 50 ML (optional)
12. 50-mL plastic centrifuge tubes
13. Nylon filter, 2 µm, 25 mm, Whatman GD/X 25
14. Waters Oasis HLB SPE, 60 mg, 3cc, 30 µm
15. Extraction solvent (50 mM acetic acid/10 mM Na_2EDTA): mix 2.9 mL acetic acid and 3.7 g Na_2EDTA in 200-mL of purified water.
16. 50 ng/ml IS fortified extraction solvent: dilute IS 20 µg/ml mixed isotope internal standard, prepared in step C.2.a, 400:1 using extraction solvent, prepared in step A.15, e.g. 2.5 ml (IS 20 µg/ml) to 1000 ml extraction solvent
17. Mobile phase A (4 mM tetrabutylammonium formate)
    a. Add 10.0 ml of 0.4 M TBAOH to ~900 mL HPLC water, and adjust the pH to 2.8±0.05 using formic acid (~ 3 ml). OR
    b. Add 1.20 g TBA acetate in 1 L HPLC water; and adjust the pH to 2.8±0.05 using formic acid (~2 mL). OR
    c. 4 ml 1M TBuAA in 1 L HPLC water; and adjust the pH to 2.8±0.05 using formic acid (~2 mL).

B. Standard Reference Materials

1. Glyphosate
2. Glufosinate
3. AMPA
4. N-acetyl-glyphosate, available from EPA and Toronto Research Chemicals (TRC No A178245)
5. Glyphosate-^{13}C
6. Glufosinate-D^3

C. Standard Solutions

1. General instructions
    a. Unless otherwise indicated prepare standards in DI water
    b. Store standard solutions in plastic containers because glass can leach standard reference material from solution. Use of glass volumetric flasks for standard preparation is OK if solution is removed from the glassware after preparation.
    c. Do not store standards prepared with water or aqueous media in the freezer.

2. Stock standards 1 mg/ml
   a. Includes all native and isotopic standards listed in Section B
   b. Prepare individual stock standard for each compound
3. Isotopic working solutions
   a. IS 20 µg/ml mixed isotope internal standard
      i) Combine isotopes Glyphosate-^{13}C and Glufosinate-D^3 (step B.5 & 6)
      ii) Dilute 1 mg/ml stock isotope internal standards, prepared in step C.2, 50:1
4. Intermediate mixed standards
   a. 50 µg/ml mixed native standard
      i) Combine native 1 mg/ml stock standards, prepared in step C.2
      ii) Include glyphosate, glufosinate, AMPA, and N-acetyl-glyphosate (Step B.1-4)
      iii) Dilute 20:1
   b. 5.0 µg/ml mixed native standard
      i) Dilute 50 µg/ml mixed standard, prepared in step C.4.a, 10:1
   c. 1.0 µg/ml mixed native standard
      i) Dilute50 µg/ml mixed standard, prepared in step C.4.a, 50:1
5. LC-MS/MS calibration standard 50 ng/ml
   a. Dilute 5.0 µg/ml mixed native standard, prepared in step C.4.b, 100:1, using 50 ng/ml IS fortified extraction solvent (A.16)

D. **Equipment and Instrumentation**
1. Genogrinder
2. Centrifuge
3. Pipettes
4. LC-MS/MS
   a. Shimadzu HPLC system: two LC-20AD pumps, Sil-20AC autosampler, CTO-20AC column oven
      NOTE: Replace all metal LC tubing with PEEK tubing between the autosampler and injection valve because glyphosate can be retained on metal surfaces.
   b. AB model 5500, or 6500, Q-TRAP mass spectrometer
   c. HPLC column: Phenomenex Luna C8(2), 100 Å, 5 µm, 150 x 4.6 mm, Phenomenex 00F-4249-E0
   d. HPLC guard column: Phenomenex guard column KrudKatcher P/N AFO-8497

      NOTE: Install peek tubing between the autosampler and column because metal can affect glyphosate and glufosinate chromatography

E. **Extraction Procedure**
1. 5 g sample + 25 ml 50 ng/ml IS fortified extraction solvent prepared in step A.15
   For dry products containing less than 50 % moisture: 2 g sample plus 10 ml 50 ng/ml IS fortified extraction solvent prepared in step A.15 for dry products
2. Add 10 ml PE or MeCl as needed for fatty or dirty matrices
3. Shake @ 1000 for 10 min
4. Centrifuge at ≥ 3000 rpm for 5 min
5. Filter aqueous extract thru HLB SPE cartridge
6. Filter for injection (could be included with SPE step)

7. Sample concentration: 0.2 g/ml

F. **LC-MS/MS method**

LC Parameters		Gradient	
Column:	Phenomenex Luna C8(2), 150 x 4.6 mm, 5 µm OR Phenomenex Luna C8, 150 x 2 mm, 5 µm, with Phenomenex KrudKatcher guard column	**Time**	**MPB**
MP A:	4 mM tetrabutlyammonium formate + 0.1 % formic acid in water (pH 2.8±0.05)	0.00	5
MP B:	MeCN	1.00	5
Flow:	0.45 mL/min (4.6 mm column)	5.00	90
	0.3 mL/min (2.0 mm column)	7.00	90
Inj Vol:	10 µL	8.00	5
Temp	40 °C	14.00	5

MS/MS Parameters (5500 & 6500)

Q1	Q3	RT	Transition	DP	EP	CE	CXP
110	63	2.5	AMPA 1	-40	-11	-30	-9
110	79	2.5	AMPA 2	-40	-11	-34	-9
110	81	2.5	AMPA 3	-40	-11	-34	-9
112	63	2.5	AMPA IS	-60	-11	-26	-9
180	63	4.0	Glufosinate 1	-60	-11	-66	-9
180	95	4.0	Glufosinate 2	-40	-11	-19	-5
180	85	4.0	Glufosinate 3	-60	-11	-25	-9
183	63	4.0	Glufosinate IS	-60	-11	-40	-9
168	63	5.0	Glyphosate 1	-30	-11	-28	-9
168	79	5.0	Glyphosate 2	-30	-11	-56	-9
168	150	5.0	Glyphosate 3	-30	-11	-16	-9
171	63	5.0	Glyphosate IS	-30	-11	-28	-9
210	63	6.0	N-acetyl glyphosate 1	-85	-11	-40	-13
210	124	6.0	N-acetyl glyphosate 2	-85	-11	-17	-13
210	79	6.0	N-acetyl glyphosate 3	-85	-11	-50	-13

MS Parameters

CUR	25
CAD	MEDIUM

FDA Glyphosate Method

IS	-4000
GAS 1	65
GAS 2	65
TEM	450 ˚C (6500) 650 ˚C (5500)
Q1	UNIT
Q3	UNIT

FDA Glyphosate Method

A. Reagents and Supplies

1. Acetonitrile, HPLC grade
2. Petroleum ether
3. Methylene chloride
4. Water, HPLC grade
5. Formic acid, 98% solution
6. Acetic acid
7. Ammonium formate
8. Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA)
9. Tetrabutylammonium hydroxide (TBAOH) titrant, 0.4 M in Water, HPLC Grade, ACROS Organics
10. Tetrabutylammonium acetate (TBuAA), Aldrich No. 335991-10G (optional)
11. Tetrabutylammonium acetate 1 M (TBuAA 1M), Aldrich No. 401803 – 50 ML (optional)
12. 50-mL plastic centrifuge tubes
13. Nylon filter, 2 µm, 25 mm, Whatman GD/X 25
14. Waters Oasis HLB SPE, 60 mg, 3cc, 30 µm
15. Extraction solvent (50 mM acetic acid/10 mM Na_2EDTA): mix 2.9 mL acetic acid and 3.7 g Na_2EDTA in 200-mL of purified water.
16. 50 ng/ml IS fortified extraction solvent: dilute IS 20 µg/ml mixed isotope internal standard, prepared in step C.2.a, 1:400 using extraction solvent, prepared in step A.15, e.g. 2.5 ml (IS 20 µg/ml) to 1000 ml extraction solvent
17. Mobile phase A (4 mM tetrabutylammonium formate)
    a. Add 10.0 ml of 0.4 M TBAOH to ~900 mL HPLC water, and adjust the pH to 2.8±0.05 using formic acid (~ 3 ml). OR
    b. Add 1.20 g TBA acetate in 1 L HPLC water; and adjust the pH to 2.8±0.05 using formic acid (~2 mL). OR
    c. 4 ml 1M TBuAA in 1 L HPLC water; and adjust the pH to 2.8±0.05 using formic acid (~2 mL).

B. Standard Reference Materials

1. Glyphosate
2. Glufosinate
3. AMPA
4. N-acetyl-glyphosate, available from EPA and Toronto Research Chemicals (TRC No A178245)
5. Glyphosate-^{13}C
6. Glufosinate-D^3

C. Standard Solutions

1. General instructions
    a. Unless otherwise indicated prepare standards in DI water
    b. Store standard solutions in plastic containers because glass can leach standard reference material from solution. Use of glass volumetric flasks for standard preparation is OK if solution is removed from the glassware after preparation.
    c. Do not store standards prepared with water or aqueous media in the freezer.

2. Stock standards 1 mg/ml
   a. Includes all native and isotopic standards listed in Section B
   b. Prepare individual stock standard for each compound
3. Isotopic working solutions
   a. IS 20 µg/ml mixed isotope internal standard
      i) Combine isotopes Glyphosate-^{13}C and Glufosinate-D^3 (step B.5 & 6)
      ii) Dilute 1 mg/ml stock isotope internal standards, prepared in step C.2, 1:50
4. Intermediate mixed standards
   a. 50 µg/ml mixed native standard
      i) Combine native 1 mg/ml stock standards, prepared in step C.2
      ii) Include glyphosate, glufosinate, AMPA, and N-acetyl-glyphosate (Step B.1-4)
      iii) Dilute 1:20
   b. 5.0 µg/ml mixed native standard
      i) Dilute 50 µg/ml mixed standard, prepared in step C.4.a, 1:10
   c. 1.0 µg/ml mixed native standard
      i) Dilute50 µg/ml mixed standard, prepared in step C.4.a, 1:50
5. LC-MS/MS calibration standard 50 ng/ml
   a. Dilute 5.0 µg/ml mixed native standard, prepared in step C.4.b, 1:100, using 50 ng/ml IS fortified extraction solvent (A.16)

D. **Equipment and Instrumentation**
1. Genogrinder
2. Centrifuge
3. Pipettes
4. LC-MS/MS
   a. Shimadzu HPLC system: two LC-20AD pumps, Sil-20AC autosampler, CTO-20AC column oven
      NOTE: Replace all metal LC tubing with PEEK tubing between the autosampler and injection valve because glyphosate can be retained on metal surfaces.
   b. AB model 5500, or 6500, Q-TRAP mass spectrometer
   c. HPLC column: Phenomenex Luna C8(2), 100 Å, 5 µm, 150 x 4.6 mm, Phenomenex 00F-4249-E0
   d. HPLC guard column: Phenomenex guard column KrudKatcher P/N AFO-8497

      NOTE: Install peek tubing between the autosampler and column because metal can affect glyphosate and glufosinate chromatography

E. **Extraction Procedure**
1. 5 g sample + 25 ml 50 ng/ml IS fortified extraction solvent prepared in step A.15
   For dry products containing less than 50 % moisture: 2 g sample plus 10 ml 50 ng/ml IS fortified extraction solvent prepared in step A.15 for dry products
2. Add 10 ml PE or $MeCl_2$ as needed for fatty or dirty matrices
3. Shake @ 1000 for 10 min
4. Centrifuge at ≥ 3000 rpm for 5 min
5. Filter aqueous extract thru HLB SPE cartridge
6. Filter for injection (could be included with SPE step)

7. Sample concentration: 0.2 g/ml

F. **LC-MS/MS method**

LC Parameters		Gradient	
Column:	Phenomenex Luna C8(2), 150 x 4.6 mm, 5 µm OR Phenomenex Luna C8, 150 x 2 mm, 5 µm, with Phenomenex KrudKatcher guard column	**Time**	**MPB**
MP A:	4 mM tetrabutlyammonium formate + 0.1 % formic acid in water (pH 2.8±0.05)	0.00	5
MP B:	MeCN	1.00	5
Flow:	0.45 mL/min (4.6 mm column)	5.00	90
	0.3 mL/min (2.0 mm column)	7.00	90
Inj Vol:	10 µL	8.00	5
Temp	40 °C	14.00	5

MS/MS Parameters (5500 & 6500)

Q1	Q3	RT	Transition	DP	EP	CE	CXP
110	63	2.5	AMPA 1	-40	-11	-30	-9
110	79	2.5	AMPA 2	-40	-11	-34	-9
110	81	2.5	AMPA 3	-40	-11	-34	-9
112	63	2.5	AMPA IS	-60	-11	-26	-9
180	63	4.0	Glufosinate 1	-60	-11	-66	-9
180	95	4.0	Glufosinate 2	-40	-11	-19	-5
180	85	4.0	Glufosinate 3	-60	-11	-25	-9
183	63	4.0	Glufosinate IS	-60	-11	-40	-9
168	63	5.0	Glyphosate 1	-30	-11	-28	-9
168	79	5.0	Glyphosate 2	-30	-11	-56	-9
168	150	5.0	Glyphosate 3	-30	-11	-16	-9
171	63	5.0	Glyphosate IS	-30	-11	-28	-9
210	63	6.0	N-acetyl glyphosate 1	-85	-11	-40	-13
210	124	6.0	N-acetyl glyphosate 2	-85	-11	-17	-13
210	79	6.0	N-acetyl glyphosate 3	-85	-11	-50	-13

MS Parameters

CUR	(b) (5)
CAD	MEDIUM

FDA Glyphosate Method

A. Reagents and Supplies

1. Acetonitrile, HPLC grade
2. Petroleum ether
3. Methylene chloride
4. Water, HPLC grade
5. Formic acid, 98% solution
6. Acetic acid
7. Ammonium formate
8. Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA)
9. Tetrabutylammonium hydroxide (TBAOH) titrant, 0.4 M in Water, HPLC Grade, ACROS Organics
10. Tetrabutylammonium acetate (TBuAA), Aldrich No. 335991-10G (optional)
11. Tetrabutylammonium acetate 1 M (TBuAA 1M), Aldrich No. 401803 – 50 ML (optional)
12. 50-mL plastic centrifuge tubes
13. Filter, (b)(5) µm, (b)(5) mm,
14. Waters Oasis HLB SPE, 60 mg, 3cc, 30 µm
15. Extraction solvent (50 mM acetic acid/10 mM Na_2EDTA): mix 2.9 mL acetic acid and 3.7 g Na_2EDTA in 200-mL of purified water.
16. 50 ng/ml IS fortified extraction solvent: dilute IS 20 µg/ml mixed isotope internal standard, prepared in step C.2.a, 1:400 using extraction solvent, prepared in step A.15, e.g. 2.5 ml (IS 20 µg/ml) to 1000 ml extraction solvent
17. Mobile phase A (4 mM tetrabutylammonium formate)
    a. Add 10.0 ml of 0.4 M TBAOH to ~900 mL HPLC water, and adjust the pH to 2.8±0.05 using formic acid (~ 3 ml). OR
    b. Add 1.20 g TBA acetate in 1 L HPLC water; and adjust the pH to 2.8±0.05 using formic acid (~2 mL). OR
    c. 4 ml 1M TBuAA in 1 L HPLC water; and adjust the pH to 2.8±0.05 using formic acid (~2 mL).

B. Standard Reference Materials

1. Glyphosate
2. Glufosinate
3. AMPA
4. N-acetyl-glyphosate, available from EPA and Toronto Research Chemicals (TRC No A178245)
5. Glyphosate-^{13}C
6. Glufosinate-D^3

C. Standard Solutions

1. General instructions
    a. Unless otherwise indicated prepare standards in DI water
    b. Store standard solutions in plastic containers because glass can leach standard reference material from solution. Use of glass volumetric flasks for standard preparation is OK if solution is removed from the glassware after preparation.
    c. Do not store standards prepared with water or aqueous media in the freezer.

FDA Glyphosate Method

A. Reagents and Supplies

1. Acetonitrile, HPLC grade
2. Petroleum ether
3. Methylene chloride
4. Water, HPLC grade
5. Formic acid, 98% solution
6. Acetic acid
7. Ammonium formate
8. Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA)
9. Tetrabutylammonium hydroxide (TBAOH) titrant, 0.4 M in Water, HPLC Grade, ACROS Organics
10. Tetrabutylammonium acetate (TBuAA), Aldrich No. 335991-10G (optional)
11. Tetrabutylammonium acetate 1 M (TBuAA 1M), Aldrich No. 401803 – 50 ML (optional)
12. 50-mL plastic centrifuge tubes
13. Filter, 0.2 µm, 25 mm, nylon
14. Waters Oasis HLB SPE, 60 mg, 3cc, 30 µm
15. Extraction solvent (50 mM acetic acid/10 mM Na_2EDTA): mix 2.9 mL acetic acid and 3.7 g Na_2EDTA in 1000-mL of purified water.
16. 50 ng/ml IS fortified extraction solvent: dilute IS 20 µg/ml mixed isotope internal standard, prepared in step C.2.a, 1:400 using extraction solvent, prepared in step A.15, e.g. 2.5 ml (IS 20 µg/ml) to 1000 ml extraction solvent
17. Mobile phase A (4 mM tetrabutylammonium formate)
    a. Add 10.0 ml of 0.4 M TBAOH to ~900 mL HPLC water, and adjust the pH to 2.8±0.05 using formic acid (~ 3 ml). OR
    b. Add 1.20 g TBA acetate in 1 L HPLC water; and adjust the pH to 2.8±0.05 using formic acid (~2 mL). OR
    c. 4 ml 1M TBuAA in 1 L HPLC water; and adjust the pH to 2.8±0.05 using formic acid (~2 mL).

B. Standard Reference Materials

1. Glyphosate
2. Glufosinate
3. AMPA
4. N-acetyl-glyphosate, available from EPA and Toronto Research Chemicals (TRC No A178245)
5. Glyphosate-^{13}C
6. Glufosinate-D_3

C. Standard Solutions

1. General instructions
    a. Unless otherwise indicated prepare standards in DI water
    b. Store standard solutions in plastic containers because glass can leach standard reference material from solution. Use of glass volumetric flasks for standard preparation is OK if solution is removed from the glassware after preparation.

c. Store standard solutions in a refrigerator. Do not store standards prepared with water or aqueous media in the freezer.

2. Stock standards 1 mg/ml
   a. Includes all native and isotopic standards listed in Section B
   b. Prepare individual stock standard for each compound
3. Isotopic working solutions
   a. IS 20 µg/ml mixed isotope internal standard
      i) Combine isotopes Glyphosate-^{13}C and Glufosinate-D^3 (step B.5 & 6)
      ii) Dilute 1 mg/ml stock isotope internal standards, prepared in step C.2, 1:50
4. Intermediate mixed standards
   a. 50 µg/ml mixed native standard
      i) Combine native 1 mg/ml stock standards, prepared in step C.2
      ii) Include glyphosate, glufosinate, AMPA, and N-acetyl-glyphosate (Step B.1-4)
      iii) Dilute 1:20
   b. 5.0 µg/ml mixed native standard
      i) Dilute 50 µg/ml mixed standard, prepared in step C.4.a, 1:10
   c. 1.0 µg/ml mixed native standard
      i) Dilute50 µg/ml mixed standard, prepared in step C.4.a, 1:50
5. LC-MS/MS calibration standard 50 ng/ml
   a. Dilute 5.0 µg/ml mixed native standard, prepared in step C.4.b, 1:100, using 50 ng/ml IS fortified extraction solvent (A.16)

D. **Equipment and Instrumentation**

1. Genogrinder
2. Centrifuge
3. Pipettes
4. LC-MS/MS
   a. Shimadzu HPLC system: two LC-20AD pumps, Sil-20AC autosampler, CTO-20AC column oven
      NOTE: Replace all metal LC tubing with PEEK tubing between the autosampler and injection valve because glyphosate can be retained on metal surfaces.
   b. AB model 5500, or 6500, Q-TRAP mass spectrometer
   c. HPLC column: Phenomenex Luna C8(2), 100 Å, 5 µm, 150 x 4.6 mm, Phenomenex 00F-4249-E0; Or, Phenomenex Luna C8, 100 Å, 5 µm, 150 x 2 mm, Phenomenex 00F-4040-B0
   d. HPLC guard column: Phenomenex guard column KrudKatcher P/N AFO-8497

      NOTE: Install peek tubing between the autosampler and column because metal can affect glyphosate and glufosinate chromatography

E. **Extraction Procedure**

1. 5 g sample + 25 ml 50 ng/ml IS fortified extraction solvent prepared in step A.15
   For dry products containing less than 50 % moisture: 2 g sample plus 10 ml 50 ng/ml IS fortified extraction solvent prepared in step A.15 for dry products
2. Add 10 ml PE, or $MeCl_2$, for matrices containing more than 3 % fat.
3. Shake @ 1000 shakes per min for 10 min

4. Centrifuge at ≥ 3000 rpm for 5 min to achieve phase separation. NOTE: When using PE to remove lipid co-extractants high fat matrices, the PE will be the top layer. When using $MeCl_2$, the $MeCl_2$ will be the bottom layer.
5. Filter aqueous extract thru unconditioned HLB SPE cartridge, limit filter volume to less than 2 mls. Note: When using PE cleanup withdraw the aqueous extract from below the top PE layer.
6. Filter for injection (could be included with SPE step)
7. Sample concentration: 0.2 g/ml; i.e. 5g/25 ml or 2g/10 ml (for dry products)

F. **LC-MS/MS Method**

	LC Parameters	**Gradient**	
Column :	Phenomenex Luna C8(2), 150 x 4.6 mm, 5 µm OR Phenomenex Luna C8, 150 x 2 mm, 5 µm Guard column: Phenomenex KrudKatcher	**Time**	**MPB**
MP A:	4 mM tetrabutylammonium formate + 0.1 % formic acid in water (pH 2.8±0.05)	0.00	5
MP B:	MeCN	1.00	5
Flow:	0.45 mL/min (4.6 mm column)	5.00	90
	0.3 mL/min (2.0 mm column)	7.00	90
Inj Vol:	10 µL	8.00	5
Temp	40 °C	14.00	5
Divert Valve	Divert flow from mass spectrometer about 30 seconds before the first analyte and 60 seconds after the last analyte elutes		

MS/MS Parameters (5500 & 6500)

Q1	Q3	RT	Transition	DP*	EP	CE	CXP
110	63	1.3	AMPA 1	-40	-11	-30	-9
110	79	1.3	AMPA 2	-40	-11	-34	-9
110	81	1.3	AMPA 3	-40	-11	-34	-9
112	63	2.5	AMPA IS	-60	-11	-26	-9
180	63	3.0	Glufosinate 1	-60	-11	-66	-9
180	95	3.0	Glufosinate 2	-40	-11	-19	-5
180	85	3.0	Glufosinate 3	-60	-11	-25	-9
183	63	3.0	Glufosinate IS	-60	-11	-40	-9
168	63	4.4	Glyphosate 1	-30	-11	-28	-9
168	79	4.4	Glyphosate 2	-30	-11	-56	-9
168	150	4.4	Glyphosate 3	-30	-11	-16	-9
171	63	4.4	Glyphosate IS	-30	-11	-28	-9

210	150	5.3	N-acetyl glyphosate 1	-20 (-40)	-11	-20	-13
210	63	5.3	N-acetyl glyphosate 2	-20 (-40)	-11	-40	-13
210	168	5.3	N-acetyl glyphosate 3	-20 (-40)	-11	-18	-13

**DP: if more than one DP is provided the first is optimized for the 6500 and the DP in () is optimized for the 5500*

MS Parameters

Ionization:	Ionspray in negative ionization mode		
CUR:	35	TEM:	450 °C (6500)
CAD:	medium		650 °C (5500)
IS:	-4000	Q1:	unit
GAS 1 & 2:	65	Q3:	unit

G. **Quantitation of Residues**

1. Calibrate instrument using single level calibration standard at 50 ng/ml
2. Calibrate using internal standard calibration for glyphosate, glufosinate and AMPA
   a. Assign internal standard calibration standards
      i) Glyphosate: Glyphosate-^{13}C
      ii) Glufosinate: Glufosinate-D_3
      iii) AMPA: Glyphosate-^{13}C
3. Calibrate using external calibration for N-acetylglyphosate
4. Reportable residues must meet the identification criteria provided in Appendix A "Identification of Residues" in ORA-LAB.10
5. Quantitate residues per instructions in Appendix B "Quantitation of Residues" in ORA-LAB.10. Give preference to quantitation using the primary MS/MS transition, e.g. "Glyphosate 1", however, use of secondary transitions for quantitation may be advisable if/when matrix coextractants interfere with the primary transition response.

7. Sample concentration: 0.2 g/ml

F. **LC-MS/MS method**

LC Parameters		Gradient	
Column:	Phenomenex Luna C8(2), 150 x 4.6 mm, 5 µm OR Phenomenex Luna C8, 150 x 2 mm, 5 µm, with Phenomenex KrudKatcher guard column	**Time**	**MPB**
MP A:	4 mM tetrabutlyammonium formate + 0.1 % formic acid in water (pH 2.8±0.05)	0.00	5
MP B:	MeCN	1.00	5
Flow:	0.45 mL/min (4.6 mm column)	5.00	90
	0.3 mL/min (2.0 mm column)	7.00	90
Inj Vol:	10 µL	8.00	5
Temp	40 °C	14.00	5

MS/MS Parameters (5500 & 6500)

Q1	Q3	RT	Transition	DP	EP	CE	CXP
110	63	2.5	AMPA 1	-40	-11	-30	-9
110	79	2.5	AMPA 2	-40	-11	-34	-9
110	81	2.5	AMPA 3	-40	-11	-34	-9
112	63	2.5	AMPA IS	-60	-11	-26	-9
180	63	4.0	Glufosinate 1	-60	-11	-66	-9
180	95	4.0	Glufosinate 2	-40	-11	-19	-5
180	85	4.0	Glufosinate 3	-60	-11	-25	-9
183	63	4.0	Glufosinate IS	-60	-11	-40	-9
168	63	5.0	Glyphosate 1	-30	-11	-28	-9
168	79	5.0	Glyphosate 2	-30	-11	-56	-9
168	150	5.0	Glyphosate 3	-30	-11	-16	-9
171	63	5.0	Glyphosate IS	-30	-11	-28	-9

(b) (5)

MS Parameters

CUR (b) (5)

CAD MEDIUM

FDA Glyphosate Method

A. Reagents and Supplies

1. Acetonitrile, HPLC grade
2. Petroleum ether
3. Methylene chloride
4. Water, HPLC grade
5. Formic acid, 98% solution
6. Acetic acid
7. Ammonium formate
8. Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA)
9. Tetrabutylammonium hydroxide (TBAOH) titrant, 0.4 M in Water, HPLC Grade, ACROS Organics
10. Tetrabutylammonium acetate (TBuAA), Aldrich No. 335991-10G (optional)
11. Tetrabutylammonium acetate 1 M (TBuAA 1M), Aldrich No. 401803 – 50 ML (optional)
12. 50-mL plastic centrifuge tubes
13. Filter, 2 µm, 25 mm,
14. Waters Oasis HLB SPE, 60 mg, 3cc, 30 µm
15. Extraction solvent (50 mM acetic acid/10 mM Na_2EDTA): mix 2.9 mL acetic acid and 3.7 g Na_2EDTA in 1000-mL of purified water.
16. 50 ng/ml IS fortified extraction solvent: dilute IS 20 µg/ml mixed isotope internal standard, prepared in step C.2.a, 1:400 using extraction solvent, prepared in step A.15, e.g. 2.5 ml (IS 20 µg/ml) to 1000 ml extraction solvent
17. Mobile phase A (4 mM tetrabutylammonium formate)
    a. Add 10.0 ml of 0.4 M TBAOH to ~900 mL HPLC water, and adjust the pH to 2.8±0.05 using formic acid (~ 3 ml). OR
    b. Add 1.20 g TBA acetate in 1 L HPLC water; and adjust the pH to 2.8±0.05 using formic acid (~2 mL). OR
    c. 4 ml 1M TBuAA in 1 L HPLC water; and adjust the pH to 2.8±0.05 using formic acid (~2 mL).

B. Standard Reference Materials

1. Glyphosate
2. Glufosinate
3. AMPA
4. N-acetyl-glyphosate, available from EPA and Toronto Research Chemicals (TRC No A178245)
5. Glyphosate-^{13}C
6. Glufosinate-D^3

C. Standard Solutions

1. General instructions
    a. Unless otherwise indicated prepare standards in DI water
    b. Store standard solutions in plastic containers because glass can leach standard reference material from solution. Use of glass volumetric flasks for standard preparation is OK if solution is removed from the glassware after preparation.
    c. Do not store standards prepared with water or aqueous media in the freezer.

2. Stock standards 1 mg/ml
   a. Includes all native and isotopic standards listed in Section B
   b. Prepare individual stock standard for each compound
3. Isotopic working solutions
   a. IS 20 µg/ml mixed isotope internal standard
      i) Combine isotopes Glyphosate-^{13}C and Glufosinate-D^3 (step B.5 & 6)
      ii) Dilute 1 mg/ml stock isotope internal standards, prepared in step C.2, 1:50
4. Intermediate mixed standards
   a. 50 µg/ml mixed native standard
      i) Combine native 1 mg/ml stock standards, prepared in step C.2
      ii) Include glyphosate, glufosinate, AMPA, and N-acetyl-glyphosate (Step B.1-4)
      iii) Dilute 1:20
   b. 5.0 µg/ml mixed native standard
      i) Dilute 50 µg/ml mixed standard, prepared in step C.4.a, 1:10
   c. 1.0 µg/ml mixed native standard
      i) Dilute50 µg/ml mixed standard, prepared in step C.4.a, 1:50
5. LC-MS/MS calibration standard 50 ng/ml
   a. Dilute 5.0 µg/ml mixed native standard, prepared in step C.4.b, 1:100, using 50 ng/ml IS fortified extraction solvent (A.16)

D. **Equipment and Instrumentation**
1. Genogrinder
2. Centrifuge
3. Pipettes
4. LC-MS/MS
   a. Shimadzu HPLC system: two LC-20AD pumps, Sil-20AC autosampler, CTO-20AC column oven
      NOTE: Replace all metal LC tubing with PEEK tubing between the autosampler and injection valve because glyphosate can be retained on metal surfaces.
   b. AB model 5500, or 6500, Q-TRAP mass spectrometer
   c. HPLC column: Phenomenex Luna C8(2), 100 Å, 5 µm, 150 x 4.6 mm, Phenomenex 00F-4249-E0
   d. HPLC guard column: Phenomenex guard column KrudKatcher P/N AFO-8497

      NOTE: Install peek tubing between the autosampler and column because metal can affect glyphosate and glufosinate chromatography

E. **Extraction Procedure**
1. 5 g sample + 25 ml 50 ng/ml IS fortified extraction solvent prepared in step A.15
   For dry products containing less than 50 % moisture: 2 g sample plus 10 ml 50 ng/ml IS fortified extraction solvent prepared in step A.15 for dry products
2. Add 10 ml PE, or $MeCl_2$, as needed for fatty or dirty matrices
3. Shake @ 1000 for 10 min
4. Centrifuge at ≥ 3000 rpm for 5 min
5. Filter aqueous extract thru HLB SPE cartridge, limit filter volume to less than 2 mls.
6. Filter for injection (could be included with SPE step)

7. Sample concentration: 0.2 g/ml

F. **LC-MS/MS method**

LC Parameters		Gradient	
Column:	Phenomenex Luna C8(2), 150 x 4.6 mm, 5 µm OR Phenomenex Luna C8, 150 x 2 mm, 5 µm, with Phenomenex KrudKatcher guard column	**Time**	**MPB**
MP A:	4 mM tetrabutlyammonium formate + 0.1 % formic acid in water (pH 2.8±0.05)	0.00	5
MP B:	MeCN	1.00	5
Flow:	0.45 mL/min (4.6 mm column)	5.00	90
	0.3 mL/min (2.0 mm column)	7.00	90
Inj Vol:	10 µL	8.00	5
Temp	40 °C	14.00	5

MS/MS Parameters (5500 & 6500)

Q1	Q3	RT	Transition	DP*	EP	CE	CXP
110	63	1.3	AMPA 1	-40	-11	-30	-9
110	79	1.3	AMPA 2	-40	-11	-34	-9
110	81	1.3	AMPA 3	-40	-11	-34	-9
112	63	2.5	AMPA IS	-60	-11	-26	-9
180	63	3.0	Glufosinate 1	-60	-11	-66	-9
180	95	3.0	Glufosinate 2	-40	-11	-19	-5
180	85	3.0	Glufosinate 3	-60	-11	-25	-9
183	63	3.0	Glufosinate IS	-60	-11	-40	-9
168	63	4.4	Glyphosate 1	-30	-11	-28	-9
168	79	4.4	Glyphosate 2	-30	-11	-56	-9
168	150	4.4	Glyphosate 3	-30	-11	-16	-9
171	63	4.4	Glyphosate IS	-30	-11	-28	-9
210	150	5.3	N-acetyl glyphosate 1	-20 (-40)	-11	-20	-13
210	63	5.3	N-acetyl glyphosate 2	-20 (-40)	-11	-40	-13
210	168	5.3	N-acetyl glyphosate 3	-20 (-40)	-11	-18	-13

**DP: if more than one DP is provided the first is optimized for the 6500 and the DP in () is optimized for the 5500*

MS Parameters	
CUR	35

FDA Glyphosate Method

CAD	MEDIUM
IS	-4000
GAS 1	65
GAS 2	65
TEM	450 ˚C (6500) 650 ˚C (5500)
Q1	UNIT
Q3	UNIT

210	150	5.3	N-acetyl glyphosate 1	-20 (-40)	-11	-20	-13
210	63	5.3	N-acetyl glyphosate 2	-20 (-40)	-11	-40	-13
210	168	5.3	N-acetyl glyphosate 3	-20 (-40)	-11	-18	-13

**DP: if more than one DP is provided the first is optimized for the 6500 and the DP in () is optimized for the 5500*

MS Parameters

Ionization: Ionspray in negative ionization mode

(b) (5)

G. **Quantitation of Residues**

(b) (5)

H. **Placeholder**

FDA Glyphosate Method

A. Reagents and Supplies

1. Acetonitrile, HPLC grade
2. Petroleum ether
3. Methylene chloride
4. Water, HPLC grade
5. Formic acid, 98% solution
6. Acetic acid
7. Ammonium formate
8. Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA)
9. Tetrabutylammonium hydroxide (TBAOH) titrant, 0.4 M in Water, HPLC Grade, ACROS Organics
10. Tetrabutylammonium acetate (TBA acetate), Aldrich No. 335991-10G
11. 50-mL plastic centrifuge tubes
12. Waters Oasis HLB SPE, 60 mg, 3cc, 30 µm
13. Extraction solvent (50 mM acetic acid/10 mM Na_2EDTA): mix 572 µL acetic acid and 0.74 g Na_2EDTA in 200-mL of purified water.
14. Mobile phase A (4 mM tetrabutylammonium formate)
    a. Add 10.0 ml of 0.4 M TBAOH to ~900 mL HPLC water, and adjust the pH to 2.8±1 using formic acid (~ 3 ml). OR
    b. Add 1.20 g TBA acetate in 1 L HPLC water; and adjust the pH to 2.8±1 using formic acid (~2 mL).

B. Standard Reference Materials

1. Glyphosate
2. Glufosinate
3. AMPA
4. Glyphosate-^{13}C
5. Glufosinate-D^3
6. N-acetyl-glyphosate, available from Toronto Research Chemicals (TRC No A178245), or Santa Cruz BioTech (SCBT No. sc-479500)

C. Standard Solutions

(b) (5)

D. **Equipment and Instrumentation**

1. Genogrinder
2. Centrifuge
3. Pipettes
4. LC-MS/MS
   a. Shimadzu HPLC system: two LC-20AD pumps, Sil-20AC autosampler, CTO-20AC column oven
      NOTE: Replace all metal LC tubing with PEEK tubing between the autosampler and injection valve because glyphosate can be retained on metal surfaces.
   b. AB 6500 Q-TRAP mass spectrometer
   c. HPLC column: Phenomenex Luna C8(2), 100 Å, 5 µm, 150 x 4.6 mm, Phenomenex 00F-4249-E0
   d. HPLC guard column: Phenomenex guard column KrudKatcher P/N AFO-8497

E. **Extraction Procedure**

1. 5 g sample + 25 ml extraction solvent
   2g sample plus 10 ml extraction solvent for dry products
2. Add 10 ml PE or MeCl to fatty matrices
3. Spike with isotopes @ 200 ng/g (could be included in the extraction solvent)
4. Shake @ 1000 for 10 min
5. Centrifuge at ≥ 3000 rpm for 5 min
6. Filter aqueous extract thru HLB SPE cartridge
7. Filter for injection (could be included with SPE step)
8. Sample concentration: 0.2 g/ml

F. **LC-MS/MS method**

LC Parameters		**Gradient**	
Column:	Phenomenex Luna C8(2), 150 x 4.6 mm, 5 µm, with Phenomenex KrudKatcher guard column	**Time**	**MPB**
MP A:	4 mM tetrabutlyammonium formate + 0.1 % formic acid in water (pH 2.8±1)	0.00	5
MP B:	MeCN	1.00	5
Flow:	0.6 mL/min (4.6 mm column)	5.00	95
Inj Vol:	10 µL	6.50	95
Temp	45 °C	6.60	5
		10.00	5

MS/MS Parameters

Q1	**Q3**	**RT**	**Transition**	**DP**	**EP**	**CE**	**CXP**
110	63	2.5	AMPA 1	-40	-11	-30	-9

110	79	2.5	AMPA 2	-40	-11	-34	-9
112	63	2.5	AMPA IS	-60	-11	-26	-9
180	63	4.0	Glufosinate 1	-60	-11	-66	-9
180	95	4.0	Glufosinate 2	-40	-11	-24	-5
180	85	4.0	Glufosinate 3	-60	-11	-25	-9
183	63	4.0	Glufosinate IS	-60	-11	-40	-9
168	63	5.0	Glyphosate 1	-30	-11	-28	-9
168	79	5.0	Glyphosate 2	-30	-11	-56	-9
168	150	5.0	Glyphosate 3	-30	-11	-16	-9
171	63	5.0	Glyphosate IS	-30	-11	-28	-9
210	63	6.0	N-acetyl glyphosate 1	-85	-11	-40	-13
210	124	6.0	N-acetyl glyphosate 2	-85	-11	-17	-13
210	79	6.0	N-acetyl glyphosate 3	-85	-11	-50	-13

MS Parameters

CUR	25
CAD	MEDIUM
IS	-4000
GAS 1	65
GAS 2	65
TEM	650 ℃
Q1	UNIT
Q3	UNIT

Memorandum

TO: Greg Mercer, Chair, Pesticides Technical Advisory Group (TAG), and Chris Sack, Study Organizer

FROM: Shaun MacMahon, Chair, Chemistry Methods Validation Subcommittee (CMVS)

RE: MLV Proposal, "Determination of Glyphosate and Glufosinate Residues in Food"

DATE: 8/9/2017

The CMVS has reviewed your submission of a multi-laboratory validation report for the method, "Determination of Glyphosate and Glufosinate Residues in Food." While the results are very encouraging, the enclosed report summarizes the findings of the subcommittee and includes comments and suggestions which need to be addressed before the study can be approved as a Level III Multi-Laboratory Validation.

CMVS Response to MLV Report

Method Title: Determination of Glyphosate and Glufosinate Residues in Food

TAG Chair: Greg Mercer (ORA)
MLV POC: Chris Sack (CFSAN)

On 7/21/2017, a report describing the multi-laboratory validation (MLV) was sent to the Chemistry Method Validation Subcommittee (CMVS) by Chris Sack. The following is an evaluation of the MLV report/manuscript. The enclosed report and attached MLV report summarize the findings of the subcommittee. While the results are encouraging, these comments and suggestions need to be addressed before the study can be approved as a Level III Multi-Laboratory Validation.

The criteria considered by the CMVS in evaluating submissions of *completed method validation packages* include the following:

- **Has the validation study demonstrated that the method is "fit for intended use"? Does the method clearly show that the chemical(s)/organism(s) in the scope can be recovered and detected in all relevant matrices in the scope at the sensitivity required to meet regulatory and/or health/hazard thresholds?**
  The report should include information on what the target concentration or "level of concern" is for these analytes.

- **Does the validation study follow the Office of Foods' "Guidelines for Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods" and "Guidelines for the Validation of Chemical Methods for the FDA Foods Program" as appropriate? Does the validated method have properly identified acceptance criteria for the validation elements that were met?**
  The report should include reference to appropriate mass spectrometry confirmation criteria to confirm these were met for all samples.

- **Scientific recommendations of the TSC (or other SMEs), if involved in the review.**
  We understand the report was shared with the TAG, with their comments already incorporated.

- **Does the validation package follow the original proposal that was submitted and approved? Use the criteria above for proposed studies if this is not available.**
  The study appears to follow the plan as described in the original proposal. The lack of collaborative study data from NFFL and EPA has been discussed with the study organizer but should be explained in the text.

- **Quality of results obtained from the multi-laboratory validation study.**
  The results indicate the method generally performed well in the 5 participating labs. However, there are questions related to the calculation of RSD and method

uncertainty as opposed to RSD_r and RSD_R, the use of 30% uncertainty as an acceptance measure, the use of R^2 as a performance measure, the use of a single point calibration, the regulatory relevance (or lack thereof) for AMPA, the lack of discussion of confirmation of identity, and the use of a different approach for quantitation on the study samples than will be applied to regulatory samples. More detailed comments on all of these topics are included in the report itself.

Additional comments:
Include an LC-MS/MS chromatogram to demonstrate how chromatography/transitions typically look.
Describe the rationale behind using multiple extraction solvents, and whether performance between the 2 approaches is comparable.

Glyphosate Method Collaboration Report

Introduction

A multi-laboratory validation (collaboration) was conducted of a method for the determination of residue levels of glyphosate, glufosinate, and two degradants of glyphosate N-acetylglyphosate and AMPA. Single laboratory validation of the method was conducted at PSW prior to the collaboration. Seven FDA pesticide laboratories plan to participate in the collaboration eventually. Data from three laboratories (ARL, PNW, and PSW) have been received at this time; this preliminary collaboration report summarizes data submitted from those three laboratories only. A final report encompassing all participating laboratories will be issued after all data from all laboratories has been submitted.

In addition to the collaboration summary of data from all three laboratories, an abbreviated report for the single laboratory validation and each collaborating laboratory are included as attachments to the collaboration report.

Conclusion

The collaboration data indicates the method is suitable for the purpose of quantitative determination for residues of glyphosate, glufosinate and N-acetylglyphosate and semi quantitative determination of AMPA residues in the three primary matrix types analyzed in the FDA pesticide program, i.e., high moisture, low moisture, and high fat items. The collaboration meets all the requirements of a level three multi-laboratory validation as per the "Guidelines for Validation of Chemical Methods for the FDA FVM Program, 2nd Edition.

Protocols and Procedure

Commodities were selected to represent the three major food commodity types analyzed in the FDA pesticide program, i.e. grain corn for dry products, carrots for high moisture products, and avocados for high lipid commodities. Composites of each of these three study matrices were prepared, composited, and distributed to the participating laboratories (PNW, PSW, KAN, ARL, SRL, NRL and CFSAN). Note: avocados were prepared without the outer peel. Each lab analyzed all matrices fortified with each analyte at the fortification levels in replicate as listed below:

i none: 2x
ii 0.050 ppm: 2x
iii 0.250 ppm: 2x
iv 0.500: 2x

Each lab was additionally sent two samples previously found to contain incurred glyphosate residues when analyzed at SRL using the method described in LIB 4596, i.e., ground grain corn in which 0.04 ppm was found and ground soy beans in which 4.5 ppm was found.

Glyphosate Method Collaboration Report

A detailed protocol is provided in attachment A and the method is provided in attachment B.

PSW conducted a single laboratory validation (SLV) of the procedure using the same procedure and collaboration protocol. The SLV results and protocols are reported in the C attachments.

Prior to conducting multiple laboratory method validation each participating laboratory was required to demonstrate proficiency with the procedure. Instrument proficiency was demonstrated conducting system suitability tests that included determination of accuracy, precision, linearity and LOQ by preparing and injecting standards. Results of the system suitability testing are reported with the attached individual laboratory reports (attachments C, F, G and H).

The concentrations and spike recoveries were calculated by single level calibration using average responses of matrix matched standards bracketing the samples and prepared at the same concentration as the spiked sample. For glyphosate and glufosinate residue levels were calculated using corresponding isotopic internal standards added to the extraction solvent prior to analysis. AMPA residues were calculated against the glyphosate isotopic internal standard. Residues of N-acetylglyphosate were calculated using external standard calibration.

The mean recoveries for all three spike levels (50, 250, and 500 ng/g) were calculated by matrix for each laboratory. The overall mean, RSD and method uncertainty (MU) of all three laboratories was calculated for each matrix. The linearity coefficient of determination (R^2) was calculated from the concentrations found at each level for each matrix and laboratory by squaring the Excel correlation function (Correl); the average R^2 of the three laboratories is reported in Table 1. Method specificity was evaluated by the analysis of control matrices. Acceptable validation specifications for the collaboration study are listed below.

Specificity: No residues found in blank control matrices

Recovery: 70-120 % RSD: 15% MU: 30% R^2: 0.990

Results and Discussion

The method collaboration results in this report were provided by three of the participating laboratories: ARL, PNW and PSW. Table 1 contains the summary statistical analysis of all collaboration analyses; results that did not meet specifications are highlighted in red font. Scatter plots of the recoveries are provided in attachment D. No residues were found in the control samples analyzed for each matrix. All results for glyphosate, glufosinate, and N-acetylglyphosate were within the validation specifications. The linearity of the AMPA results did not meet the specification of R2 = 0.99 in any of the three matrices studied, however all were above 0.95. One lab reported low recoveries (48.6 % and 61.3 %) of AMPA in avocado and carrot, respectively.

Glyphosate Method Collaboration Report

Table 1. Summary data includes the average spike recovery for each lab, overall average recovery, RSD, method uncertainty (MU) of the spike recoveries and the average coefficient of determination (R^2) of the spike concentrations.

Matrix	ARL	PNW	PSW	Mean	RSD	MU	R^2
Glyphosate							
Avocado	85.3	87.2	96.6	89.7	6.8	14.3	0.9990
Carrot	80.0	85.9	83.7	83.2	5.7	12.0	0.9995
Corn	91.4	95.1	101.8	96.1	5.4	11.4	0.9995
Glufosinate							
Avocado	82.9	87.0	94.4	88.1	6.7	14.0	0.9970
Carrot	81.0	90.4	84.6	85.3	5.8	12.2	0.9991
Corn	98.4	101.4	102.0	100.6	2.2	4.6	0.9994
N-acetylglyphosate							
Avocado	85.7	90.3	106.3	94.1	11.3	23.8	0.9941
Carrot	79.7	86.7	97.7	88.0	10.9	23.0	0.9965
Corn	93.1	94.4	117.9	101.8	12.0	25.4	0.9979
AMPA	(b) (5)						(b) (5)
Avocado		87.3	85.9	74.0	13.9	27.8	
Carrot		83.4	90.9	78.5	9.7	19.4	
Corn	95.8	76.5	90.3	87.5	9.2	18.4	

The matrix effect for each analyte/matrix combination was evaluated by calculating residue concentrations using both matrix matched standards and standards prepared in solvent and comparing the slopes of the corresponding linearity charts. Results of the matrix study are tabulated in Table 2 and linearity charts for each analyte/matrix combination are provided in attachment E. Results indicate none of the matrices in the study had much effect on the determination of glyphosate, glufosinate and N-acetylglyphosate. However, all three matrices had a significant impact on residues of AMPA with matrix effects of 391 % in avocado, 327 % in carrot, and 455 % in corn. These results also reflect the advantage of using isotopically labelled internal standards.

Table 2. Matrix effects as percentages of slope ratios of residues calculated for the three spike levels using standards prepared in solvent vs matrix extracts

Compound	Avocado	Carrot	Corn
Glyphosate	91.1	102.2	100.7
Glufosinate	89.4	90.5	103.3
N-acetylglyphosate	108.1	103.1	101.3
AMPA	391	327	455

Each laboratory analyzed a corn sample and a soy sample previously analyzed and found to contain incurred residue of glyphosate. Results of the incurred residue analysis, tabulated in Table 3, are in excellent agreement.

Table 3. Incurred residues (ppb) in corn and soy samples.

Matrix	Original	ARL	PNW	PSW	Mean	RSD
Corn	40	36	35	46	39.3	(12.7)
Soy	4500	4290	4610	4620	4510	(3.4)

For the method collaboration study spike recoveries were calculated based upon a single level calibration at the same concentration as the spike level, i.e., the 50 ng/g spikes were calculated based upon calibration at 50 ng/g equivalence, or 10 ng/ml. Once implemented for routine analysis calibration will be conducted at a single level equivalent to 250 ng/g in the sample. In Table 4 the relative percent difference (RPD) of spike recoveries from the collaboration and the same spike recoveries calculated using a single level standard at concentration equivalent to 250 ng/g. Very low RPDs demonstrate the linearity of the method and accuracy of residue levels calculated from a single level calibration.

Table 4. Relative Percent Difference (RPD) of average recoveries for all levels and laboratories calculated based upon a single level calibration at 250 ng/g vs. calibration per each individual spike level.

Matrix	Single Level	Per Level	RPD	Single Level	Per Level	RPD
	Glyphosate			*Glufosinate*		
Avocado	90.1	89.7	0.4	87.6	88.1	0.6
Carrot	84.7	83.2	1.7	86.8	85.3	1.7
Corn	98.4	96.1	2.4	101.2	100.6	0.6
	N-acetylglyphosate			*AMPA*		
Avocado	87.6	96.9	10.1	65.9	74	11.5
Carrot	86.8	88	1.4	76.9	78.5	2.0
Corn	101.2	101.8	0.6	90.6	87.5	3.4

Glyphosate Method Collaboration Report

Attachments

A. Collaboration Protocol
B. Analytical Method
C. Single Laboratory Validation
    C_1 SLV Method Recovery Charts
    C_2 SLV Method Linearity Charts
D. Method Collaboration Recovery Charts
E. Method Collaboration Matrix Effects Charts
F. PSW Collaboration Data and System Suitability
    F_1 PSW Recovery Charts
    F_2 PSW Linearity Charts
G. PNW Collaboration Data and System Suitability
    G_1 PNW Recovery Charts
    G_2 PNW Linearity Charts
H. ARL Collaboration Data and System Suitability
    H_1 ARL Recovery Charts
    H_2 ARL Linearity Charts

Collaboration Protocol

Matrices: corn (dry), carrot (high moisture), avocado (high fat)

Analyses:

Recovery Study

Level	N*
Control	2
Spike 50	2
Spike 250	2
Spike 500	2

** replicates per matrix*

Incurred Residues

Matrix	Level
Corn	~40 ng/g
Soybean	~4.5 µg/g

Preparation of Standards: Prepare calibration/fortification standards in both solvent and in matrix extracts and listed below.

Calibration Standards in Solvent				Matrix Calibration Standards			
Std Conc (ng/ml)	Spk Std[1] Conc (µg/ml)	Spk Std Volume Added (µl)	Dilution[2] Volume (ml)	Std Conc (ng/ml)	Spk Std[1] Conc (µg/ml)	Spk Std Volume Added (µl)	Dilution[3] Volume (ml)
	corn (2 g sample)						
10	1	100	10	10	1	50	5
50	5	100	10	50	5	50	5
100	5	200	10	100	5	100	5
	carrot/avocado (5 g sample)						
10	5	50	25	10	1	100	10
50	5	250	25	50	5	100	10
100	50	50	25	100	50	20	10

[1] *Prepare mixed native standards as directed in method step C.4*

[2] *Dilute with 50 ng/ml IS fortified extraction solvent*

[3] *Dilute with control sample matrix*

Fortification Procedure:

Spike Level (ng/g)	Spk Std Conc (µg/ml)	Volume Added (µl)
corn (2 g/sample)		
50	1	100
250	5	100
500	5	200

Collaboration Protocol

carrot/avocado (5 g/sample)		
50	5	50
250	5	250
500	50	50

Extraction Cleanup for Avocado:

Follow method as written. Re the cleanup option for avocadoes; i.e. dichloromethane (DCM) vs petroleum ether (PE) three ORA labs agreed to use DCM and the remaining three ORA labs agreed to use PE. CFSAN can choose either.

DCM	PE
ARL	PNW
SRL	PSW
KAN	NRL

LCMS Transition Names:

AMPA[110-63] 1
AMPA[110-79] 2
AMPA[110-81] 3
Glu[180-63] 1
Glu[180-95] 2
Glu[180-85] 3
Glu[183-63] IS
Gly[168-63] 1
Gly[168-79] 2
Gly[168-150] 3
Gly[171-63] IS
N-acetyl[210-150] 1
N-acetyl[210-63] 2
N-acetyl[210-168] 3

LCMS Calibration: Calibrate using single level calibration for each spike level. Assign the internal standards as below.

Analyte	Internal Standard
Glyphosate:	Glyphosate-^{13}C
N-acetylglyphosate:	Glyphosate-^{13}C
AMPA:	Glyphosate-^{13}C
Glufosinate:	Glufosinate-D^{3}

Collaboration Protocol

Inj Sequence: Group by spike level. Assign Sample Name to Sample description and the Sample Types and Actual Concentrations listed in the table below.

Description	Sample Name	Sample Type	Actual Conc
50 ng/g spike level			
10 ng/ml calibration std in solvent	CalStd10	Standard	50
10 ng/ml calibration std in solvent	CalStd10	Standard	50
10 ng/ml corn matrix calibration std	MatStd10 Corn	QC	50
Corn control	Control Corn	Unknown	
Corn spike 50 #1	Spk50-1 Corn	QC	50
Corn spike 50 #2	Spk50-2 Corn	QC	50
Corn incurred residue	Corn Incur	Unknown	
10 ng/ml corn matrix calibration std	MatStd10 Corn	QC	50
10 ng/ml calibration std in solvent	CalStd10	Standard	50
10 ng/ml carrot matrix calibration std	MatStd10 Carrot	QC	50
Carrot control	Control Carrot	Unknown	
Carrot spike 50 #1	Spk50-1 Carrot	QC	50
Carrot spike 50 #2	Spk50-2 Carrot	QC	50
10 ng/ml carrot matrix calibration std	MatStd10 Carrot	QC	50
10 ng/ml calibration std in solvent	CalStd10	Standard	50
10 ng/ml avocado matrix calibration std	MatStd10 Avocado	QC	50
Avocado control	Control Avocado	Unknown	
Avocado spike 50 #1	Spk50-1 Avocado	QC	50
Avocado spike 50 #2	Spk50-2 Avocado	QC	50
10 ng/ml avocado matrix calibration std	MatStd10 Avocado	QC	50
10 ng/ml calibration std in solvent	CalStd10	Standard	50
250 ng/g spike level			
50 ng/ml calibration std in solvent	CalStd50	Standard	250
50 ng/ml calibration std in solvent	CalStd50	Standard	250
50 ng/ml corn matrix calibration std	MatStd50 Corn	QC	250
Corn spike 250 #1	Spk250-1 Corn	QC	250
Corn spike 250 #2	Spk250-2 Corn	QC	250
50 ng/ml corn matrix calibration std	MatStd50 Corn	QC	250
50 ng/ml calibration std in solvent	CalStd50	Standard	250
50 ng/ml carrot matrix calibration std	MatStd50 Carrot	QC	250
Carrot spike 250 #1	Spk250-1 Carrot	QC	250
Carrot spike 250 #2	Spk250-2 Carrot	QC	250
50 ng/ml carrot matrix calibration std	MatStd50 Carrot	QC	250
50 ng/ml calibration std in solvent	CalStd50	Standard	250
50 ng/ml avocado matrix calibration std	MatStd50 Avocado	QC	250
Avocado spike 250 #1	Spk250-1 Avocado	QC	250
Avocado spike 250 #2	Spk250-2 Avocado	QC	250
50 ng/ml avocado matrix calibration std	MatStd50 Avocado	QC	250
50 ng/ml calibration std in solvent	CalStd50	Standard	250

Collaboration Protocol

500 ng/g spike level

100 ng/ml calibration std in solvent	CalStd100	Standard	500
100 ng/ml calibration std in solvent	CalStd100	Standard	500
100 ng/ml corn matrix calibration std	MatStd100 Corn	QC	500
Corn spike 500 #1	Spk250-1 Corn	QC	500
Corn spike 500 #2	Spk250-2 Corn	QC	500
100 ng/ml corn matrix calibration std	MatStd100 Corn	QC	500
100 ng/ml calibration std in solvent	CalStd100	Standard	500
100 ng/ml carrot matrix calibration std	MatStd100 Carrot	QC	500
Carrot spike 500 #1	Spk250-1 Carrot	QC	500
Carrot spike 500 #2	Spk250-2 Carrot	QC	500
100 ng/ml carrot matrix calibration std	MatStd100 Carrot	QC	500
100 ng/ml calibration std in solvent	CalStd100	Standard	500
100 ng/ml avocado matrix calibration std	MatStd100 Avocado	QC	500
Avocado spike 500 #1	Spk250-1 Avocado	QC	500
Avocado spike 500 #2	Spk250-2 Avocado	QC	500
100 ng/ml avocado matrix calibration std	MatStd100 Avocado	QC	500
100 ng/ml calibration std in solvent	CalStd100	Standard	500
100 ng/ml soy matrix calibration std	MatStd100 Soy	QC	500
Soy control	Control Corn	Unknown	
Soy incurred residue	Soy Incur	Unknown	
Soy incurred residue Dil 1-10	Soy Incur (1-10)	Unknown	
100 ng/ml soy matrix calibration std	MatStd100 Soy	QC	500
100 ng/ml calibration std in solvent	CalStd100	Standard	500

Data: Provide the following data fields when reporting results

Index
Sample Name
Sample Type
Dilution Factor
Peak Name (Transition Name)
Peak Area
IS Peak Area
Retention Time (RT)
Actual Concentration (Spk level or Std conc)
Calculated concentration

Glyphosate Method Collaboration Report Attachment B

Analytical Method

A. Reagents and Supplies

1. Acetonitrile, HPLC grade
2. Petroleum ether
3. Methylene chloride
4. Water, HPLC grade
5. Formic acid, 98% solution
6. Acetic acid
7. Ammonium formate
8. Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA)
9. Tetrabutylammonium hydroxide (TBAOH) titrant, 0.4 M in Water, HPLC Grade, ACROS Organics
10. Tetrabutylammonium acetate (TBuAA), Aldrich No. 335991-10G (optional)
11. Tetrabutylammonium acetate 1 M (TBuAA 1M), Aldrich No. 401803 – 50 ML (optional)
12. 50-mL plastic centrifuge tubes
13. Filter, 0.2 µm, 25 mm, nylon
14. Waters Oasis HLB SPE, 60 mg, 3cc, 30 µm
15. Extraction solvent (50 mM acetic acid/10 mM Na_2EDTA): mix 2.9 mL acetic acid and 3.7 g Na_2EDTA in 1000-mL of purified water.
16. 50 ng/ml IS fortified extraction solvent: dilute IS 20 µg/ml mixed isotope internal standard, prepared in step C.2.a, 1:400 using extraction solvent, prepared in step A.15, e.g. 2.5 ml (IS 20 µg/ml) to 1000 ml extraction solvent
17. Mobile phase A (4 mM tetrabutylammonium formate)
    a. Add 10.0 ml of 0.4 M TBAOH to ~900 mL HPLC water, and adjust the pH to 2.8±0.05 using formic acid (~ 3 ml). OR
    b. Add 1.20 g TBA acetate in 1 L HPLC water; and adjust the pH to 2.8±0.05 using formic acid (~2 mL). OR
    c. 4 ml 1M TBuAA in 1 L HPLC water; and adjust the pH to 2.8±0.05 using formic acid (~2 mL).

B. Standard Reference Materials

1. Glyphosate
2. Glufosinate
3. AMPA
4. N-acetyl-glyphosate, available from EPA and Toronto Research Chemicals (TRC No A178245)
5. Glyphosate-^{13}C
6. Glufosinate-D^3

C. Standard Solutions

1. General instructions
    a. Unless otherwise indicated prepare standards in DI water
    b. Store standard solutions in plastic containers because glass can leach standard reference material from solution. Use of glass volumetric flasks for standard preparation is OK if solution is removed from the glassware after preparation.
    c. Store standard solutions in a refrigerator. Do not store standards prepared with water or aqueous media in the freezer.
2. Stock standards 1 mg/ml

a. Includes all native and isotopic standards listed in Section B
b. Prepare individual stock standard for each compound

3. Isotopic working solutions
   a. IS 20 µg/ml mixed isotope internal standard
      i) Combine isotopes Glyphosate-^{13}C and Glufosinate-D^3 (step B.5 & 6)
      ii) Dilute 1 mg/ml stock isotope internal standards, prepared in step C.2, 1:50
4. Intermediate mixed standards
   a. 50 µg/ml mixed native standard
      i) Combine native 1 mg/ml stock standards, prepared in step C.2
      ii) Include glyphosate, glufosinate, AMPA, and N-acetyl-glyphosate (Step B.1-4)
      iii) Dilute 1:20
   b. 5.0 µg/ml mixed native standard
      i) Dilute 50 µg/ml mixed standard, prepared in step C.4.a, 1:10
   c. 1.0 µg/ml mixed native standard
      i) Dilute50 µg/ml mixed standard, prepared in step C.4.a, 1:50
5. LC-MS/MS calibration standard 50 ng/ml
   a. Dilute 5.0 µg/ml mixed native standard, prepared in step C.4.b, 1:100, using 50 ng/ml IS fortified extraction solvent (A.16)

D. **Equipment and Instrumentation**

1. Genogrinder
2. Centrifuge
3. Pipettes
4. LC-MS/MS
   a. Shimadzu HPLC system: two LC-20AD pumps, Sil-20AC autosampler, CTO-20AC column oven
      NOTE: Replace all metal LC tubing with PEEK tubing between the autosampler and injection valve because glyphosate can be retained on metal surfaces.
   b. AB model 5500, or 6500, Q-TRAP mass spectrometer
   c. HPLC columns: Phenomenex Luna C8(2), 100 Å, 5 µm, 150 x 4.6 mm, Phenomenex No. 00F-4249-E0; Or Phenomenex Luna C8, 100 Å, 5 µm, 150 x 2 mm, Phenomenex No. 00F-4040-B0
   d. HPLC guard column: Phenomenex guard column KrudKatcher P/N AFO-8497

      NOTE: Install peek tubing between the autosampler and column because metal can affect glyphosate and glufosinate chromatography

E. **Extraction Procedure**

1. 5 g sample + 25 ml 50 ng/ml IS fortified extraction solvent prepared in step A.15
   For dry products containing less than 50 % moisture: 2 g sample plus 10 ml 50 ng/ml IS fortified extraction solvent prepared in step A.15 for dry products
2. Add 10 ml PE, or $MeCl_2$, for matrices containing more than 3 % fat.
3. Shake @ 1000 shakes per min for 10 min
4. Centrifuge at ≥ 3000 rpm for 5 min NOTE: When using PE to remove lipid co-extractants high fat matrices, the PE will be the top layer. When using $MeCl_2$, the $MeCl_2$ will be the bottom layer in the centrifuge tube.

Analytical Method

5. Filter aqueous extract thru HLB SPE cartridge, limit filter volume to less than 2 mls.
6. Filter for injection (could be included with SPE step)
7. Sample concentration: 0.2 g/ml

F. **LC-MS/MS method**

LC Parameters		Gradient	
Column:	Phenomenex Luna C8(2), 150 x 4.6 mm, 5 µm OR Phenomenex Luna C8, 150 x 2 mm, 5 µm Guard Column: Phenomenex KrudKatcher	**Time**	**MPB**
MP A:	4 mM tetrabutlyammonium formate + 0.1 % formic acid in water (pH 2.8±0.05)	0.00	5
MP B:	MeCN	1.00	5
Flow:	0.45 mL/min (4.6 mm column)	5.00	90
	0.3 mL/min (2.0 mm column)	7.00	90
Inj Vol:	10 µL	8.00	5
Temp	40 °C	14.00	5
Divert Valve	Divert flow from mass spectrometer about 30 seconds before the first analyte and 60 seconds after the last analyte elutes		

MS/MS Parameters (5500 & 6500)

Q1	Q3	RT	Transition	DP*	EP	CE	CXP
110	63	1.3	AMPA 1	-40	-11	-30	-9
110	79	1.3	AMPA 2	-40	-11	-34	-9
110	81	1.3	AMPA 3	-40	-11	-34	-9
112	63	2.5	AMPA IS	-60	-11	-26	-9
180	63	3.0	Glufosinate 1	-60	-11	-66	-9
180	95	3.0	Glufosinate 2	-60	-11	-19	-5
180	85	3.0	Glufosinate 3	-60	-11	-25	-9
183	63	3.0	Glufosinate IS	-60	-11	-40	-9
168	63	4.4	Glyphosate 1	-30	-11	-28	-9
168	79	4.4	Glyphosate 2	-30	-11	-56	-9
168	150	4.4	Glyphosate 3	-30	-11	-16	-9
171	63	4.4	Glyphosate IS	-30	-11	-28	-9
210	150	5.3	N-acetyl glyphosate 1	-20 (-40)	-11	-20	-13
210	63	5.3	N-acetyl glyphosate 2	-20 (-40)	-11	-40	-13
210	168	5.3	N-acetyl glyphosate 3	-20 (-40)	-11	-18	-13

**DP: if more than one DP is provided the first is optimized for the 6500 and the DP in () is optimized for the 5500*

Analytical Method

MS Parameters

Ionization:	Ionspray in negative ionization mode		
CUR:	35	TEM:	450 °C (6500)
CAD:	medium		650 °C (5500)
IS:	-4000	Q1:	unit
GAS 1 & 2:	65	Q3:	unit

G. **Quantitation of Residues**

1. Calibrate instrument using single level calibration standard at 50 ng/ml
2. Calibrate using internal standard calibration for glyphosate, glufosinate and AMPA
   a. Assign internal standard calibration standards
      i) Glyphosate: Glyphosate-^{13}C
      ii) Glufosinate: Glufosinate-D_3
      iii) AMPA: Glyphosate-^{13}C
3. Calibrate using external calibration for N-acetylglyphosate
4. Reportable residues must meet the identification criteria provided in Appendix A “Identification of Residues” in ORA-LAB.10
5. Quantitate residues per instructions in Appendix B “Quantitation of Residues” in ORA-LAB.10. Give preference to quantitation using the primary MS/MS transition, e.g. “Glyphosate 1”, however, use of secondary transitions for quantitation may be advisable if/when matrix coextractants interfere with the primary transition response.

Single Laboratory Validation

The PSW laboratory conducted single laboratory validation (SLV) for the procedure "Analysis of Glyphosate in Food by HPLC-MS/MS" (Att. B). Standards were prepared as per glyphosate procedure (Att. B) at 1, 2, 10, 50, 100, 200, 250, 350, 400 and 500 ng/ml in extraction solvent fortified at 50 ng/ml with isotopic internal standards. The matrices studied were the collaboration samples of corn, carrot and avocado. Recovery studies were conducted using the calibration protocols and analysis sequences prescribed in the collaboration protocol (Att. A). Each matrix was analyzed as an unfortified control and fortified in duplicate at three different levels: 50, 250, and 500 ng/g; i.e. six analyses per matrix, 21 analyses altogether. For the MDL study each of the three matrices was fortified at 20 ng/ml and seven replicates were analyzed per the instructions of 40 CFR 136 Appendix B.

Prior to starting the collaboration, instrument system suitability (SS) was demonstrated. Standards were injected at concentrations of 10, 50, 100, 200, 350, and 500 ng/ml to determine accuracy and linearity. Five replicates of the 50 ng/ml standard were injected to determine precision. The instrument LOQ was determined as per ORA-LAB.10 by injecting a 2 ng/ml standard in solvent and determining the S/N of the quantifier and qualifier ions. The LOQ was calculated as the lowest level where the S/N of the quantifier ion ≥ 10 and the S/N of the qualifier ion ≥ 3. Results for the instrument system suitability study are listed in the table below.

SS Factor	Glyphosate		Glufosinate		AMPA		N-acetylglyphosate	
LOQ (ng/mL)	0.3		0.3		0.5		0.2	
Precision (RSD)	99.1	(1.4)	99.8	(2.3)	97.7	(2.1)	102.3	(1.2)
Accuracy (R^2)	100.4	(0.9997)	104.4	(0.9996)	96.1	(0.9998)	96.6	(0.9998)

For the recovery study the average recovery, RSD, method uncertainty (MU), and the coefficient of determination (R^2) for all levels was determined for each matrix and overall. MU at the 95 % confidence level was calculated as 2 * the RSD as prescribed in ORA-LAB.5.4.6. Linearity (R^2) was calculated by squaring the Excel correlation function (Correl) of the spike level and calculated concentrations of the spiked samples. The method LOQ was determined by multiplying the standard deviation of the concentrations of seven replicate 20 ng/ml spikes per matrix by 10. For the overall method LOQ the standard deviation was calculated by adding the variances and degrees of freedom of the individual matrix concentrations taking the square root. Specificity was determined by the analysis of the control samples. Acceptable method validation specifications for each method performance metric are listed below.

Recovery: 70-120 % RSD: 15% MU: 30%
R^2: 0.990 LOQ: ≤ 10 ng/g

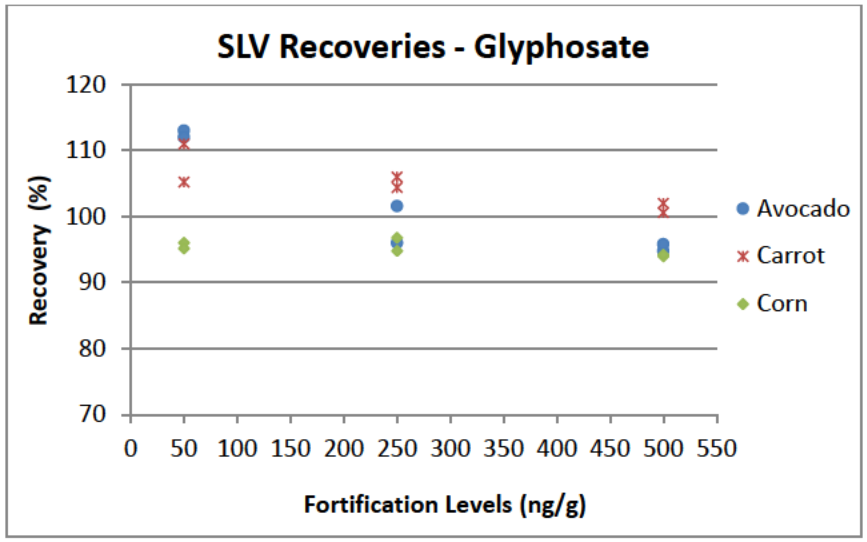
Results of the SLV are summarized in the Table C1 below; results that were not within validation specifications are indicated in red font. Scatter plots of recoveries and linearity charts for each analyte are provided in attachments C_1 and C_2. Results for both of the pesticides, glyphosate and glufosinate met all validation performance specifications and results for the glyphosate degradant N-acetylglyphosate met all specifications with the exception of the R^2 of

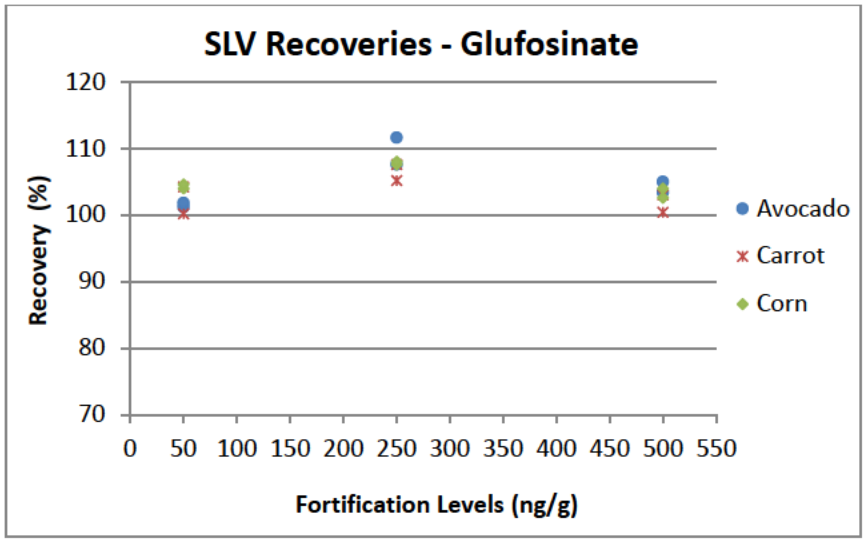
0.9871 for avocado recoveries was just below the specification of 0.99. Recoveries of the glyphosate degradant AMPA were very low, averaging 19.8 %, however it did meet most of the other specifications. AMPA will be considered qualitative and will not be reported for routine analyses

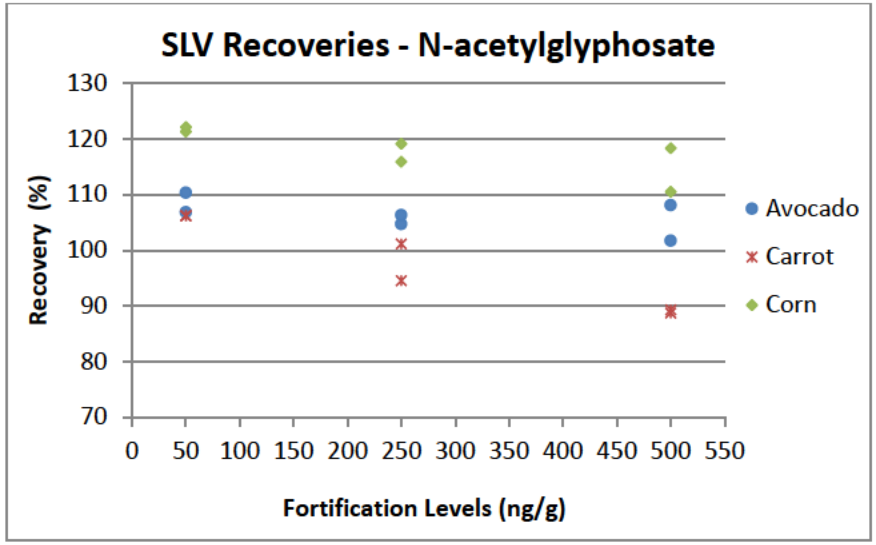
Table 1. Summary data includes the average, RSD, method uncertainty (MU) and coefficient of determination (R^2) from the recovery study and method limit of quantitation (LOQ) from the LOQ study.

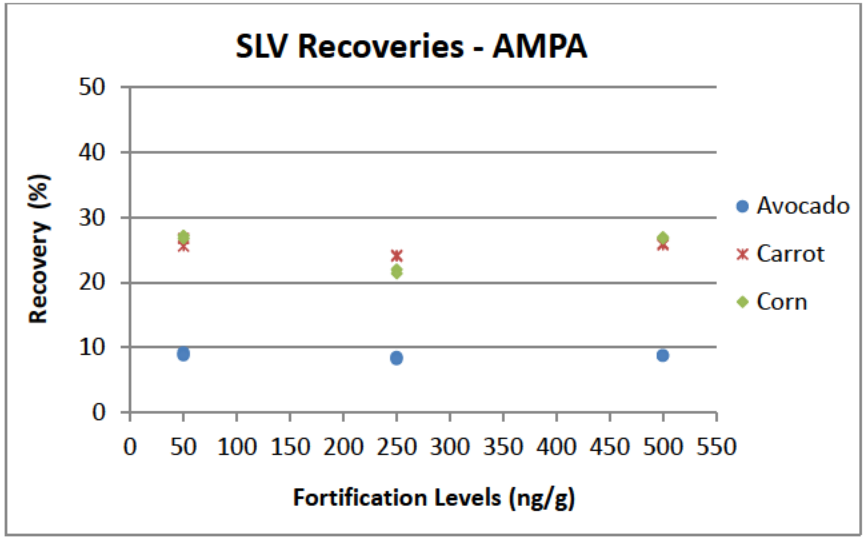
	Recoveries				Limits
Matrix	**Average**	**RSD**	**MU**	**R^2**	**LOQ**
Glyphosate					
Avocado	102.2	8.2	21.0	0.9993	3.5
Carrot	104.9	3.5	8.9	0.9994	7.5
Corn	95.2	1.1	2.9	0.9998	5.2
Overall	100.7	6.5	13.6	0.9957	5.7
Glufosinate					
Avocado	105.1	3.7	9.6	0.9984	7.4
Carrot	103.4	2.8	7.1	0.9986	8.8
Corn	105.1	2.1	5.3	0.9991	10
Overall	104.6	2.9	6.1	0.9984	8.8
N-acetylglyphosate					
Avocado	106.3	2.8	7.1	0.9976	8.4
Carrot	97.7	8.2	21.0	0.9965	4.4
Corn	117.9	3.6	9.2	0.9968	7.6
Overall	107.3	9.3	19.6	0.9681	7.0
AMPA					
Avocado	(b) (5)	3.8	9.9	0.9986	6.1
Carrot		4.3	11.0	0.9978	9.9
Corn		10.8	27.8	(b) (5)	3.9
Overall		(b) (5)	87.6	(b) (5)	7.1

Single Laboratory Validation – Method Recovery Charts

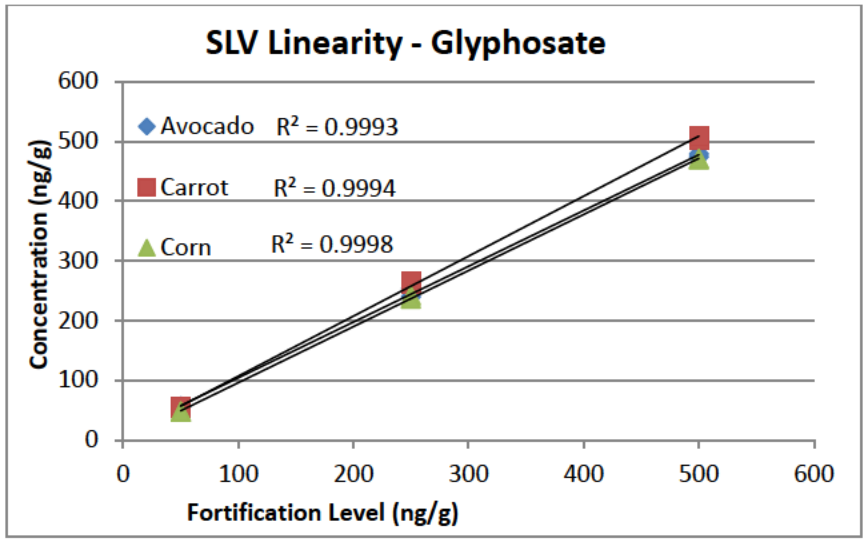


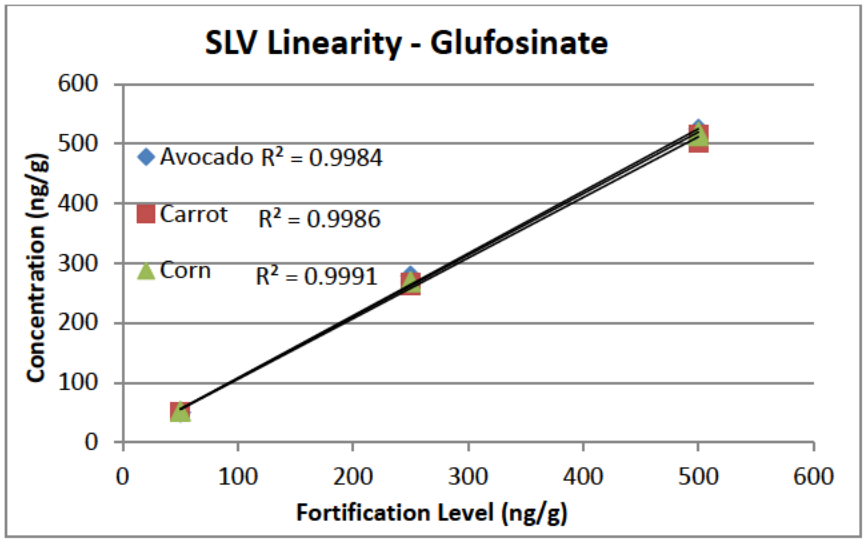


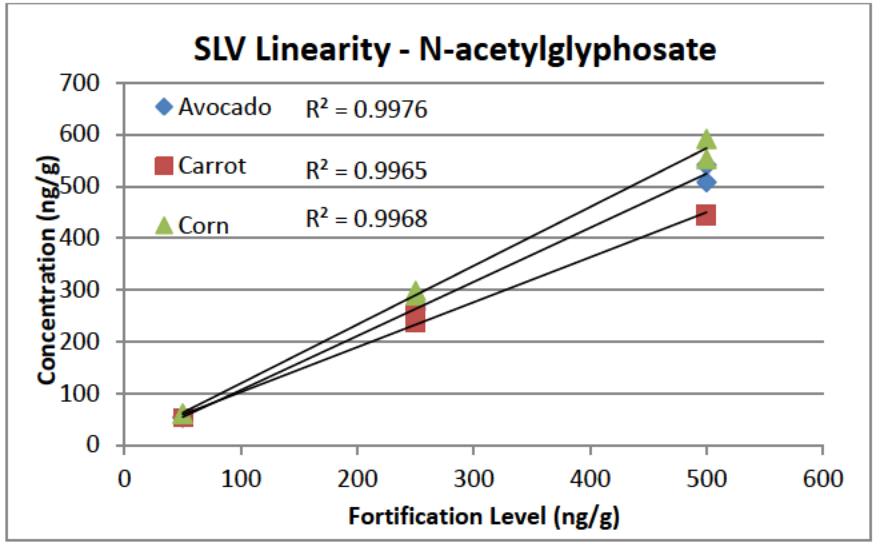


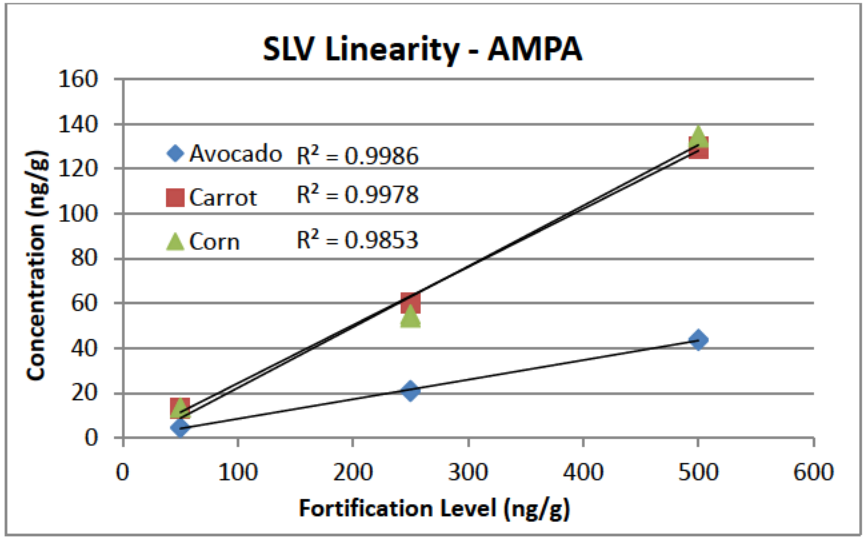


Single Laboratory Validation – Method Linearity Charts

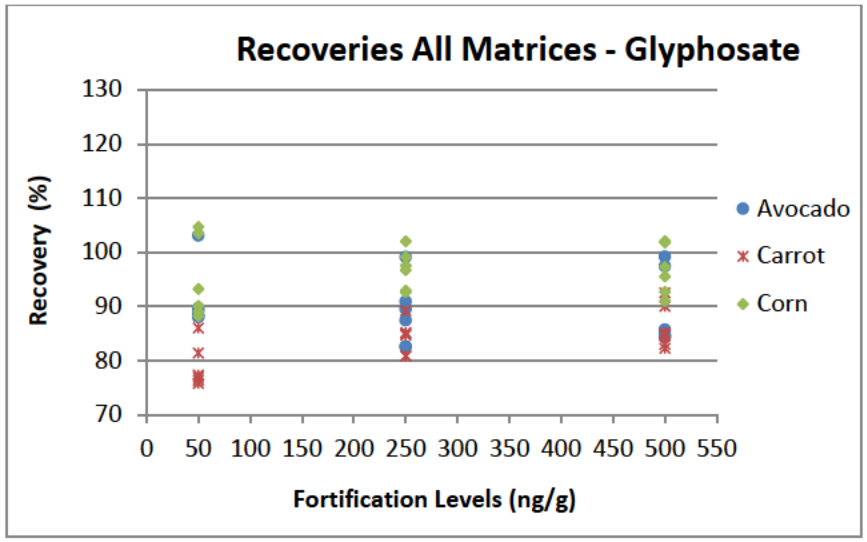


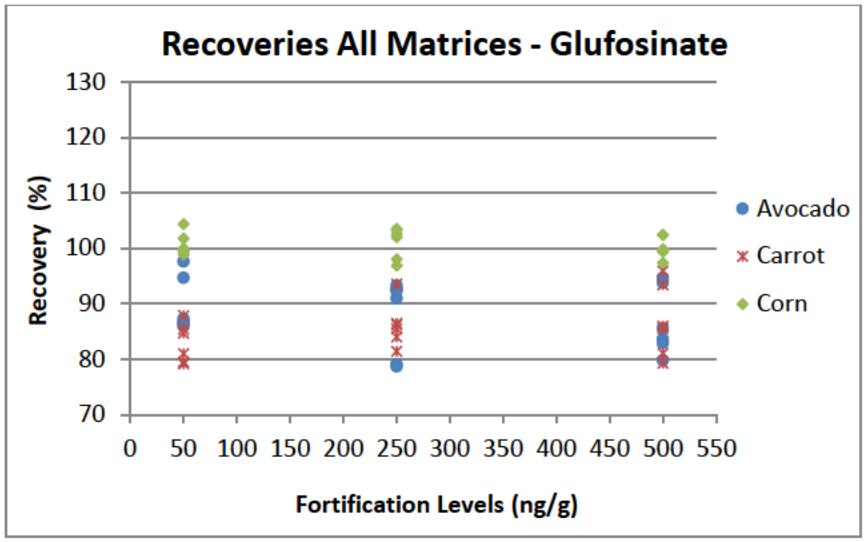


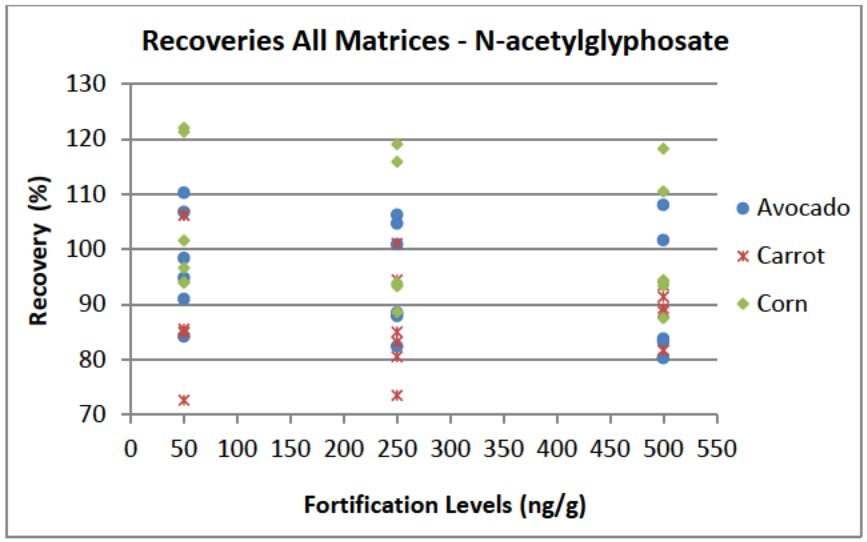


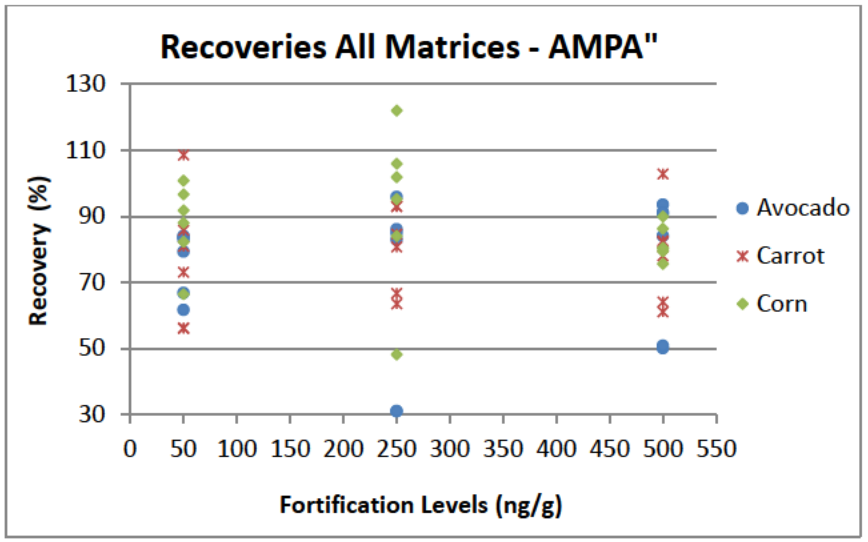


Method Collaboration Recovery Charts

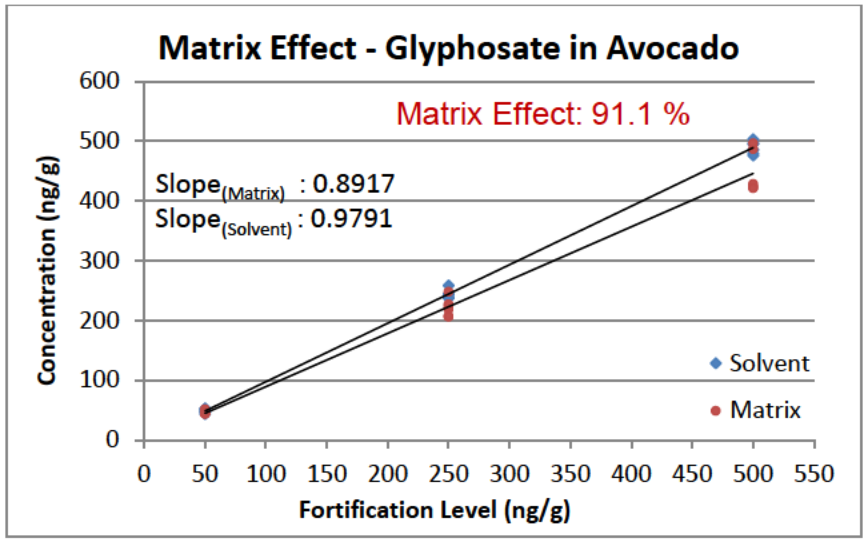


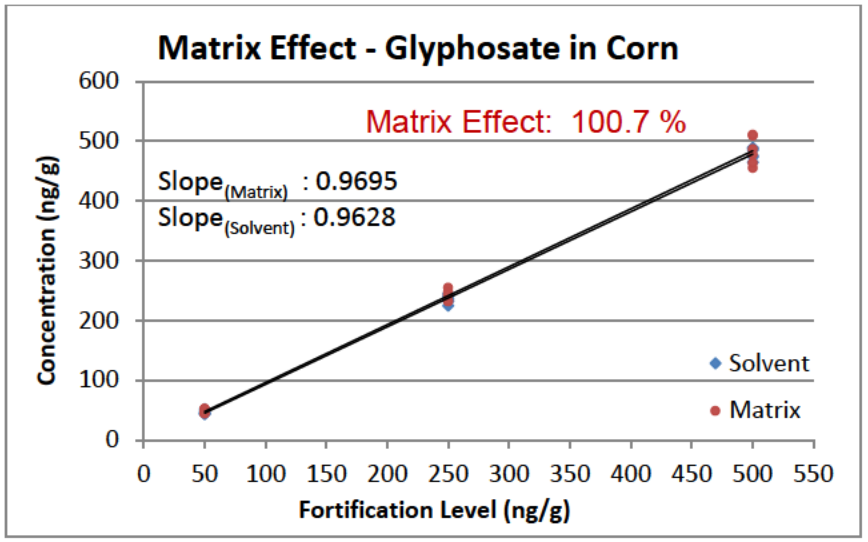


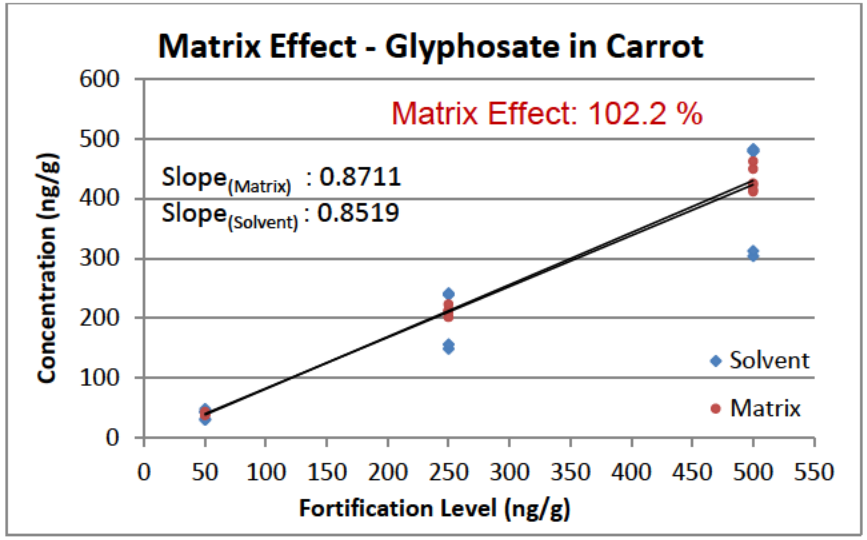


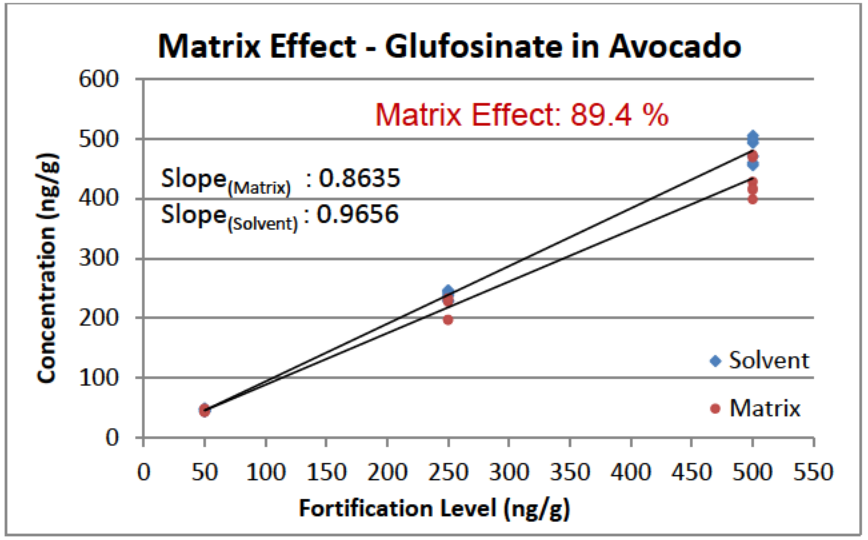


Method Collaboration Matrix Effects Charts

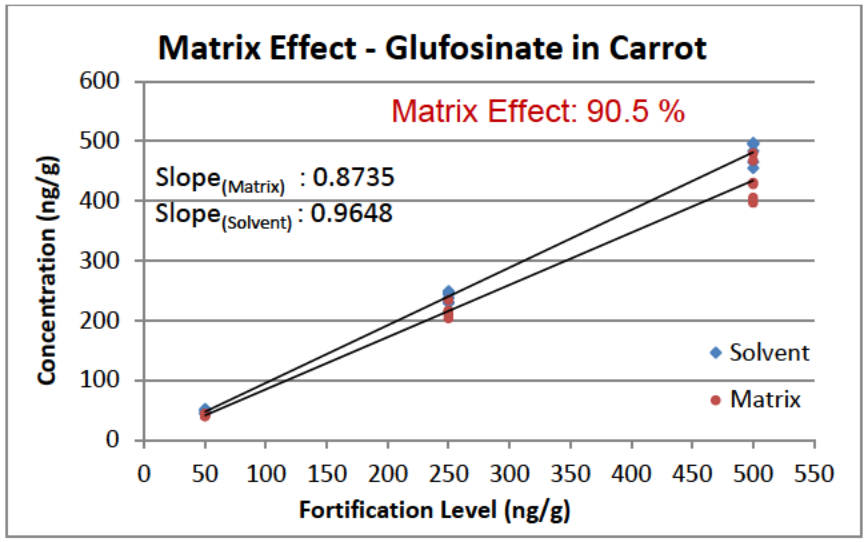


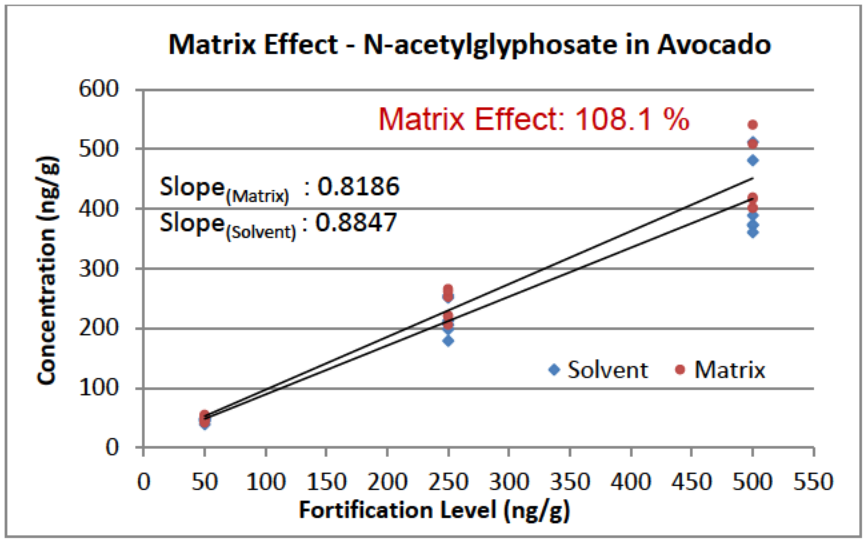


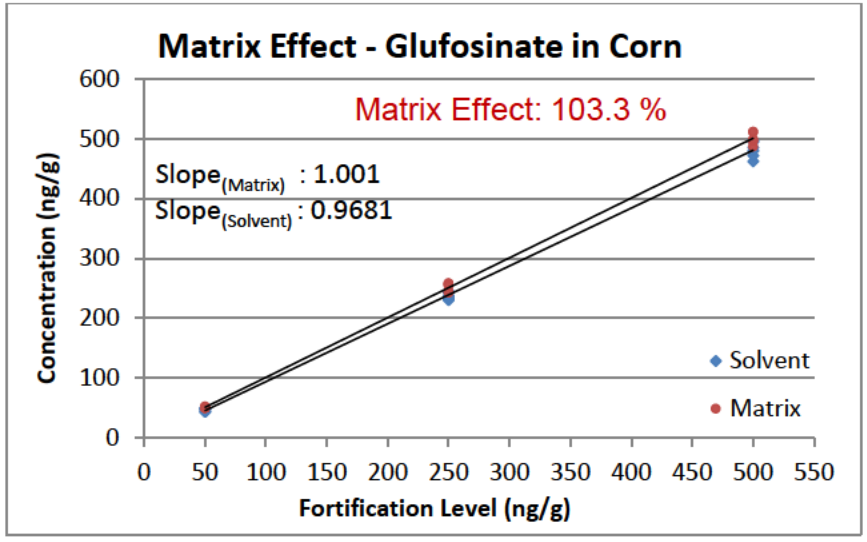


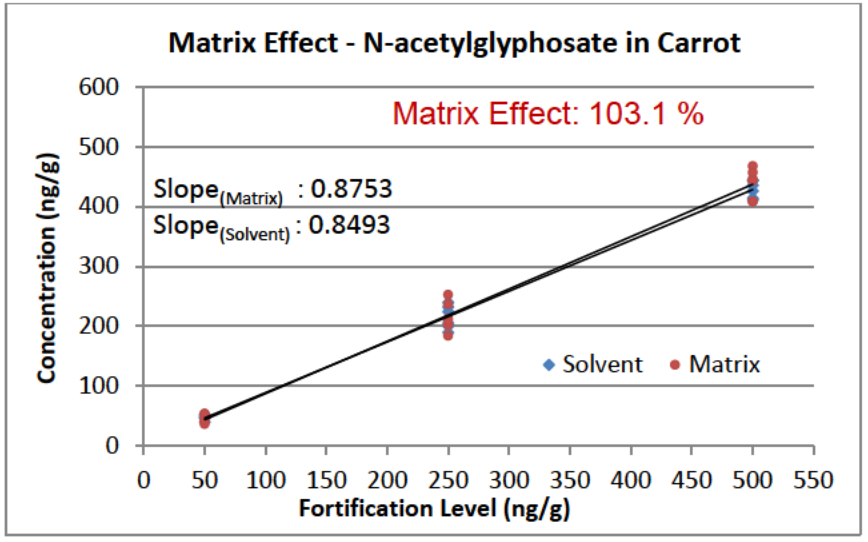


Method Collaboration Matrix Effects Charts

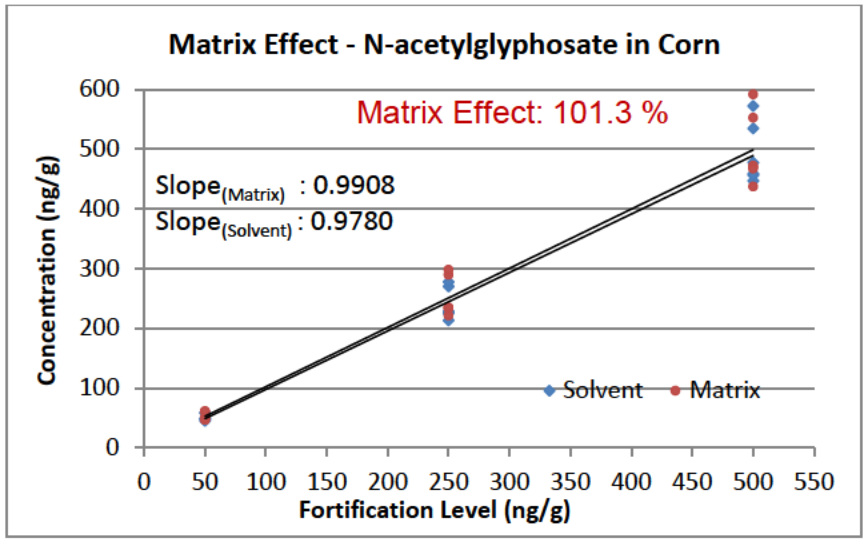


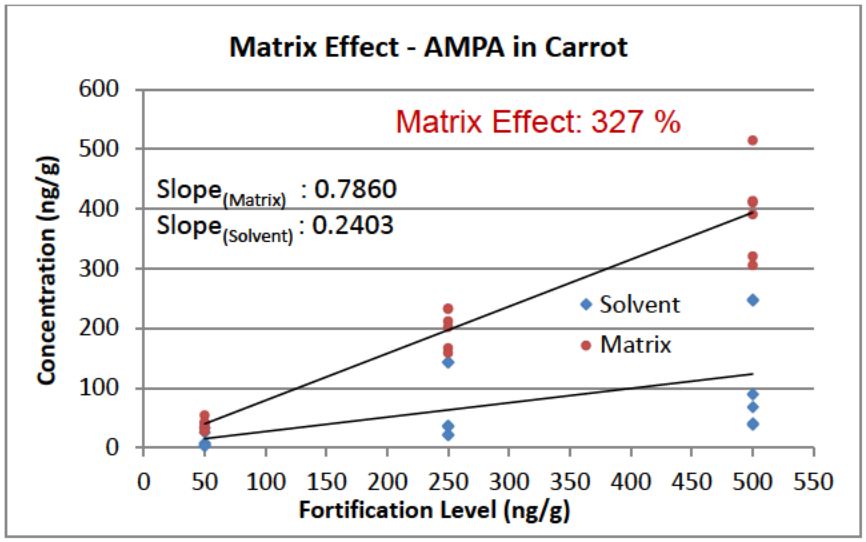


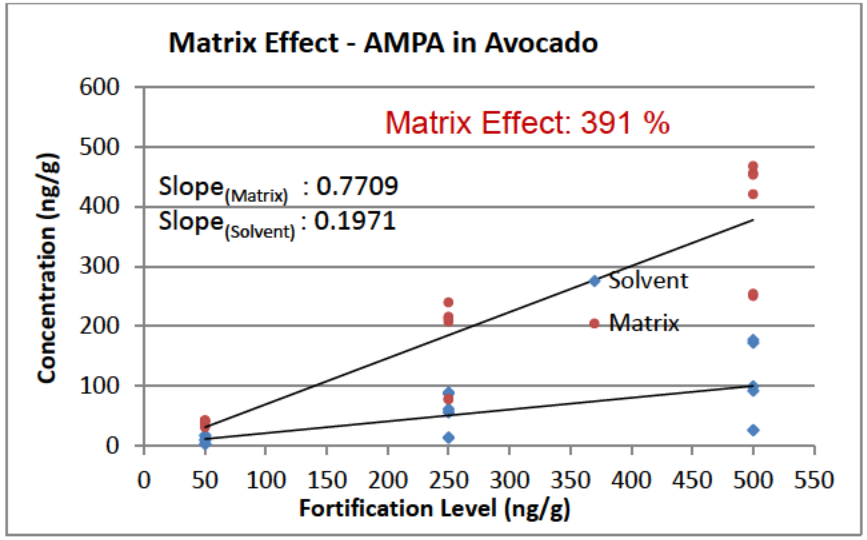


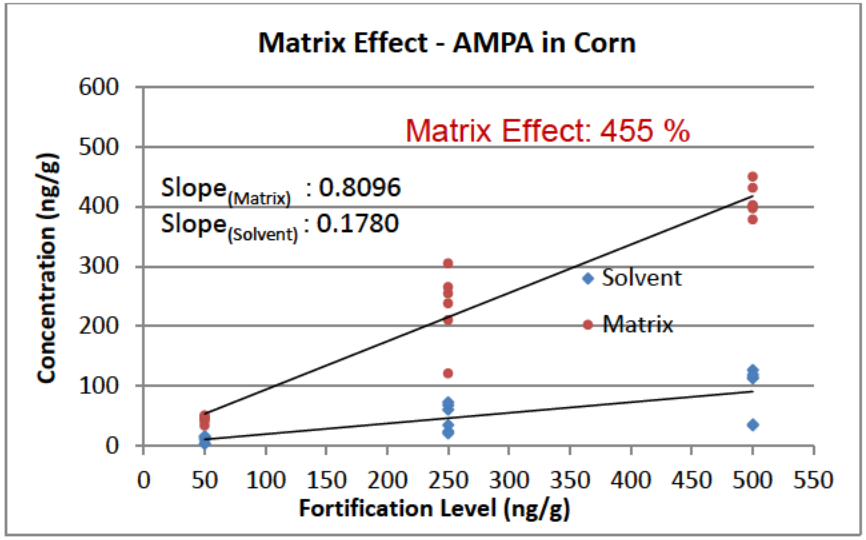


Method Collaboration Matrix Effects Charts









PSW Collaboration Data and System Suitability

All data and derived statistics in this attachment are from the method collaboration analyses conducted at PSW only. Results of the instrument system suitability and method collaboration verify that PSW is able to the method proficiently.

Instrument System Suitability

Prior to starting the collaboration instrument system suitability (SS) was demonstrated. Standards were injected at concentrations of 10, 50, 100, 200, 350, and 500 ng/ml to determine accuracy and linearity. Five replicates of the 50 ng/ml standard were injected to determine precision. The instrument LOQ was determined as per ORA-LAB.10 by injecting a 2 ng/ml standard and determining the S/N of the quantifier and qualifier ions. The LOQ was calculated as the lowest level where the S/N of the quantifier ion ≥ 10 and the S/N of the qualifier ion ≥ 3. Results for the instrument system suitability study are listed in the table below. Criteria for instrument system suitability are tabulated below.

LOQ (ng/ml)	Precision (RSD)	Accuracy (%)	Linearity (R^2)
≤ 2	≤ 10	90 - 110	0.995

Results for the instrument system suitability study, listed in the table below, are all within acceptable criteria.

SS Factor	Glyphosate		Glufosinate		AMPA		N-acetylglyphosate	
LOQ (ng/mL)	0.3		0.3		0.5		0.2	
Precision (RSD)	99.1	(1.4)	99.8	(2.3)	97.7	(2.1)	102.3	(1.2)
Accuracy (R^2)	100.4	(0.9997)	104.4	(0.9996)	96.1	(0.9998)	96.6	(0.9998)

Method Collaboration

The method and collaboration protocol are described in attachments A and B, respectively. The mean, RSD, method uncertainty (MU) of the recoveries for all three spike levels (50, 250, and 500 ng/g) were determined by matrix and overall. The linearity coefficient of determination (R^2) was calculated from the concentrations found at each level for each matrix by squaring the Excel correlation function (Correl). Statistics for all matrices were calculated from the whole set of data without correction for matrix bias. Acceptable method validation specifications for the collaboration study are listed below.

Recovery: 70-120 % RSD: 15% MU: 30% R^2: 0.990

Method collaboration results contributed by PSW are summarized in the Table F1 below; results that did not meet specifications are highlighted in red font. Scatter plots of the recoveries and linearity charts are provided in attachments F_1 and F_2, respectively. All results were within the validation specifications, with the exception of the R^2 for AMPA in corn of 0.9721 was just below the 0.99 specification.

PSW Collaboration Data and System Suitability

Table F1. Summary data includes the mean, RSD, method uncertainty (MU) of spike recoveries and coefficient of determination (R^2) of the three spike levels for each matrix.

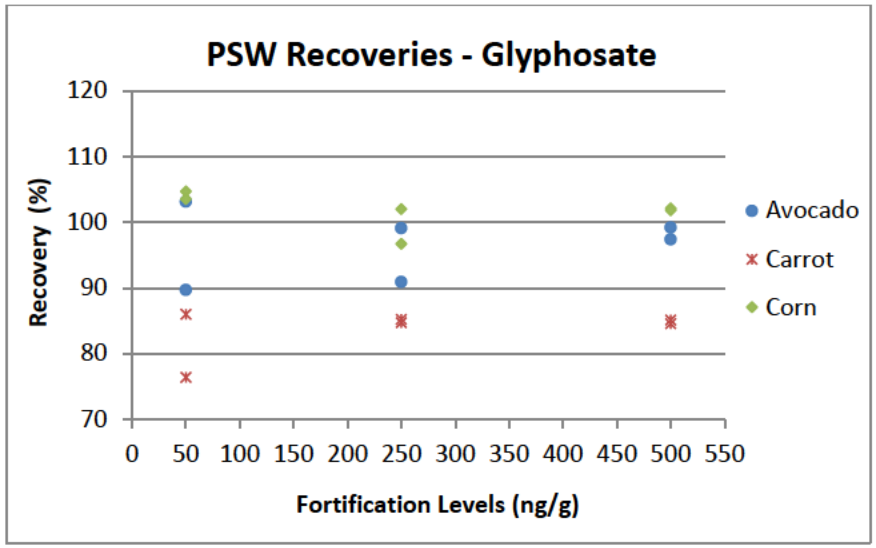
Matrix	N	Mean	RSD	MU	R^2
Glyphosate					
Avocado	6	96.6	5.4	13.9	0.9982
Carrot	6	83.7	4.3	11	0.9999
Corn	6	101.8	2.7	6.9	0.9993
Glufosinate					
Avocado	6	94.4	1.8	4.7	0.9998
Carrot	6	84.6	3.0	7.7	0.9999
Corn	6	102.0	1.9	4.9	0.9995
N-acetylglyphosate					
Avocado	6	106.3	2.8	7.1	0.9976
Carrot	6	97.7	8.2	21	0.9965
Corn	6	117.9	3.6	9.2	0.9968
AMPA					
Avocado	6	85.9	6.3	16.1	0.9971
Carrot	6	90.9	10.9	28.1	0.9943
Corn	6	90.3	11.2	28.9	0.9721

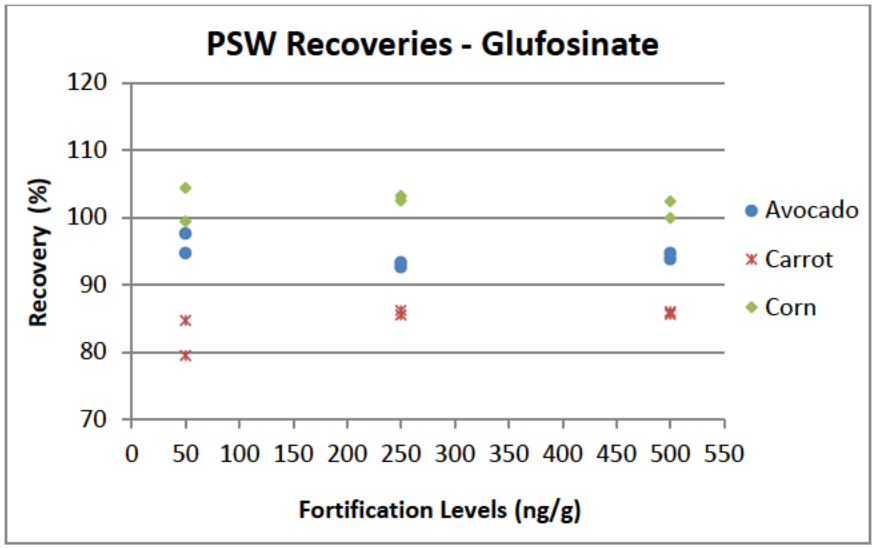
Analysis of Incurred Residues

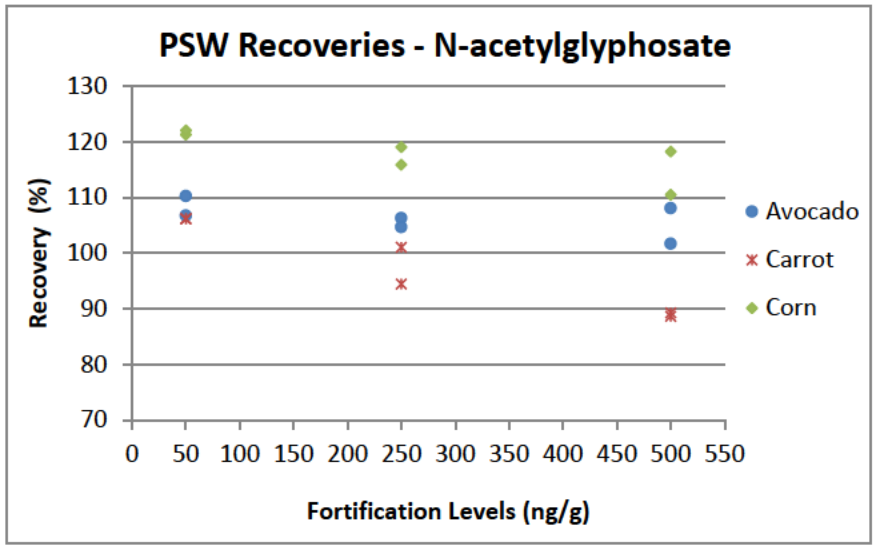
Results of the analysis of corn and soy containing incurred glyphosate residues are tabulated below. PSW findings are consistent with the range of residues levels reported from four different laboratories.

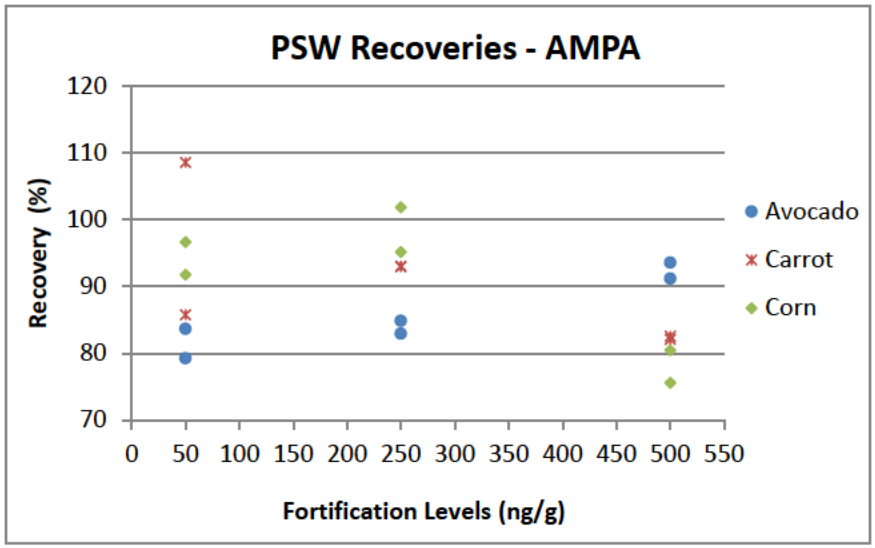
Matrix	Range	PSW
Corn	35-46	46
Soy	4290-4620	4620

PSW Collaboration Data and System Suitability – Recovery Charts

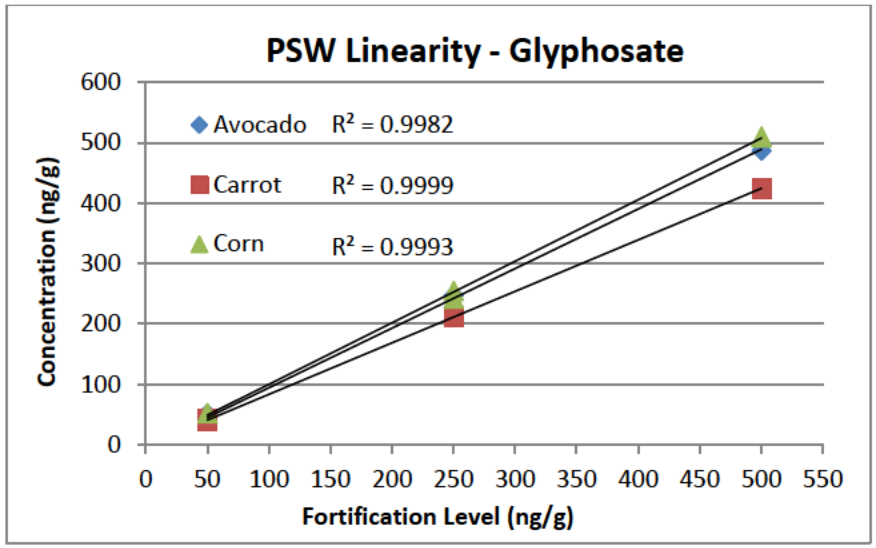


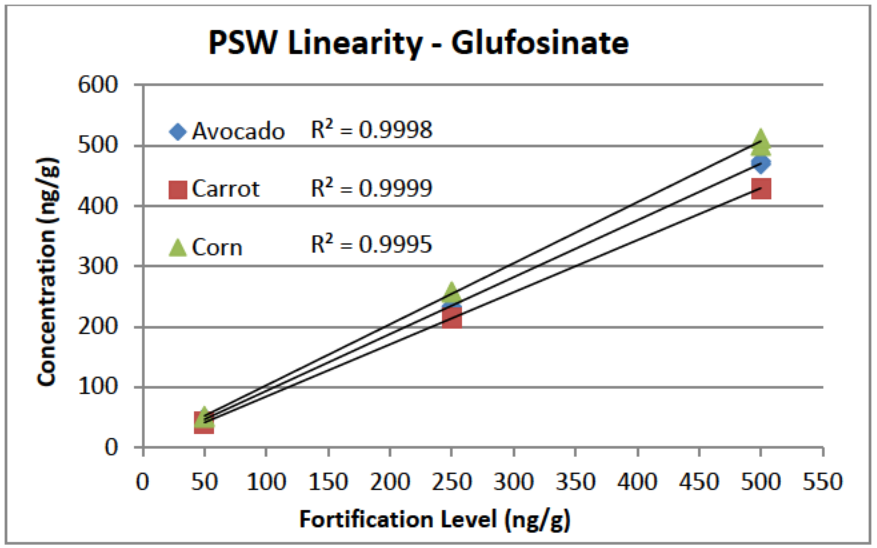


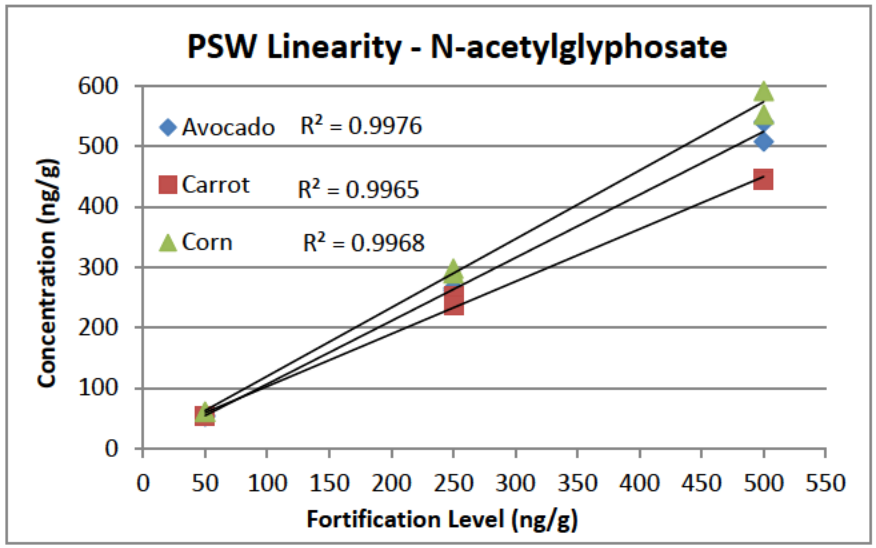


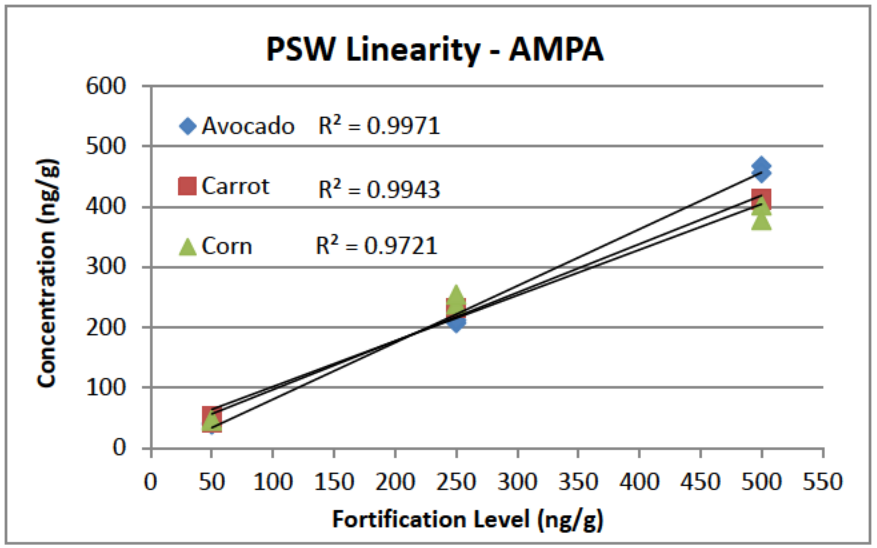


PSW Collaboration Data and System Suitability – Linearity Charts









PNW Collaboration Data and System Suitability

All data and derived statistics in this attachment are from the method collaboration analyses conducted at PNW only. Results of the instrument system suitability and method collaboration verify that PNW is able to the method proficiently.

Instrument System Suitability

Prior to starting the collaboration instrument system suitability (SS) was demonstrated. Standards were prepared and injected at concentrations of 1, 2, 5 10, 25, 50, 100, 200, 500, and 1000 ng/ml to determine accuracy and linearity; the standards at concentrations of 500 and 1000 ng/ml were not included in the accuracy and linearity calculations. Seven replicates of the 50 ng/ml standard were injected to determine precision. The instrument LOQ was determined as per ORA-LAB.10 by injecting standards at concentrations of 1, 2, 5, and 50 ng/ml and determining the S/N of the quantifier and qualifier ions. The LOQ was calculated as the lowest level where the S/N of the quantifier ion ≥ 10 and the S/N of the qualifier ion ≥ 3. Criteria for instrument system suitability are tabulated below.

LOQ (ng/ml)	Precision (RSD)	Accuracy (%)	Linearity (R^2)
≤ 2	≤ 10	90 - 110	0.995

Results for the instrument system suitability study, listed in the table below, are all within acceptable criteria with the exception of the LOQ for N-acetylglphosate at 6 ng/ml exceeded the maximum acceptable level of 2 ng/ml.

SS Factor	Glyphosate		Glufosinate		AMPA		N-acetylglyphosate	
LOQ (ng/mL)	0.4		1.4		2		6	
Precision (RSD)	98.4	(2.8)	96.2	(0.7)	96.4	(3.3)	97.2	(6.7)
Accuracy (R^2)	101	(0.9998)	99.4	(0.9999)	98.9	(0.9999)	101.1	(0.9998)

Method Collaboration

The method and collaboration protocol are described in attachments A and B, respectively. The mean, RSD, method uncertainty (MU) of the recoveries for all three spike levels (50, 250, and 500 ng/g) were determined by matrix and overall. The linearity coefficient of determination (R^2) was calculated from the concentrations found at each level for each matrix by squaring the Excel correlation function (Correl). Statistics for all matrices were calculated from the whole set of data without correction for matrix bias. Acceptable method validation specifications for the collaboration study are listed below.

Recovery: 70-120 % RSD: 15% MU: 30% R^2: 0.990

Method collaboration results contributed by PNW are summarized in the Table G1 below; results that did not meet specifications are highlighted in red font. Scatter plots of recoveries and

linearity charts for each analyte are provided in attachments G_1 and G_2. All results were within the validation specifications, with the exception of the R^2 of 0.9871 for N-acetylglyphosate, the R^2 of 0.9556 and 0.9571 for AMPA in carrot and corn, respectively, were just below the 0.99 specification. The precision and MU for AMPA in corn, 23.2 and 46.4 % also did not meet specifications of 15 and 30 %, respectively.

Table G1. Summary data includes the mean, RSD, method uncertainty (MU) of spike recoveries and coefficient of determination (R^2) of the three spike levels for each matrix.

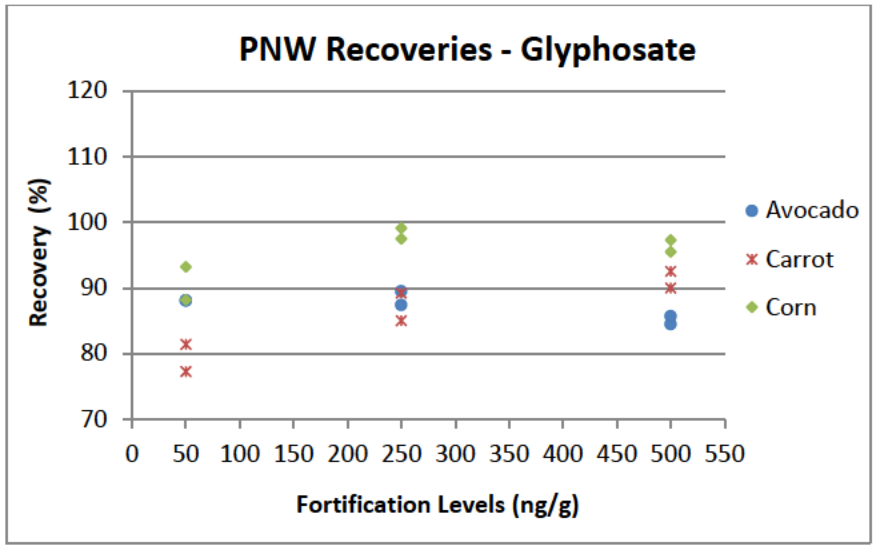
Matrix	N	Mean	RSD	MU	R^2
Glyphosate					
Avocado	6	87.2	2.1	5.4	0.9992
Carrot	6	85.9	6.7	17.3	0.9988
Corn	6	95.1	4.2	10.7	0.9994
Glufosinate					
Avocado	6	87.0	5.1	13.1	0.9925
Carrot	6	90.4	4.8	12.4	0.9981
Corn	6	101.4	1.6	4.2	0.9993
N-acetylglyphosate					
Avocado	6	90.3	9.0	23.2	(b) (5)
Carrot	6	86.7	5.5	14.3	0.9957
Corn	6	94.4	1.3	3.2	1.0000
AMPA					
Avocado	6	87.3	5.7	14.7	0.9938
Carrot	6	83.4	12.3	31.7	(b) (5)
Corn	6	76.5	(b) (5)	59.6	(b) (5)

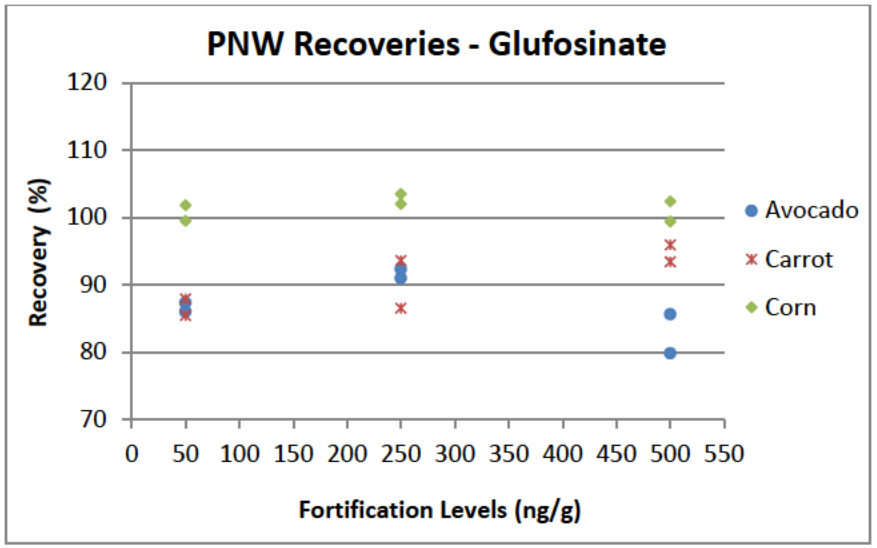
Analysis of Incurred Residues

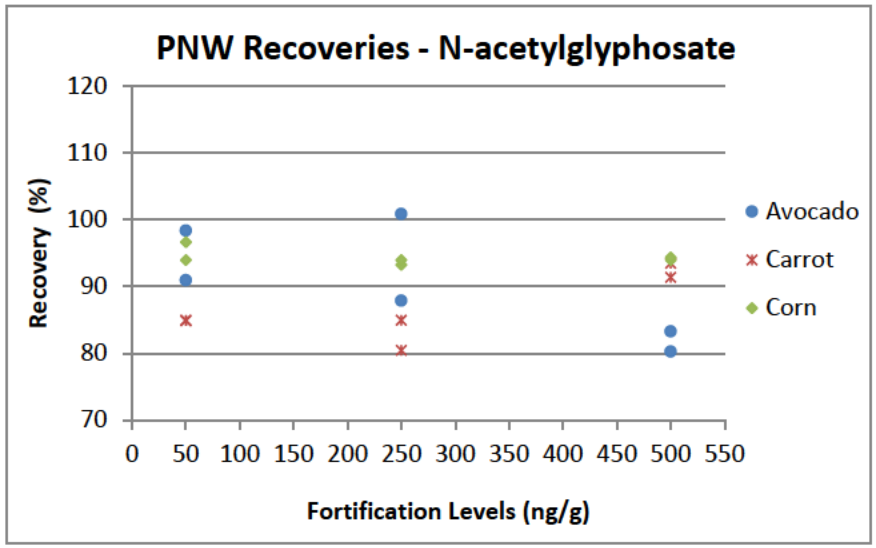
Results of the analysis of corn and soy containing incurred glyphosate residues are tabulated below. PNW findings are consistent with the range of residues levels reported from four different laboratories.

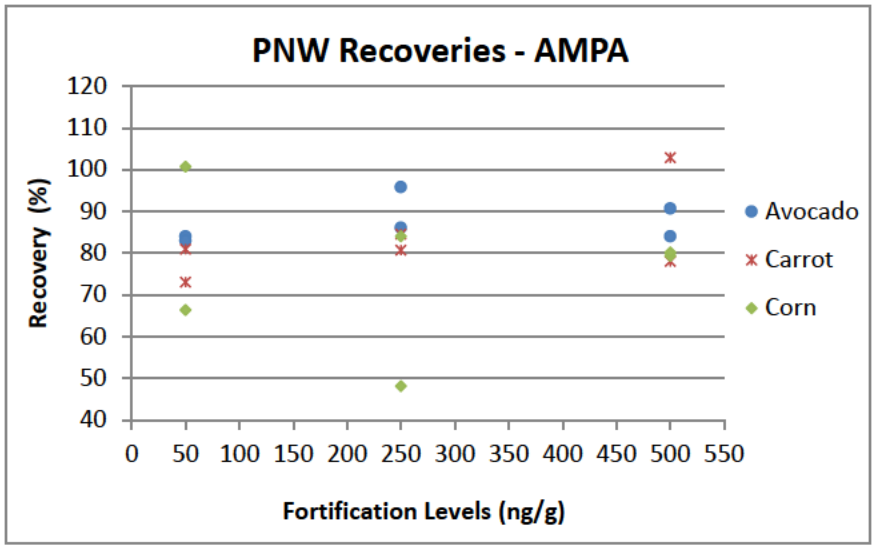
Matrix	Range	PNW
Corn	35-46	35
Soy	4290-4620	4610

PNW Collaboration Data and System Suitability – Recovery Charts

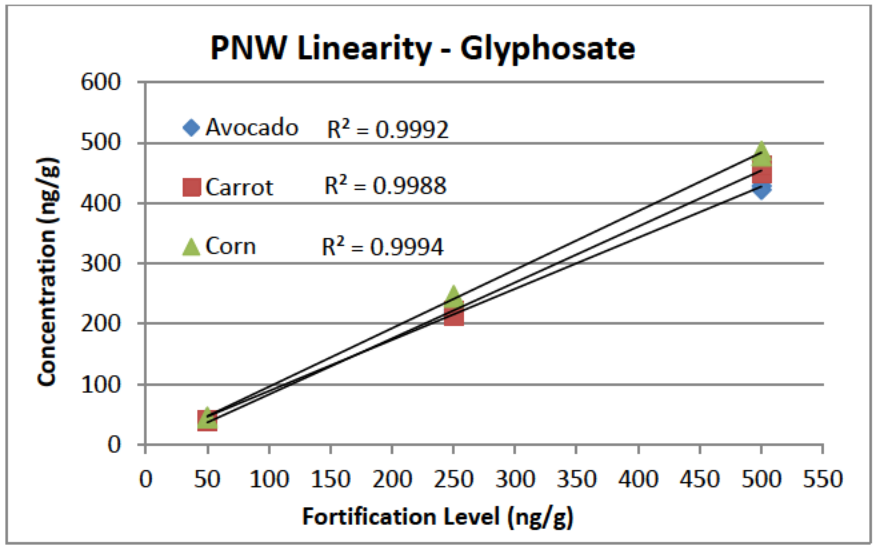


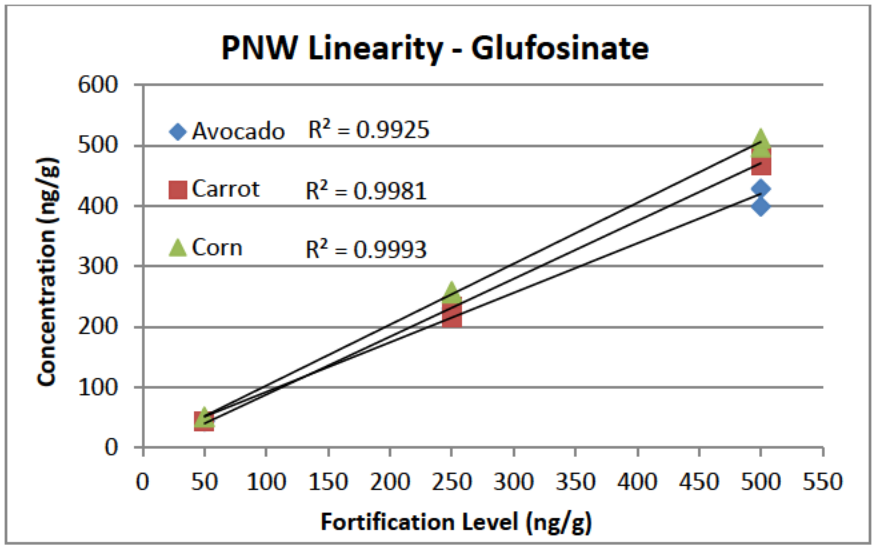


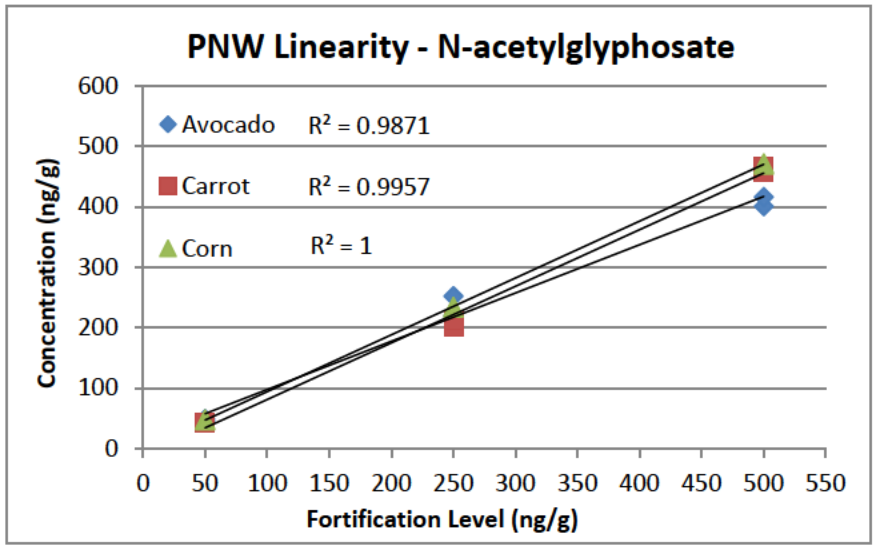


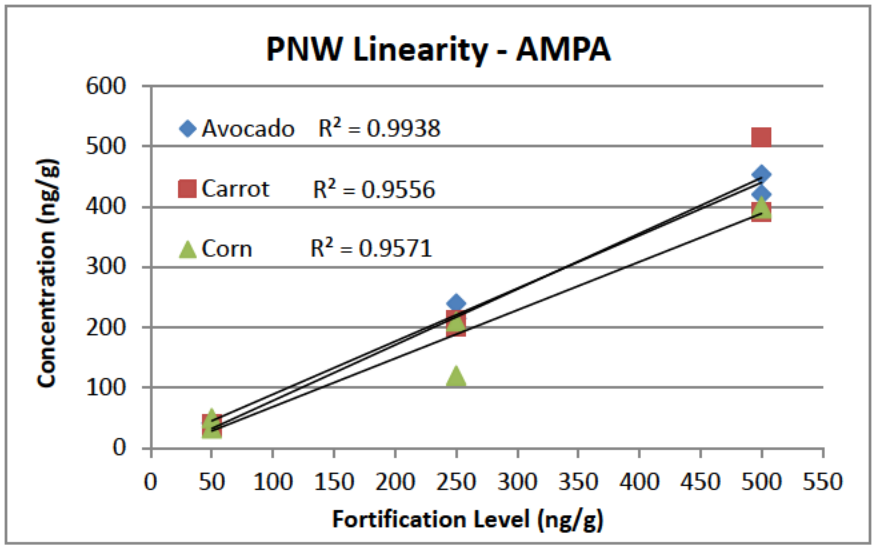


PNW Collaboration Data and System Suitability – Linearity Charts









ARL Collaboration Data and System Suitability

All data and derived statistics in this attachment are from the method collaboration analyses conducted at ARL only. Results of the instrument system suitability and method collaboration verify that ARL is able to the method proficiently.

Instrument System Suitability

Prior to starting the collaboration instrument system suitability (SS) was demonstrated. Standards were prepared and injected at concentrations of 1, 2, 5, 10, 25, 50, 100, 250, 500 and 1000 ng/ml to determine accuracy and linearity. Eight replicates of the 50 ng/ml standard were injected to determine precision. The instrument LOQ was determined as per ORA-LAB.10 by injecting standards at concentrations of 1, 2, 5, and 10 ng/ml and determining the S/N of the quantifier and qualifier ions. The LOQ was calculated as the lowest level where the S/N of the quantifier ion ≥ 10 and the S/N of the qualifier ion ≥ 3. Results for the instrument system suitability study are listed in the table below. Criteria for instrument system suitability are tabulated below.

LOQ (ng/ml)	Precision (RSD)	Accuracy (%)	Linearity (R^2)
≤ 2	≤ 10	90 - 110	0.995

Results for the instrument system suitability study, listed in the table below, are all within acceptable criteria.

SS Factor	Glyphosate		Glufosinate		AMPA		N-acetylglyphosate	
LOQ (ng/mL)	0.2		0.3		0.2		1.8	
Precision (RSD)	100.0	(1.1)	100.0	(1.0)	100.0	(1.8)	100.0	(1.9)
Accuracy (R^2)	102.8	(0.9998)	99.3	(0.9999)	106.7	(0.9996)	99.8	(0.9998)

Method Collaboration

The method and collaboration protocol are described in attachments A and B, respectively. Results from the analysis of spiked avocado, carrot, and corn matrices are summarized in Table E1. The mean, RSD, method uncertainty (MU) of the recoveries for all three spike levels (50, 250, and 500 ng/g) were determined by matrix and overall. The linearity coefficient of determination (R^2) was calculated from the concentrations found at each level for each matrix by squaring the Excel correlation function (Correl). Statistics for all matrices were calculated from the whole set of data without correction for matrix bias. Acceptable method validation specifications for the collaboration study are listed below.

Recovery: 70-120 % RSD: 15% MU: 30% R^2: 0.990

ARL Collaboration Data and System Suitability

Method collaboration results contributed by ARL are summarized in the Table H1 below; results that did not meet specifications are highlighted in red font. Scatter plots of individual recoveries and linearity charts for each matrix are provided in attachments H_1 and H_2, respectively. All results were within the validation specifications for glyphosate, glufosinate and the N-acetylglyphosate. Almost all results for AMPA failed validation specifications.

Table H1. Summary data includes the mean, RSD, method uncertainty (MU) of spike recoveries and coefficient of determination (R^2) of the three spike levels for each matrix.

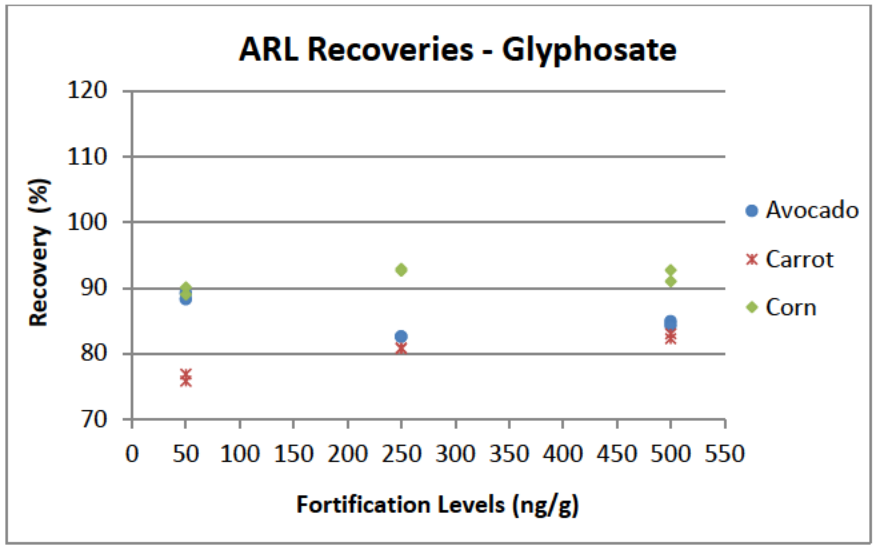
Matrix	N	Mean	RSD	MU	R^2
Glyphosate					
Avocado	6	85.3	3.3	8.5	0.9996
Carrot	6	80.0	3.7	9.4	0.9999
Corn	6	91.4	1.8	4.5	0.9997
Glufosinate					
Avocado	6	82.9	4.2	10.7	0.9987
Carrot	6	81.0	2.2	5.6	0.9991
Corn	6	98.4	1.2	3.1	0.9997
N-acetylglyphosate					
Avocado	6	85.7	6.1	15.7	0.9975
Carrot	6	79.7	6.7	17.2	0.9972
Corn	6	93.1	5.4	13.8	0.9968
AMPA					
Avocado	6	48.6	30.9	79.4	0.9324
Carrot	6	61.3	7.1	18.2	0.9972
Corn	6	95.8	15.9	40.8	0.9587

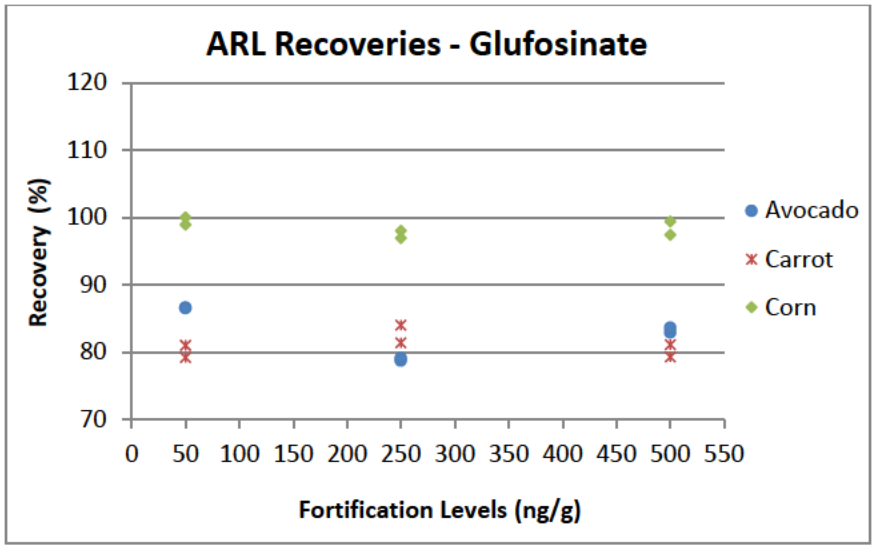
Analysis of Incurred Residues

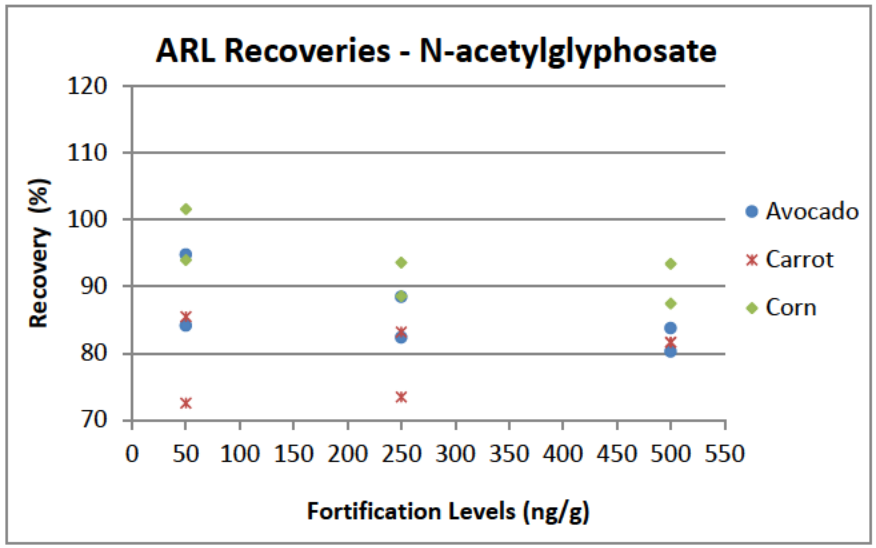
Results of the analysis of corn and soy containing incurred glyphosate residues are tabulated below. ARL findings are consistent with the range of residues levels reported from four different laboratories.

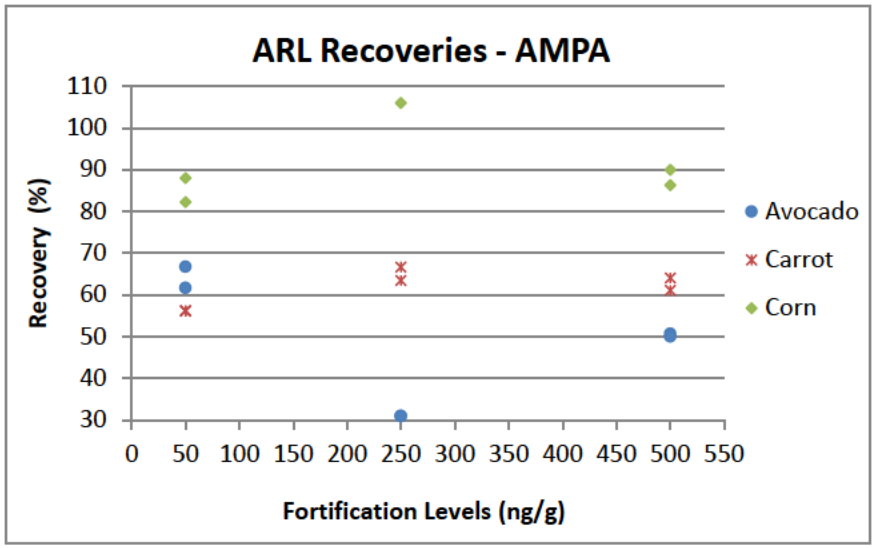
Matrix	Range	ARL
Corn	35-46	36
Soy	4290-4620	4290

ARL Collaboration Data and System Suitability – Recovery Charts

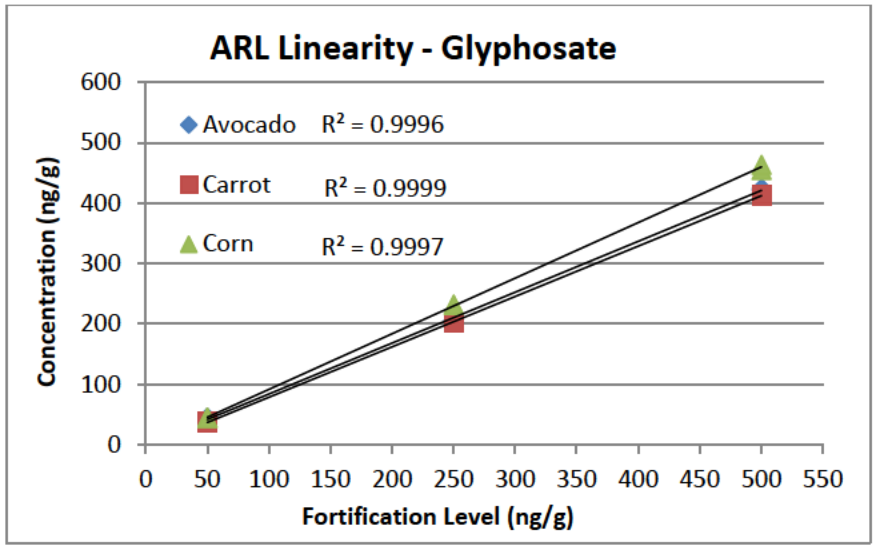


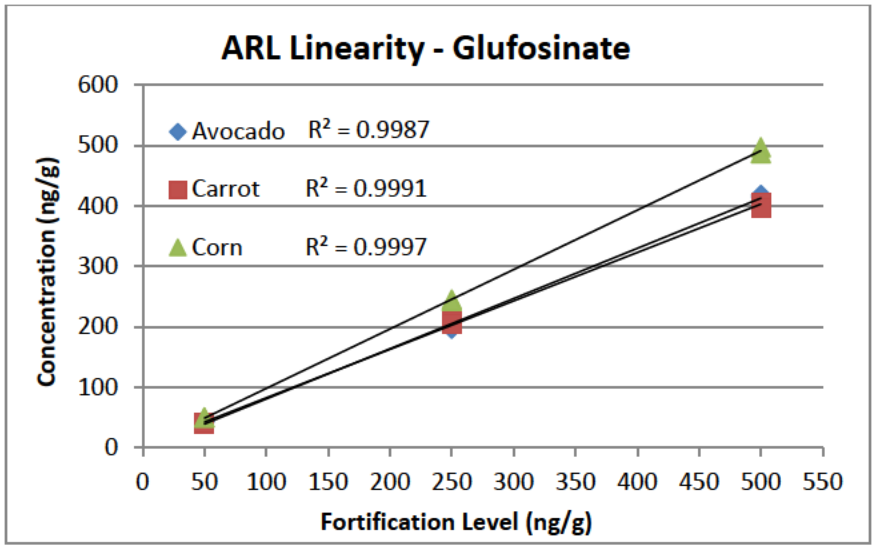


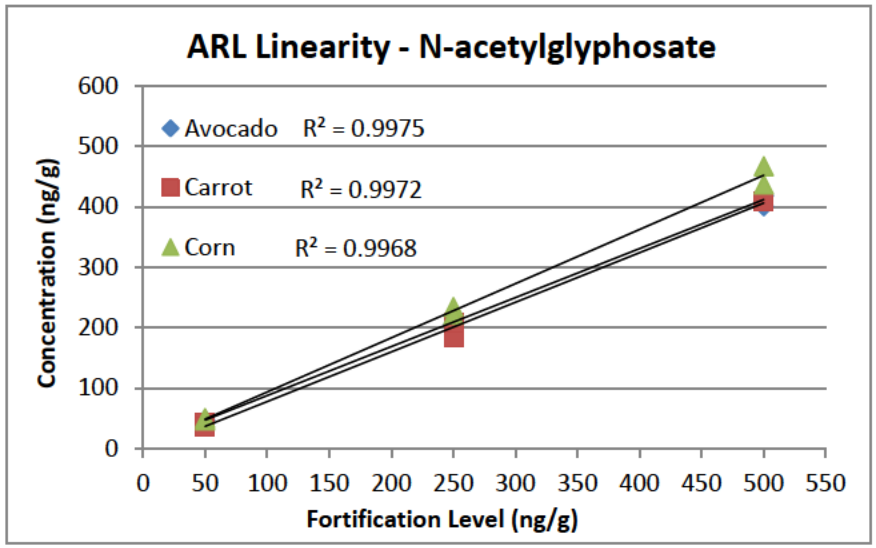


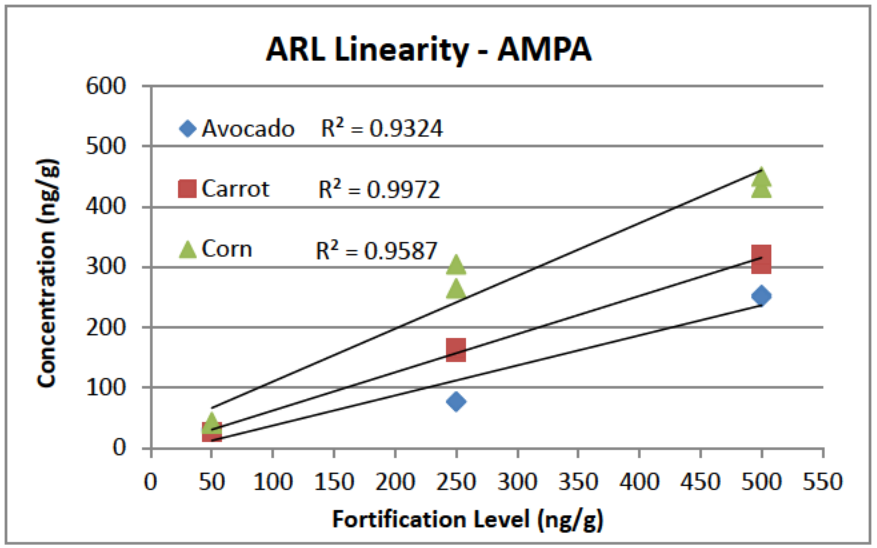


ARL Collaboration Data and System Suitability – Linearity Charts

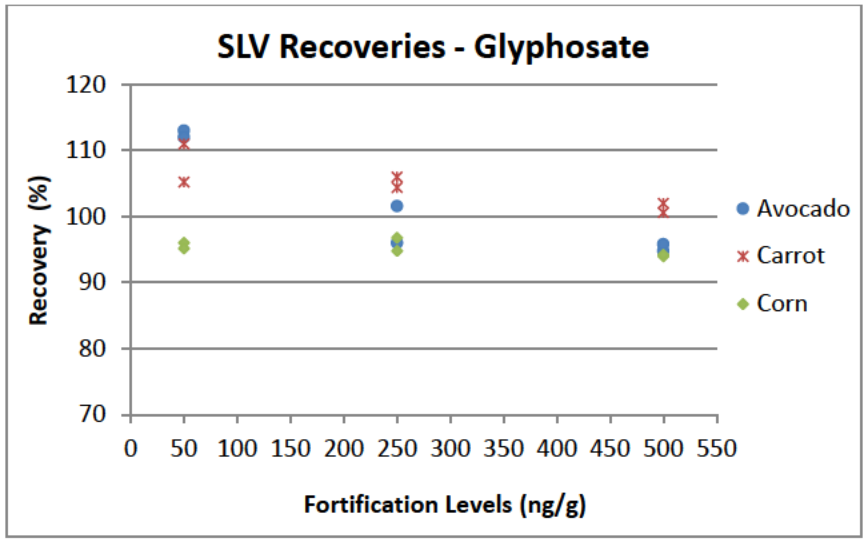


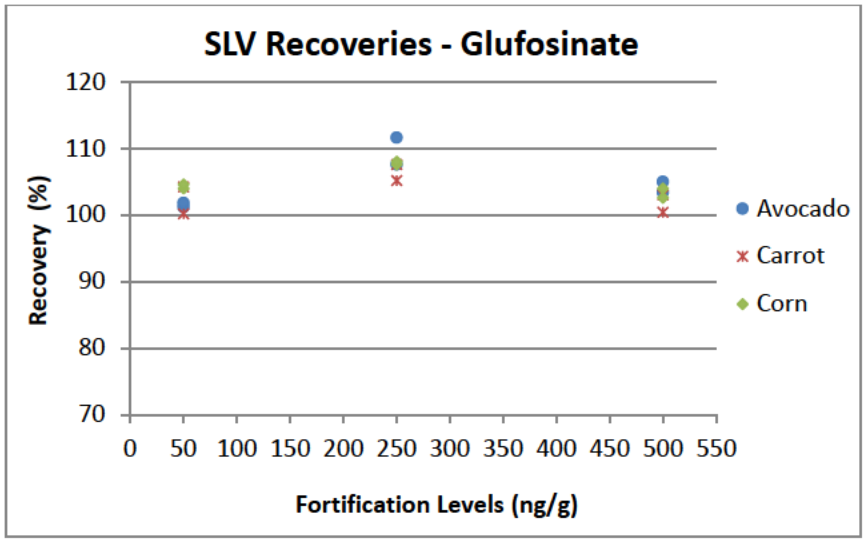


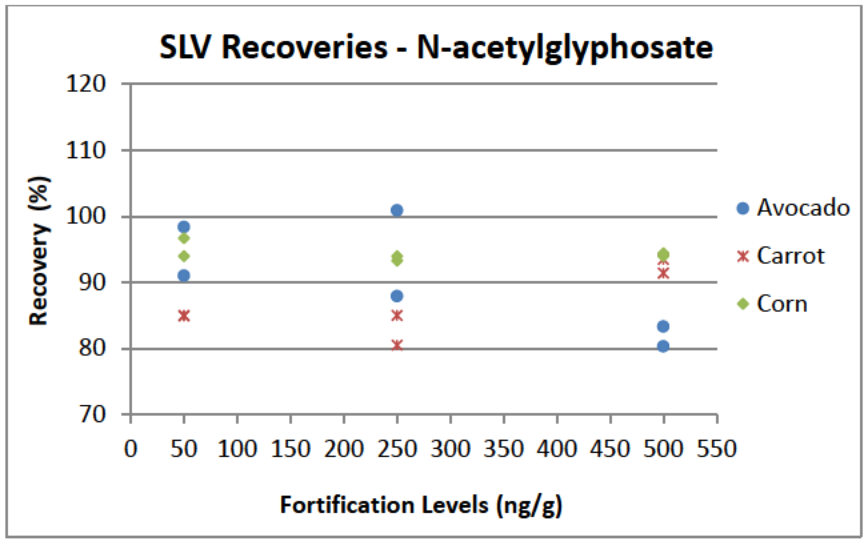


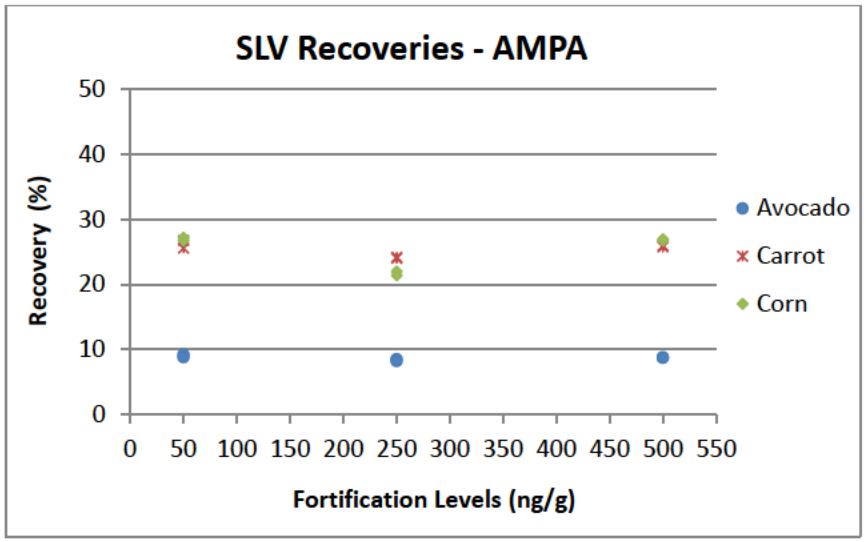


Single Laboratory Validation – Method Recovery Charts

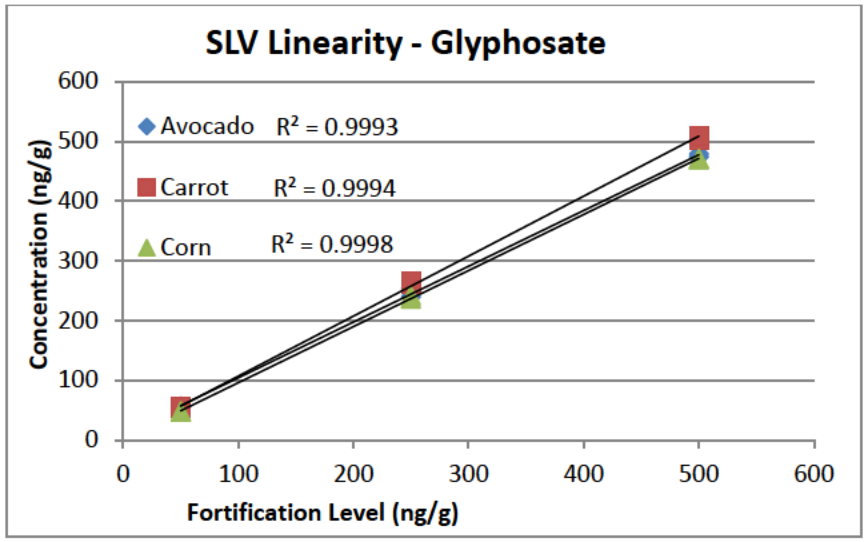


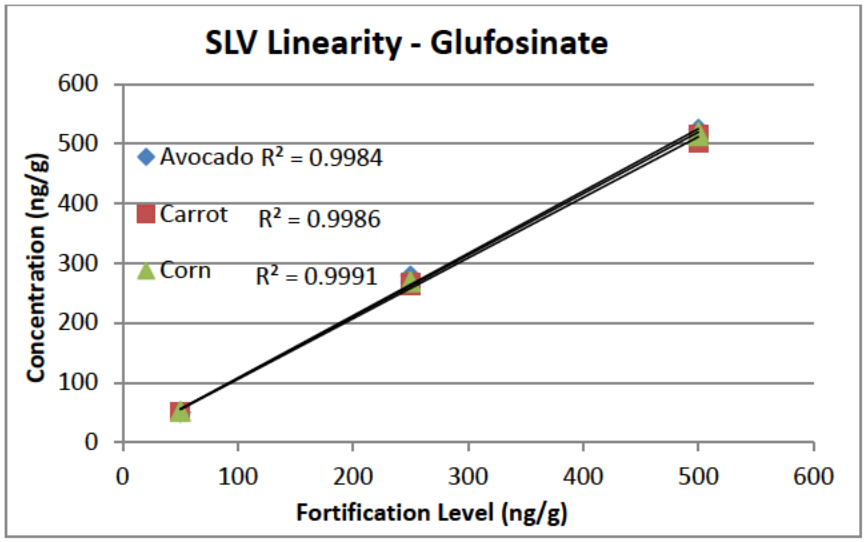


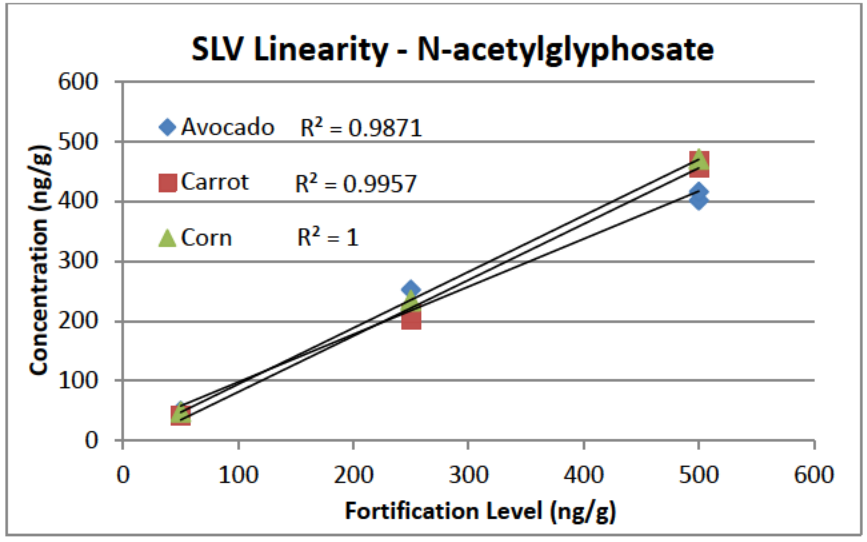


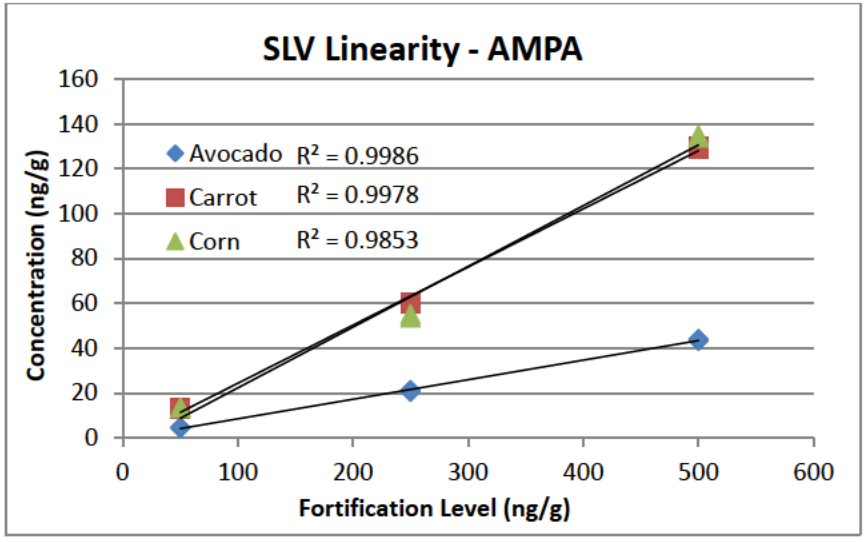


Single Laboratory Validation – Method Linearity Charts









PSW Collaboration Data and System Suitability

Table F1. Summary data includes the mean, RSD, method uncertainty (MU) of spike recoveries and coefficient of determination (R^2) of the three spike levels for each matrix.

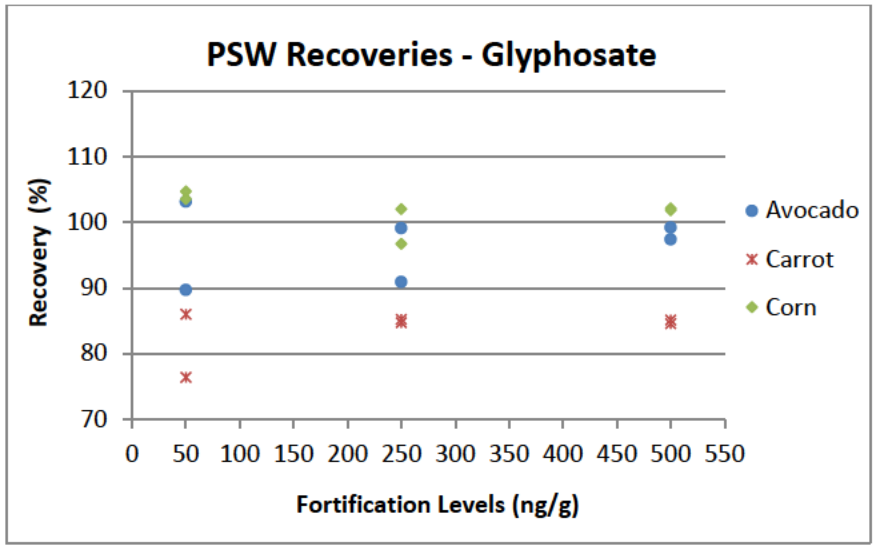
Matrix	N	Mean	RSD	MU	R^2
Glyphosate					
Avocado	6	96.6	5.4	10.8	0.9982
Carrot	6	83.7	4.3	8.6	0.9999
Corn	6	101.8	2.7	5.4	0.9993
Glufosinate					
Avocado	6	94.4	1.8	3.7	0.9998
Carrot	6	84.6	3.0	6.0	0.9999
Corn	6	102.0	1.9	3.8	0.9995
N-acetylglyphosate					
Avocado	6	90.3	9	18.1	0.9976
Carrot	6	86.7	5.5	11.1	0.9965
Corn	6	94.4	1.3	2.5	0.9968
AMPA					
Avocado	6	85.9	6.3	12.5	0.9971
Carrot	6	90.9	10.9	21.9	0.9943
Corn	6	90.3	11.2	22.5	0.9721

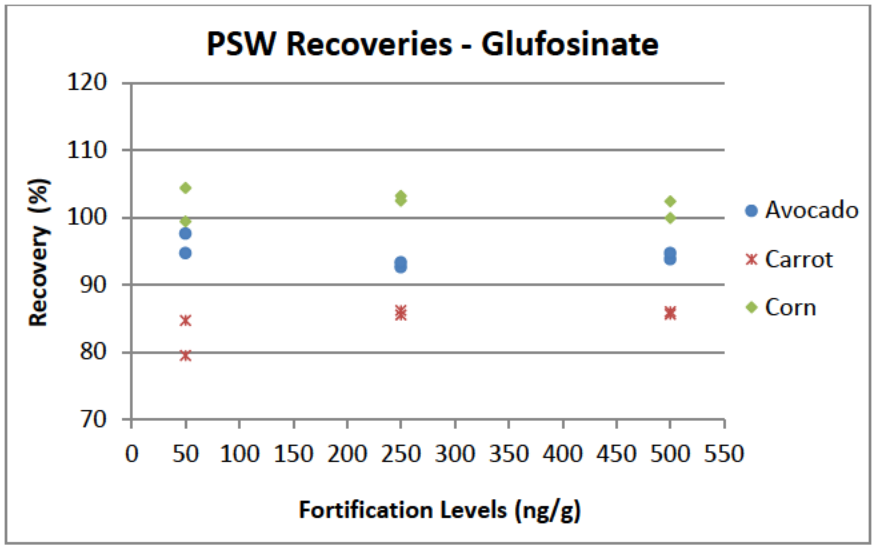
Analysis of Incurred Residues

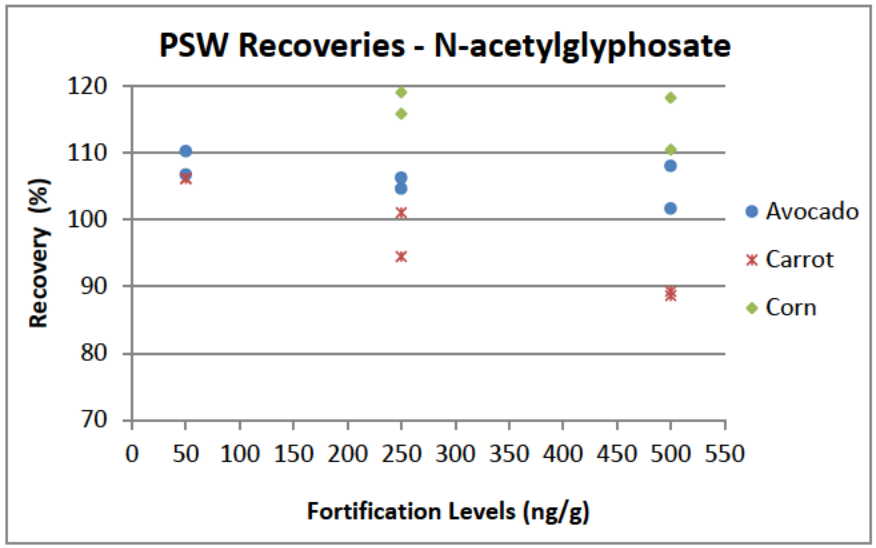
Results of the analysis of corn and soy containing incurred glyphosate residues are tabulated below. PSW findings are consistent with the range of residues levels reported from four different laboratories.

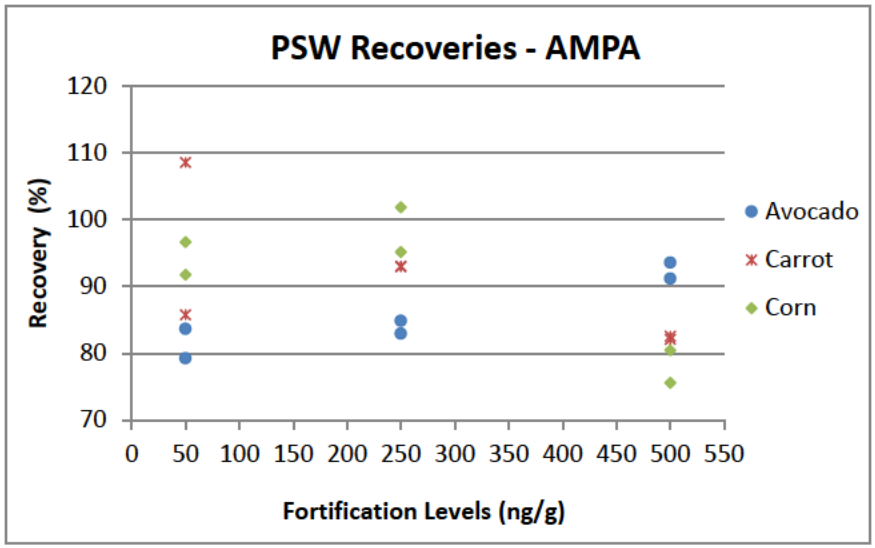
Matrix	Range	PSW
Corn	35-46	46
Soy	4290-4620	4620

PSW Collaboration Data and System Suitability – Recovery Charts









linearity charts for each analyte are provided in attachments G_1 and G_2. All results were within the validation specifications, with the exception of the R^2 of (b) (4) for N-acetylglyphosate, the R^2 of (b) (4) and (b) (4) for AMPA in carrot and corn, respectively, were just below the 0.99 specification. The precision and MU for AMPA in corn, (b) (4) and (b) (4) % also did not meet specifications of 15 and 30 %, respectively.

Table G1. Summary data includes the mean, RSD, method uncertainty (MU) of spike recoveries and coefficient of determination (R^2) of the three spike levels for each matrix.

Matrix	N	Mean	RSD	MU	R^2
Glyphosate					
Avocado	6	87.2	2.1	4.2	0.9992
Carrot	6	85.9	6.7	13.4	0.9988
Corn	6	95.1	4.2	8.3	0.9994
Glufosinate					
Avocado	6	87.0	5.1	10.2	0.9925
Carrot	6	90.4	4.8	9.7	0.9981
Corn	6	101.4	1.6	3.2	0.9993
N-acetylglyphosate					
Avocado	6	90.3	9.0	18.1	(b) (4)
Carrot	6	86.7	5.5	11.1	0.9957
Corn	6	94.4	1.3	2.5	1.0000
AMPA					
Avocado	6	87.3	5.7	11.5	0.9938
Carrot	6	83.4	12.3	24.6	(b) (4)
Corn	6	76.5	(b) (4)	(b) (4)	(b) (4)

Analysis of Incurred Residues

Results of the analysis of corn and soy containing incurred glyphosate residues are tabulated below. PNW findings are consistent with the range of residues levels reported from four different laboratories.

Matrix	Range	PNW
Corn	35-46	35
Soy	4290-4620	4610

ARL Collaboration Data and System Suitability

All data and derived statistics in this attachment are from the method collaboration analyses conducted at ARL only. Results of the instrument system suitability and method collaboration verify that ARL is able to the method proficiently.

Instrument System Suitability

Prior to starting the collaboration instrument system suitability (SS) was demonstrated. Standards were prepared and injected at concentrations of 1, 2, 5, 10, 25, 50, 100, 250, 500 and 1000 ng/ml to determine accuracy and linearity. Eight replicates of the 50 ng/ml standard were injected to determine precision. The instrument LOQ was determined as per ORA-LAB.10 by injecting standards at concentrations of 1, 2, 5, and 10 ng/ml and determining the S/N of the quantifier and qualifier ions. The LOQ was calculated as the lowest level where the S/N of the quantifier ion ≥ 10 and the S/N of the qualifier ion ≥ 3. Results for the instrument system suitability study are listed in the table below. Criteria for instrument system suitability are tabulated below.

LOQ (ng/ml)	Precision (RSD)	Accuracy (%)	Linearity (R^2)
≤ 2	≤ 10	90 - 110	0.995

Results for the instrument system suitability study, listed in the table below, are all within acceptable criteria.

SS Factor	Glyphosate		Glufosinate		AMPA		N-acetylglyphosate	
LOQ (ng/mL)	0.2		0.3		0.2		1.8	
Precision (RSD)	100.0	(1.0)	100.0	(1.0)	100.0	(1.8)	100.0	(1.7)
Accuracy (R^2)	102.8	(0.9998)	99.3	(0.9999)	106.7	(0.9996)	99.8	(0.9998)

Method Collaboration

The method and collaboration protocol are described in attachments A and B, respectively. Results from the analysis of spiked avocado, carrot, and corn matrices are summarized in Table E1. The mean, RSD, method uncertainty (MU) of the recoveries for all three spike levels (50, 250, and 500 ng/g) were determined by matrix and overall. The linearity coefficient of determination (R^2) was calculated from the concentrations found at each level for each matrix by squaring the Excel correlation function (Correl). Statistics for all matrices were calculated from the whole set of data without correction for matrix bias. Acceptable method validation specifications for the collaboration study are listed below.

Recovery: 70-120 % RSD: 15% MU: 30% R^2: 0.990

ARL Collaboration Data and System Suitability

Method collaboration results contributed by ARL are summarized in the Table H1 below; results that did not meet specifications are highlighted in red font. Scatter plots of individual recoveries and linearity charts for each matrix are provided in attachments H_1 and H_2, respectively. All results were within the validation specifications for glyphosate, glufosinate and the N-acetylglyphosate. Almost all results for AMPA failed validation specifications.

Table H1. Summary data includes the mean, RSD, method uncertainty (MU) of spike recoveries and coefficient of determination (R^2) of the three spike levels for each matrix.

Matrix	N	Mean	RSD	MU	R^2
Glyphosate					
Avocado	6	85.3	3.3	6.6	0.9996
Carrot	6	80.0	3.7	7.3	0.9999
Corn	6	91.4	1.8	3.5	0.9997
Glufosinate					
Avocado	6	82.9	4.2	8.3	0.9987
Carrot	6	81.0	2.2	4.3	0.9991
Corn	6	98.4	1.2	2.4	0.9997
N-acetylglyphosate					
Avocado	6	85.7	6.1	12.2	0.9975
Carrot	6	79.7	6.7	13.4	0.9972
Corn	6	93.1	5.4	10.7	0.9968
AMPA					
Avocado	6	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Carrot	6	(b) (4)	7.1	14.1	0.9972
Corn	6	95.8	(b) (4)	(b) (4)	(b) (4)

Analysis of Incurred Residues

Results of the analysis of corn and soy containing incurred glyphosate residues are tabulated below. ARL findings are consistent with the range of residues levels reported from four different laboratories.

Matrix	Range	ARL
Corn	35-46	36
Soy	4290-4620	4290

July 21, 2017

Glyphosate Method Collaboration Report

Introduction

A multi-laboratory validation (collaboration) was conducted of a method for the determination of residue levels of glyphosate, glufosinate, and two degradants of glyphosate N-acetylglyphosate and AMPA. Single laboratory validation of the method, conducted at PSFFL, is reported separately. Five FDA pesticide laboratories participated in the collaboration: PSFFL, PNL, ARKL, KCL and CFSAN. This collaboration report summarizes data submitted from all five participating laboratories. In addition to the summary of collaboration data, abbreviated reports for each collaborating laboratory are included as attachments to the collaboration report.

Conclusion

The collaboration data indicates the method is suitable for the purpose of quantitative determination for residues of glyphosate, glufosinate and N-acetylglyphosate and semi quantitative determination of AMPA residues in the three primary matrix types analyzed in the FDA pesticide program, i.e., high moisture, low moisture, and high fat items. The collaboration meets all the requirements of a level three multi-laboratory validation as per the "Guidelines for Validation of Chemical Methods for the FDA FVM Program, 2nd Edition.

Protocols and Procedure

Commodities were selected to represent the three major food commodity types analyzed in the FDA pesticide program, i.e. grain corn for dry products, carrots for high moisture products, and avocados for high lipid commodities. Composites of each of these three study matrices were prepared, composited, and distributed to the participating laboratories (PNL, PSFFL, ARKL, KCL, and CFSAN). Note: avocados were prepared without the outer peel. Each lab analyzed all matrices fortified with each analyte at the fortification levels in replicate as listed below:

i none: 1x
ii 0.050 ppm: 2x
iii 0.250 ppm: 2x
iv 0.500 ppm: 2x

Each lab was additionally sent two samples previously found to contain incurred glyphosate residues when analyzed at SRL using the method described in LIB 4596, i.e., ground grain corn in which 0.04 ppm was found and ground soy beans in which 4.5 ppm was found.

A detailed protocol is provided in attachment A and the method is provided in attachment B.

PSFFL conducted a single laboratory validation (SLV) of the procedure using the same procedure and collaboration protocol. The SLV results and protocols are reported separately.

Prior to conducting multiple laboratory method validation each participating laboratory was required to demonstrate proficiency with the procedure. Instrument proficiency was

demonstrated conducting system suitability tests that included determination of accuracy, precision, linearity and LOQ by preparing and injecting standards. Results of the system suitability testing are reported with the attached individual laboratory reports (attachments E - I).

The concentrations and spike recoveries were calculated by single level calibration using average responses of matrix matched standards bracketing the samples and prepared at the same concentration as the spiked sample. For glyphosate and glufosinate residue levels were calculated using corresponding isotopic internal standards added to the extraction solvent prior to analysis. With the exception of CFSAN, the AMPA residues were calculated against the glyphosate isotopic internal standard. CFSAN used isotopically labelled AMPA to calculate their residue AMPA levels. Residues of N-acetylglyphosate were calculated using external standard calibration.

The mean recoveries for all three spike levels (50, 250, and 500 ng/g) were calculated by matrix for each laboratory. The overall mean, RSD and method uncertainty (MU) of all three laboratories was calculated for each matrix. MU was calculated at the 95 % confidence level using the Student T distribution corresponding to the degrees of freedom of the number of repetitions conducted. The linearity coefficient of determination (R^2) was calculated from the concentrations found at each level for each matrix and laboratory by squaring the Excel correlation function (Correl); the average R^2 of the three laboratories is reported in Table 1. Method specificity was evaluated by the analysis of control matrices. Acceptable validation specifications for the collaboration study are listed below.

Specificity: No residues found in blank control matrices

Recovery: 70-120 % RSD: 15% MU: 30% R^2: 0.990

Glyphosate Method Collaboration Report

Results and Discussion

The method collaboration results in this report were provided by the five participating laboratories: PSFFL, PNL, ARKL, KCL and CFSAN. Table 1 contains the summary statistical analysis of all collaboration analyses; results that did not meet specifications are highlighted in red font. Scatter plots of the recoveries are provided in attachment D. No residues were found in the control samples analyzed for each matrix. All results for glyphosate, glufosinate, and N-acetylglyphosate were within the validation specifications. The average linearity of the AMPA results was just below the specification of $R^2 \geq 0.99$ in carrots at 0.9892. One lab reported low recoveries (48.6 % and 61.3 %) of AMPA in avocado and carrot, respectively. Those low AMPA recoveries resulted in corresponding RSDs and MUs that did meet the specifications of 15 and 30 %, respectively.

Table 1. Summary data includes the average spike recovery for each lab, overall average recovery, RSD, method uncertainty (MU) of the spike recoveries and the average coefficient of determination (R^2) of the spike concentrations.

	Mean Spike Recoveries Per Lab					**Accuracy, Precision, and Linearity - All Labs**			
Matrix	**PSFFL**	**PNL**	**ARKL**	**KCL**	**CFSAN**	**Mean**	**RSD**	**MU**	**R^2**
Glyphosate									
Avocado	96.6	87.2	85.3	88.5	83.5	88.2	6.1	12.6	0.9979
Carrot	83.7	85.9	80.0	83.1	80.4	82.6	5.3	10.8	0.9968
Corn	101.8	95.1	91.4	97.4	96.4	96.4	5.0	10.3	0.9986
Glufosinate									
Avocado	94.4	87.0	82.9	88.3	83.2	87.2	6.0	12.3	0.9958
Carrot	84.6	90.4	81.0	83.7	80.4	84.0	5.6	11.4	0.9956
Corn	102.0	101.4	98.4	98.0	99.5	99.9	2.3	4.7	0.9994
N-acetylglyphosate									
Avocado	106.3	90.3	85.7	89.4	80.9	90.5	12.0	24.6	0.9924
Carrot	97.7	86.7	79.7	85.6	83.7	86.7	9.8	20.0	0.9941
Corn	117.9	94.4	93.1	97.9	95.1	99.7	10.4	21.2	0.9986
AMPA									
Avocado	85.9	87.3	(b) (4)	87.6	83.9	78.7	(b) (4)	(b) (4)	0.9984
Carrot	90.9	83.4	(b) (4)	85.2	80.8	80.3	(b) (4)	(b) (4)	(b) (4)
Corn	90.3	76.5	95.8	97.0	93.8	90.7	14.4	29.5	0.9995

Glyphosate Method Collaboration Report

Matrix Effects

The matrix effect for each analyte/matrix combination was evaluated by calculating residue concentrations using both matrix matched standards and standards prepared in solvent and comparing the slopes of the corresponding linearity charts. Results of the matrix study are tabulated in Table 2 and linearity charts for each analyte/matrix combination are provided in attachment E. Results indicate none of the matrices in the study had much effect on the determination of glyphosate, glufosinate and N-acetylglyphosate. However, all three matrices had a significant impact on residues of AMPA with matrix effects of 391 % in avocado, 327 % in carrot, and 455 % in corn. These results also reflect the advantage of using isotopically labelled internal standards.

Table 2. Matrix effects as percentages of slope ratios of residues calculated for the three spike levels using standards prepared in solvent vs matrix extracts

Compound	Avocado	Carrot	Corn
Glyphosate	89.0	91.6	99.8
Glufosinate	87.8	87.1	102.8
N-acetylglyphosate	117.9	103.1	104.2
AMPA	261	327	283

Analysis of Proficiency Samples

Each laboratory analyzed a corn sample and a soy sample previously analyzed and found to contain incurred residue of glyphosate. Results of the incurred residue analysis, tabulated in Table 3, are in excellent agreement.

Table 3. Incurred residues (ppb) in corn and soy samples.

Matrix	Mean	(± 2SD Range)	PSFFL	PNL	ARKL	KCL	CFSAN
Corn	40.5	(30.7 - 50.3)	46.5	35.3	36.2	40.1	44.4
Soy	4260	(3530 - 4990)	4620	4610	4290	3920	3850

Calculation of Residues Levels Using a Single Vs Multiple Calibration Levels

For the method collaboration study spike recoveries were calculated based upon a single level calibration at the same concentration as the spike level, i.e., the 50 ng/g spikes were calculated based upon calibration at 10 ng/ml (equivalent to 50 ng/g in sample), the 250 ng/g spikes were calculated based upon calibration at 50 ng/ml (equivalent to 250 ng/g in sample), and the 500 ng/g spikes were calculated based upon calibration at 100 ng/ml (equivalent to 500 ng/g in sample). However, once implemented for routine analysis, calibration will be conducted at a single level of 50 ng/ml (equivalent to 250 ng/g in the sample). In Table 4 the relative percent difference (RPD) of spike recoveries from the collaboration and the same spike recoveries

calculated using a single level standard at 50 ng/ml. Extremely low RPDs demonstrate the linearity of the method and accuracy of residue levels calculated from a single level calibration.

Table 4. Relative Percent Difference (RPD) of average recoveries for all levels and laboratories calculated based upon a single level calibration at 250 ng/g vs. calibration per each individual spike level.

Matrix	Single Level	Per Level	RPD	Single Level	Per Level	RPD
	Glyphosate			*Glufosinate*		
Avocado	88.7	88.2	0.6	87.5	87.2	0.4
Carrot	85.0	82.6	2.9	85.5	84.0	1.7
Corn	97.9	96.4	1.5	100.8	99.9	0.9
	N-acetylglyphosate			*AMPA*		
Avocado	90.8	90.5	0.3	72.7	78.7	7.8
Carrot	85.7	86.7	1.1	82.1	80.3	2.2
Corn	98.9	99.7	0.8	88.2	90.7	2.7

Attachments

A. Collaboration Protocol
B. Analytical Method
C. Method Collaboration Recovery Charts
D. Method Collaboration Matrix Effects Charts
E. PSFFL Collaboration Data and System Suitability
    - E_1 PSFFL Recovery Charts
    - E_2 PSFFL Linearity Charts
F. PNL Collaboration Data and System Suitability
    - F_1 PNL Recovery Charts
    - F_2 PNL Linearity Charts
G. ARKL Collaboration Data and System Suitability
    - G_1 ARKL Recovery Charts
    - G_2 ARKL Linearity Charts
H. KCL Collaboration Data and System Suitability
    - H_1 KCL Recovery Charts
    - H_2 KCL Linearity Charts
I. CFSAN Collaboration Data
    - I_1 CFSAN Recovery Charts
    - I_2 CFSAN Linearity Charts

Collaboration Protocol

carrot/avocado (5 g/sample)		
50	5	50
250	5	250
500	50	50

Extraction Cleanup for Avocado:

Follow method as written. Re the cleanup option for avocadoes; i.e. dichloromethane (DCM) vs petroleum ether (PE) three ORA labs agreed to use DCM and the remaining three ORA labs agreed to use PE. CFSAN can choose either.

DCM	PE
ARKL	PNL
SRL	PSFFL
KAN	NRL

LCMS Transition Names:

AMPA[110-63] 1
AMPA[110-79] 2
AMPA[110-81] 3
Glu[180-63] 1
Glu[180-95] 2
Glu[180-85] 3
Glu[183-63] IS
Gly[168-63] 1
Gly[168-79] 2
Gly[168-150] 3
Gly[171-63] IS
N-acetyl[210-150] 1
N-acetyl[210-63] 2
N-acetyl[210-168] 3

LCMS Calibration: Calibrate using single level calibration for each spike level. Assign the internal standards as below.

Analyte	Internal Standard
Glyphosate:	Glyphosate-^{13}C
N-acetylglyphosate:	Glyphosate-^{13}C
AMPA:	Glyphosate-^{13}C
Glufosinate:	Glufosinate-D^{3}

Ruggedness/Robustness

To show ruggedness of the method resulting from changes in the sample matrix, PSFFL-Pesticide conducted spike recoveries utilizing banana, rice, 3%milk, soymilk, and chicken eggs and for robustness by repeating the carrots, corn and avocado recoveries. See Representative Commodities Table for matrix categories. These spike matrix recoveries were conducted by members of the PSFFL-Pesticide section chemists and results were used to validate PSFFL chemists on the Glyphosate method.

Matrix Blanks and reagent blanks detected no target compounds and were free of interferences affecting the analytical signal.

Quantitative determination for glyphosate and glufosinate were done on all commodities. Only avocado, eggs, corn and rice were spike with AMPA (milk, banana, soymilk and carrots were not). Glyphosate N-acetylglyphosate was not used in ruggedness/robustness study. Chromatograms are located in Glyphosate database CD.

.

Analyte Name	Average % Recovery
Glyphosate	98
Glyfosinate	103
AMPA	93

The average % recoveries were calculated to be 98%, 103% and 93% which is acceptable.

The method was demonstrated to have ruggedness and robustness for glyphosate, glufosinate and AMPA.

Representative Commodities Table

Matrix Categories	Commodity Groups	Typical Commodity Categories	Representative Commodities
High-moisture products	High water content	Pome fruit	
		Stone fruit	
		Tropical and subtropical fruit	banana
		Fruiting vegetables/cucurbits	
		Brassica vegetables	
		Legume vegetables	
		leaf vegetables	
		Stem and stalk vegetables	
		forage crops	
		Alliums	
		Root and tuber vegetables	carrots
	High acid content and high water content	Citrus fruit	
		Small fruit and berries	
Low-moisture products	High sugar and low water content	Honey, dried fruit	
	High starch and/or protein content and low water and fat content	Dry legume vegetables/pulses	
		Cereal grain and products thereof	rice, corn
Fatty-Food products	Meat (muscle) and seafood	fish	
		crustaceans	
	High oil content and very low water content	Tree nuts	
	High oil content and intermediate water content	Oily fruits and products	avocado
	Milk and milk products	Milk	3%milk, soymilk
		cheese	
		Dairy products	
	Eggs	eggs	Chicken eggs
	Fat from food of animal origin	Milk fat	
		Fish oil	

Ruggedness/Robustness Recovery

Product	Analyte Name	RT (min)	Cal Conc (ng/mL)	Rec (%)
3% Milk	Glyphosate	5.8	8.57	85.7
3% Milk	Glyphosate	5.81	12.6	126
3% Milk	Glyphosate	5.8	14.2	142
3% Milk	Glyphosate	5.8	99.2	99.2
3% Milk	Glyphosate	5.8	103	103
3% Milk	Glyphosate	5.8	104	104
3% Milk	Glyphosate	5.81	1040	104
3% Milk	Glyphosate	5.8	1040	104
3% Milk	Glyphosate	5.8	1080	108
avocado	Glyphosate	5.74	1.92	19.2
avocado	Glyphosate	5.73	2.08	20.8
avocado	Glyphosate	5.73	2.42	24.2
avocado	Glyphosate	5.73	33.6	67.1
avocado	Glyphosate	5.74	34.4	68.9
avocado	Glyphosate	5.73	35.7	71.5
avocado	Glyphosate	5.73	337	67.4
avocado	Glyphosate	5.73	338	67.5
avocado	Glyphosate	5.73	372	74.4
banana	Glyphosate	5.84	9.79	97.9
banana	Glyphosate	5.86	11	110
banana	Glyphosate	5.81	13.6	136
banana	Glyphosate	5.83	98.1	98.1
banana	Glyphosate	5.9	104	104
banana	Glyphosate	5.82	117	117
banana	Glyphosate	5.83	1060	106
banana	Glyphosate	5.82	1270	127
banana	Glyphosate	5.83	1370	137
carrots	Glyphosate	5.83	7.42	74.2
carrots	Glyphosate	5.82	7.78	77.8
carrots	Glyphosate	5.81	8.24	82.4
carrots	Glyphosate	5.82	64.6	64.6
carrots	Glyphosate	5.83	72.9	72.9
carrots	Glyphosate	5.82	104	104
carrots	Glyphosate	5.81	955	95.5
carrots	Glyphosate	5.82	963	96.3
carrots	Glyphosate	5.82	977	97.7
CORN	Glyphosate	5.74	35.7	71.4

CORN	Glyphosate	5.76	39.2	78.4
CORN	Glyphosate	5.76	47.4	94.9
CORN	Glyphosate	5.74	50.6	101
CORN	Glyphosate	5.73	50.6	101
CORN	Glyphosate	5.75	52.2	104
CORN	Glyphosate	5.75	90.2	180
CORN	Glyphosate	5.75	140	69.8
CORN	Glyphosate	5.74	255	128
CORN	Glyphosate	5.73	361	72.2
CORN	Glyphosate	5.73	553	111
eggs	Glyphosate	5.75	11.1	111
eggs	Glyphosate	5.75	11.2	112
eggs	Glyphosate	5.97	11.4	114
eggs	Glyphosate	5.77	13.2	132
eggs	Glyphosate	5.75	13.5	135
eggs	Glyphosate	5.86	14.4	144
eggs	Glyphosate	5.88	14.7	147
eggs	Glyphosate	5.93	90	90
eggs	Glyphosate	5.74	92.3	92.3
eggs	Glyphosate	5.74	93.9	93.9
eggs	Glyphosate	5.83	107	107
eggs	Glyphosate	5.83	107	107
eggs	Glyphosate	5.83	125	125
eggs	Glyphosate	5.83	890	89
eggs	Glyphosate	5.75	962	96.2
eggs	Glyphosate	5.74	996	99.6
eggs	Glyphosate	5.82	1270	127
eggs	Glyphosate	5.82	1290	129
rice	Glyphosate	5.86	9.5	95
rice	Glyphosate	5.85	9.56	95.6
rice	Glyphosate	5.86	11.3	113
rice	Glyphosate	5.86	85.1	85.1
rice	Glyphosate	5.86	105	105
rice	Glyphosate	5.86	107	107
rice	Glyphosate	5.87	1020	102
rice	Glyphosate	5.87	1040	104
rice	Glyphosate	5.86	1190	119
soymilk	Glyphosate	5.82	8.17	81.7
soymilk	Glyphosate	5.81	8.91	89.1
soymilk	Glyphosate	5.9	11.2	112
soymilk	Glyphosate	5.81	71.9	71.9

soymilk	Glyphosate	5.87	74	74
soymilk	Glyphosate	5.9	92.8	92.8
soymilk	Glyphosate	5.85	720	72
soymilk	Glyphosate	5.82	764	76.4
soymilk	Glyphosate	5.9	828	82.8
			Glyphosate Average % Recovery =	98

Ruggedness/Robustness Recovery

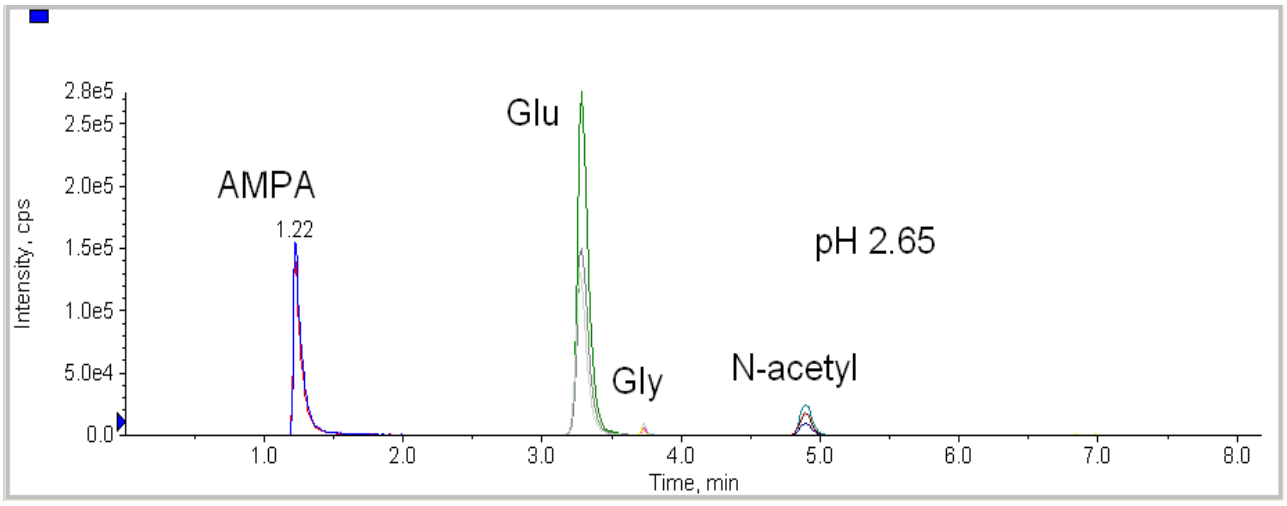
Product	Analyte Name	RT (min)	Cal Conc (ng/mL)	Rec (%)
3% Milk	Glufosinate	5.42	8.66	86.6
3% Milk	Glufosinate	5.44	9.35	93.5
3% Milk	Glufosinate	5.41	10.4	104
3% Milk	Glufosinate	5.42	85.7	85.7
3% Milk	Glufosinate	5.42	87.1	87.1
3% Milk	Glufosinate	5.42	88.7	88.7
3% Milk	Glufosinate	5.42	852	85.2
3% Milk	Glufosinate	5.42	933	93.3
3% Milk	Glufosinate	5.42	1060	106
avocado	Glufosinate	5.5	36.8	73.6
avocado	Glufosinate	5.49	39.1	78.3
avocado	Glufosinate	5.5	40.5	81
avocado	Glufosinate	5.5	415	83
avocado	Glufosinate	5.5	448	89.5
avocado	Glufosinate	5.5	454	90.8
banana	Glufosinate	5.48	18.7	187
banana	Glufosinate	5.48	20.3	203
banana	Glufosinate	5.51	21.1	211
banana	Glufosinate	5.52	107	107
banana	Glufosinate	5.52	112	112
banana	Glufosinate	5.51	128	128
banana	Glufosinate	5.52	884	88.4
banana	Glufosinate	5.52	1040	104
banana	Glufosinate	5.51	1220	122
carrots	Glufosinate	5.45	9.52	95.2
carrots	Glufosinate	5.46	13.1	131
carrots	Glufosinate	5.45	14.2	142
carrots	Glufosinate	5.46	95.4	95.4
carrots	Glufosinate	5.44	104	104
carrots	Glufosinate	5.45	105	105
carrots	Glufosinate	5.45	1090	109
carrots	Glufosinate	5.45	1130	113
carrots	Glufosinate	5.45	1220	122
CORN	Glufosinate	5.51	39.5	78.9
CORN	Glufosinate	5.52	41.9	83.8
CORN	Glufosinate	5.51	42.2	84.3
CORN	Glufosinate	5.51	42.3	84.6
CORN	Glufosinate	5.5	42.9	85.8
CORN	Glufosinate	5.5	43.4	86.8
CORN	Glufosinate	5.51	46	92

CORN	Glufosinate	5.51	180	90.1
CORN	Glufosinate	5.5	182	91.2
CORN	Glufosinate	5.5	417	83.5
CORN	Glufosinate	5.5	453	90.5
eggs	Glufosinate	5.51	9.75	97.5
eggs	Glufosinate	5.51	9.86	98.6
eggs	Glufosinate	5.51	9.94	99.4
eggs	Glufosinate	5.5	10.7	107
eggs	Glufosinate	5.51	11.9	119
eggs	Glufosinate	5.49	12.1	121
eggs	Glufosinate	5.53	12.2	122
eggs	Glufosinate	5.5	91.7	91.7
eggs	Glufosinate	5.6	97	97
eggs	Glufosinate	5.48	97.9	97.9
eggs	Glufosinate	5.5	98.1	98.1
eggs	Glufosinate	5.48	104	104
eggs	Glufosinate	5.48	113	113
eggs	Glufosinate	5.51	877	87.7
eggs	Glufosinate	5.51	952	95.2
eggs	Glufosinate	5.49	991	99.1
eggs	Glufosinate	5.49	1020	102
eggs	Glufosinate	5.49	1030	103
rice	Glufosinate	5.51	8.98	89.8
rice	Glufosinate	5.51	9.23	92.3
rice	Glufosinate	5.52	10.5	105
rice	Glufosinate	5.51	91.6	91.6
rice	Glufosinate	5.51	97.3	97.3
rice	Glufosinate	5.51	109	109
rice	Glufosinate	5.52	993	99.3
rice	Glufosinate	5.51	1040	104
rice	Glufosinate	5.52	1550	155
soymilk	Glufosinate	5.46	8.6	86
soymilk	Glufosinate	5.45	10.3	103
soymilk	Glufosinate	5.52	14.8	148
soymilk	Glufosinate	5.51	68.6	68.6
soymilk	Glufosinate	5.45	76.2	76.2
soymilk	Glufosinate	5.47	95.5	95.5
soymilk	Glufosinate	5.51	866	86.6
soymilk	Glufosinate	5.46	983	98.3
soymilk	Glufosinate	5.46	1100	110
			Glufosinate Average % Recovery	103

Ruggedness/Robustness Recovery

Product	Analyte Name	RT (min)	Cal Conc (ng/mL)	Rec (%)
avocado	AMPA	3.2	30.5	60.9
avocado	AMPA	3.19	34.2	68.4
avocado	AMPA	3.2	35.8	71.6
avocado	AMPA	3.2	361	72.3
avocado	AMPA	3.2	383	76.6
avocado	AMPA	3.19	405	81
CORN	AMPA	3.21	50.2	100
CORN	AMPA	3.22	50.7	101
CORN	AMPA	3.23	51.4	103
CORN	AMPA	3.22	51.5	103
CORN	AMPA	3.22	52.3	105
CORN	AMPA	3.22	52.7	105
CORN	AMPA	3.22	55.3	111
CORN	AMPA	3.22	188	94
CORN	AMPA	3.22	197	98.5
CORN	AMPA	3.22	446	89.3
CORN	AMPA	3.22	468	93.6
eggs	AMPA	3.14	8.62	86.2
eggs	AMPA	3.16	9.04	90.4
eggs	AMPA	3.21	9.24	92.4
eggs	AMPA	3.2	10.6	106
eggs	AMPA	3.2	12	120
eggs	AMPA	3.15	12.3	123
eggs	AMPA	3.21	12.7	127
eggs	AMPA	3.16	66.8	66.8
eggs	AMPA	3.16	70.9	70.9
eggs	AMPA	3.21	92.6	92.6
eggs	AMPA	3.2	95.4	95.4
eggs	AMPA	3.2	97.7	97.7
eggs	AMPA	3.15	98.2	98.2
eggs	AMPA	3.2	912	91.2
eggs	AMPA	3.2	951	95.1
eggs	AMPA	3.16	987	98.7
eggs	AMPA	3.17	993	99.3
eggs	AMPA	3.16	1170	117
rice	AMPA	3.14	7.62	76.2
rice	AMPA	3.14	8.2	82
rice	AMPA	3.13	8.48	84.8

rice	AMPA	3.14	82.1	82.1
rice	AMPA	3.14	82.1	82.1
rice	AMPA	3.14	83.5	83.5
rice	AMPA	3.14	783	78.3
rice	AMPA	3.14	807	80.7
rice	AMPA	3.14	1250	125
			AMPA Average % Recovery	93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Glu
Gly
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2.0
3.0
4.0
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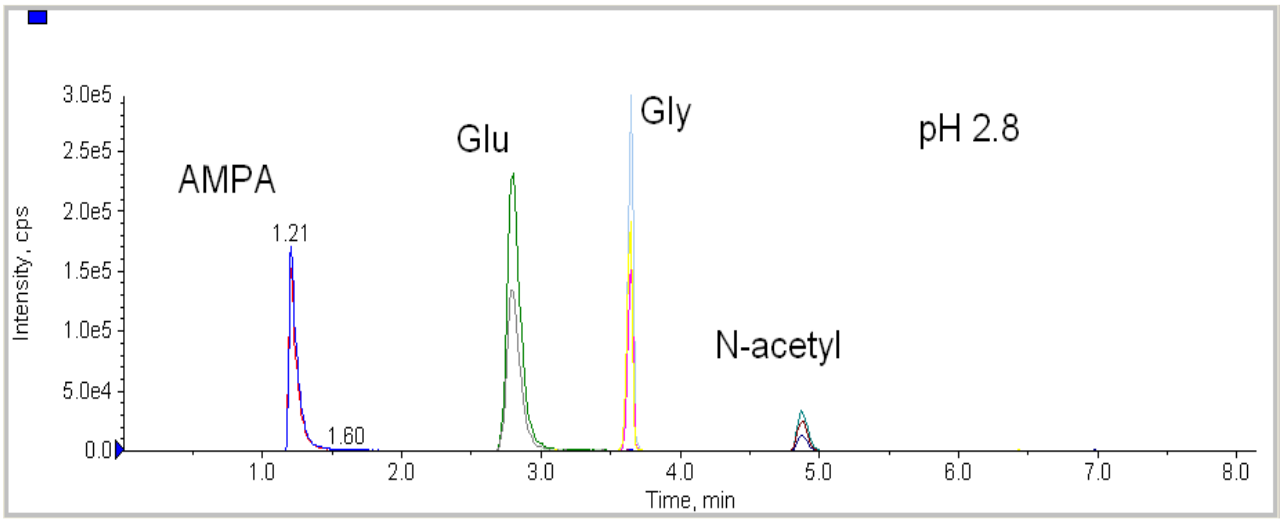
6.0
7.0
8.0
Time, min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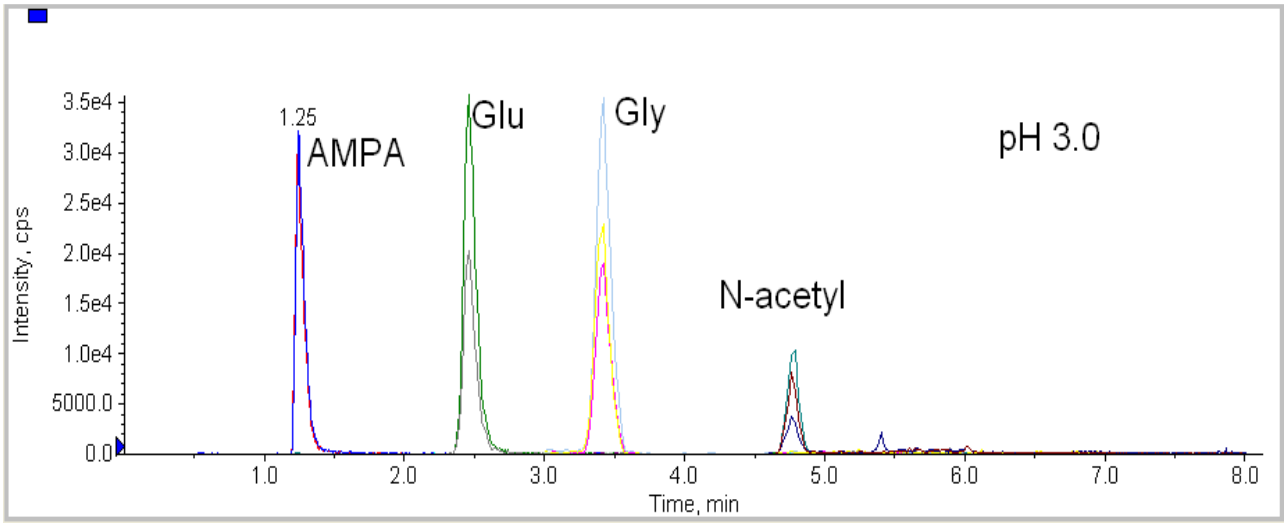
1.60
Glu
Gly
N-acetyl
pH 2.8
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
Time, min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AMPA
Glu
Gly
N-acetyl
pH 3.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
Time, min

From: Sack, Chris A
To: Shireen, Kaniz F
Cc: Robin, Lauren (Posnick); South, Paul; Islam, Mohammed R; McLaughlin, Michael A
Subject: RE: New Issue for Eggs and milk
Date: Wednesday, May 31, 2017 4:23:00 PM

Hi Kaniz,

Just had an excellent conversation with LA lab and they will do the work necessary to validate the glyphosate method for milk and eggs. If you can just have the collectors hold off for a month, or so, then provide instructions to ship all milk and egg samples to the LA lab. I think we are good to go.

Thanks,

Chris

Ph: 240-402-2464

From: Sack, Chris A
Sent: Wednesday, May 31, 2017 3:26 PM
To: Shireen, Kaniz F
Subject: RE: New Issue for Eggs and milk

One thing is for sure Kaniz, we cannot at this time analyze milk and eggs. So, you can at least contact the collectors and tell them to hold off until we have worked through the issue. I will be in contact.

Chris

Ph: 240-402-2464

From: Shireen, Kaniz F
Sent: Wednesday, May 31, 2017 2:40 PM
To: Sack, Chris A
Subject: RE: New Issue for Eggs and milk

Chris:
At this moment, I am little confused reading all emails.
I'll wait until OFS decide what would you like to do. Andrew said that he is fine if samples are not analyzed.
Paul wants to continue with sample collection and analysis.

I'll wait to inform ORA until I know exactly what we would do.

Thanks, Kaniz

From: Sack, Chris A
Sent: Wednesday, May 31, 2017 3:04 PM
To: South, Paul; Robin, Lauren (Posnick); Shireen, Kaniz F
Subject: RE: New Issue for Eggs and milk

Kaniz,

Let me know what is decided. If we analyzed the milk and egg samples, I will need to set up a matrix extension validation for both.

Chris

Ph: 240-402-2464

From: South, Paul
Sent: Wednesday, May 31, 2017 2:02 PM
To: Robin, Lauren (Posnick); Shireen, Kaniz F; Sack, Chris A
Subject: RE: New Issue for Eggs and milk

Let's collect the remaining egg and milk samples and analyze when methods are available.

From: Robin, Lauren (Posnick)
Sent: Wednesday, May 31, 2017 2:55 PM
To: South, Paul; Shireen, Kaniz F; Sack, Chris A
Subject: RE: New Issue for Eggs and milk

From: South, Paul
Sent: Wednesday, May 31, 2017 2:48 PM
To: Robin, Lauren (Posnick); Shireen, Kaniz F; Sack, Chris A
Subject: RE: New Issue for Eggs and milk

How long will it take to have the method for milk and eggs up and running?

From: Robin, Lauren (Posnick)
Sent: Wednesday, May 31, 2017 2:46 PM
To: South, Paul; Shireen, Kaniz F; Sack, Chris A
Subject: RE: New Issue for Eggs and milk

This is a matrix extension, the MLV is complete. Chris said by the end of June earlier. We also emailed Mike McL to see if he agrees.

From: South, Paul
Sent: Wednesday, May 31, 2017 2:44 PM
To: Robin, Lauren (Posnick); Shireen, Kaniz F; Sack, Chris A
Subject: RE: New Issue for Eggs and milk

How long will it take to get a validated method?

From: Robin, Lauren (Posnick)
Sent: Wednesday, May 31, 2017 2:42 PM
To: Shireen, Kaniz F; Sack, Chris A
Cc: South, Paul
Subject: FW: New Issue for Eggs and milk

I disagree – the optics are bad. We have been saying that we will restart the assignment for all four commodities, right?

From: Sack, Chris A
Sent: Wednesday, May 31, 2017 2:35 PM
To: Robin, Lauren (Posnick)
Subject: FW: New Issue for Eggs and milk

FYI. It looks like we won't have to analyze any more milk and eggs.

Chris

Ph: 240-402-2464

From: Sack, Chris A
Sent: Wednesday, May 31, 2017 1:30 PM
To: Shireen, Kaniz F
Subject: RE: New Issue for Eggs and milk

Wow, that makes everything much simpler. Have you notified ORA?

Chris

Ph: 240-402-2464

From: Shireen, Kaniz F
Sent: Wednesday, May 31, 2017 1:19 PM
To: Sack, Chris A
Subject: FW: New Issue for Eggs and milk

Chris:
It seems Andrew's group is okay if lab is unable to analyze egg and milk samples.

Thanks, Kaniz

From: Yeung, Andrew
Sent: Wednesday, May 31, 2017 1:18 PM
To: Shireen, Kaniz F
Cc: Sheehan, John
Subject: RE: New Issue for Eggs and milk

Hi Kaniz,

Thanks for letting me know. With only 16 outstanding samples for each commodity, we are ok not to pursue them.

Andrew

From: Shireen, Kaniz F
Sent: Wednesday, May 31, 2017 12:25 PM
To: Yeung, Andrew
Subject: FW: New Issue for Eggs and milk

Hey Andrew:
Please read Chris's email below and let know your thoughts.