From Page No. ____

3/1/16

- Finished blowing down remaining samples for Day 1 soil.
- Started blowing down samples for Day 2 soil.

Pollen samples

-Weighed out remaining pollen samples from Nuc box #7 on bag #186 - #203. Roughly 2 grams ~~each~~ into 15mL centrifuge tu

3/2/16

Ran new tetraconazole standard & old tetraconazole method on GC/MS and couldn't see any peak not sure, it used to be a great peak

GC/MS ions

Metolachlor	-	162, 238
TDN	-	208, 57
BIF	-	181, 165, 166
PERMETHRIN	-	183, 163
TETRA	-	336

- Removed Day 3 soil samples from freezer to thaw
- Spiked samples with 10uL of 100ppm tetraconazole & 225ppm perm
- Added 5mL MeOH → vortexed → sonicated for 30 min → vortexed → centrifuged for 20min @ 3250rpm
- Transferred top organic MeOH layer to a 20mL scintillation vial
- Repeated above step 2 more times
- Placed samples under nitrogen to blow down.

- To Day 2 samples + some Day 1 soil samples that were blown down to
  - Added 10mL MQ → vortexed → added 3mL MTBE → vortex → Na_2SO_4 small scoop → vortex
  - Transferred top organic layer to a 2mL centrifuge tube pre-labled

To Page No.

Project No. _____

TITLE _____ Book No. _____

From Page No. ____

3/2/16

- Centrifuged 2mL tubes for 15min @ 14000rpm
- Transferred 1mL to a pre-labeled GC vial - capped & place in fr

3/3/16

- Placed remaining Day 3 soil samples under nitrogen to blow dow to 1mL. Ran out of N_2 gas :: - Placed back in the fridge

- To Day 3 soil samples that were blown to 1mL from yesterday
  - Added 10mL MQ → vortexed → added 3mL MTBE → vortexed → added small amount of Na_2SO_4 → vortexed
  - Transferred top organic layer to a prelabeled 2mL centrifuge tube - centrifuged for 15min @ 14000rpm
  - Transferred a 1mL aliquot to a pre-labeled GC vial
  - Placed in freezer

3/14/16

Pollen Samples - to the Nuc box #7 - #186-#203.
- Spiked with 2uL of 1000ppm thiamethoxam D3
- Added 2mL MQ to each sample + vortexed for 2 minutes → well mix
- Placed on speed vac overnight to remove MQ
- Probably should centrifuge after this step to get H_2O to top

LF · BIF 2014 - Chopped/homogenized remaining Leopard frogs exposed to BIF in
- Spiked with 10uL of 33.3 ppm permethrin
- Placed in freezer + then freeze dryer.

LF Biomarker - Thawed LF Biomarkers Day 3 - control 17-24 + TBmax
- Chopped/homogenized
- Recapped + placed in freezer.

LF biomarkers livers - extracted livers Day 1 + Day 2 following procedure on pg. 154 To Page No. –

Witnessed & Understood by me,	Date	Invented by:	Date
		Recorded by:	

3/15/16

LF BIF 2014 - Samples were capped & removed from freeze dryer

Pollen
- Samples were removed from Speed Vac
- Added 12mL of ETOAc to each sample
- Vortexed until well-mixed
- Placed on wrist-shaker for ~~2~~ 2 hours
- Centrifuged for 15 min @ 3250 rpm
- Transferred supernatant to a clean 15mL Centrifuge tube
- Placed samples in fridge

LF Biomarkers soil - Placed Day 1 + Day 2 on ~~gc~~/MS with a new/old method

Metolachlor	-	15.991 min	- 162, 238 m/z
TDN	-	16.202 min	- 57, 208 m/z
Tetra	-	16.546 min	- 336, 338 m/z
BIF	-	19.912 min	- 165, 166, 181 m/z
Per (chiral 2 peaks → *)	-	21.767	- 183, 165, 163 m/z

Method → Pest_biomarkers_sim

Ramp	Temp	Hold
	80	2.00
10.00	300	6.00 / 30.0 min

Witnessed & Understood by me, | Date | Invented by: | Date

3/18/16

- Cut up more frogs from Biomarker cocktail exposure 2016
- Spiked with 10 uL of 1000ppm tetraconazole + 1 uL of 225ppm of permethrin

3/21/16

- Biomarkers cocktail exposure frogs were placed on freeze dryer
- Honeybees from 2015 - NurseBees Bag #2-#5 were uncapped + placed on freeze dryer overnight

3/22/16

- Calibrate the ToF samples TF
- Placed more bees on the freeze dryer overnight
- Blow down remaining pollen samples under nitrogen
- Blow down remaining biomarker cocktail soil samples Day 3

3/23/16

- Placed pollen samples on Speedvac for the day to remove residual water and ~~EtOAc~~ EtOAc.

3/24/16

- Reconstituted pollen samples with 1 mL 10% ACN: H_2O and sonicated for 30 min.

- Transferred the pollen sample to a 2mL centrifuge tube with a 0.2um filter
- Centrifuge for ~30min. @ 14000rpm
- Removed filter + transferred sample to pre-labeled GC vial
  - some samples still had a gooey sludge at bottom so it was also added that's why volume is so high + has #2

Witnessed & Understood by me,	Date	Invented by:	Date
		Recorded by:	

Project No. _____

TITLE _____ Book No. _____

From Page No. ____

3/28/16

- The first 2 bags of ~~bees~~ nurse bees #31 - #88 were weighed out into pre-labeled quart size bags and their weight was recorded tried to get as close to 3 grams

3/30/16

- Trying 2 different methods of extracting honey bees
  1.) Added 3 grams bees, 2 grams of diatomaceous earth and 2 mL of 1000ppm thiamethoxam D3
  2. crush up with a rolling pin
  3. transfer to a pre-labeled 15mL centrifuge tube
  4 Squirted in ~2mL of EtOH and hand shake ~~bag~~ bag to remove residuals
  5 - Pour EtOH into 15mL centrifuge tube
  6 - Place on speedvac ~~for several hours~~ overnight
  2.) Same as above except stop 3 before adding EtOH

~~Samples~~

4/4/16

~~Cocktail~~ Biomarker Bifenthrin 2014
- extracted LF control, LF 0.1, LF 1 + LF 10 ppb following original extraction method.
- extracted BA control, + BA 1.0 ppb
  - Added 7.5mL of MeOH to LF + 5mL MeOH to BA
  - sonicate for 30minutes
  - vortex → centrifuge for 20minutes
  - Transfer supernatant to a 20mL scint vial
  - Repeat extraction w/ 5mL MeOH add supernatant to scint vial
  - Blow down under N_2 gas until 1mL
  - Added 10mL MQ → vortex, add 3mL MTBE → vortex add Na_2SO_4 →
  - Transferred top organic layer to a 2mL centrifuge tube
  - Centrifuge for 15min @ 14,000 rpm
  - Transfer 1mL aliquot to a pre-labeled GC vial

To Page No. .

Witnessed & Understood by me,	Date	Invented by:	Date
		Recorded by:	

4/7/16

Cocktail Biomarker 2016

- For the remaining sample that needed to be blown down under nitrogen until 1mL left.

- Added 10mL MQ → vortex
- Added 3mL MTBE → vortex
- Added scoop of Na_2SO_4 → vortex
- Transfered top organic layer to a prelabeled 2mL centrifuge tube
- Centrifuge for 15min @ 14000rpm
- Transferred 1mL aliquot to a prelabeled 6 Cmal
- Place in freezer

4/8/16

- Made up new standards for Bifenthrin Biomarker Study 2014
- samples were all spiked with 10uL of 33.3̄ ppm
  33.3̄ (10uL) = 3000uL (x) x = 0.111ppm (3mL of MTBE)

- For Standards -
  0.500ppm (x) = 1mL (0.111 ppm) x = 222uL

- Took 222uL of each standard
- Blow down under nitrogen gas
- Reconstitute in 1mL of MTBE
- Place in freezer

- Weighed out remaining ~~NBees~~ nurse bees from nuc 2015 into Biplex bags

- Ran Bifenthrin 2014 - LF+BA (BIF) frags on GCMS singlequad 160408A under DAG/Biomarkers

Witnessed & Understood by me,	Date	Invented by:	Date
		Recorded by:	

4/8/16

- Made up new neonic standards:

Neonic Standard

Thiamethoxam - 1.91 mg
acetamiprid - 2.99 mg
Dinotefuran - 1.88 mg
Clothiandin - 2.00 mg
Imidacloprid - 1.00 mg
thiacloprid - 5.13 mg
Nitenpyram - 1.64 mg

in 1.64 mL ACN

1000ppm (x) = 6mL (1ppm) x = 6uL in 5994uL 10% ACN

1ppm (x) = 6mL (0.5ppm)
0.5ppm (x) = 6mL (0.25ppm) x = 3mL
0.25ppm (x) = 6mL (0.125ppm)
0.125ppm (x) = 6mL (0.0625ppm)
0.0625ppm (x) = 6mL (0.03125ppm)
0.03125ppm (x) = 6mL (0.015625ppm)
0.015625ppm (x) = 6mL (0.00782ppm)
0.00782ppm (x) = 6mL (0.0039ppm)

- Spiked each standard with 6uL of 1000ppm thiamethoxam - D3

- Except the last standard 0.0039 had 12uL because there was 6mL

Witnessed & Understood by me,	Date	Invented by:	Date
		Recorded by:	

Project No. _____

TITLE _____ Book No. _____

From Page No. ____

4/11/16

Nurse Bees - Samples 50 + 88 were placed on ASE (~~accelerate~~
(accelerated solvent extractor)
- Using 60% MeOH : H_2O.
- Sample took 20 min.

- Transferred sample to a SS cell 10mL with a filter on both end caps
- Packed sample down in cell
- Topped the extra space off with quartz - OTTAWA sand
- Ran sample on ASE

- Samples were placed in fridge overnight.

~~[illegible]~~

- BIF Biomarker 2014 - samples were hard to see on GC/MS so they were blown down to dryness + reconstituted in 200uL of MTBE + reran on GC/MS.
  - 10ppb samples looked good, but couldn't see 1 or 0.1ppb. :-

4/12/16

- ~~[illegible]~~ Nurse Bees
- Tried to extract through a filter because they had weird composition
- Took too long, so Matthew added EtOAc - b/c he thought it would be immiscible - wrong!
- Placed samples on speedvac overnight

Took 2 new nurse bee samples
- Spiked w/ 2mL of IS
- 2 scoops of diatamaceous earth (2g)
- smashed up sample with rolling pin + transferred to 15mL centrifuge tube along with an EtOH rinse.
- Placed on speedvac until dry

To Page No. ____

Witnessed & Understood by me,	Date	Invented by:	Date
		Recorded by:	

From Page No. ____

4/15/16

- Chopped up Day 2 frogs from Mixture/cocktail Biomarker 2016 Study
  - Spiked with 10mL of 1000ppm tetraconazole for all samples
  - Spiked with 10mL of 225ppm permethrin for Bifenthrin samples
- Placed in freezer

4/18/16

- Placed cocktail biomarker samples on freeze-dryer
- Chopped up more Day 2 + Day 1 cocktail biomarker frogs
  - Spiked with 10mL of 1000ppm tetraconazole for all samples
  - Spiked with 10mL of ~~225~~ ppm permethrin for bifenthrin samples
  - Placed in freezer
- Moved Keri's soil samples in 20mL MeOH to the freezer to freeze them and place on freeze-dryer instead of SPE.

4/19/16

- Placed more frog samples on freeze-dryer
- Keri's samples didn't freeze last night so they must be in 100% MeOH @ 24mL - So they are going to be blown down under nitrogen to 4mL and then transfer to GC vial w/ 3 rinses
- Extracting Cocktail Biomarker Day 3 + Day 1 samples Met 10, TB 10, BIF MAX following frog procedure on pg. 186

To Page No. ____

Witnessed & Understood by me,	Date	Invented by:	Date
		Recorded by:	

Project No. _____

TITLE _____ Book No. _____

From Page No. ____

4/20/16

- Placed more frogs - Biomarker cocktail 2016 on freeze-dryer
- Extracted Cocktail Biomarker 2016 frog samples following procedure on pg. 186 - Met max, BIF max, MB 10, BIF 10, TMB max
- Finished blowing down Keri's August sediment samples. However there is still 5mL which I think is water

4/21/16

- Finished blowing down + extracting more cocktail biomarker 2016 frogs following procedure on pg. 186

4/22/16

- Following procedure on pg. 186 finished blowing down + extracting cocktail biomarker 2016 frogs

4/25/16

- Finished chopping up remaining cocktail biomarker 2016 frogs (Josi
- Honeybees
  - Spiked with 2uL of 1000ppm thiamethoxam D3
  - Added 2 scoops of diatomaceous earth
  - Crushed everything with a rolling pin
  - Transferred sample to a 15mL prelabeled centrifuge tube
  - Added a small volume of EtOH to bag - hand massage
  - Transfer EtOH to centrifuge tube too
  - Place in speedvac overnight

To Page No. -

Witnessed & Understood by me,	Date	Invented by:	Date
		Recorded by:	

From Page No. ____

4/12/16

Nurse Bees ASE

- Both caps were fitted with filter paper.
- Add scoop of Alumina to the bottom of SS cell.
- Packed sample into cell
- Topped off with quartz OTTAWA sand
- Ran on ASE w/ method #1

- Collected sample in 60mL scint vial
- Vial was placed on speedvac overnight.

- Samples NB 65, 88, 43, 50

  - To #43 + 65 added 1mL of 100% ACN → vortex + sonicate for 30min.
  - Transferred to 2mL GC vial
  - Added 1mL EtOH - vortex + sonicate for 30min
  - Transfer to GC vial - blow to dryness under N_2 gas
  - Reconstitute with 1mL 10% ACN: H_2O
    - vortex + place through 0.2um filter centrifuge tube
  - Transfer to GC vial

  - To #50 + 88
    - vortex + sonicate for 30min with EtOH
    - Transfer to a 2mL centrifuge tube w/ 0.2um filter
    - Centrifuge @ 13500rpm for 15min
    - Transfer to GC vial

Witnessed & Understood by me,	Date	Invented by:	Date
		Recorded by:	