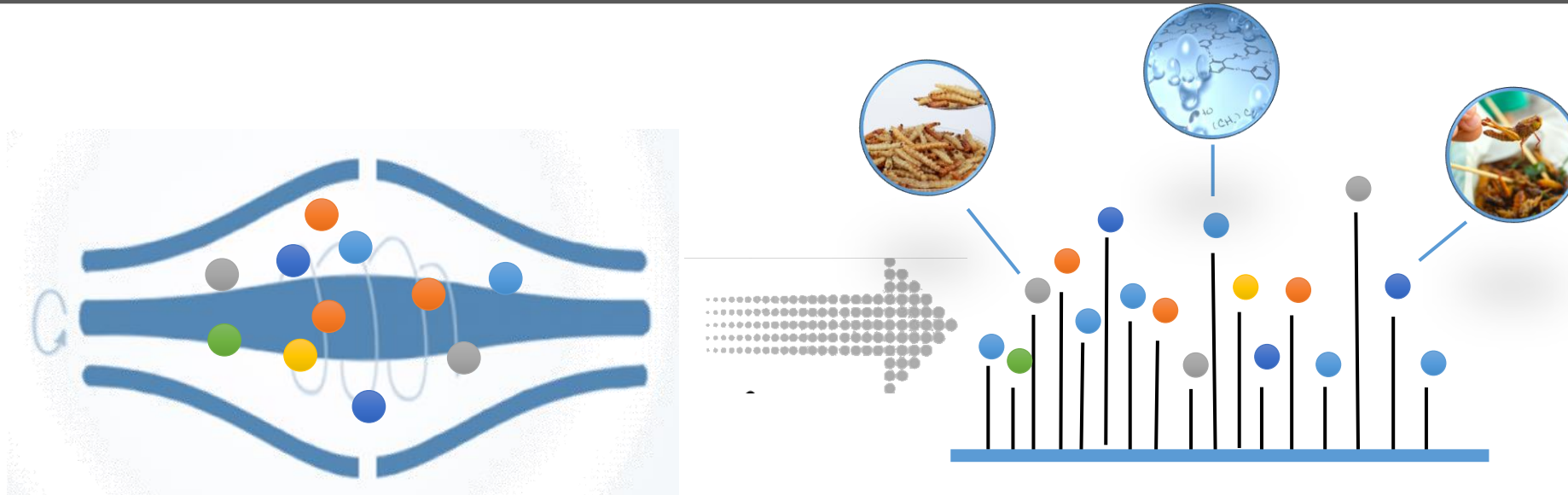
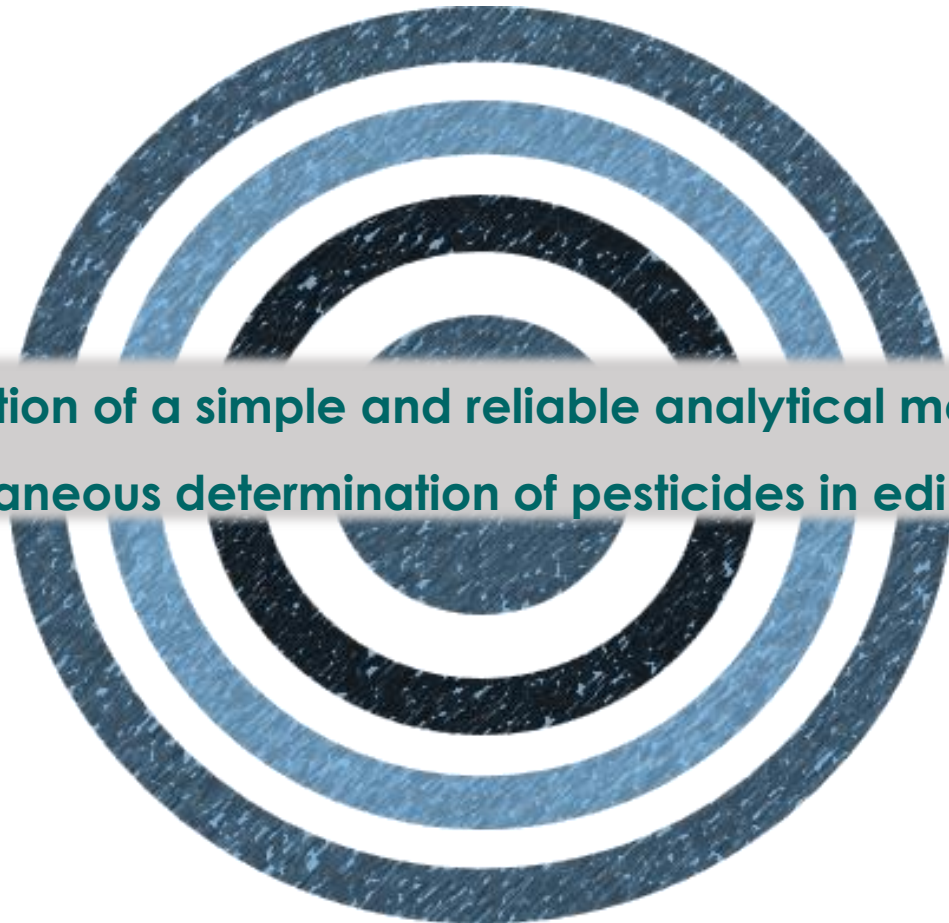


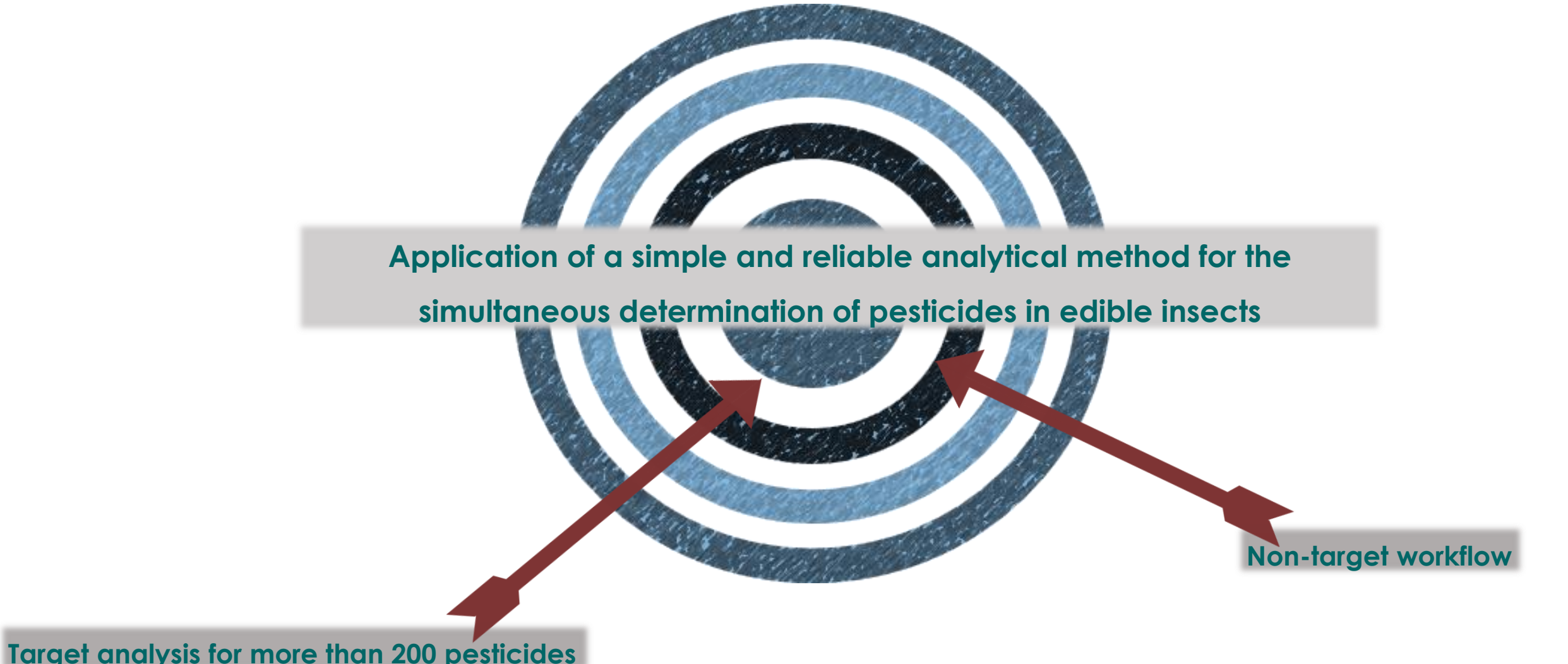
# Multiresidue Determination of Current-used Pesticides in Edible Insects by Orbitrap HRMS Target, Suspect and Non-target Approaches



L.-A. Koronaiou, D. Heath, E. Heath, N. Ogrinc, L. Kouřimská, M. Kulma, D.A. Lambropoulou



**Application of a simple and reliable analytical method for the simultaneous determination of pesticides in edible insects**



Application of a simple and reliable analytical method for the simultaneous determination of pesticides in edible insects

Target analysis for more than 200 pesticides

Non-target workflow

# ● Aim of the study

## CONTAMINANTS FROM MANY SOURCES

Pollution, agricultural run-off, chemical spills etc

## EDIBLE INSECTS



## SAMPLE COLLECTION

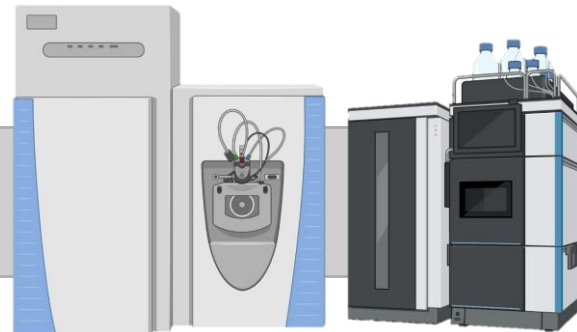
Samples are collected in the field and sent to the lab

## SAMPLE EXTRACTION

Remove interferences prior to analysis



## ANALYSIS BY LC-MS/MS



Introduction



**Selection of studied insects**  
**HRMS application**

Experimental



Method application  
Suspect screening and non-target workflow

Results &  
Discussion



Application results  
General remarks

Conclusions



Basic conclusions &  
experiments in progress



- ❑ Increased population
- ❑ Food scarcity
- ❑ Search for alternative protein sources

# Entomophagy

- ✓ High nutritional content
- ✓ Environmental sustainability
- ✓ Recently introduced in Western countries

- ❑ Currently no authorized pesticides for use in edible insects in the EU

# Entomophagy



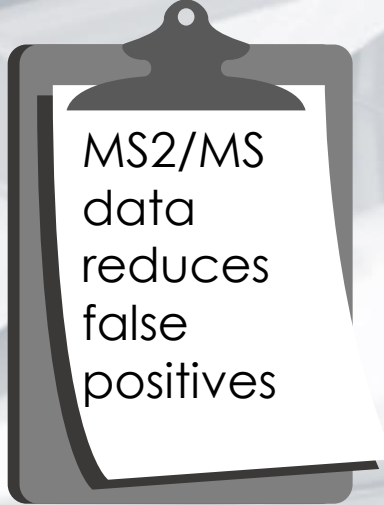
- ✓ The EU and EFSA are in the process of developing a legal framework for novel foods
- ✓ EFSA is evaluating potential risks associated with pesticide residues in edible insects



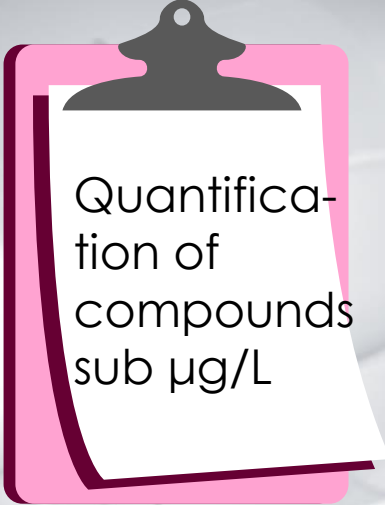
# Why HRMS?




Alleviates matrix effect of complex samples




MS2/MS data reduces false positives



Quantification of compounds sub  $\mu\text{g/L}$



Less sample preparation and faster results



Retrospective analysis of data and confirmation of results



# Orbitrap Q Exactive Focus

Scan speed up to 12 Hz

Resolution up to 70000 (FWHM), at  $m/z$  200

Mass accuracy: sub ppm for routine analysis

Mass range:  $m/z$  50-2000

Simultaneous acquisitions (eg. FS, FS-ddMS, SIM, PRM, vDIA)

Dynamic linear range

HCD fragmentation

Switching polarity within a single run

Introduction



Selection of target analytes  
HRMS application challenges

Experimental



**Method application**  
**Suspect screening and non-target workflow**

Results &  
Discussion



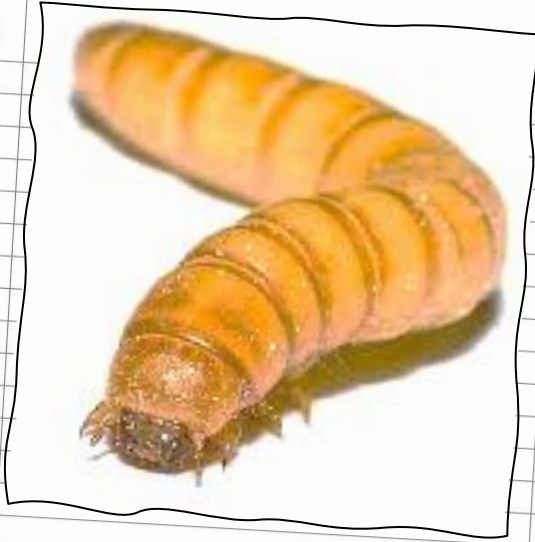
Application results  
General remarks

Conclusions

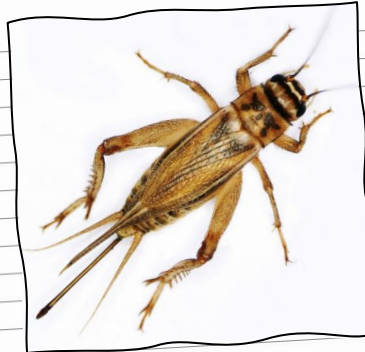


Basic conclusions &  
experiments in progress

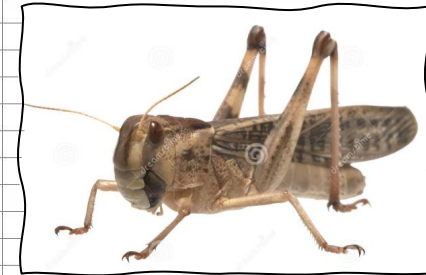
# Studied edible insects



- Phylum: Arthropoda
- Class: Insecta
- Order: Coleoptera
- Family: Tenebrionidae
- Genus: Tenebrio
- Species: Tenebrio molitor



- Phylum: Arthropoda
- Class: Insecta
- Order: Orthoptera
- Suborder: Ensifera
- Family: Gryllidae
- Genus: Acheta
- Species: A. domestica






- Phylum: Arthropoda
- Class: Insecta
- Order: Orthoptera
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- Family: Gryllidae
- Genus: Acheta
- Species: A. domestica

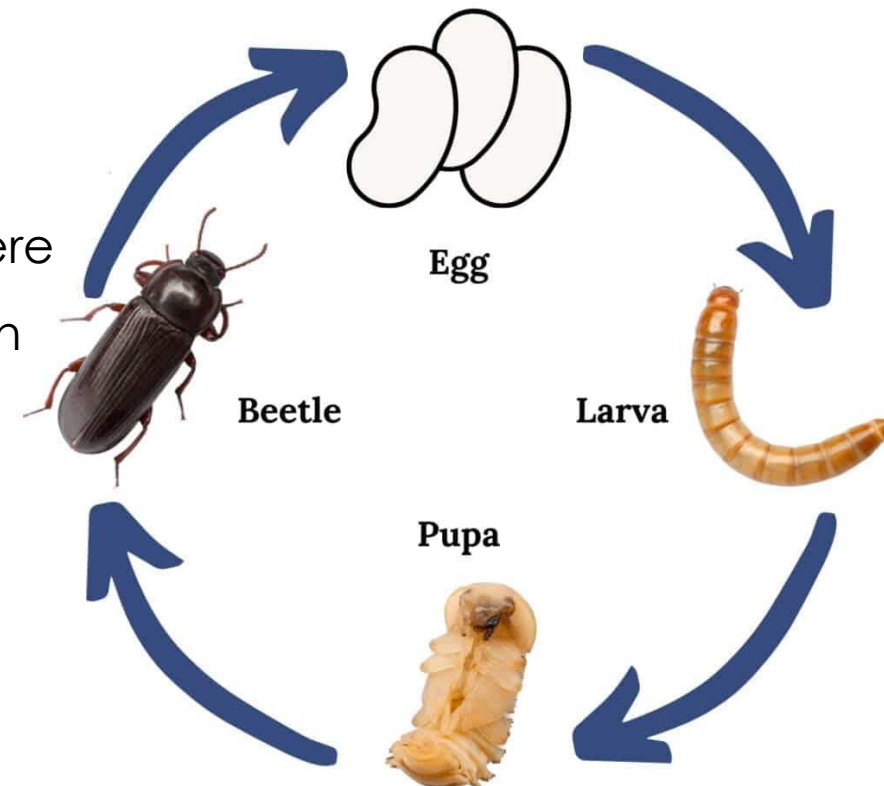
# Studied edible insects



- Phylum: Arthropoda
- Class: Insecta
- Order: Coleoptera
- Family: Tenebrionidae
- Genus: Tenebrio
- Species: Tenebrio molitor

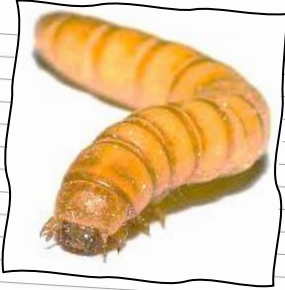
-  Tenebrio molitor (Freeze Dried)
-  Feed for Tenebrio molitor 4:1 Oats/Bran
-  Tenebrio molitor frozen whole

- 2017: approved as food in Switzerland
- 2021: dried mealworms were authorized as novel food in the EU

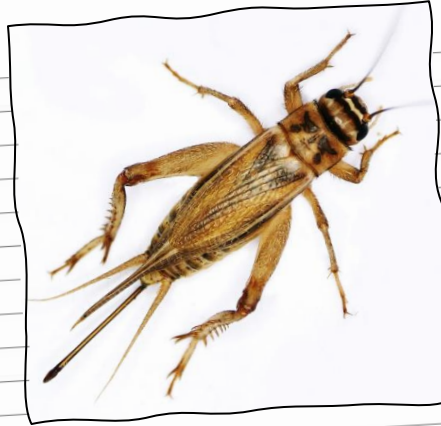




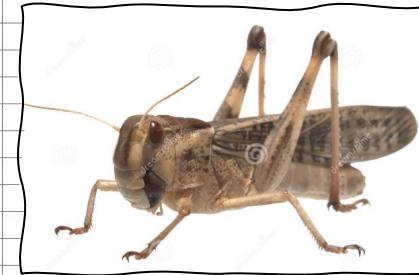
# Studied edible insects



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# Studied edible insects



- Phylum: Arthropoda
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- Family: Gryllidae
- Genus: Acheta
- Species: *A. domesticus*


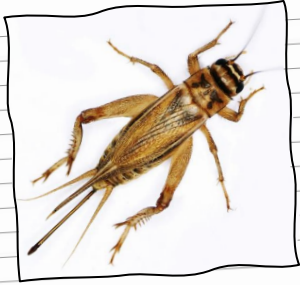
- 🍷 Acheta Domesticus (Freeze Dried)
- 🍷 Feed for Acheta Domesticus Oats
- 🍷 Acheta Domesticus frozen whole

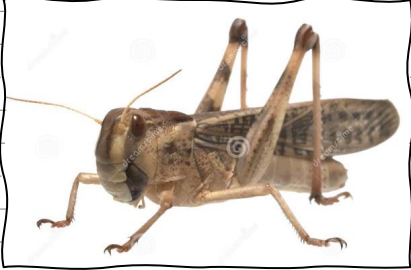
- ❑ 2017: approved as food in Switzerland
- ❑ 2022: house cricket is officially approved for use in food products in the EU



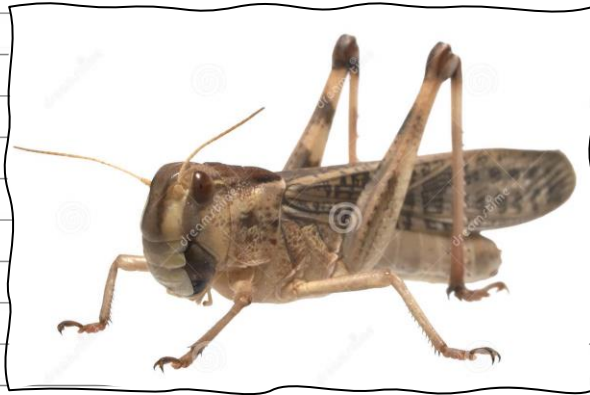
2nd ISO-FOOD Symposium  
Portorož, Slovenia  
April 24 - 26, 2023

# Studied edible insects

	
<ul style="list-style-type: none"><li>○ Phylum: Arthropoda</li><li>○ Class: Insecta</li><li>○ Order: Orthoptera</li><li>○ Family: Acrididae</li><li>○ Genus: Locusta</li><li>○ Species: Locusta migratoria</li></ul>	<ul style="list-style-type: none"><li>○ Phylum: Arthropoda</li><li>○ Class: Insecta</li><li>○ Order: Orthoptera</li><li>○ Suborder: Ensifera</li><li>○ Family: Gryllidae</li><li>○ Genus: Acheta</li><li>○ Species: A. domesticus</li></ul>


<ul style="list-style-type: none"><li>○ Phylum: Arthropoda</li><li>○ Class: Insecta</li><li>○ Order: Orthoptera</li><li>○ Suborder: Caelifera</li><li>○ Family: Acrididae</li><li>○ Genus: Locusta</li><li>○ Species: L. migratoria</li></ul>

# Studied edible insects



- Phylum: Arthropoda
- Class: Insecta
- Order: Orthoptera
- Suborder: Caelifera
- Family: Acrididae
- Genus: Locusta
- Species: *L. migratoria*



Locusta migratoria (freeze-dried)



Feed for Locusta migratoria Wheat Bran



Feed for Locusta migratoria Chicken feed



Grass collected for additional feed for Locusta migratoria



Waste from Locusta migratoria, Frass

- 2017: approved as food in Switzerland
- 2021: green light for the EU Commission to authorize the placing on the market of migratory locust as a food





## Extraction

1 g freeze-dried sample

6 mL ACN

+0.1 % FA for pesticides

Vortex 1 min

Salts: 1 g MgSO<sub>4</sub>, 0.25 g NaCl

Shake manually 30 s Vortex 1 min

Centrifugation 5 min, 4000 rpm

Collect the upper layer, evaporation to 2 mL

## Clean-up

Vortex 1 min

50 mg PSA, 100 mg C18

upper layer

Centrifugation 5 min, 4000 rpm

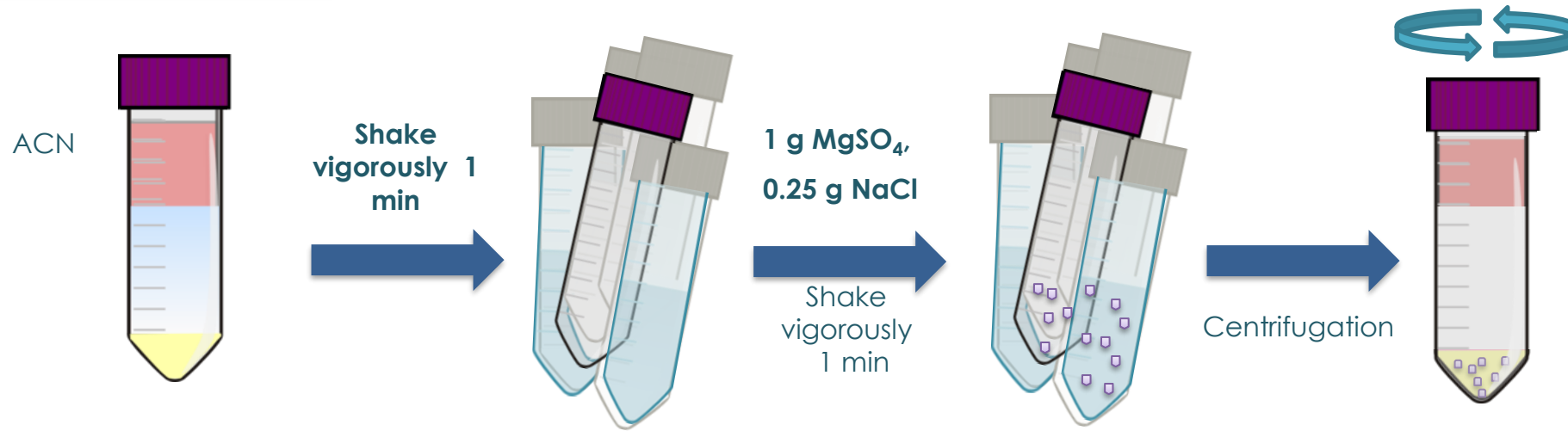
Evaporation - reconstitution  
250 µL MeOH:H<sub>2</sub>O, 50:50

Injection to LC-Orbitrap MS/MS

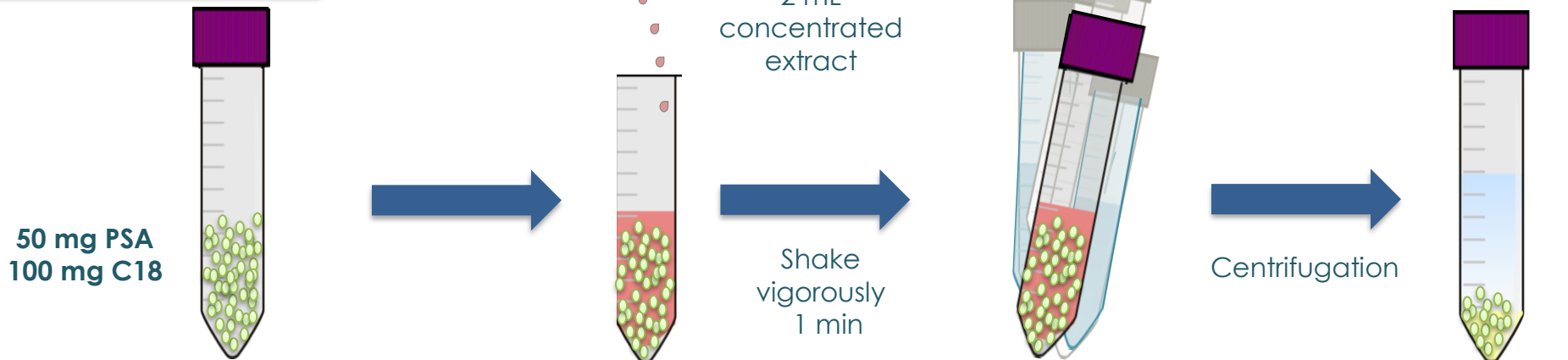
Poma, G. et al. (2022) 'First insights into the occurrence of pesticide residues in edible insects from sub-Saharan African countries', Journal of Environmental Exposure Assessment, doi:10.20517/jeea.2022.25

# Modified QuEChERS

## Step 1: Extraction



## Step 2: Clean-up



2nd ISO-FOOD Symposium  
Portorož, Slovenia  
April 24 - 26, 2023

Poma, G. et al. (2022) 'First insights into the occurrence of pesticide residues in edible insects from sub-Saharan African countries', *Journal of Environmental Exposure Assessment*, doi:10.20517/jeea.2022.25

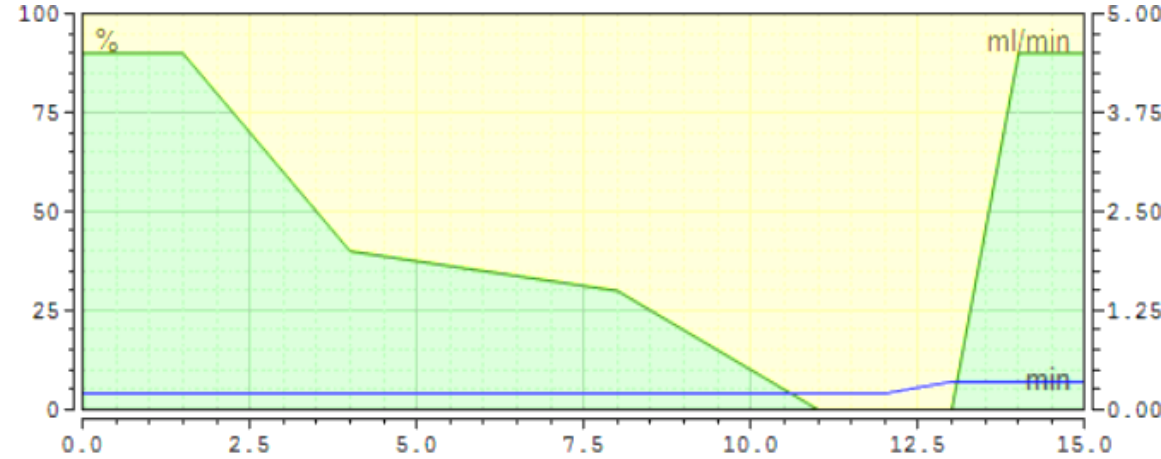
# ● LC-Orbitrap MS/MS method

- LC System: Vanquish Flex (Thermo)
- Column: Thermo Hypersil Gold aQ C18 (50 x 2.1, 1.9  $\mu\text{m}$ )
- Elution solvents (A) H<sub>2</sub>O+0,1% FA and (B) MeOH+0,1% FA
- Flow rate: 200 $\mu\text{L min}^{-1}$
- Injection volume: 5 $\mu\text{L}$
- Ion Max heated electrospray ionization (HESI-II) probe in switching ionization mode
- MS/MS System: Q Exactive Focus Orbitrap MS (Thermo Scientific)
- Software: Xcalibur 4.1 and Trace Finder 4.1 EFS



# LC-Orbitrap MS/MS method

15 min



Time (min)	Flow (mL min <sup>-1</sup> )	MeOH + 0.1% FA	H <sub>2</sub> O + 0.1% FA
0.0	0.200	10.0	90.0
1.5	0.200	10.0	90.0
4.0	0.200	60.0	40.0
8.0	0.200	70.0	30.0
11.0	0.200	100.0	0.0
13.0	0.200	100.0	0.0
14.0	0.350	10.0	90.0
15.0	0.350	10.0	90.0



# ● LC-Orbitrap MS/MS method

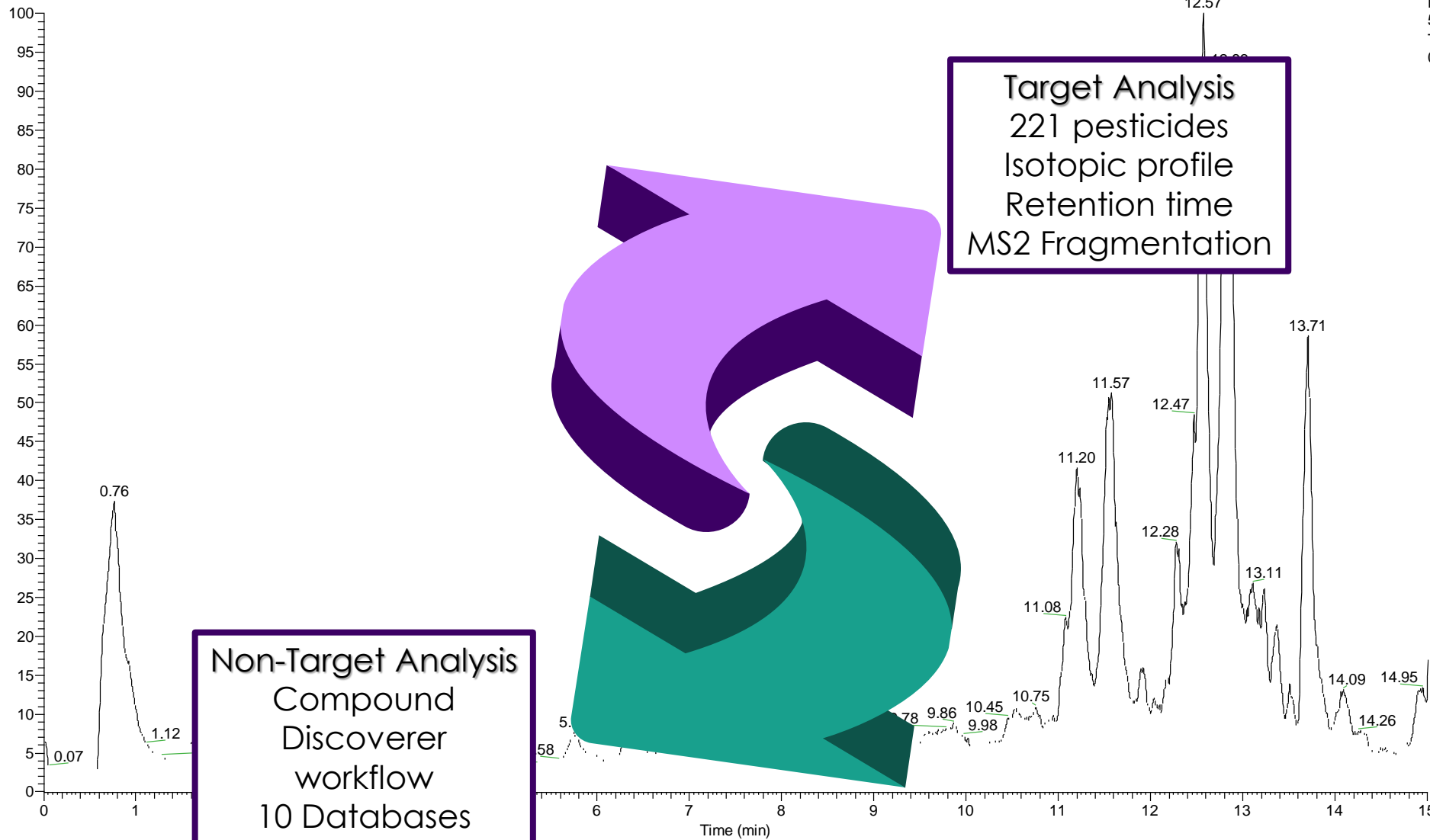
HESI Parameters	Value
Tube lens (V)	110
Sheath gas flow rate	45
Auxiliary gas flow rate	10
Sweep gas flow rate	2
Spray voltage ( kV )	2.5
Cap. Temperature (°C)	320
S-lens RF	50
Heater temperature (°C)	400

MS Parameters	Value
Polarity	Switching
Resolution	70000
Scan Range	100–1000 <i>m/z</i>
AGC Target	10 <sup>6</sup>
Maximum IT	auto
Microscan	1
MS/MS Parameters	Value
Resolution	17500
Isolation window	1 <i>m/z</i>
CE/(N)CE	15-30-50
AGC target	2×10 <sup>5</sup>
Maximum IT	auto
Apex trigger	2-5 s

# LC-Orbitrap MS/MS data

RT: 0.00 - 15.00 SM: 11G

NL:  
5.02E9  
TIC MS  
002



Non-Target Analysis  
Compound  
Discoverer  
workflow  
10 Databases

Target Analysis  
221 pesticides  
Isotopic profile  
Retention time  
MS2 Fragmentation

Acetochlor	Bupirimate	Clethodim	Dimethomorph	Fenazaquin	Hexaconazole	Mefenacet	Nitenpyram	Prochloraz	Pyrimethanil	Terbufos	Zoxamide
Acibenzolar-S-methyl	Buprofezin	Clomazone	Dimoxystrobin	Fenbuconazole	Hexaflumuron	Mepanipyrim	Nitralin	Prodiamine	Quinalphos	Terbumeton	
Alachlor	Butafenacil	Clothianidin	Diniconazole	Fenhexamid	Hexazinone	Mepronil	Norflurazon	Profenofos	Quinoxifen	Terbutylazine	
Aldicarb	Butocarboxim	Coumaphos	Dioxacarb	Fenpropimorph	Hexythiazox	Mesotrione	Novaluron	Prometon	Rotenone	Terbutryn	
Aldicarb-sulfone	Carbaryl	Cyazofamid	Diphenamid	Fenpyroximate	Imazalil	Metaflumizone	Nuarimol	Prometryn	Secbumeton	Tetrachlorvinphos	
Allethrin	Carbetamide	Cycloate	Diphenylamine	Fenthion	Imidacloprid	Metalaxyl	Oxadiazon	Propachlor	Siduron	Tetraconazole	
Ametryn	Carbofuran	Cycluron	Diuron	Flonicamid	Indoxacarb	Metazachlor	Oxadixyl	Propanil	Simetryn	Thiacloprid	
Aminocarb	Carbophenothion	Cymoxanil	Edifenphos	Fluazifop-P-butyl	Ipconazole	Metconazole	Paclobutrazol	Propargite	Spinosad A	Thidiazuron	
Atrazine	Carboxin	Cyproconazole	Emamectin Benzoate	Flufenacet	Iprovalicarb	Methabenzthiazuron	Parathion	Propham	Spinosad D	Thiobencarb	
Azinphos-methyl	Carfentrazone-ethyl	Cyprodinil	Epoxiconazole	Fluometuron	Isazofos	Methacrifos	Penconazole	Propiconazole	Spirodiclofen	Tolclofos-methyl	
Azoxystrobin	Chlorantraniliprole	DEET	Eprinomectin	Fluoxastrobin	Isocarbophos	Methiocarb	Pencycuron	Propisochlor	Spirotetramat	Triadimefon	
Benalaxyl	Chlorfenapyr	Desmedipham	Etaconazol	Fluridone	Isoprocarb	Methomyl	Pendimethalin	Propoxur	Spiroxamine	Triadimenol	
Bendiocarb	Chlorfenvinphos, B-	Diallate	Ethion	Flusilazole	Isopropalin	Methoprotryne	Phenmedipham	Propyzamide	Sulfadiazine	Triallate	
Benzoximate	Chlorfluazuron	Diazinon	Ethiprole	Flutolanil	Isoproturon	Methoxyfenozide	Phorate	Pymetrozine	Sulfentrazone	Triazophos	
Bitertanol	Chloroxuron	Diclobutrazol	Ethirimol	Flutriafol	Kresoxim-methyl	Metobromuron	Phosalone	Pyracarbolid	Sulfotep	Tricyclazole	
Boscalid	Chlorpropham	Dicrotophos	Ethofumesate	Fonofos	Lenacil	Metolachlor	Picoxystrobin	Pyraclofos	Sulprofos	Trifloxystrobin	
Bromfenvinphos-ethyl	Chlorpyrifos	Diethofencarb	Etoxazole	Forchlorfenuron	Linuron	Mevinphos-Cis_Trans	Piperonyl-butoxide	Pyraclostrobin	Tebuconazole	Triflumizole	
Bromfenvinphos-methyl	Chlorpyrifos-methyl	Difenoconazole	Fenamidone	Furalaxyl	Lufenuron	Monocrotophos	Pirimicarb	Pyrazophos	Tebufenozide	Triflumuron	
Bromuconazole, cis-	Chlorthiophos	Diflubenzuron	Fenamiphos	Furathiocarb	Malathion	Myclobutanil	Pirimiphos-ethyl	Pyridaben	Tebufenpyrad	Triticonazole	
Bromuconazole, trans-	Chlortoluron	Dimethachlor	Fenarimol	Halofenozide	Mandipropamid	Neburon	Pirimiphos-methyl	Pyridaphenthion	Tebuthiuron	Vamidothion	

Acetochlor	Bupirimate	Clethodim	Dimethomorph	Fenazaquin	Hexaconazole	Mefenacet	Nitenpyram	Prochloraz	Pyrimethanil	Terbufos	Zoxamide
Acibenzolar-S-methyl	Buprofezin	Clomazone	Dimoxystrobin	Fenbuconazole	Hexaflumuron	Mepanipyrim	Nitralin	Prodiamine	Quinalphos	Terbumeton	
Alachlor	Butafenacil	Clothianidin	Diniconazole	Fenhexamid	Hexazinone	Mepronil	Norflurazon	Profenofos	Quinoxyfen	Terbutylazine	
Aldicarb	Butocarboxim	Coumaphos	Dioxacarb	Fenpropimorph	Hexythiazox	Mesotrione	Novaluron	Prometon	Rotenone	Terbutryn	
Aldicarb-sulfone	Carbaryl	Cyazofamid	Diphenamid	Fenpyroximate	Imazalil	Metaflumizone	Nuarimol	Prometryn	Secbumeton	Tetrachlorvinphos	
Allethrin	Carbetamide	Cycloate	Diphenylamine	Fenthion	Imidacloprid	Metalaxyl	Oxadiazon	Propachlor	Siduron	Tetraconazole	
Ametryn	Carbofuran	Cycluron							Simetryn	Thiacloprid	
Aminocarb	Carbophenothion	Cymoxanil							Spinosad A	Thidiazuron	
Atrazine	Carboxin	Cyproconazole							Spinosad D	Thiobencarb	
Azinphos-methyl	Carfentrazone-ethyl	Cyprodinil							Spirodiclofen	Tolclofos-methyl	
Azoxystrobin	Chlorantraniliprole	DEET							Spirotetramat	Triadimefon	
Benalaxyl	Chlorfenapyr	Desmedipham							Spiroxamine	Triadimenol	
Bendiocarb	Chlorfenvinphos, B-	Diallate							Sulfadiazine	Triallate	
Benzoximate	Chlorfluazuron	Diazinon							Sulfentrazone	Triazophos	
Bitertanol	Chloroxuron	Diclobutrazol							Sulfotep	Tricyclazole	
Boscalid	Chlorpropham	Dicrotophos							Sulprofos	Trifloxystrobin	
Bromfenvinphos-ethyl	Chlorpyrifos	Diethofencarb							Tebuconazole	Triflumizole	
Bromfenvinphos-methyl	Chlorpyrifos-methyl	Difenoconazole							Tebufenozide	Triflumuron	
Bromuconazole, cis-	Chlorthiophos	Diflubenzuron	Fenamiphos	Furathiocarb	Malathion	Myclobutanil	Pirimiphos-ethyl	Pyridaben	Tebufenpyrad	Triticonazole	
Bromuconazole, trans-	Chlortoluron	Dimethachlor	Fenarimol	Halofenozide	Mandipropamid	Neburon	Pirimiphos-methyl	Pyridaphenthion	Tebuthiuron	Vamidothion	

**221 pesticides:**

Herbicides: 61

Plant activator: 1

Insecticides: 86

Fungicides: 63

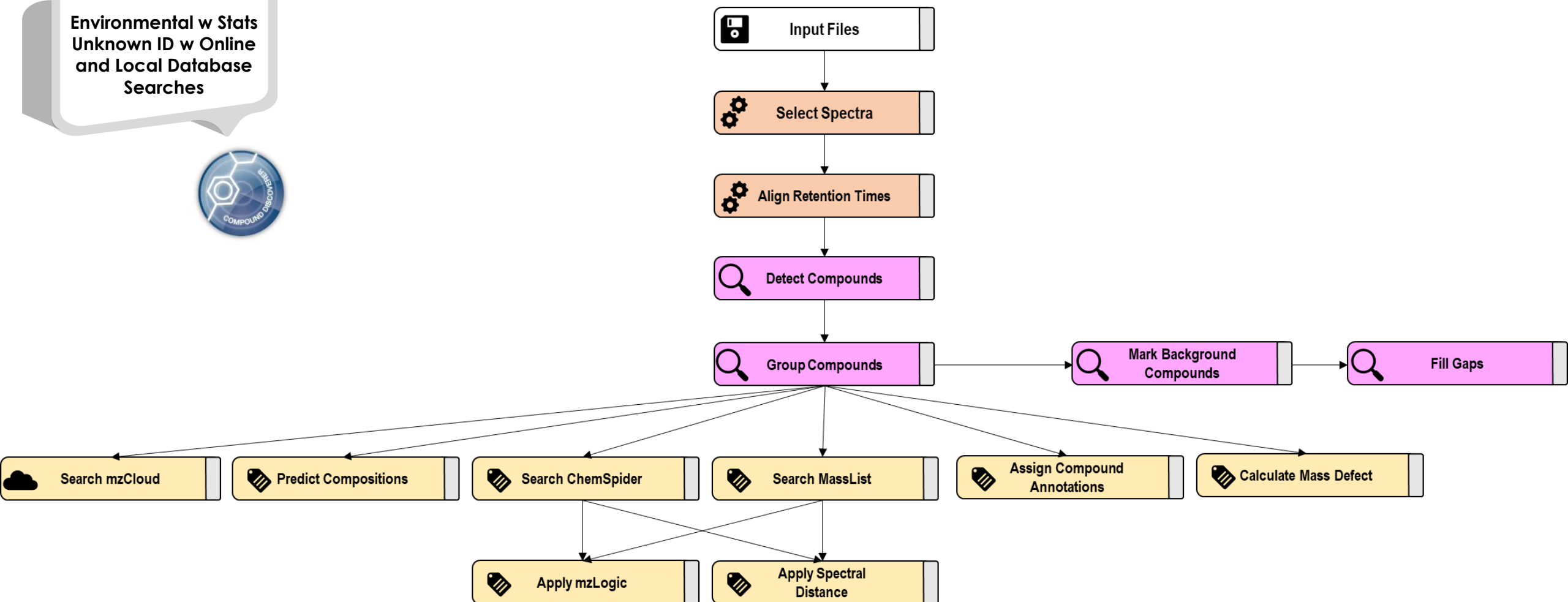
Acaricides: 7

Plant grow regulators: 2

Antibacterial: 1

# Non-target workflow

Environmental w Stats  
Unknown ID w Online  
and Local Database  
Searches





Environmental w Stats  
Unknown ID w Online  
and Local Database  
Searches



## Selected databases:

CAS Common Chemistry

EAWAG Biocatalysis/Biodegradation Database

EPA DSSTox

EPA Toxcast

EU-OpenScreen

FDA

FDA UNII – NLM

Food and Agriculture Organization of the United Nations

FoodB

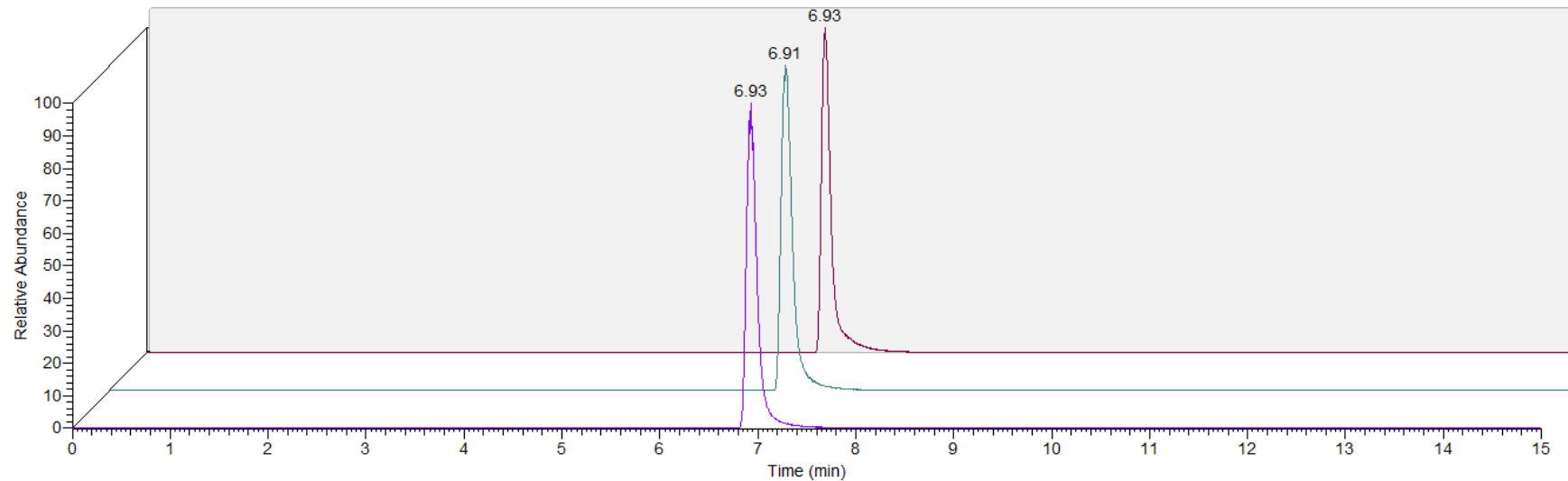
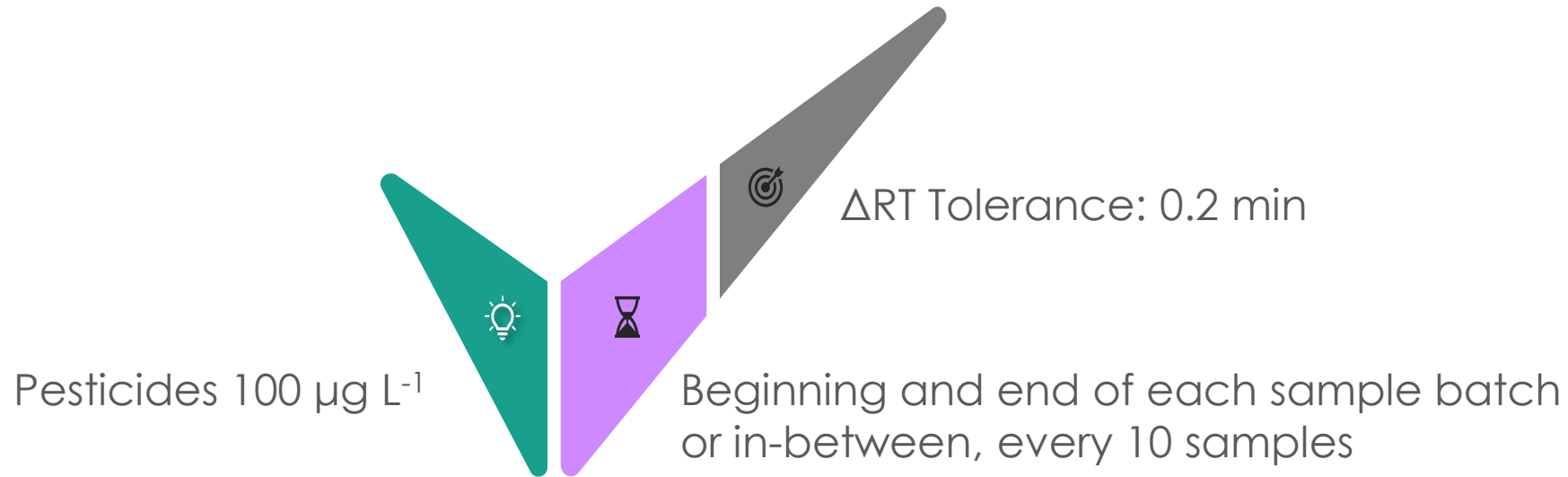
Pesticide Common Names

Search mzCloud

Predict Compositions

Fill Gaps

Calculate Mass Defect



Introduction



Selection of target analytes  
HRMS application challenges

Experimental



Method application  
Suspect screening and non-target workflow

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Discussion



**Application results**  
**General remarks**

Conclusions



Basic conclusions &  
experiments in progress



## Detected:

- ✓ Ametryn
- ✓ DEET
- ✓ Dicrotophos
- ✓ Diniconazole
- ✓ Diphenylamine
- ✓ Isoprocarb
- ✓ Isopropalin
- ✓ Oxadixyl
- ✓ Pendimethalin
- ✓ Pirimiphos-methyl

# Target Analysis

Compounds				Sample Results											
Flags	Compound	RT	Type	Filename	Calculated Amt	Area	Actual RT	RT	RT Delta	Peak Label	Num Isotopes Matched	Isotopic Pattern Score (%)	Formula		
79	Diflubenzuron	9.32	Target Compound	001	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
80	Dimethachlor	7.25	Target Compound	002	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
81	Dimethoate	6.34	Target Compound	003	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
82	Dimethomorph	8.28	Target Compound	004	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
83	Dimoxystrobin	9.38	Target Compound	005	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
84	Diniconazole	10.55	Target Compound	006	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
85	Dinotefuran	4.28	Target Compound	007	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
86	Dioxacarb	6.23	Target Compound	008	-0.137	28997	10.54	10.55	-0.02	T1	0 of 2	0	5H17C		
87	Diphenamid	7.27	Target Compound	009	-0.132	41632	10.56	10.55	0.01	T1	0 of 2	0	5H17C		
88	Diphenylamine	8.59	Target Compound	010	11.498	30504783	10.56	10.55	0.01	T1	7 of 10	78	5H17C		
89	Diuron	7.25	Target Compound	011	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
90	Duloxetine	8.01	Target Compound	012	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
91	Edifenphos	11.29	Target Compound												

**Compound Details**

010 Diniconazole m/z: 326.0821

RT: 10.56  
AA: 30504783  
AH: 3470839

m/z: 326.0821  
Apex RT: 10.56 Left RT: 10.43 Right RT: 10.91

**Calibration Curve**

Diniconazole

Y = 2.619e6X + 3.873e5; R<sup>2</sup>: 0.9997; Origin: Ignore; W: 1/X; Area

Area

ug/L

**Fragments**

Minimum # of fragments needed: 1

010 # 3648 RT: 10.54  
F: FTMS + c ESI sid=5.00 d Full ms2 326. ...

Intensity

m/z

- #1: 308.07150
- #2: 252.00900
- #3: 158.97630
- #4: 70.03990

**Isotope**

Scan #: 3621-3707 RT: 10.43 - 10.91 AV 010  
F: FTMS + c ESI sid=5.00 Full ms [100.00 ...

Relative Intensity

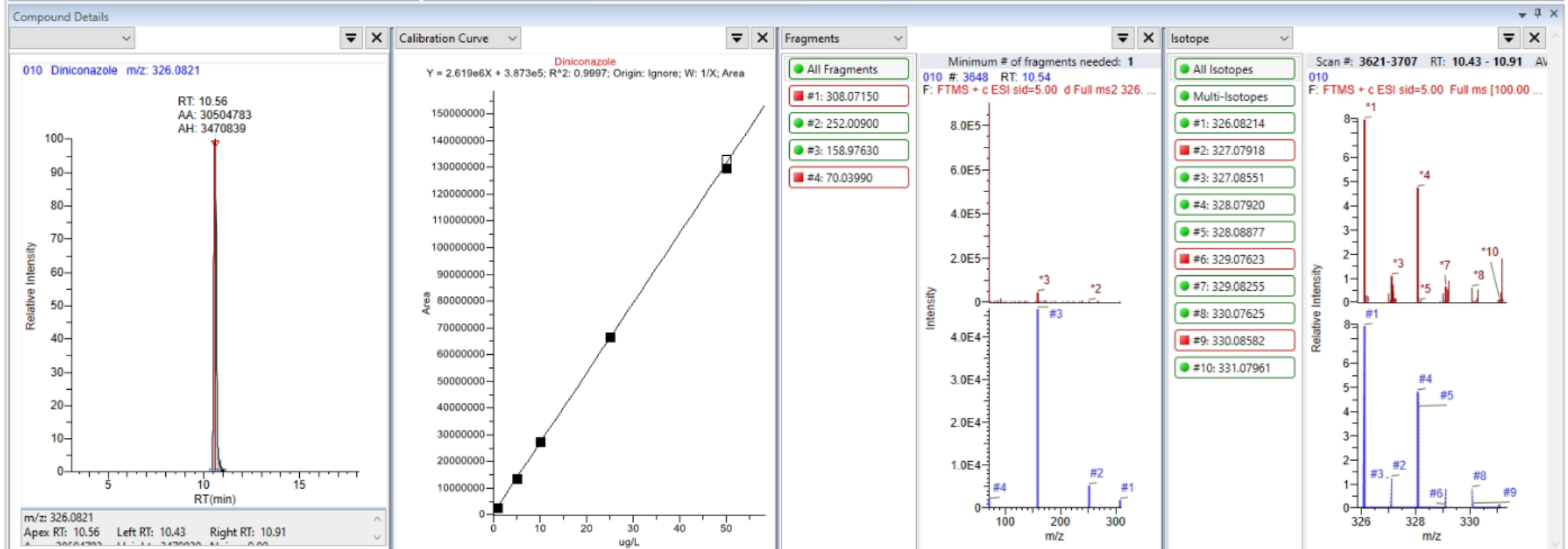
m/z

- #1: 326.08214
- #2: 327.07918
- #3: 327.08551
- #4: 328.07920
- #5: 328.08877
- #6: 329.07623
- #7: 329.08255
- #8: 330.07625
- #9: 330.08582
- #10: 331.07961



# Target Analysis

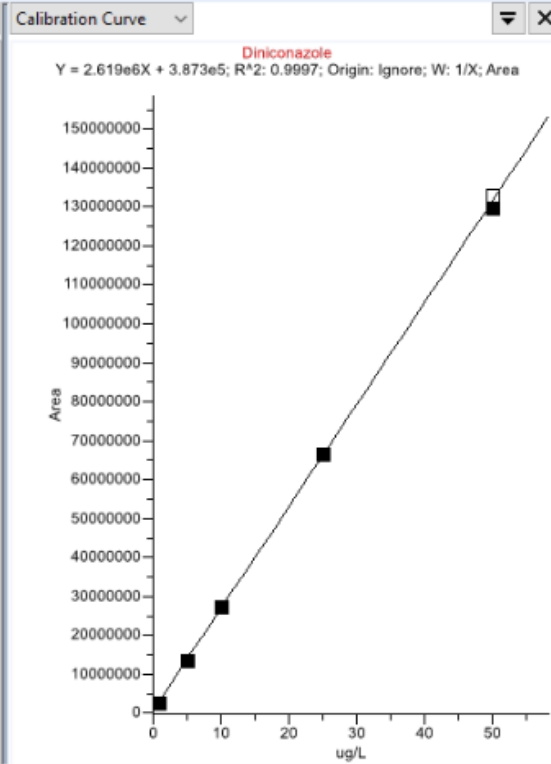
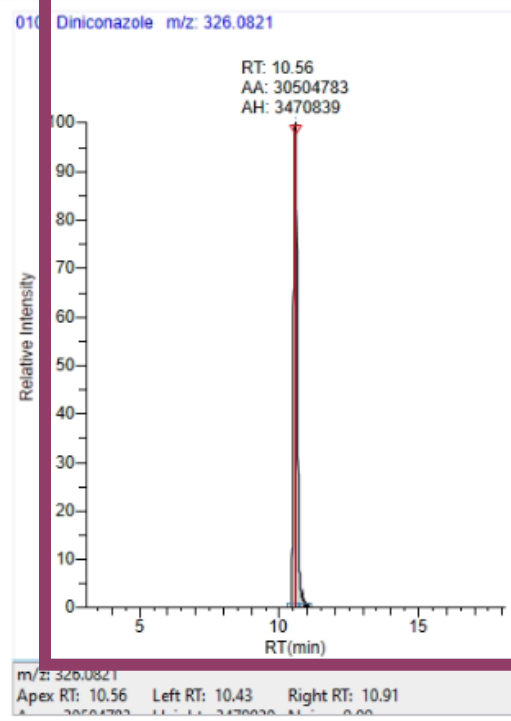
Compounds				Sample Results											
Flags	Compound	RT	Type	Filename	Calculated Amt	Area	Actual RT	RT	RT Delta	Peak Label	Num Isotopes Matched	Isotopic Pattern Score (%)	Formula		
	Diflubenzuron	9.32	Target Compound	001	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
	Dimethachlor	7.25	Target Compound	002	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
⚠	Dimethoate	6.34	Target Compound	003	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
⚠	Dimethomorph	8.28	Target Compound	004	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
	Dimethylsulfoni...	9.38	Target Compound	005	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
	Diniconazole	10.55	Target Compound	006	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
	Diniconazole	10.55	Target Compound	007	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
⚠	Dioxacarb	6.23	Target Compound	008	-0.137	28997	10.54	10.55	-0.02	T1	0 of 2	0	5H17C		
⚠	Diphenamid	7.27	Target Compound	009	-0.132	41632	10.56	10.55	0.01	T1	0 of 2	0	5H17C		
	Diphenylamine	8.59	Target Compound	010	11.498	30504783	10.56	10.55	0.01	T1	7 of 10	78	5H17C		
	Diuron	7.25	Target Compound	011	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
⚠	Duloxetine	8.01	Target Compound	012	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
	Edifenphos	11.29	Target Compound												



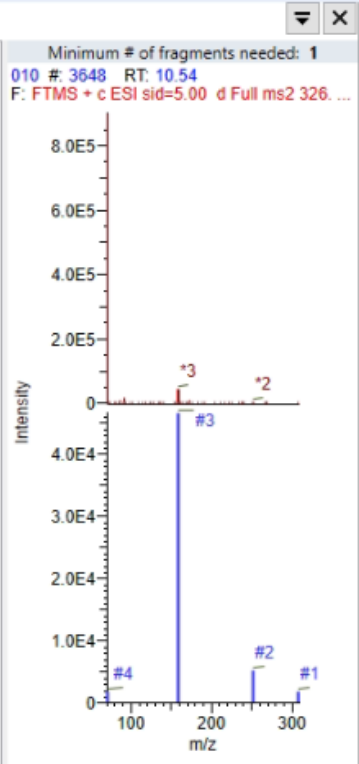
# Target Analysis

Compounds				Sample Results											
Flags	Compound	RT	Type	Filename	Calculated Amt	Area	Actual RT	RT	RT Delta	Peak Label	Num Isotopes Matched	Isotopic Pattern Score (%)	Formula		
	Diflubenzuron	9.32	Target Compound	001	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
	Dimethachlor	7.25	Target Compound	002	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
⚠	Dimethoate	6.34	Target Compound	003	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
⚠	Dimethomorph	8.28	Target Compound	004	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
⚠	Dimoxystrobin	9.38	Target Compound	005	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
	<b>Diniconazole</b>	<b>10.55</b>	<b>Target Compound</b>	<b>006</b>	<b>N/F</b>	<b>N/F</b>	<b>N/F</b>	<b>10.55</b>	<b>N/F</b>	<b>T1</b>	<b>N/A</b>	<b>N/A</b>	<b>5H17C</b>		
⚠	Dinotefuran	4.28	Target Compound	007	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
⚠	Dioxacarb	6.23	Target Compound	008	-0.137	28997	10.54	10.55	-0.02	T1	0 of 2	0	5H17C		
⚠	Diphenamid	7.27	Target Compound	009	-0.132	41632	10.56	10.55	0.01	T1	0 of 2	0	5H17C		
	Diphenylamine	8.59	Target Compound	010	11.498	30504783	10.56	10.55	0.01	T1	7 of 10	78	5H17C		
	Diuron	7.25	Target Compound	011	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
⚠	Duloxetine	8.01	Target Compound	012	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		

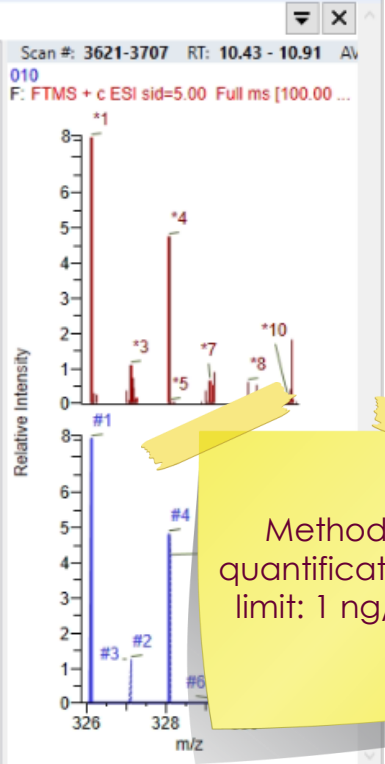
Quan Peak



- Fragments
- All Fragments
  - #1: 308.07150
  - #2: 252.00900
  - #3: 158.97630
  - #4: 70.03990



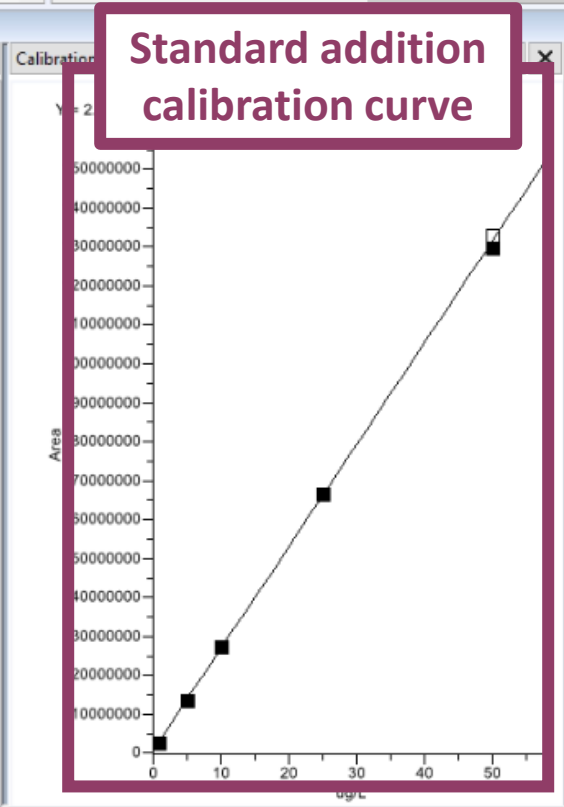
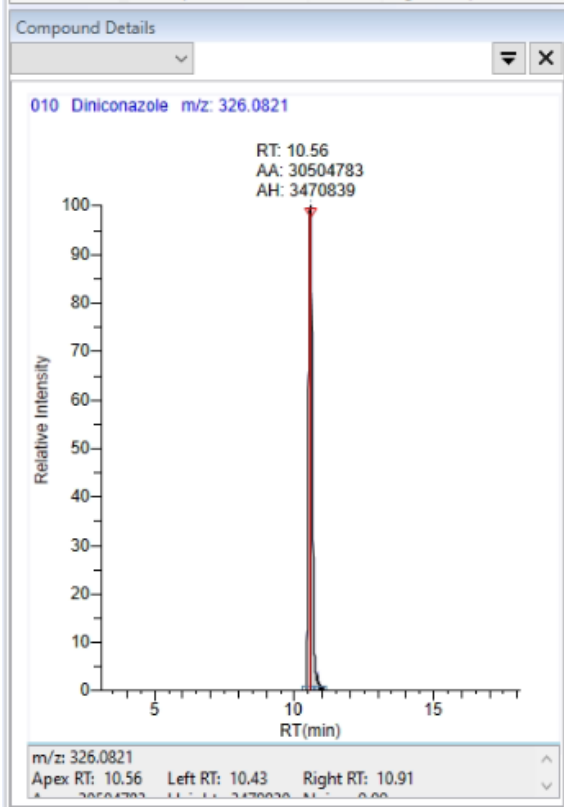
- Isotope
- All Isotopes
  - Multi-Isotopes
  - #1: 326.08214
  - #2: 327.07918
  - #3: 327.08551
  - #4: 328.07920
  - #5: 328.08877
  - #6: 329.07623
  - #7: 329.08255
  - #8: 330.07625
  - #9: 330.08582
  - #10: 331.07961



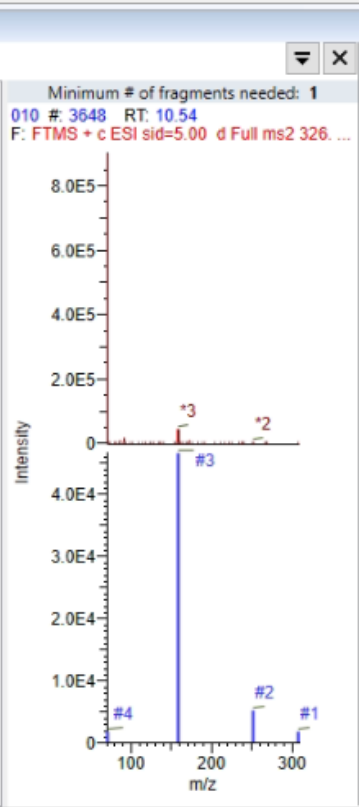
Method quantification limit: 1 ng/g

# Target Analysis

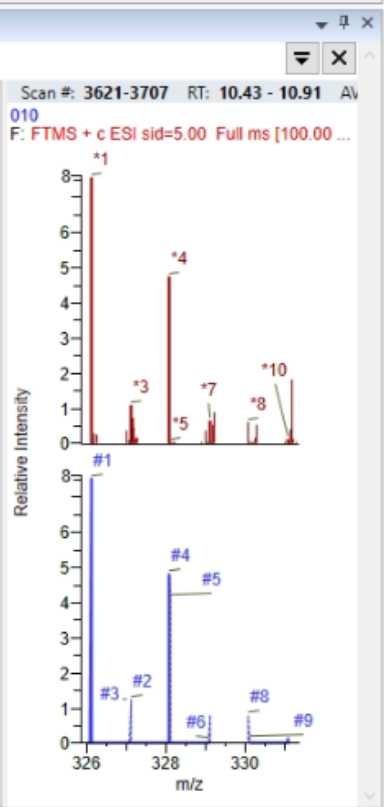
Compounds				Sample Results											
Flags	Compound	RT	Type	Filename	Calculated Amt	Area	Actual RT	RT	RT Delta	Peak Label	Num Isotopes Matched	Isotopic Pattern Score (%)	Formula		
	Diflubenzuron	9.32	Target Compound	001	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
	Dimethachlor	7.25	Target Compound	002	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
⚠	Dimethoate	6.34	Target Compound	003	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
⚠	Dimethomorph	8.28	Target Compound	004	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
⚠	Dimoxystrobin	9.38	Target Compound	005	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
	Diniconazole	10.55	Target Compound	006	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
⚠	Dinotefuran	4.28	Target Compound	007	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
⚠	Dioxacarb	6.23	Target Compound	008	-0.137	28997	10.54	10.55	-0.02	T1	0 of 2	0	5H17C		
⚠	Diphenamid	7.27	Target Compound	009	-0.132	41632	10.56	10.55	0.01	T1	0 of 2	0	5H17C		
	Diuron	8.59	Target Compound	010	11.498	30504783	10.56	10.55	0.01	T1	7 of 10	78	5H17C		
	Duron	7.25	Target Compound	011	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
⚠	Duloxetine	8.01	Target Compound	012	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
	Edifenphos	11.29	Target Compound												



- Fragments
- All Fragments
  - #1: 308.07150
  - #2: 252.00900
  - #3: 158.97630
  - #4: 70.03990



- Isotope
- All Isotopes
  - Multi-Isotopes
  - #1: 326.08214
  - #2: 327.07918
  - #3: 327.08551
  - #4: 328.07920
  - #5: 328.08877
  - #6: 329.07623
  - #7: 329.08255
  - #8: 330.07625
  - #9: 330.08582
  - #10: 331.07961



# Target Analysis

Compounds				Sample Results											
Flags	Compound	RT	Type	Filename	Calculated Amt	Area	Actual RT	RT	RT Delta	Peak Label	Num Isotopes Matched	Isotopic Pattern Score (%)	Formula		
79	Diflubenzuron	9.32	Target Compound	001	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
80	Dimethachlor	7.25	Target Compound	002	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
81	Dimethoate	6.34	Target Compound	003	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
82	Dimethomorph	8.28	Target Compound	004	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
83	Dimoxystrobin	9.38	Target Compound	005	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
84	Diniconazole	10.55	Target Compound	006	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
85	Dinotefuran	4.28	Target Compound	007	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
86	Dioxacarb	6.23	Target Compound	008	-0.137	28997	10.54	10.55	-0.02	T1	0 of 2	0	5H17C		
87	Diphenamid	7.27	Target Compound	009	-0.132	41632	10.56	10.55	0.01	T1	0 of 2	0	5H17C		
88	Diphenylamine	8.59	Target Compound	010	11.498	30504783	10.56	10.55	0.01	T1	7 of 10	78	5H17C		
89	Diuron	7.25	Target Compound	011	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
90	Duloxetine	8.01	Target Compound	012	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
91	Edifenphos	11.29	Target Compound												

**Compound Details**

010 Diniconazole m/z: 326.0821

RT: 10.56  
AA: 30504783  
AH: 3470839

m/z: 326.0821  
Apex RT: 10.56 Left RT: 10.43 Right RT: 10.91

**Calibration Curve**

Diniconazole

Y = 2.619e6X + 3.873e5; R<sup>2</sup>: 0.9997; Origin: Ignore; W: 1/X; Area

**Fragments**

- All Fragments
- #1: 308.07150
- #2: 252.00900
- #3: 158.97630
- #4: 70.03990

**Isotope**

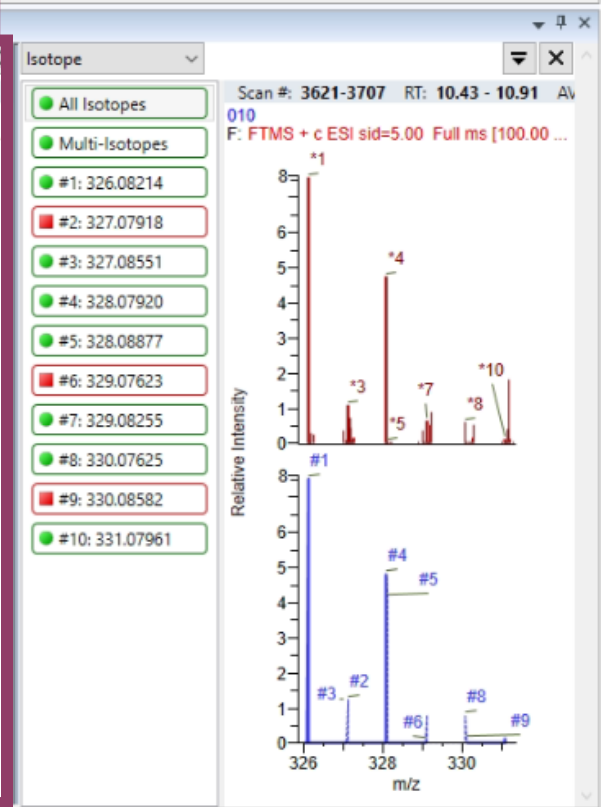
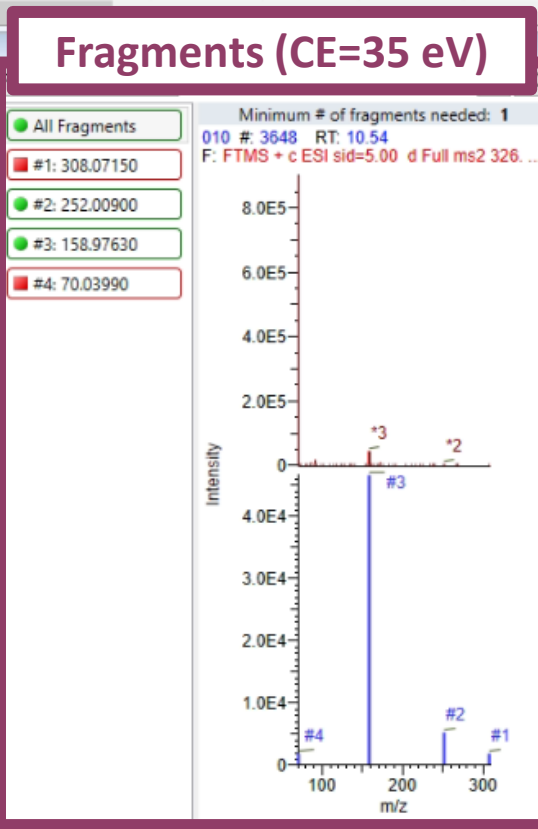
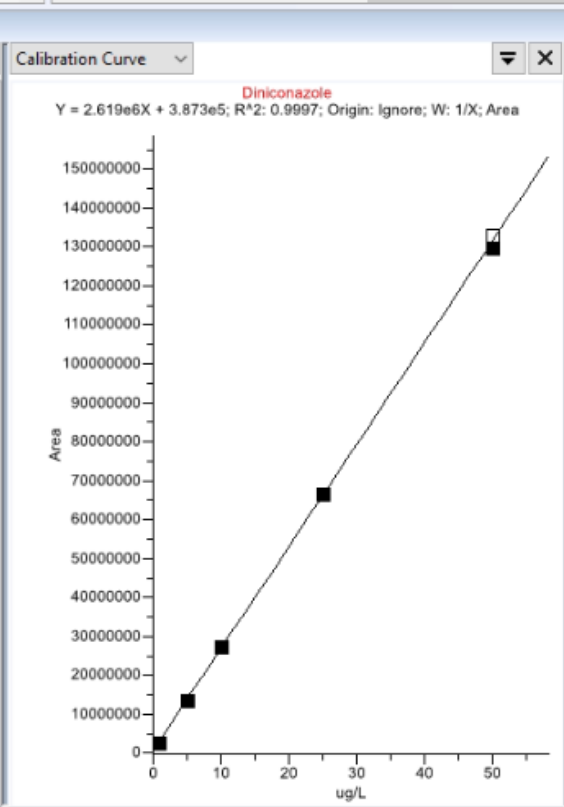
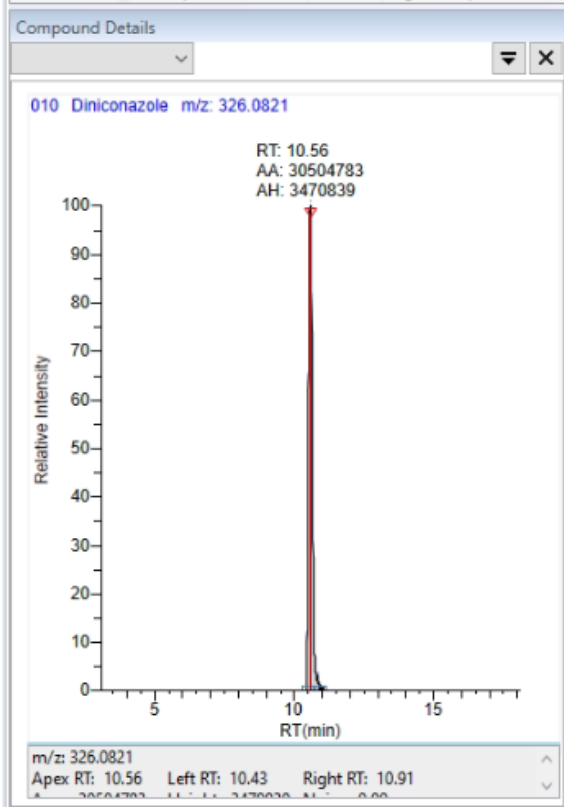
Minimum # of fragments needed: 1

010 # 3648 RT: 10.54  
F: FTMS + c ESI sid=5.00 d Full ms2 326...

Isotopic Profile

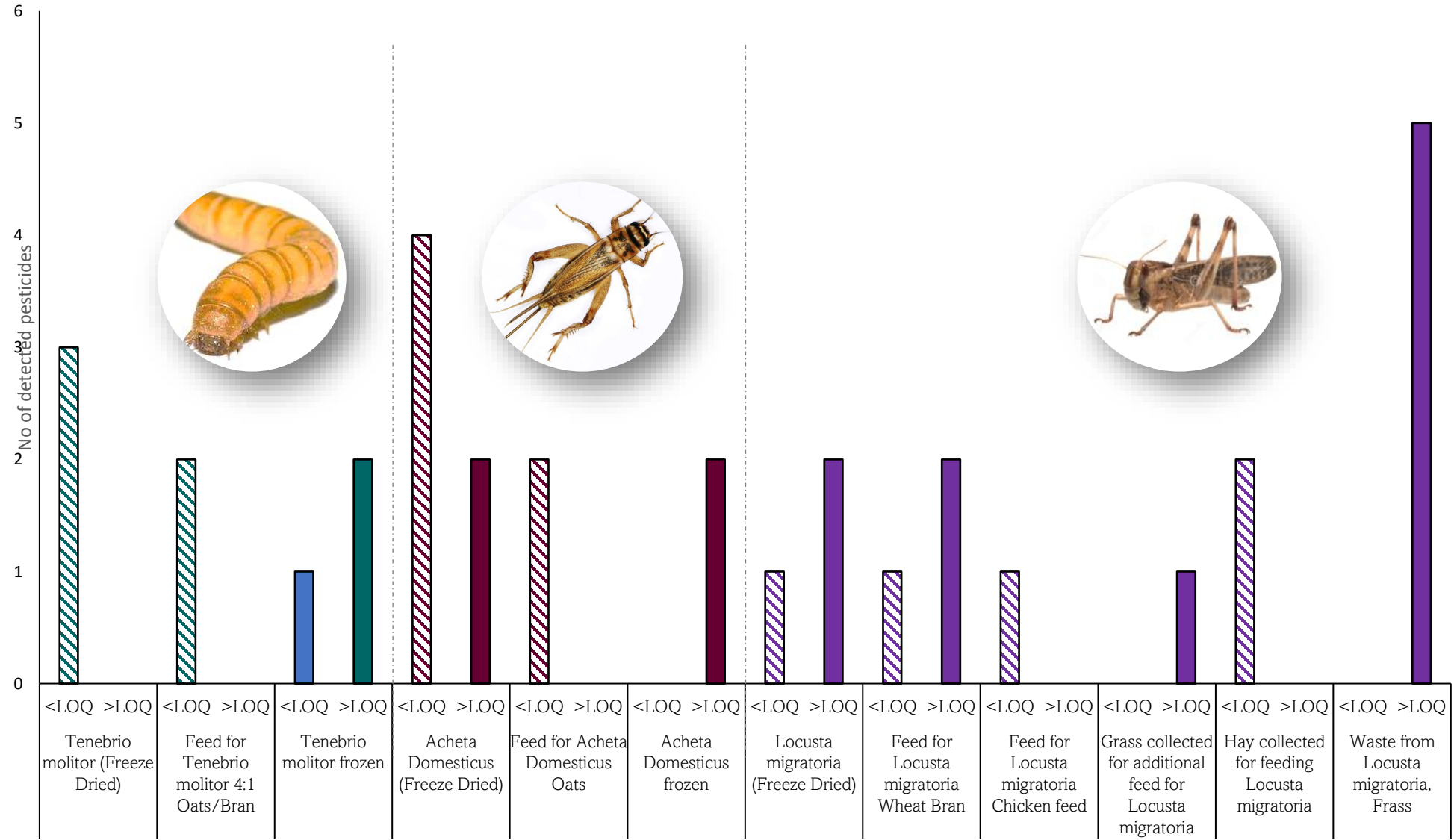
# Target Analysis

Compounds				Sample Results											
Flags	Compound	RT	Type	Filename	Calculated Amt	Area	Actual RT	RT	RT Delta	Peak Label	Num Isotopes Matched	Isotopic Pattern Score (%)	Formula		
79	Diflubenzuron	9.32	Target Compound	001	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
80	Dimethachlor	7.25	Target Compound	002	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
81	Dimethoate	6.34	Target Compound	003	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
82	Dimethomorph	8.28	Target Compound	004	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
83	Dimoxystrobin	9.38	Target Compound	005	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
84	Diniconazole	10.55	Target Compound	006	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
85	Dinotefuran	4.28	Target Compound	007	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
86	Dioxacarb	6.23	Target Compound	008	-0.137	28997	10.54	10.55	-0.02	T1	0 of 2	0	5H17C		
87	Diphenamid	7.27	Target Compound	009	-0.132	41632	10.56	10.55	0.01	T1	0 of 2	0	5H17C		
88	Diphenylamine	8.59	Target Compound	010	11.498	30504783	10.56	10.55	0.01	T1	7 of 10	78	5H17C		
89	Diuron	7.25	Target Compound	011	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
90	Duloxetine	8.01	Target Compound	012	N/F	N/F	N/F	10.55	N/F	T1	N/A	N/A	5H17C		
91	Edifenphos	11.29	Target Compound												





# Target Analysis







**Tenebrio molitor**

## Tenebrio molitor

	Pesticides (ng/g)				
	Ametryn	DEET	Diniconazole	Diphenylamine	Isopropalin
Tenebrio molitor (Freeze Dried)	<LOQ	n.d.	<LOQ	n.d.	<LOQ
Feed for Tenebrio molitor 4:1 Oats/Bran	n.d.	<LOQ	n.d.	<LOQ	n.d.
Tenebrio molitor frozen	<LOQ	n.d.	<b>6.17</b>	n.d.	<b>14.93</b>



## Tenebrio molitor

	Pesticides (ng/g)				
	<b>Ametryn</b>	<b>DEET</b>	<b>Diniconazole</b>	<b>Diphenylamine</b>	<b>Isopropalin</b>
Tenebrio molitor (Freeze Dried)	<LOQ	n.d.	<LOQ	n.d.	<LOQ
Feed for Tenebrio molitor 4:1 Oats/Bran	n.d.	<LOQ	n.d.	<LOQ	n.d.
Tenebrio molitor frozen	<LOQ	n.d.	<b>6.17</b>	n.d.	<b>14.93</b>



## Tenebrio molitor

Quantified only in frozen whole sample

	Pesticides (ng/g)				
	Ametryn	DEET	Diniconazole	Diphenylamine	Isopropalin
Tenebrio molitor (Freeze Dried)	<LOQ	n.d.	<LOQ	n.d.	<LOQ
Feed for Tenebrio molitor 4:1 Oats/Bran	n.d.	<LOQ	n.d.	<LOQ	n.d.
Tenebrio molitor frozen	<LOQ	n.d.	<b>6.17</b>	n.d.	<b>14.93</b>







**Tenebrio molitor**





**Acheta Domesticus**



## Acheta Domesticus

	Pesticides (ng/g)					
	Ametryn	DEET	Diphenylamine	Isoproc carb	Isopropalin	Pendimethalin
Acheta Domesticus (Freeze Dried)	<LOQ	<LOQ	<LOQ	<b>3.00</b>	<LOQ	<b>6.90</b>
Feed for Acheta Domesticus Oats	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.
Acheta Domesticus frozen	n.d.	n.d.	n.d.	n.d.	<b>7.47</b>	<b>10.88</b>



## Acheta Domesticus

	Pesticides (ng/g)					
	Ametryn	DEET	Diphenylamine	Isoproc carb	Isopropalin	Pendimethalin
Acheta Domesticus (Freeze Dried)	<LOQ	<LOQ	<LOQ	<b>3.00</b>	<LOQ	<b>6.90</b>
Feed for Acheta Domesticus Oats	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.
Acheta Domesticus frozen	n.d.	n.d.	n.d.	n.d.	<b>7.47</b>	<b>10.88</b>



## Acheta Domesticus

	Pesticides (ng/g)					
	Ametryn	DEET	Diphenylamine	Isoproc carb	Isopropalin	Pendimethalin
Acheta Domesticus (Freeze Dried)	<LOQ	<LOQ	<LOQ	3.00	<LOQ	6.90
Feed for Acheta Domesticus Oats	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.
Acheta Domesticus frozen	n.d.	n.d.	n.d.	n.d.	7.47	10.88



## Acheta Domesticus

Higher concentrations in frozen whole samples

	Pesticides (ng/g)					
	Ametryn	DEET	Diphenylamine	Isoproc carb	Isopropalin	Pendimethalin
Acheta Domesticus (Freeze Dried)	<LOQ	<LOQ	<LOQ	<b>3.00</b>	<LOQ	<b>6.90</b>
Feed for Acheta Domesticus Oats	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.
Acheta Domesticus frozen	n.d.	n.d.	n.d.	n.d.	<b>7.47</b>	<b>10.88</b>



## Acheta Domesticus

Not detected in feed

	Pesticides (ng/g)					
	Ametryn	DEET	Diphenylamine	Isoproc carb	Isopropalin	Pendimethalin
Acheta Domesticus (Freeze Dried)	<LOQ	<LOQ	<LOQ	<b>3.00</b>	<LOQ	<b>6.90</b>
Feed for Acheta Domesticus Oats	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.
Acheta Domesticus frozen	n.d.	n.d.	n.d.	n.d.	<b>7.47</b>	<b>10.88</b>





**Acheta Domesticus**





**Locusta migratoria**

## Locusta migratoria

	Pesticides (ng/g)						
	Ametryn	DEET	Dicrotophos	Diphenylamine	Isoprocarb	Oxadixyl	Pririmiphos-methyl
Locusta migratoria (Freeze Dried)	<b>1.31</b>	<LOQ	n.d.	n.d.	<b>3.39</b>	n.d.	n.d.
Feed for Locusta migratoria Wheat Bran	n.d.	<LOQ	n.d.	<b>1.19</b>	n.d.	n.d.	<b>8.36</b>
Feed for Locusta migratoria Chicken feed	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.
Grass collected for additional feed for Locusta migratoria	n.d.	<b>1.84</b>	n.d.	n.d.	n.d.	n.d.	n.d.
Hay collected for feeding Locusta migratoria	n.d.	<LOQ	n.d.	<LOQ	n.d.	n.d.	n.d.
Waste from Locusta migratoria, Frass	n.d.	<b>1.50</b>	n.d.	<b>4.23</b>	<b>10.64</b>	<b>1.22</b>	<b>1.52</b>



# Target Analysis

## Locusta migratoria

Only in waste

	Pesticides (ng/g)						
	Ametryn	DEET	Dicrotophos	Diphenylamine	Isoprocarb	Oxadixyl	Priniphos-methyl
Locusta migratoria (Freeze Dried)	<b>1.31</b>	<LOQ	n.d.	n.d.	<b>3.39</b>	n.d.	n.d.
Feed for Locusta migratoria Wheat Bran	n.d.	<LOQ	n.d.	<b>1.19</b>	n.d.	n.d.	<b>8.36</b>
Feed for Locusta migratoria Chicken feed	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.
Grass collected for additional feed for Locusta migratoria	n.d.	<b>1.84</b>	n.d.	n.d.	n.d.	n.d.	n.d.
Hay collected for feeding Locusta migratoria	n.d.	<LOQ	n.d.	<LOQ	n.d.	n.d.	n.d.
Waste from Locusta migratoria, Frass	n.d.	<b>1.50</b>	n.d.	<b>4.23</b>	<b>10.64</b>	<b>1.22</b>	<b>1.52</b>

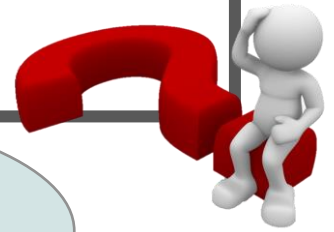
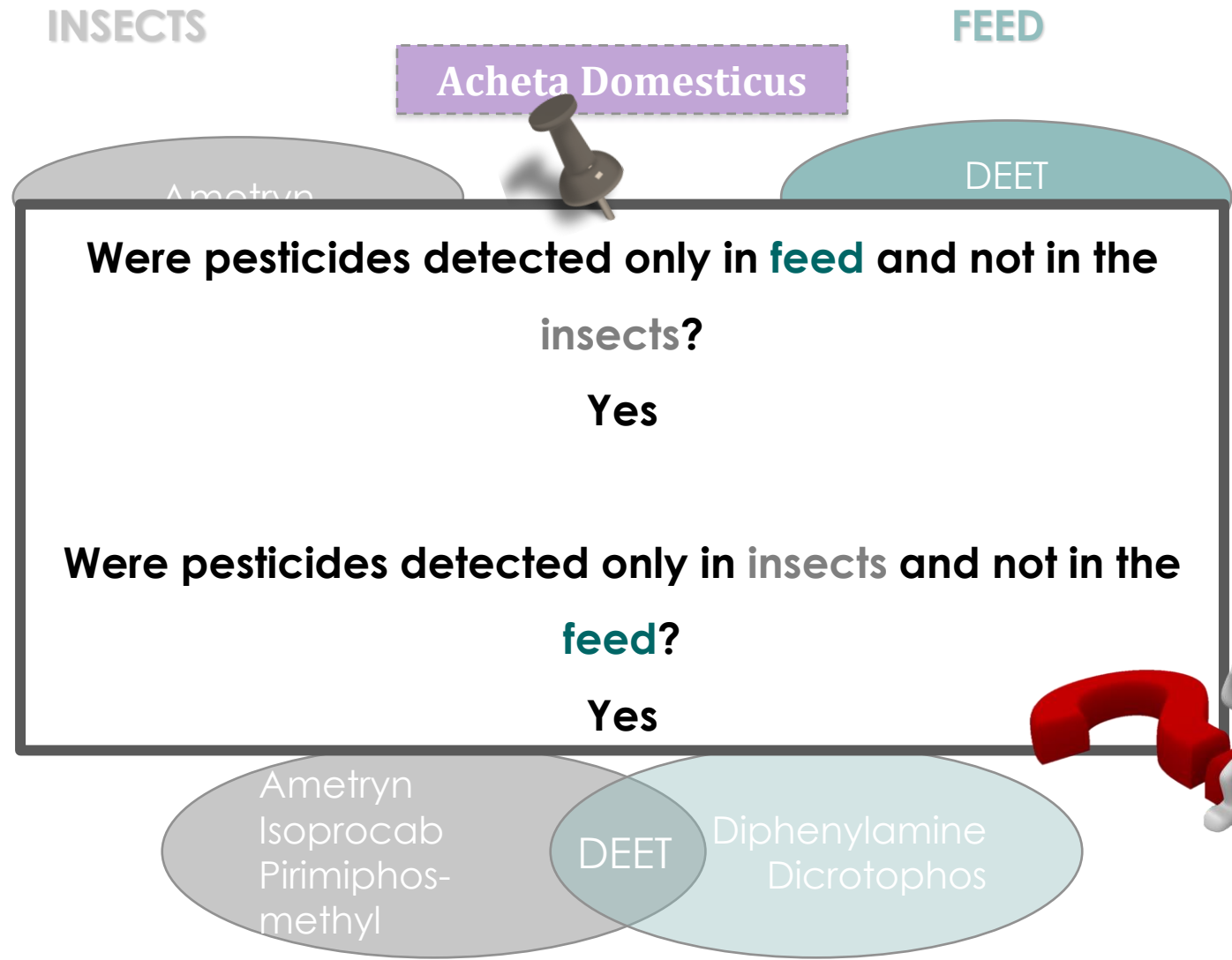


## Locusta migratoria

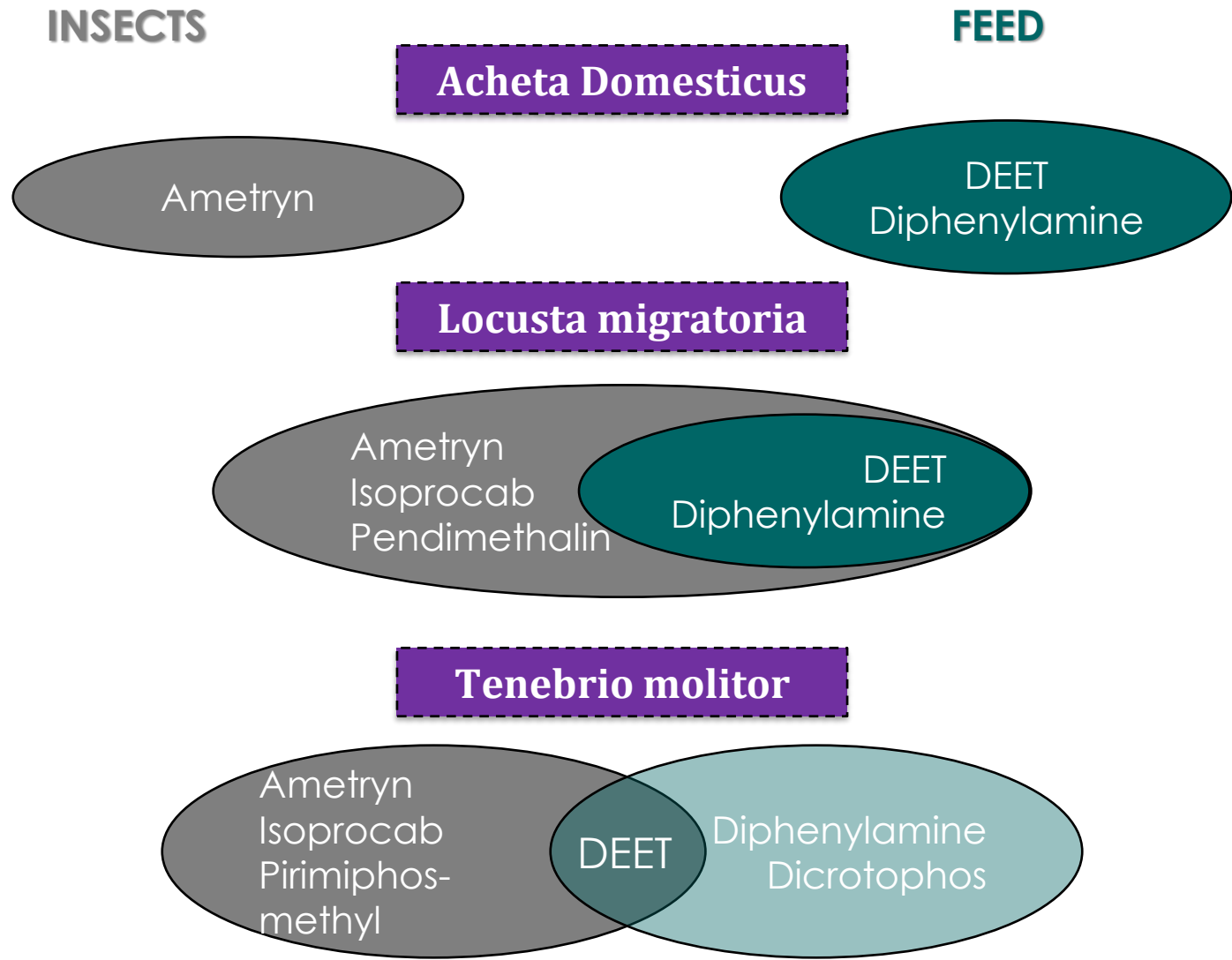
	Pesticides (ng/g)						
	Ametryn	DEET	Dicrotophos	Diphenylamine	Isoprocarb	Oxadixyl	Pririmiphos-methyl
Locusta migratoria (Freeze Dried)	<b>1.31</b>	<LOQ	n.d.	n.d.	<b>3.39</b>	n.d.	n.d.
Feed for Locusta migratoria Wheat Bran	n.d.	<LOQ	n.d.	<b>1.19</b>	n.d.	n.d.	<b>8.36</b>
Feed for Locusta migratoria Chicken feed	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.
Grass collected for additional feed for Locusta migratoria	n.d.	<b>1.84</b>	n.d.	n.d.	Highest $\Sigma$ concentration		n.d.
Hay collected for feeding Locusta migratoria	n.d.	<LOQ	n.d.	<LOQ			n.d.
Waste from Locusta migratoria, Frass	n.d.	<b>1.50</b>	n.d.	<b>4.23</b>	<b>10.64</b>	<b>1.22</b>	<b>1.52</b>



## General remarks

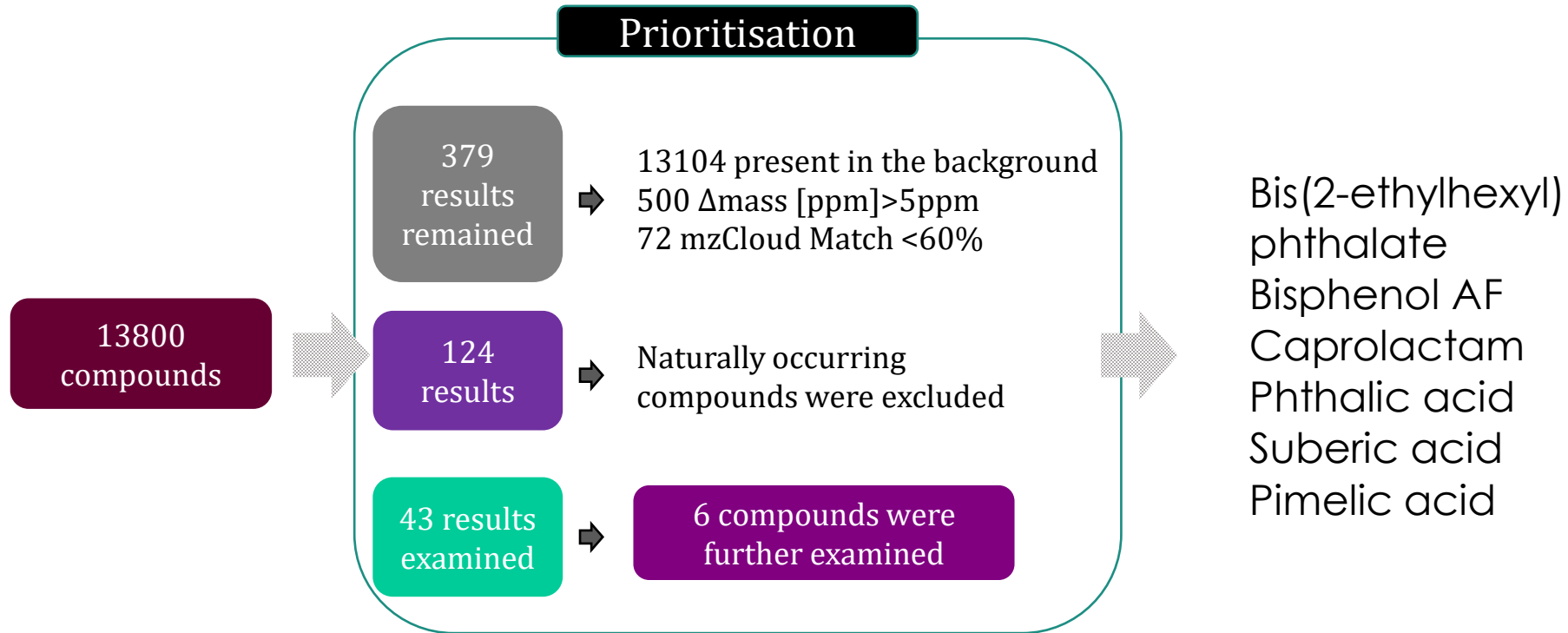


## General remarks



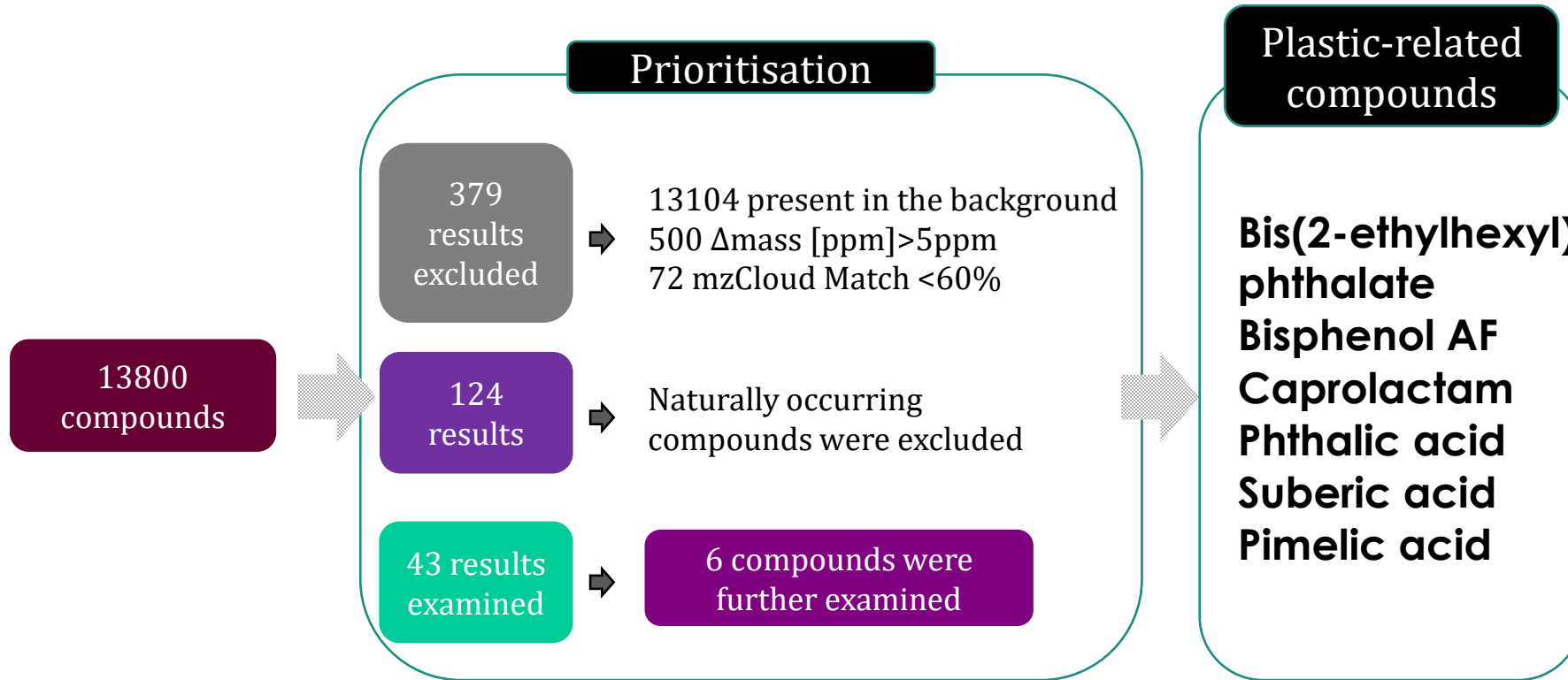


# Non-target Analysis



Level 2a  
Identification  
(Schymanski)

# Non-target Analysis



Level 2a  
Identification  
(Schymanski)

Introduction



Selection of target analytes  
HRMS application challenges

Experimental



Method application  
Suspect screening and non-target workflow

Results &  
Discussion



Application results  
General remarks

Conclusions



**Basic conclusions &  
experiments in progress**

Question	Outcome
What?	Three species of edible insects and their respective feed
Why?	Novel foods
How?	QuEChERS and UHPLC Q Exactive Focus Orbitrap MS
To what end?	Pesticides and NTA
Results?	10 pesticides and 6 plasticizers

**Investigation  
of occurrence of  
TPs and  
metabolites of  
pesticides**



**Further  
investigation of  
non target findings**







Thank you



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