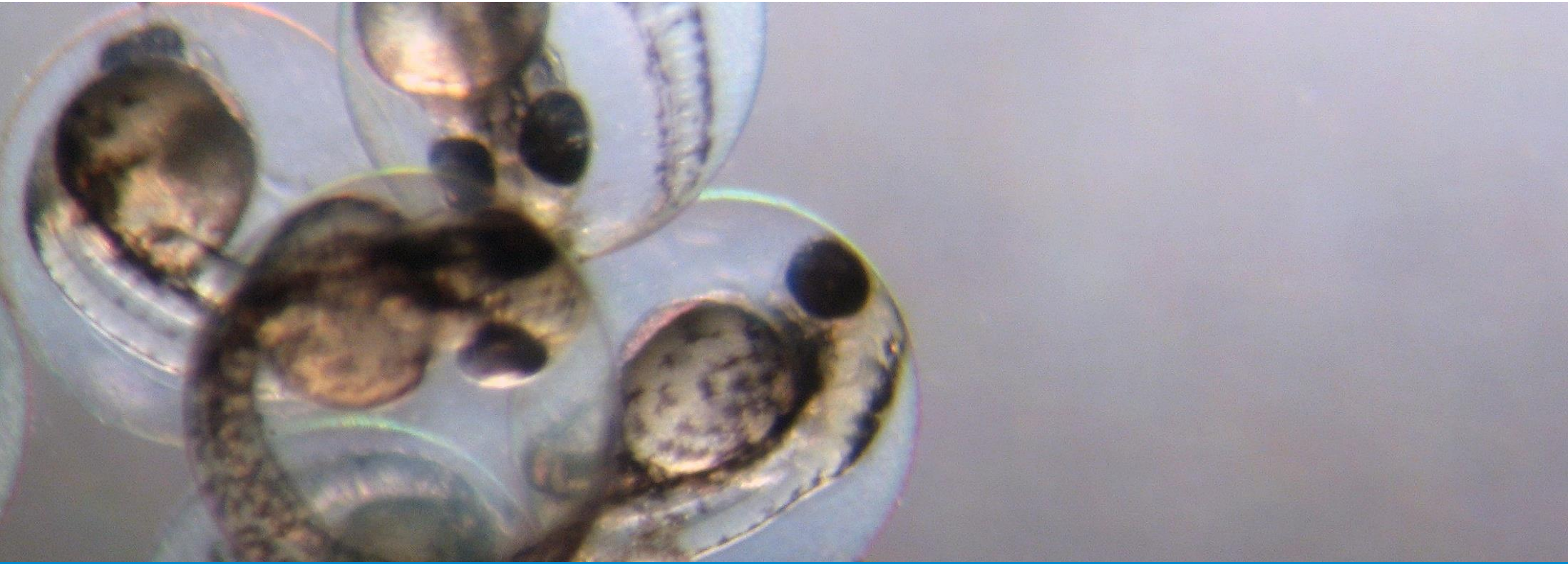
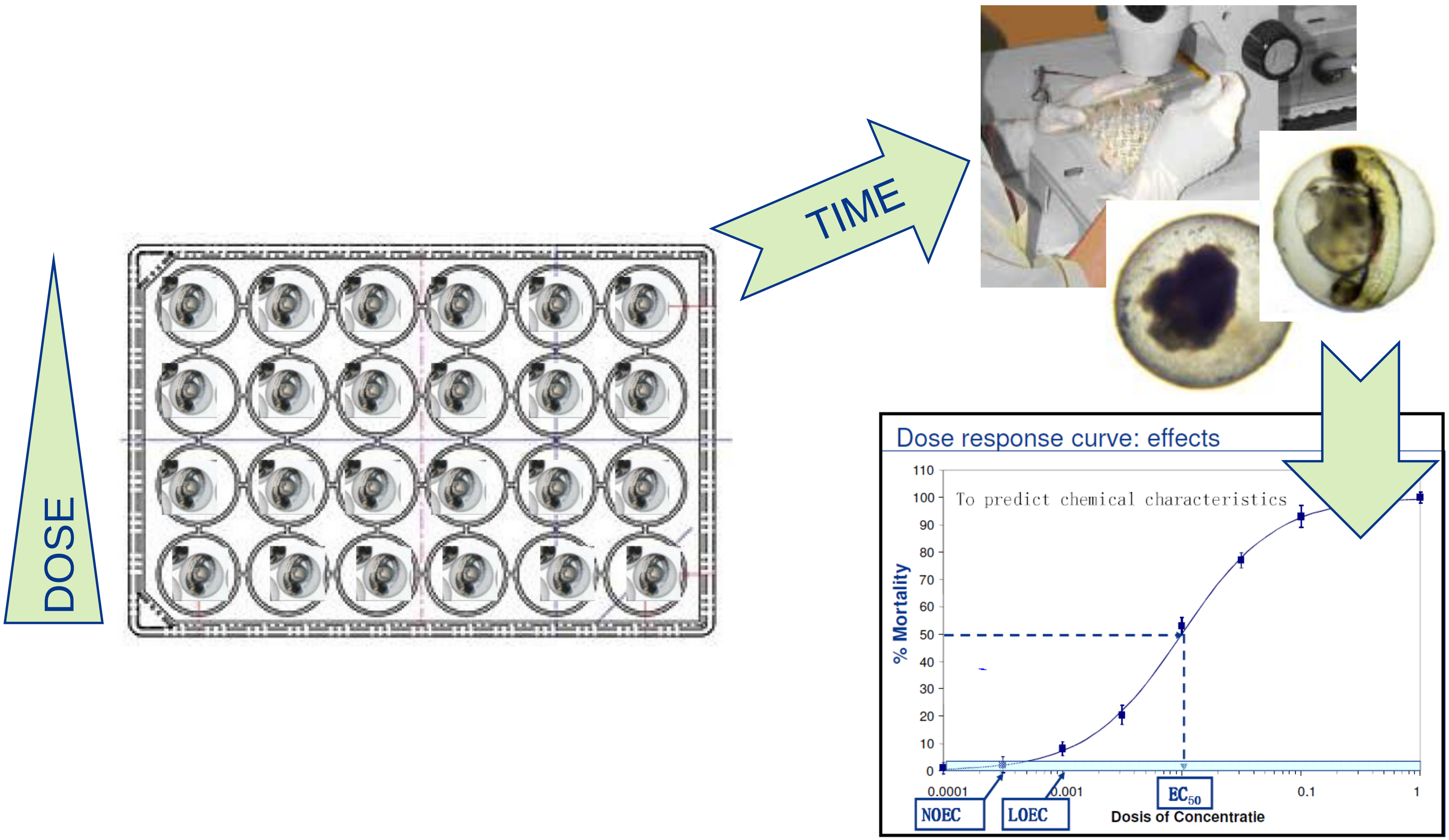


Application of novel omics tools for zebrafish (neuro-) toxicological research

Zebrafish embryo toxicity test (ZFET)

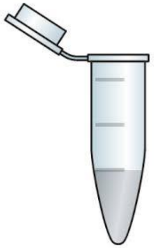


OECD 236

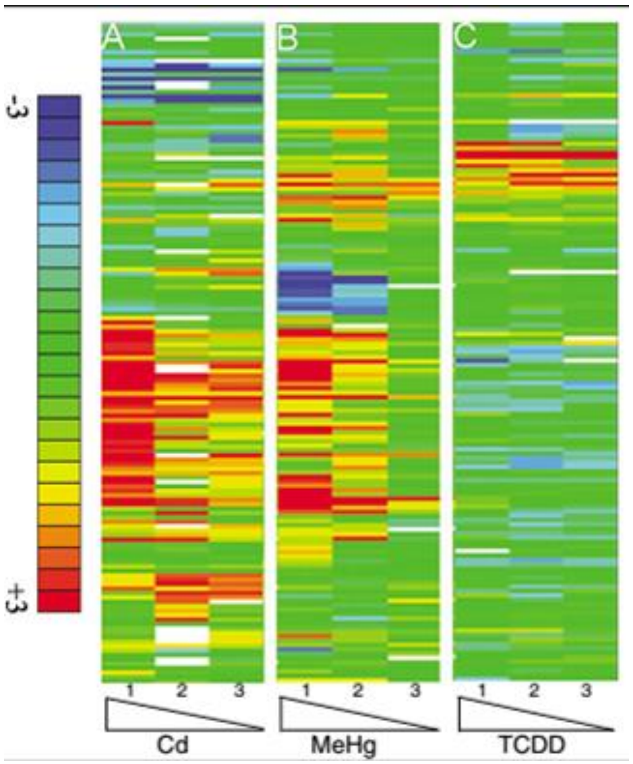
Methods to study (Neuro)toxicity

- Behavioral assays
- Monitor Heartbeat
- EROD-assay
- Transgenic zebrafish
- ….

- Acetylcholinesterase inhibition assay
- Transcriptomics
- Proteomics
- Metabolomics
- Lipidomics
- …



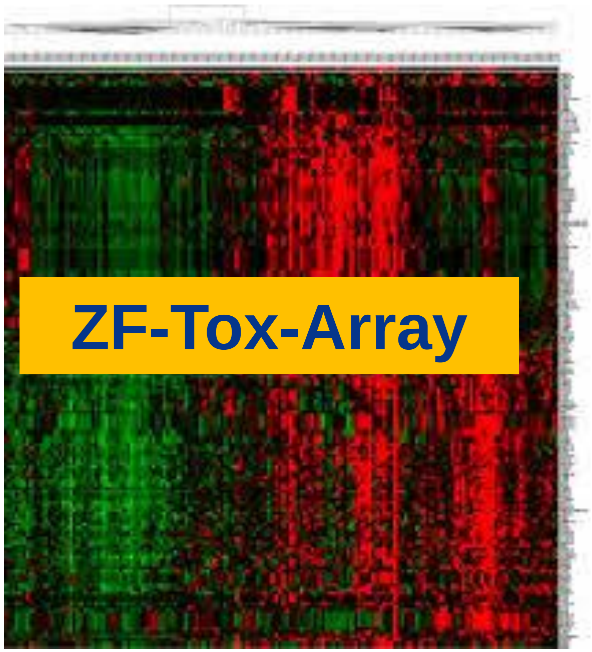
Methods to study (Neuro)toxicity



- Transcriptomics
- Proteomics
- Metabolomics
- Lipidomics

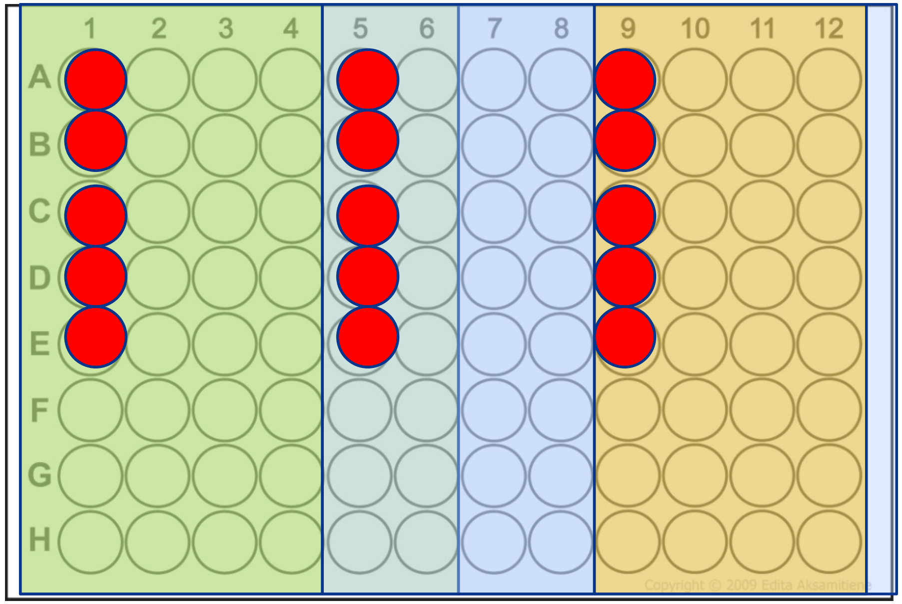
Yang et al 2007

Toxicogenomics



- 3-15 genes
- 2-10 Pathways
- Maybe novel discoveries

Tox-Array Set Up



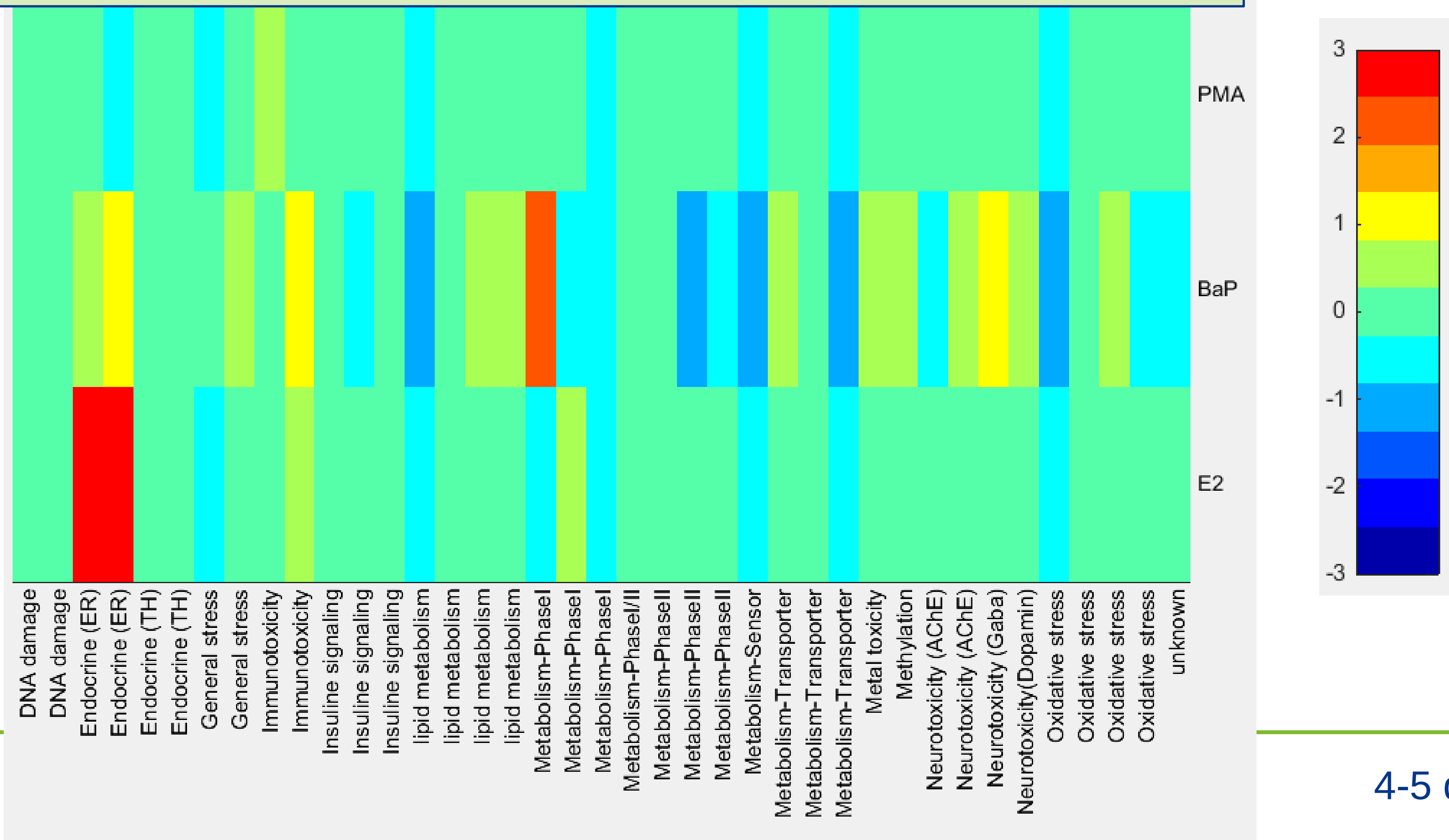
43 or 27 genes per array

Gene selection

- **Knowledge based**
  - Experience
  - From Literature – known biomarker
  - Microarray/ Sequencing studies
  - Genes involved in interesting pathways or biological processes
  - 2-3 genes per pathway or mechanism
  - Develop the gene list further

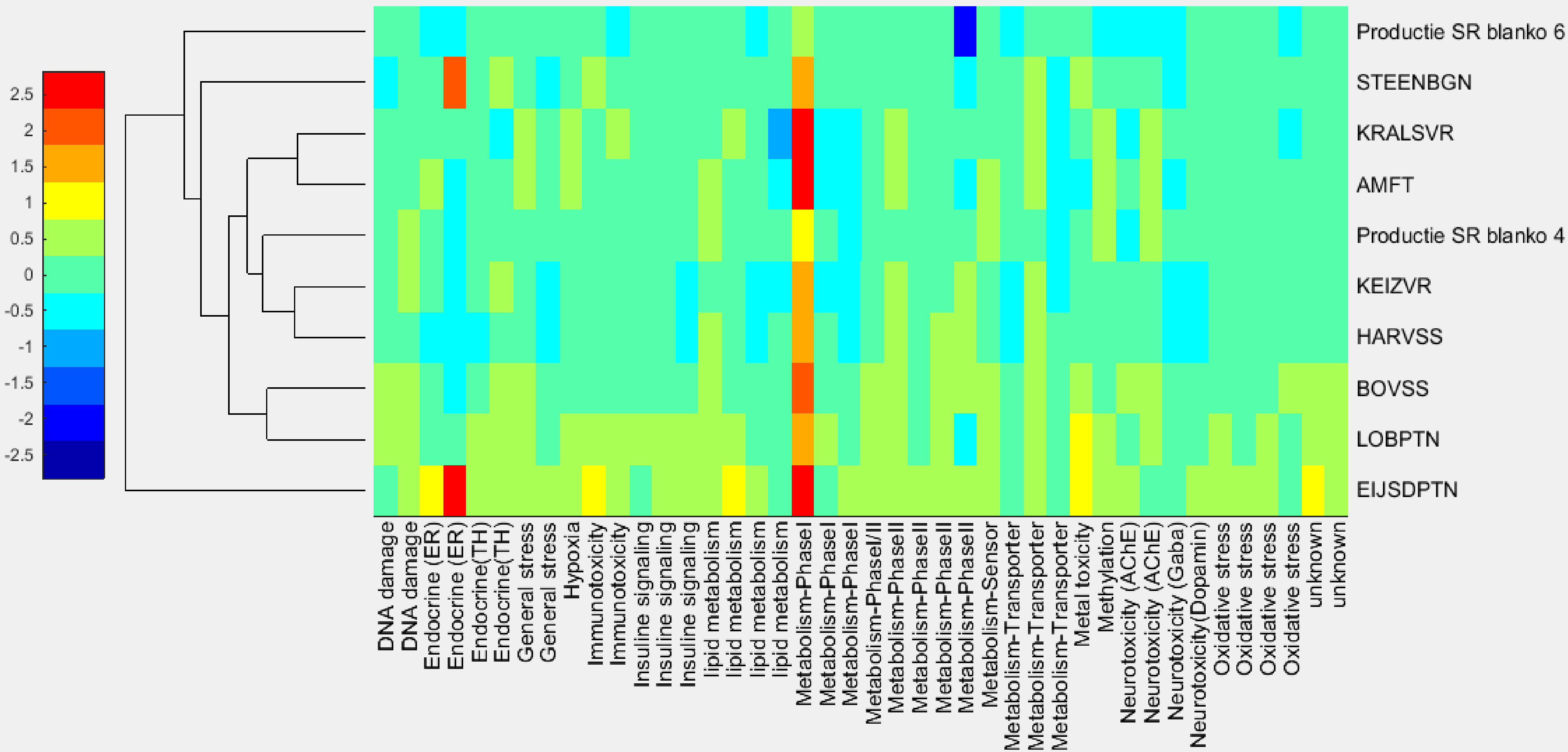
Results - Individual Compounds

TIPTOP



4-5 dpf exposure

Results - Environmental Samples (Mixtures)



4hpf -5 dpf exposure

Results - Environmental Samples (Mixtures)

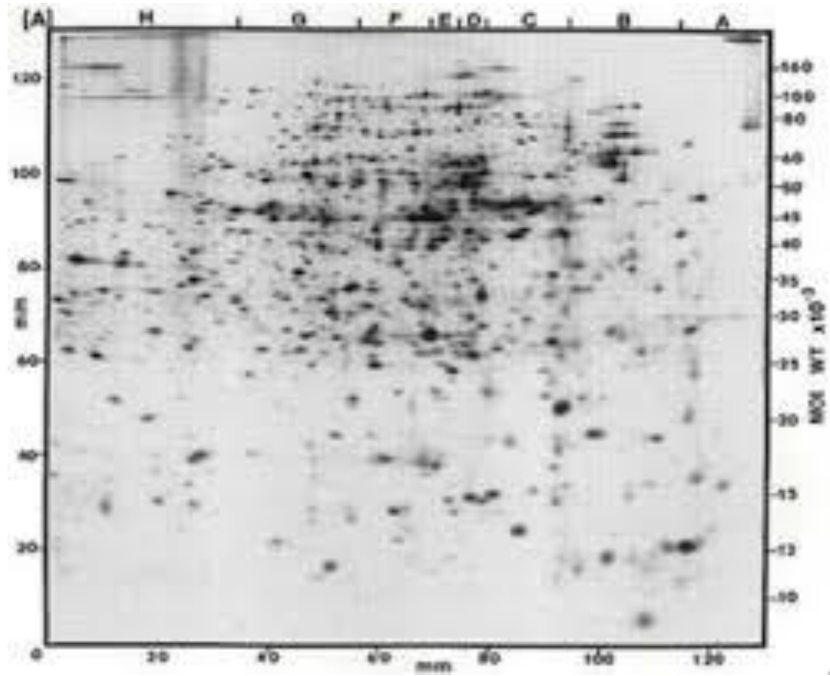
Location	DNA damage	Endocrine (ER)	Endocrine(TH)	General stress	Hypoxia	Immunotoxicity	Insuline signaling	lipid metabolism	Metabolism-PhaseI	Metabolism-PhaseII	Metabolism-Sensor	Metabolism-Transporter	Metal toxicity	Methylation	Neurotoxicity	Oxidative stress	Tox-score(0.01%)	QFET score 5 dpf (0.1%)	QFET score 5 dpf (0.01%)
Eijsden	1							1	1			1	1			1	6		
Keizersveer		1						1	1			1					4		
Bovensluis	1								1				1				3		
Steenbergen									1			1					2		
Haringvlietsluis		1					1		1								3		
Lobith									1			1	1		1		4		
WWTP Amersfoort									1	1		2					4		
WWTP Kralingseveer								1	2			2				1	6		
Productie SR blanko 4									2			1					3		
Productie SR blanko 6															1		1		

Conclusion ZF-Toxarray

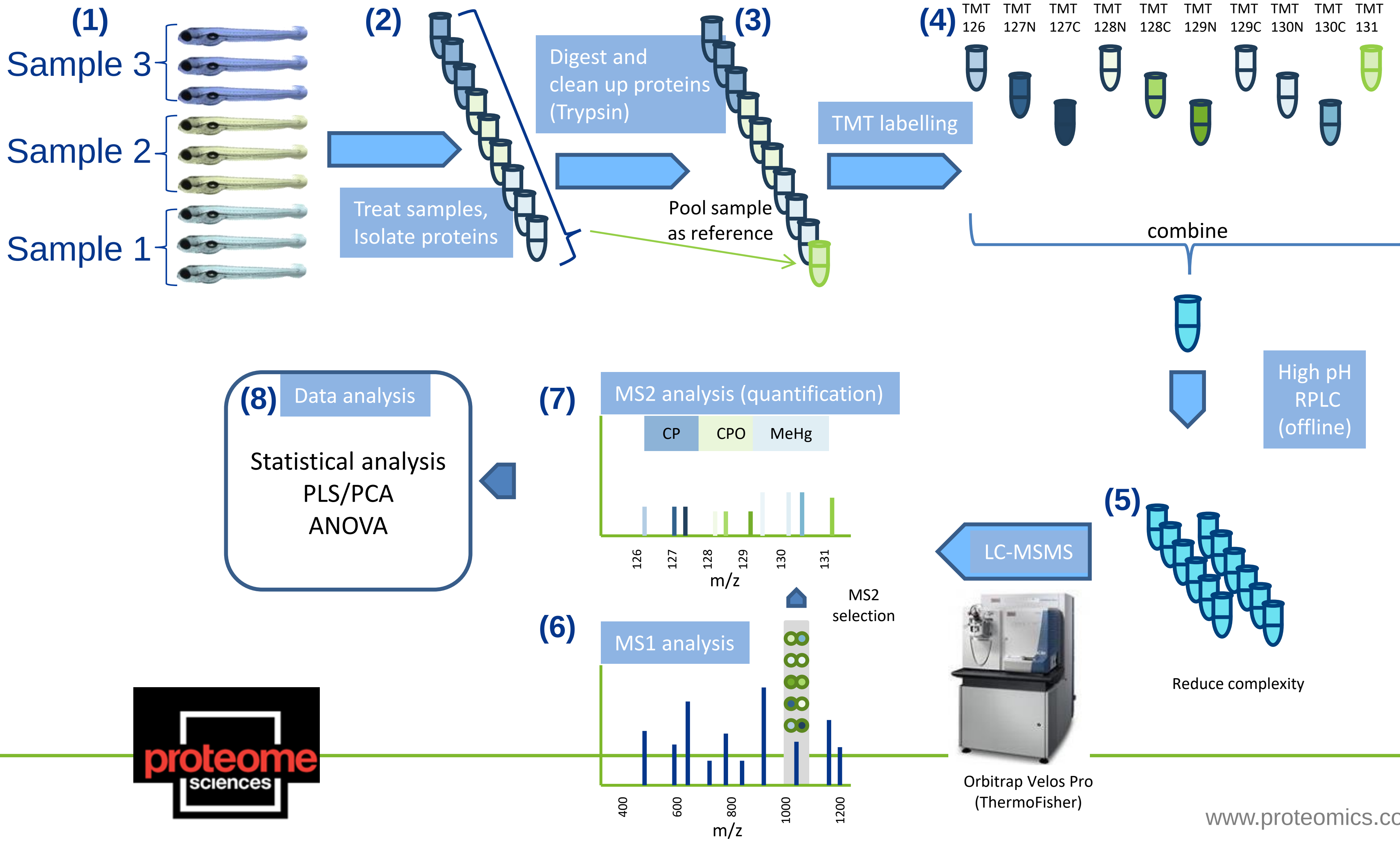
- Quick and cheap approach to do transcriptomics (10x cheaper as microarrays)
- It helps to compare and/or group
- Works for individual compounds as well as for mixture samples
- Can help to “identify” MoA
- Small amount of samples (10 embryos/larvae)

Methods to study (Neuro)toxicity

- Transcriptomics
- Proteomics
- Metabolomics
- Lipidomics

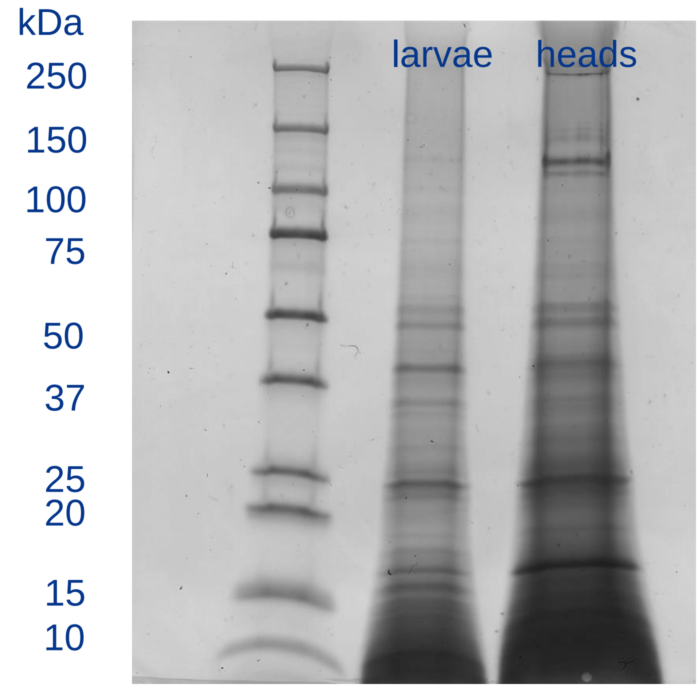


Proteomics -Tandem Mass Tags®



Proteomics -Tandem Mass Tags®

- Whole larva
  - High levels of vitellogenin
- Heads of larva
  - 676 proteins identified
  - 405 proteins characterized
  - accurate profiling of 8,291 peptides from a total of 2,396 protein groups

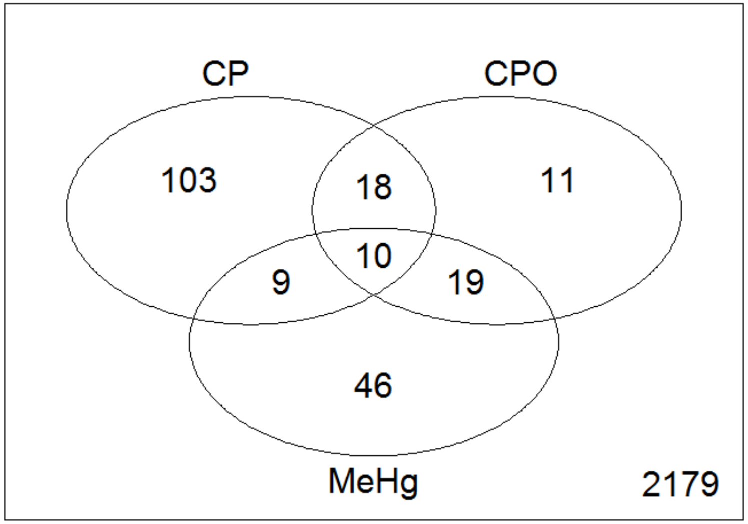


proteome sciences

Results: Comparison

Number of proteins differentially regulated than the control:

	total	down	up
MeHg 0.005 µM	84	20	64
Chlropyrifos Oxon 0.1 µM	58	7	51
Chlorpyrifos 11 µM	140	58	82



Minimum 2 peptides
BH P-value <0.3

Results - Pathways

Ion channel transport
Axon guidance
Activation of GABA B receptor
Activation of G protein gated Potassium channels
Activation of G protein gated Potassium channels (Apoptosis)
Activation of G protein gated Potassium channels

MeHg

Activation of GABA B receptor
Activation of G protein gated Potassium channels
Axon guidance
Cellular responses to stress
Cell Cycle
Cell-Cell communication
Vesicle-mediated transport
Transmembrane transport of small molecules

Chlorpyrifos

None

Chlorpyrifos Oxon

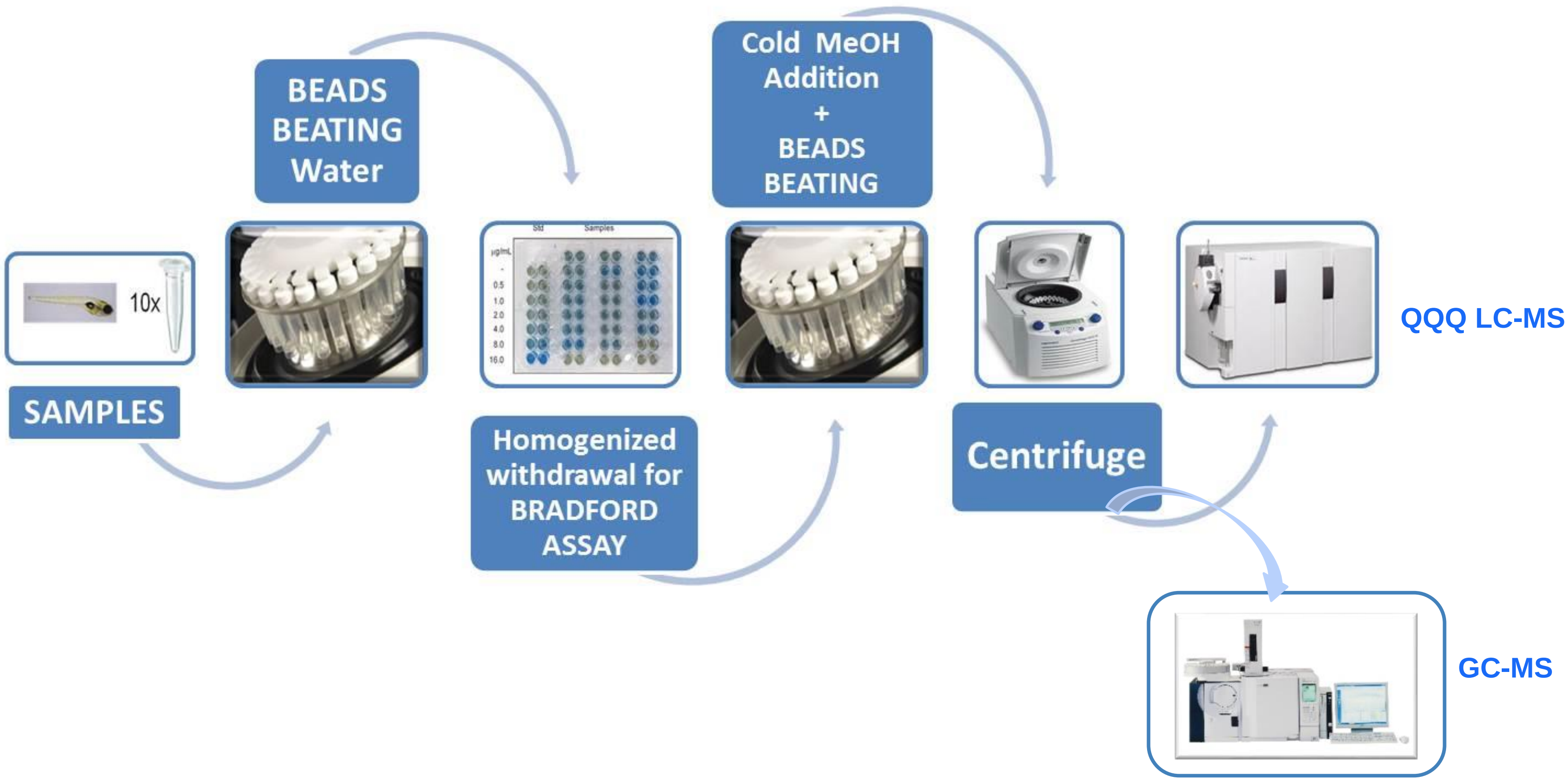
Conclusions Proteomics

- New insights into developmental neurotoxic effects on protein level
- High similarity of compounds being effective during early development vs. compounds needed to be bioactivated
- High number of proteomic changes at exposure levels where only behavioural alterations where observed
- Comparatively large amount of sample needed

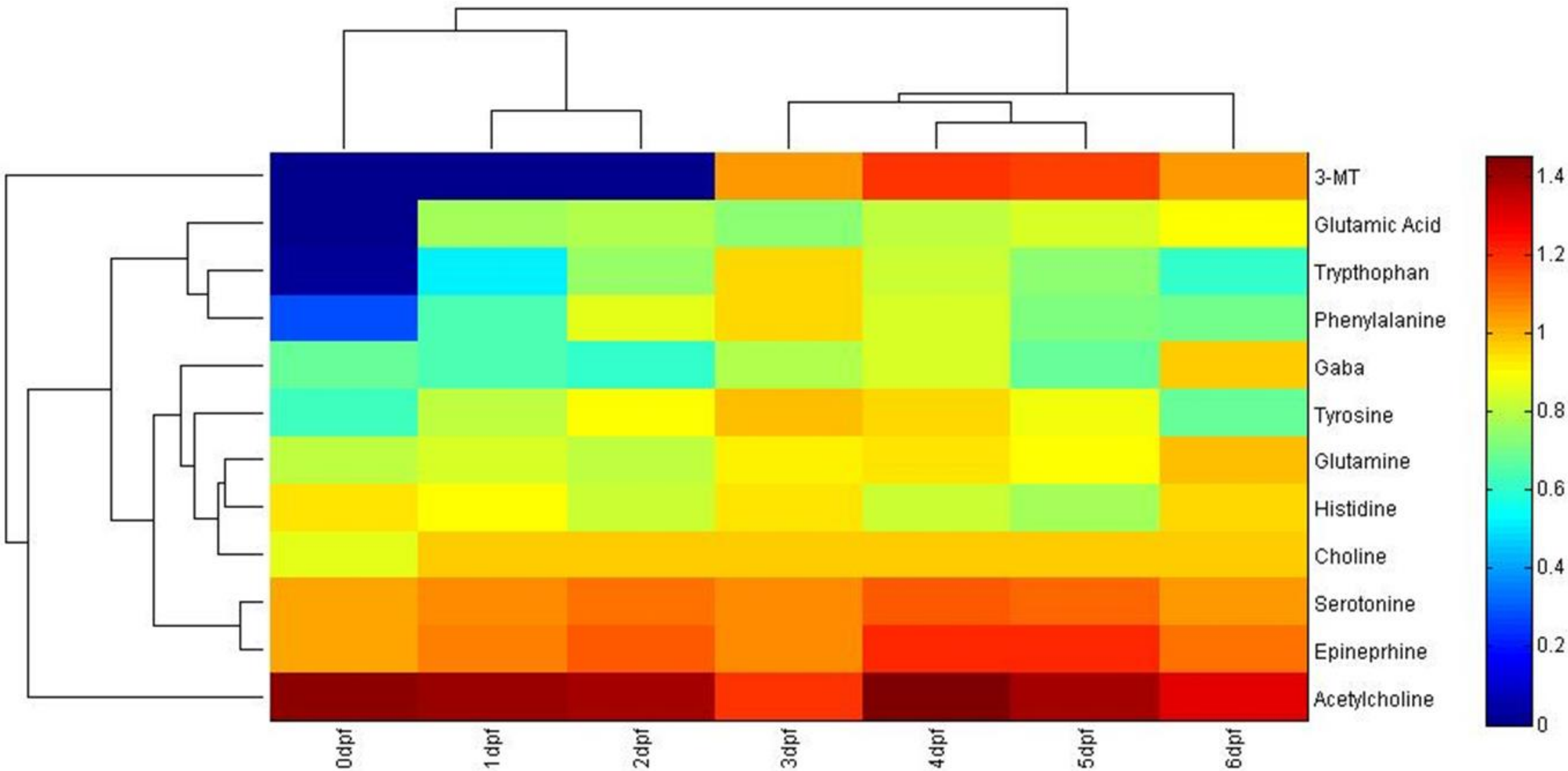
Methods to study Neurotoxicity

- Transcriptomics
- Proteomics
- Metabolomics
- Lipidomics

Metabolomics - Neurotransmitter

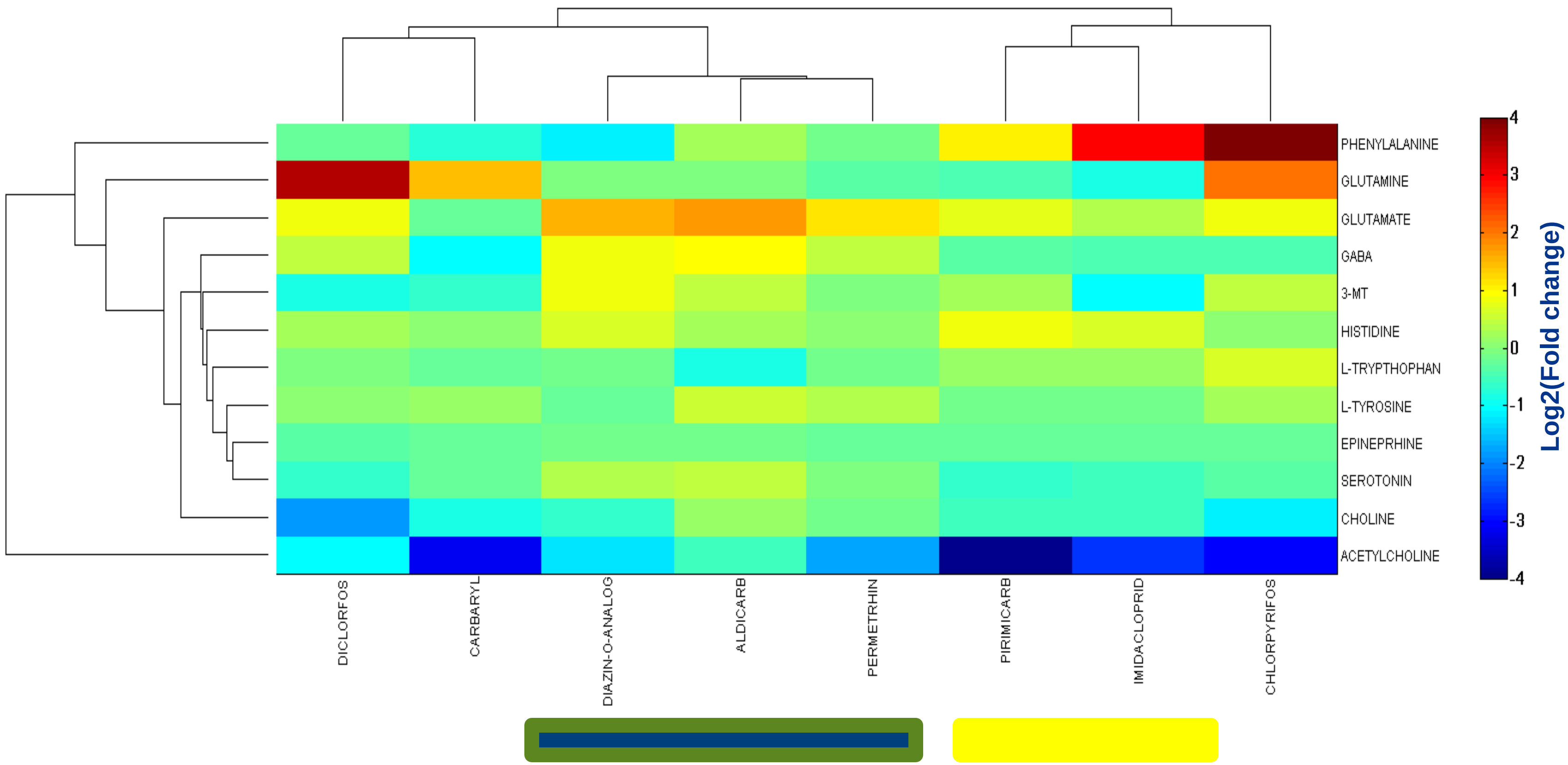


Results - Neurotransmitter fingerprint (QQQ-LC-MS)



Tufi et al 2016

Results - Neurotransmitter after exposure (QQQ-LC-MS)

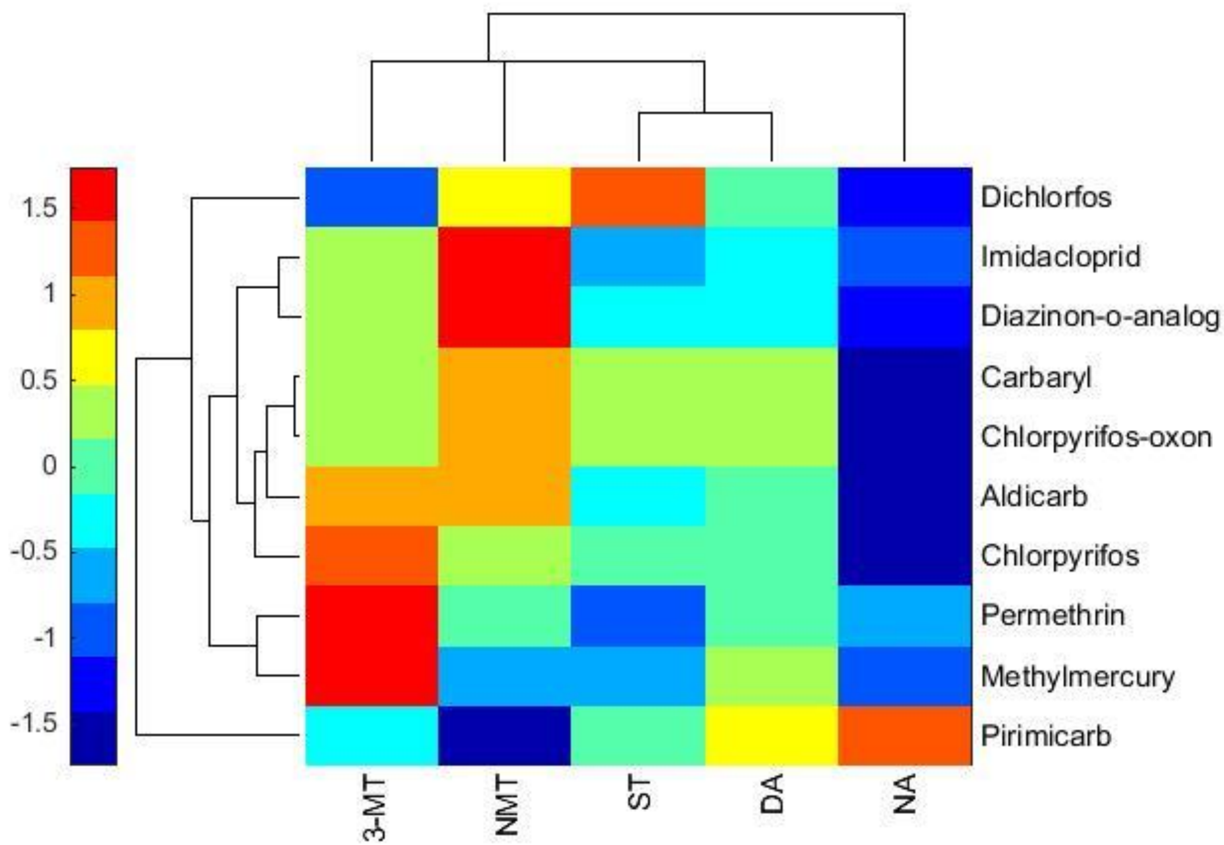


Behavioral changes only in dark period

Behavioral changes only in light period

Tufi et al 2016

Results - Monoamine Neurotransmitter (GC-MS)



dopamine (DA), norepinephrine (NA), epinephrine (EP), and serotonin (ST), metabolite 3-methoxy tyramine (3-MT), normetanephrine (NMT)

Aragon et al 2017

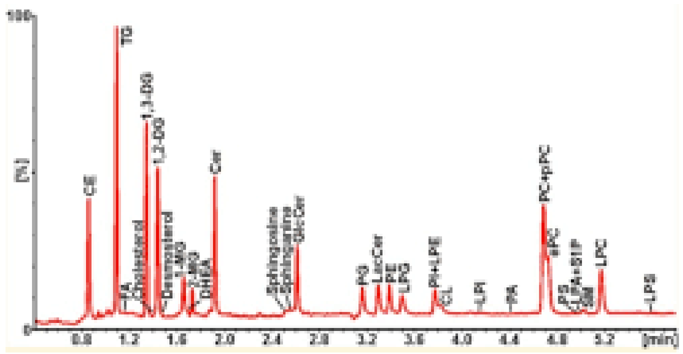
Conclusions Matabolomics

- New insights into developmental neurotoxic effects on neurotransmitter level
- We can measure precursor, neurotransmitter and metabolites of five neurotransmitter systems (dopaminergic−andrenergic, glutaminergic−GABAnergic, serotoninergic, histaminergic, and cholinergic systems), in parallel
- Changes at exposure levels where only behavioural alterations where observed
- Effects show a high similarity with the behavioural response
- Small amount of sample (10-20 embryos/larvae)

Methods to study (Neuro)toxicity

- Transcriptomics
- Proteomics
- Metabolomics
- Lipidomics

Lipidomics: HI LI C/ SFC

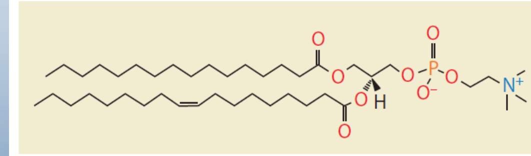


Miroslav Lisa and Michal Holčapek Anal Chem. 2015 Jun 20

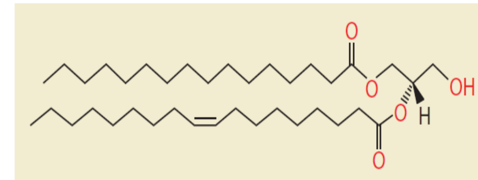
Lipids and brain function

- Brain contains large amounts and different types of lipids
- Thousands of lipids
- Neuronal function of lipids is to modify signal transduction, synaptic function, cellular signaling
- Distortion of lipid profiles

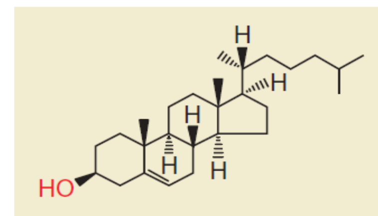
  -> disorders and diseases (Alzheimer disease)



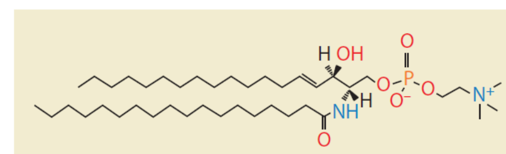
Glycerophospholipids
Glycerophosphocholines
Glycerophosphoethanolamines
Glycerophosphoserines
Glycerophosphoglycerols
Glycerophosphoglycerophosphates
Glycerophosphoinositols



Glycerolipids
Monoradylglycerols
Diradylglycerols
Triradylglycerols



Sterol lipids
Sterols



Sphingolipids
Sphingoid bases
Ceramides
Phosphosphingolipids
Neutral glycosphingolipids
Acidic glycosphingolipids
Basic glycosphingolipids

Lipidomics GC-MS

Butyrate
Hexanoate
Octanoate
Decanoate
Undecanoate
Laurate
Tridecanoate
Myristate
Myristoleic Acid
Pentadecanoate
Cis-10-Pentadecanoic Acid
Palmitate
Palmitoleate
Heptadecanoate
Cis-10-Heptadecanoic Acid
Stearate
Trans-9-Elaidic acid +...
Linolelaidic Acid +Lin...
Linoleate
Arachidate
Gamma-LInolenic Acid
Cis-11-Eicosanoate
Heneicosanoate

Cis-11,14-Eicosandieni...
Behenate
Cis-8,11,14-Eicosatrie...
Erucate
Cis-11,14,17-Eicosatri...
Tricosanoate
Cis-5,8,11,14-Eicosate...
Cis-13,16-Docosadienoi...
Lignocerate
Cis-5,8,11,14,17-Eicos...
Nevronate
Cis-4,7,10,13,16,19-Do...
Cholesterol

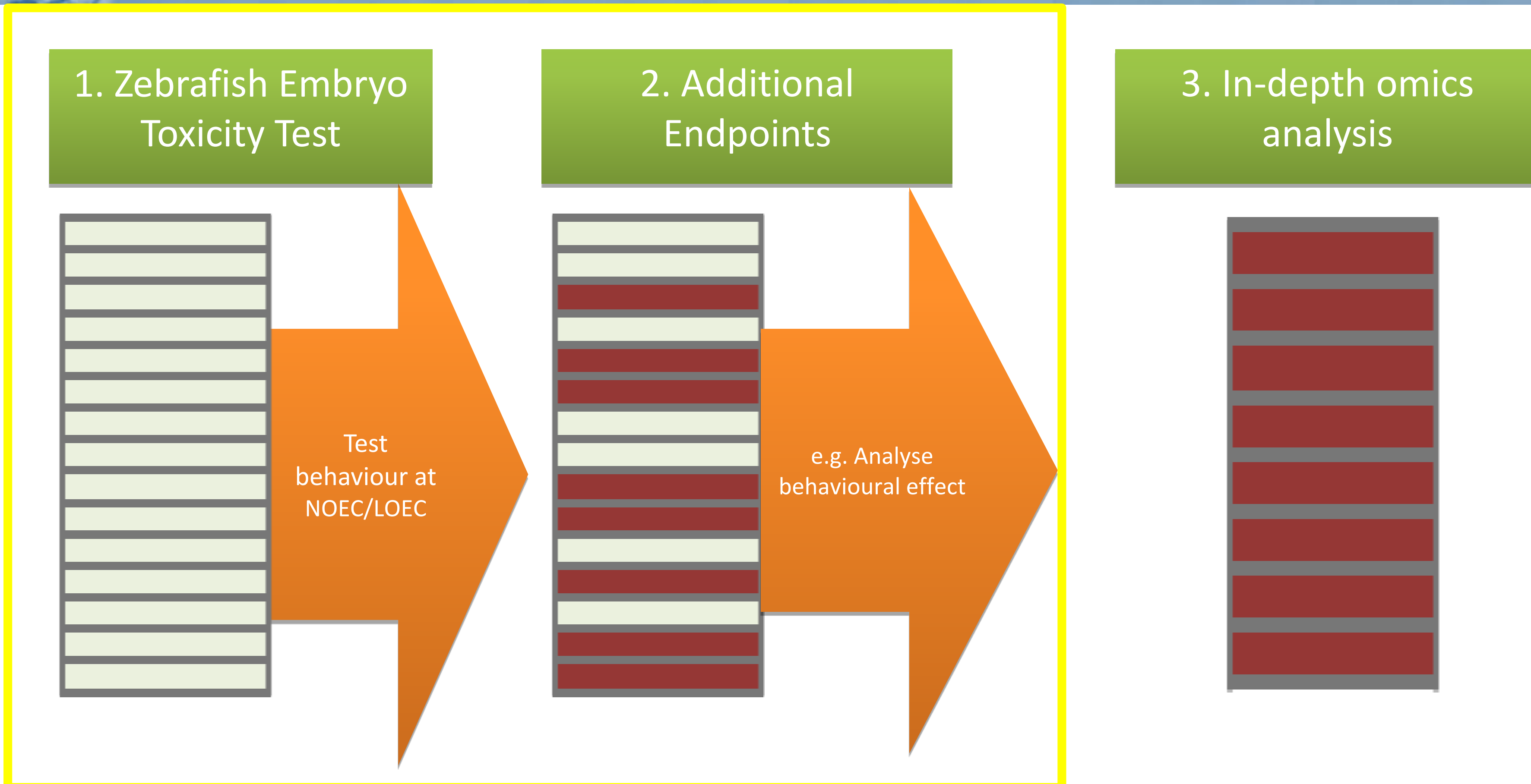
- Results - We could see changes in individual fatty acids of whole larvae after exposure

Conclusion

ZF-Omics:

- Gain new and deeper insights into
  - zebrafish development
  - toxic MoA -> could help EDA
- Easy to do (not so easy to analyse)

ZF-Smart screen



One Bioassay with a battery of endpoints

Acknowledgement

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