Identification and Structure elucidation of transformation products of Emerging Contaminants by Advanced Mass Spectrometry Based Techniques

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What Makes a “Contaminant of Emerging Concern”? 

- Over the last 15 years, the focus of chemical pollution has definitely shifted from conventional “priority” pollutants, to so-called “emerging” or “new” unregulated contaminants. Concerns during this period about the potential health and ecological impacts of exposure to emerging contaminants (ECs) have led to the establishment of new, multi-stakeholder research and testing initiatives, committees, expert groups, newsletters, databases, etc., throughout the world.

- Up to date, despite these actions, the term “emerging contaminants” remains problematic and sometimes it is difficult to determine which chemicals should or should not be classified as ECs, because they represent a changing reality, dependent on perspective as well as timing.
Emerging Contaminants
Emerging Contaminants ...  

... depend on one’s perspective
Emerging contaminants (ECs) include more than simply chemicals previously not known to occur in the environment.

ECs also include chemicals already known to occur but now displaying new characteristics not previously suspected or recognized, such as those involving:

- origin or source (e.g., via sweat and dermal transfer)
- location (e.g., “out-of-place” chemicals; “chemical weeds”)
- unusual concentrations or levels (e.g., enriched by sorption to plastics in oceans)
- transformation and fate pathways
- exposure routes
- biological effects pathways or endpoints
What Makes a “Contaminant of Emerging Concern”? 

In general, ECs are a structurally diverse and heterogeneous group of chemical compounds, which have widely varying fate properties and adverse effects on environmental ecosystems and can be classified into the following categories:

- **“new” ECs**, which are chemicals that are recently manufactured and suddenly appear everywhere, and therefore, are not currently covered by existing regulations or legislation.

- **“old” ECs**, which are the ones that were actually around for several decades, but simply were not under regular investigation or for which analytical methods did not exist until recently.

- **“ECs within complex mixtures”**, such as industrial effluents, oil residues, hospital effluent, etc. of which either the mixture itself or newly identified (subgroups) of components within may be considered ECs.
Fundamental Research Questions

Are ECs entering our environment?

- What are the sources (signatures)?
- What happens to them in the environment?
- Do they have adverse ecological health effects?
- Do unintended exposures pose a human health risk?
- How can we minimize their entry to the environment or remove them?
Emerging pollutants

Emerging pollutants

<table>
<thead>
<tr>
<th>Antibiotics</th>
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</thead>
<tbody>
<tr>
<td>1. Metronidazole</td>
</tr>
<tr>
<td>2. Sulfamethoxazole</td>
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<tr>
<td>3. Trimethoprim</td>
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<tr>
<td>4. Ciprofloxacin</td>
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<td>5. Cefotaxime</td>
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<td>6. Ofloxacin</td>
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<td>7. Erythromycin</td>
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<td>8. Tetracycline</td>
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<td>10. Norfloxacin</td>
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<td>11. Clarithromycin</td>
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<td>12. Lincomycin</td>
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<td>13. Sulfamethazine</td>
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<td>14. Sulfapyridine</td>
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<td>15. Sulfadiazine</td>
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<td>16. Sulfathiazole</td>
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<td>17. Azithromycin</td>
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<td>18. Mevastatin</td>
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<td>19. Simvastatin</td>
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<table>
<thead>
<tr>
<th>Analgesic/Anti-Inflammatory</th>
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<tbody>
<tr>
<td>20. Acetaminophen</td>
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<tr>
<td>21. Indomethacine</td>
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<tr>
<td>22. Fenoprofen</td>
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<tr>
<td>23. Codeine</td>
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<tr>
<td>24. Mefenamic A.</td>
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<tr>
<td>25. Ibuprofen</td>
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<tr>
<td>26. Ketorolac</td>
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<td>27. Naproxen</td>
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<tr>
<td>28. Diclofenac</td>
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<td>29. Ketoprofen</td>
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<td>30. Salicylic acid</td>
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<td>31. Propyphenazone</td>
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<td>32. Urbason</td>
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<th>Beta Blockers</th>
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<td>35. Atenolol</td>
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<td>36. Propranolol</td>
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<td>37. Sotalol</td>
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<td>38. Metoprolol</td>
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<td>39. Nadolol</td>
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<th>Antidepressants</th>
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<tr>
<td>47. Fluoxetine</td>
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<td>48. Paroxetine</td>
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<td>49. Venlafaxine</td>
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<td>50. Citalopram</td>
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<td>51. Amitriptyline</td>
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<td>52. Clomipramine</td>
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<th>Antiepileptic</th>
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<td>59. Carbamazepine</td>
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<td>60. Diazepam</td>
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<td>61. Primidone</td>
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<th>Antineoplastics</th>
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<tr>
<td>62. Ifosfamide</td>
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<tr>
<td>63. Cyclophosphamide</td>
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<td>64. Tamoxifen</td>
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<th>Antineoplastics</th>
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<td>65. Mepivacaine</td>
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<thead>
<tr>
<th>Anesthetics</th>
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<td>66. Methylprednisolone</td>
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<thead>
<tr>
<th>Anti-Infective</th>
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<td>67. Clotrimazole</td>
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<th>Metabolites</th>
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<tr>
<td>68. 4-Acetamidophenol</td>
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<td>69. 4-Formamidophenol</td>
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<tr>
<td>70. 4-Methylamidophenol</td>
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<tr>
<td>71. 4-Dimethylamidophenol</td>
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<tr>
<td>72. Aminophenol</td>
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<tr>
<td>73. Paraxanthine</td>
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<tr>
<td>74. Carbamazepine</td>
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<tr>
<td>75. Antipyrine</td>
</tr>
<tr>
<td>76. Fenofibrate</td>
</tr>
<tr>
<td>77. Clofibrate</td>
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<tr>
<td>78. Caffeine</td>
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<tr>
<th>Pesticides</th>
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<tr>
<td>79. Atrazine</td>
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<tr>
<td>80. Clompirphos</td>
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<tr>
<td>81. Chlorfenvinphos</td>
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<tr>
<td>82. Diuron</td>
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<tr>
<td>83. Isoproturon</td>
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<tr>
<td>84. Simazine</td>
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<td>85. Permethrin</td>
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<tr>
<th>Disinfectants</th>
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<tbody>
<tr>
<td>87. Bipheryl</td>
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<tr>
<td>88. Chlorophenol</td>
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<table>
<thead>
<tr>
<th>Others</th>
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<tbody>
<tr>
<td>89. Nicotine</td>
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<tr>
<td>90. Caffeine</td>
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<thead>
<tr>
<th>EDCs</th>
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<tbody>
<tr>
<td>86. Bisphenol-A</td>
</tr>
</tbody>
</table>
Why study these contaminants in the Environment?

- Massive and continuous use (daily) – Pseudo-persistence
- Lack of sensitive analytical methods for most compounds & scarce knowledge on their presence in the environment
- Some (priority) compounds are regulated in environmental samples

Why study these contaminants in the Environment?

- Used in high quantities
- Heterogeneous group
- Continuous discharge
- Polar compounds (generally small)
- Biologically active substances
- Complex mixtures - potential toxic effects

Lack of data to perform a reliable Environmental Risk Assessment
Transformation Products of ECs?

- Despite the increasing number of published studies covering EC input, occurrence, fate and effects, there is still a lack of understanding and knowledge about these substances in the aquatic environment.

- Even more, we know almost nothing about the impacts of the environmental exposure to trace concentrations of their *transformation products (TPs)* and/or *metabolites*, but the detection of TPs in the environment is worrying.

- TPs of ECs in aquatic environments are still rarely considered in water quality and chemical risk assessment, although they have been found in concentrations that are of concern.

- Since many different TPs can potentially be formed in the environment and analytical standards are typically lacking for these compounds, knowledge on the prevalence of TPs in aquatic environments is fragmentary.
Transformation Products (TPs)

New emerging contaminants in the water cycle
Classification of Transformation Products of ECs?

**Biotic Processes**
- Biotic TPs
  - Human metabolites
  - Animal metabolites
  - Microbial metabolites

**Abiotic Processes**
- Abiotic TPs
  - Hydrolysis
  - Photolysis
  - Chlorination
  - Ozonation
  - Advanced Oxidation

in engineered & natural systems

natural environment & water-treatment processes
Transformation Products (TPs)

![Diagram showing the transformation of saluamine to furosemide with retention times and relative intensity over incubation time.](image)
Proposed Transformation pathways

**Bezafibrate**

**Carbamazepine**

**Diclofenac**

**Furosemide**

**Hydrochlorothiazide**
Proposed Transformation pathways

Ibuprofen

Metoprolol

Propranolol
Identification and Structure elucidation of TPs

Lab experiments:
Hydrolysis, Photolysis,
Photocatalysis, Biodegradation

“Exact masses”
Chemical composition

Identification of TPs in WWTs, underground water, natural water, drinking water

MS^n – Fragmentation HR-MS, Q-MS
Simulation of the transformation processes in batch experiments under well-defined conditions with appropriate controls is a very common first approach for the identification of TPs.

Batch experiments can be applied under biotic and abiotic conditions at high concentrations of the parent ECs.
Flow chart in environmental analysis

ANALYSIS

Target compounds
LC-QqQ-MS/MS and LC-LiT-MS/MS
GC-MS(MS)
MS/MS mode (Low mass acc.)

Non target compounds
LC-TOF-MS
GC-Q-MS
Full scan mode (High mass acc.)
Identification approaches – analytical techniques

Nowadays, liquid chromatography (LC) coupled to MS (LC-MS) using a variety of mass analyzers is the technique of choice for the investigation of ECs and TPs in environmental samples. LC is a suitable chromatographic technique for polar, thermolabile compounds, thus for the identification of TPs, which are generally more polar than their parent molecules.
Flow chart of screening procedure of transformation products (TPs)

There are various workflows in the literature for the identification of TPs, depending indispensably on the instrumentation and the available software.


There are various workflows in the literature for the identification of TPs, depending indispensably on the instrumentation and the available software:

(a) target analysis, which is based on the determination of already known TPs, and identification is carried out with standard solutions;

(b) suspect screening, with a list of possible TPs assembled from the literature or from prediction models, and the samples are screened for those candidates; and,

(c) non-target screening, with identification of novel TPs being carried out with sophisticated post-acquisition data tools and supplementary analytical techniques.
Target or Non-target analysis workflow

SAMPLE

Target Analysis

- Target screening
- Quantitation

Non-Target Analysis

- Profiling
- Finger printing
- Authenticity
- Quantitation

Suspect screening Analysis
Identification of TPs

Target analysis (large list of target compounds)
- Standards available
- Standards not available

Non Target analysis
- Genuine non target
- Unknowns TPs (common fragments)
- Lab experiments (degradation/metabolism)

TPs/metabolites
- Reported
- Non Reported
Identification of TPs

Standards available

Information in database
(exact mass, fragment ions, retention time, isotope pattern)

Target analysis
(large list of target compounds)

Information obtained
• Mass accuracy (mass error)
• Isotope pattern (i-fit)
• Retention time
• Fragment ions, at HE acquisition

Analytes selection after MS acquisition

Long list of target compounds

Unequivocal identification at least two accurate-mass ions
Identification of TPs

Target analysis (large list of target compounds)

Standards not available

Information in database
- exact mass, theoretical isotope pattern, predicted Rt

Laborious and time-consuming task

Information obtained
- Mass accuracy (mass error)
- Isotope pattern (i-fit)
- Fragment ions observed
- Justification fragments (mass fragmenter)

Confirmation with standards (later stage)

Tentative identification (highly reliable)
Identification of TPs

- Genuine non target analysis
  - No selection of analytes
  - Searching for any sample component that might be “relevant”
  - Analytical challenge (little success in the environmental field)
    - Complex unknown sample matrices
    - Low analyte concentrations
    - Many peaks in the TIC (commonly no abundant peaks for environmental pollutants)
    - Selection of “relevant“ components to be investigated
    - From the information obtained (accurate-mass full-spectrum)
      - Assignment of the empirical formula
      - Searching in chemical data bases (Reaxys, ChemSpider)
      - Assignment of the chemical structure
Mass analyzers commonly employed

- Quadrupole-linear ion trap (Q-LIT)
- Linear ion trap-Orbitrap or quadrupole-Orbitrap
- Time-of-flight (TOF)
- Triple quadrupole (QQQ)
- Quadrupole time-of-flight (Q-TOF)
- Ion Trap
Which LCMS Analyzer Do I Choose?

Pure Quantitative

Detect & Quantify

Targeted screening

1 – >40 cpds

Targeted & Unknowns screening

40 – 600 cpds

Multiple cpds

Pure Qualitative

Determine structure

- Structural ID
- Compound Confirmation
- Reaction Monitoring
- Process Monitoring
- Metabolism
- Proteomics
- Metabolomics

LTQ ORBITRAP

QTRAPs

Triple Quads

Ion Traps

Exactive & Q-Exactive (Orbitrap)

Q-TOF
Flow chart of screening procedure of transformation products (TPs).

‘Known’ TPs have been confirmed or confidently identified before

Other TPs are considered ‘Unknown’
TARGET ANALYSIS
Targeted analysis using LC-MS/MS

Having two analysers increases the selectivity that ensures interfering peaks from other analytes or matrix are rarely observed

- Less isobaric interferences
- Lower limits of detection become achievable
- Direct injection of aqueous samples
- Provides a greater degree of confidence for identification
- Most common variant is the triple quadrupole
Targeted analysis using LC-MS/MS

MS/MS selected reaction monitoring (SRM)

Identification through comparing ion ratios with those from standards
# Target analysis

## Antibiotics
- Metronidazole
- Sulfamethoxazole
- Trimethoprim
- Ciprofloxacin
- Cefotaxime
- Cefoxitin
- Erythromycin
- Tetracycline
- Norfloxacin
- Clarithromycin
- Lincomycin
- Sulfamethazine
- Sulfapyridine
- Sulfadiazine
- Sulfathiazole
- Azithromycin
- Mevastatin
- Simvastatin

## Analgesic/Anti-Inflammatory
- Acetaminophen
- Indomethacin
- Fenoprofen
- Codeine
- Mefenamic Acid
- Ibuprofen
- Ketorolac
- Naproxen
- Diclofenac
- Ketoprofen
- Salicylic acid
- Propyphenazonate
- Urbonson

## Contrast media
- Iopromide
- Iopamidol

## Beta Blockers
- Atenolol
- Propranolol
- Sotalol
- Metoprolol
- Nadolol

## Antihistamines
- Famotidine
- Lansoprazole
- Ranitidine
- Omeprazole
- Loratadine

## Lipid regulators
- Fenoibrate
- Bezaflorone
- Gemfibrozil
- Pravastatin

## Diuretics
- Furosemide
- Hydrochlorothiazide

## Antidepressants
- Fluoxetine
- Paroxetine
- Venlafaxine
- Citalopram
- Amitriptyline
- Clomipramine

## Antiepileptic
- Carbamazepine
- Diazepam
- Primidone

## Antineoplastics
- Ifosfamide
- Cyclophosphamide
- Tamoxifen

## Anesthetics
- Mepivacaine

## Antifungal
- Methylprednisolone

## Anti-Infective
- Clotrimazole

## Metabolites
- 4-Acetamidobenzoic acid
- 4-Formylbenzoic acid
- 4-Methylaminoantipyrine
- 4-Dimethylaminoantipyrine
- Aminoantipyrine
- Paraxanthine
- Carbamazepine
- Antipyrine
- Fenofibric Acid
- Clofibrate acid
- Cotinine

## Pesticides
- Atrazine
- Chlorpyrifos
- Chlorfenvinphos
- Diuron
- Isoproturon
- Simazine
- Permethrin

## Disinfectants
- Bipheryl
- Chlorophene

## Others
- Nicotine
- Caffeine

## EDCs
- Bisphenol-A
Target analysis

Polar Target Compounds
# Target analysis

## Identification criteria for the SRM method by LC-QqQ-MS/MS

<table>
<thead>
<tr>
<th>Compound</th>
<th>(t_R) (min) (%RSD)</th>
<th>Product Ion (m/z)</th>
<th>Product Ion (m/z)</th>
<th>([\text{SRM2}]/[\text{SRM1}]) (%RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furosemide</td>
<td>3.7 (1.5)</td>
<td>205, [M – H – CO(_2)SO(_2)NH(_2)]^+</td>
<td>285, [M – H – CO(_2)]^+</td>
<td>0.9 (5)</td>
</tr>
<tr>
<td>Clofibric Acid</td>
<td>3.8 (0.7)</td>
<td>127, [M – H – C(_4)H(_4)ClO]^+</td>
<td>205, [M – H – CO(_2)]^+</td>
<td>0.2 (8)</td>
</tr>
<tr>
<td>Bezafibrate</td>
<td>4.1 (0.9)</td>
<td>205, [M – H – CO(_2)]^+</td>
<td>205, [M – H – CO(_2)]^+</td>
<td>0.7 (3)</td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td></td>
<td>205, [M – H – CO(_2)]^+</td>
<td>205, [M – H – CO(_2)]^+</td>
<td>0.9 (3)</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td></td>
<td>205, [M – H – CO(_2)]^+</td>
<td>205, [M – H – CO(_2)]^+</td>
<td>0.9 (3)</td>
</tr>
<tr>
<td>Naproxen</td>
<td></td>
<td>205, [M – H – CO(_2)]^+</td>
<td>205, [M – H – CO(_2)]^+</td>
<td>0.9 (3)</td>
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<tr>
<td>Diclofen</td>
<td></td>
<td>205, [M – H – CO(_2)]^+</td>
<td>205, [M – H – CO(_2)]^+</td>
<td>0.9 (3)</td>
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<tr>
<td>Fenoprofen</td>
<td></td>
<td>205, [M – H – CO(_2)]^+</td>
<td>205, [M – H – CO(_2)]^+</td>
<td>0.9 (3)</td>
</tr>
<tr>
<td>Indomethacin</td>
<td></td>
<td>205, [M – H – CO(_2)]^+</td>
<td>205, [M – H – CO(_2)]^+</td>
<td>0.9 (3)</td>
</tr>
<tr>
<td>Mefenamic acid</td>
<td></td>
<td>205, [M – H – CO(_2)]^+</td>
<td>205, [M – H – CO(_2)]^+</td>
<td>0.9 (3)</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td></td>
<td>205, [M – H – CO(_2)]^+</td>
<td>205, [M – H – CO(_2)]^+</td>
<td>0.9 (3)</td>
</tr>
<tr>
<td>Diuron</td>
<td>12.2 (0.2)</td>
<td>121, [M – H – CO(_2)C(_6)H(_12)]^+</td>
<td>127, [M – H – C(_6)H(_2)OH]^+</td>
<td>0.2 (8)</td>
</tr>
<tr>
<td>Chlorophene</td>
<td>12.3 (0.4)</td>
<td>121, [M – H – CO(_2)C(_6)H(_12)]^+</td>
<td>127, [M – H – C(_6)H(_2)OH]^+</td>
<td>0.1 (8)</td>
</tr>
</tbody>
</table>

### Identification Criteria:

- \(t_R\)
- 2 SRM transitions per compound
- \(\text{SRM2}/\text{SRM1}\) ratio
Limitations of targeted approach

- Need reference standards
- Need to program methods with RTs of analytes and specific transitions to monitor
- The targeted approach will fail to detect other contaminants present in the sample
- Unable to go back and “mine” the data later
Introducing a new screening solution for targeted and non-targeted analysis using HR-MS
Advantages of HR-MS screening?

- Over recent years use of high resolution mass spectrometry has gained in popularity as a screening tool in the environmental sector

  - Ability to perform non-targeted analysis
  - Ability to perform historical (retrospective) data review
  - Ability to perform full spectral analysis
  - Ability to screen for larger number of compounds and adducts
  - Increased specificity in complex matrices
  - Elucidation of unknowns?
Why high resolution system?

- **Definition of resolution**
  - Resolution is the capacity to differentiate 2 masses
  - $R = \frac{m}{\Delta m}$
    - $m$: mass of the first peak
    - $\Delta m$: difference of mass between two consecutive peaks

- **LC-MS low resolution is not a good tool for screening**:
  - No or few spectral databases in LC-MS
  - Mass spectrum too simple to be specific
  - Not able to dissociate 2 molecules with the same unit mass
Why high resolution system?

- Example
  - No dissociation of the 2 compounds

Need to work with high resolution systems
Exact Mass and Isobaric Compounds

<table>
<thead>
<tr>
<th>Element</th>
<th>Exact Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>1.007825</td>
</tr>
<tr>
<td>C</td>
<td>12.000000</td>
</tr>
<tr>
<td>N</td>
<td>14.003074</td>
</tr>
<tr>
<td>O</td>
<td>15.994915</td>
</tr>
</tbody>
</table>

Is a simultaneous measurement possible?

Yes, at high resolution!
Resolution (FWHM)

Thiamethoxam

\([M+H]^+ = 292.02656\]

\(\Delta m = 0.0138 \text{ amu}\)

Parathion

\([M+H]^+ = 292.04031\)

\(
\Delta m \text{ (Parathion and Thiamethoxam) is } 0.0138 \text{ amu}
\)

\(R = \frac{m}{\Delta m}
\)

\(R = \frac{292}{0.0138}
\)

\(R = 21,160\)

Mix 1:1
Resolution (FWHM)

Thiamethoxam: 
\[ [\text{M+H}]^+ = 292.02656 \]

Parathion: 
\[ [\text{M+H}]^+ = 292.04031 \]

\[ \Delta m = 0.0138 \text{ amu} \]

\[ R = \frac{m}{\Delta m} \]
\[ R = \frac{292}{0.0138} \]
\[ R = 21,160 \]

Mix 1:3
Resolution (FWHM)

Thiamethoxam

\[ [M+H]^+ = 292.02656 \]

\[ \Delta m = 0.0138 \text{ amu} \]

Parathion

\[ [M+H]^+ = 292.04031 \]

\[ R = \frac{m}{\Delta m} \]
\[ R = \frac{292}{0.0138} \]
\[ R = 21,160 \]

Measured at
\[ R = 50,000 \]

Mix 1:1
NON TARGET SCREENING
Trends in non-targeted analysis

- Transfer of methods from specific methodologies to those providing data for comparison with databases
- An alternative so-called “non-targeted” approach
  - LC-HRMS
    - Database searching via mass measurements
  - LC-HRMS/MS
    - Also provides spectral library searching
- Non-targeted acquisition but initial data processing tends to be still targeted…
Non-targeted acquisition

- Use of “high resolution” instruments
  - Time of flight (ToF) or orbitrap mass analysers
  - Full spectral information
  - High mass resolving powers and mass resolution
    - Specifications vary significantly
  - Good mass accuracy
  - Good sensitivity through improved ion optics
  - Variable acquisition speeds
High Resolution instruments


Comparison of ToF with orbitrap

<table>
<thead>
<tr>
<th>Mass analyser</th>
<th>Resolving power (x10^3)</th>
<th>Mass accuracy (ppm)</th>
<th>Acquisition speed (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q</td>
<td>3-5</td>
<td>Low</td>
<td>2-10</td>
</tr>
<tr>
<td>IT</td>
<td>4-20</td>
<td>Low</td>
<td>2-10</td>
</tr>
<tr>
<td>ToF</td>
<td>10-60</td>
<td>1-5</td>
<td>10-100</td>
</tr>
<tr>
<td>Orbitrap</td>
<td>100-240</td>
<td>1-3</td>
<td>1-5</td>
</tr>
</tbody>
</table>

Q, ToF and orbitrap also include common hybrid configurations with Q or LIT as the first mass analyser providing MS/MS or MSn capabilities
Data processing for screening

- Peak detection by extracting those ions matched with entries in a database
  - Can be pseudo molecular ions and fragments
- Recognition is based upon measurement of:
  - Accurate mass
  - Isotope pattern
  - Retention time (if available)
  - A response threshold
- Results are reported as a “hit list” with or without creating chromatographic peaks
Non target screening

- To be effective data processing must be automated and quick
- Minimise false negatives whilst generating a manageable number of false detects
  - Apply tolerances on response threshold, retention time and isotopic fit and the presence of a second diagnostic ion
- It requires more computing power and data management/storage than that traditionally associated with LC-MS analyses using QqQ instruments
- **Non-targeted workflow**
  
  - Obtain a summary of the identified compounds that are present (and absent) and determine concentration.
  
  - Provide a list of all compounds that meet user criteria (retention time, accurate mass measurement of precursor and fragments, adducts found, isotope ratios, user-defined limits).
  
  - Provide a list of spurious results (e.g., RT & accurate mass measurement shifted, isotope ratios questionable..)

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**Summary / Overview of the Results**

- Present & Absent. Quantity.
- Compounds that need reviewing.
Looking for Unknown Components...?
-
...Use a Filter Approach
Suspect screening

- Suspect screening is the technique of choice for the identification of TPs, when the confirmation of the analytes with a reference standard is impossible, but molecular formula and structure of suspected molecules can be predicted.

- In suspect screening, an important step of the identification workflow is the prediction of possible TPs using computational (in silico) prediction tools.
Suspect screening - Computational (in silico) prediction tools

- Commercially available or freely accessible programs have been applied in the prediction step on environmental analysis

  - University of Minnesota - Pathway Prediction System (UM-PPS: http://eawag-bbd.ethz.ch/)
  - CATABOL (http://oasis-lmc.org/products/models/environmental-fate-and-ecotoxicity/catabol-301c.aspx);
  - PathPred (http://www.genome.jp/tools/pathpred/);

- (CATABOL and UM-PPS predict microbial metabolic reactions based on biotransformation rules)
- PathPred is a multi-step reaction prediction server for biodegradation pathways of xenobiotic compounds and biosynthesis pathways of secondary metabolites) and,
- Meteor was built based on mammalian biotransformation reactions of common functional groups and allows prediction of the most probable TPs, providing in parallel relevant literature references.

The prediction system should be properly selected by considering the organism or the system where TPs are formed.
To conclude

Despite great progress in the last years, the identification of suspects and non target TPs is still an analytical challenge since software and methods to predict fragmentation patterns, ionization behavior, and retention time are still under development.

Furthermore, the lack of comprehensive mass spectral libraries for high-accuracy MS/MS and the limited comparability between collision-induced dissociation (CID) and higher energy collision dissociation (HCD) spectra make the identification of unknown compounds more challenging.
To conclude

Commercial software such as Mass Frontier and Mass Fragmenter are available to predict mass spectral fragments using different fragmentation rules, but they need a lot of improvement.

Advancement of predictive models and computer tools is urgently required together with innovative analytical tools, spectral databases, multivariate tools, (pattern recognition) and biodiagnostic tools (omics)

Identification & Structure elucidation strategy employing HR-MS, complementary techniques and advanced software tools is promising
Transformation Products of Emerging Contaminants in the Environment: Analysis, Processes, Occurrence, Effects and Risks

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Thank you for your attention!!!