Analysis of Pesticide Degradation Pathways

Prof. dr. Biljana Škrbić
Faculty of Technology, University of Novi Sad, Novi Sad
Pesticides are widely used for the control of weeds, diseases, and pests of cultivated plants all over the world, mainly since after the Second World War.
At present, around **2.5 million tons** of pesticides are used annually and the number of registered active substances is higher than **500**.
However, as pesticides are toxic substances that may have undesirable effects, their use has to be regulated.
Risk assessment of pesticides requires information

• on the **toxicological** and **ecotoxicological** properties of these compounds as well as

• on their **levels** in food and environmental compartments
Therefore, **reliable analytical methods** are needed to carry out the monitoring of pesticide residues in those matrices.
Consumer concerns on food safety and society awareness of chemical contaminants in the environment have increased in the past few years.

As a consequence, more restrictions in the use of chemical products have been imposed at national and international levels.
DIURON is a systemic herbicide derived from urea, relatively persistent in soil, with half-lives from 90 to 180 days.

- It shows slight acute toxicity
- is a likely carcinogen
- is a suspected endocrine disruptor
**Diuron** has a very slow rate of natural hydrolysis in a neutral solution at 25°C. However, when hydrolysis occurs the abiotic degradation in water solution is an irreversible reaction giving **3,4-DCA** as the only product.

![Chemical structure of Diuron and 3,4-DCA](image)

The project is co-financed by the European Union through the Hungary-Serbia IPA Cross-border Co-operation Programme.
3,4-DCA (3,4-dichloroaniline) is considerably more toxic than diuron itself and has higher water solubility so it can leach out from treated agricultural land. It could condensate in chloroazobenzene which is highly toxic.
Biodegradation is a major way of diuron degradation in the environment.

Many studies were conducted and showed that degradation happens under both aerobic and anaerobic conditions, under the influence of Gram positive and negative bacteria as well as some fungi.
In a major part of the published studies the accumulation of 3,4-DCA has been reported.

There are some individual microbes capable of mineralizing diuron completely without accumulation of aromatic compounds.
Bacterial degradation pathways of diuron and 3,4-dichloroaniline
Fungal degradation pathways of diuron and 3,4-dichloroaniline
It must be taken into consideration that NOT all the mechanisms involved are well known.
Carbendazim is a **systemic fungicide** widely used to control a broad range of fungal diseases in agriculture and forestry.
Carbendazim

- has slight acute toxicity
- is a possible carcinogen
- is a suspected endocrine disruptor
• It is chemically stable and relatively persistent in the environment

• It can harm the liver and endocrine system and has mutagenic and teratogenic effects on animals, even at low concentrations.

• Carbendazim has a half-life (time taken for half the sample to decay) of 3-12 months.
Microbial metabolism is the main mechanism responsible for degradation of carbendazim.

It is usually hydrolyzed to

2-AB (2-aminobenzimidazole),
BZ (benzimidazole) and
2-HB (2-hydroxybenzimidazole).
The further degradation of 2-AB or BZ was not elucidated in earlier studies.

Some bacterium strains capable of complete degradation were reported in recent studies.
Proposed degradation pathway of carbendazim by a bacterium strain
Mancozeb is a fungicide from a subclass of carbamate pesticides called dithiocarbamates.
In the soil it negatively affects microbial decomposition activities, hence the nitrogen and carbon mineralization are affected.

Mancozeb is of low soil persistence with half lives from 2 to 8 days. It has moderate mobile potential in soils due to its high adsorption coefficient.

In water it can be quickly hydrolyzed.
The main hydrolysis degradates are ethylenethiourea (ETU), ethyleneurea (EU) and ethylenebisisothiocyanate sulfide (EBIS).
Ethylenethiourea has greater tendency to be mobile due to its high water solubility and weak adsorption in soil.

While mancozeb has a relatively low toxicity, ETU is

- a suspected human carcinogen
- is on EU Priority list of endocrine disruptors
• shows toxicity to aquatic organisms and

• when administered under various toxicological experimental conditions has been shown or suspected to have caused **tumorogenic** and **teratological** effects
ETU is only weakly adsorbed to soil particles and therefore its high soil mobility makes it a potential contaminant for groundwater.
ETU can be degraded by microorganisms to ethyleneurea (EU), which is further degraded to CO₂.
2,4-Dichlorophenoxyacetic acid, more commonly referred to as 2,4-D, is one of the most widely used herbicides throughout the world.
It effectively controls unwanted and invasive weeds across agricultural fields, lawns, public parks, lakes and more. Introduced in 1946, 2,4-D is among the most rigorously researched and regulated molecules of all time.
It is classified as a "moderately hazardous" pesticide when its acute toxicity is concerned.

Direct exposure can cause
• serious eye and skin irritation
• weakness,
• diarrhea,
• respiratory tract irritation...

It is considered possibly carcinogenic to humans and an endocrine disruptor.
2,4-D has been included on the EPA list of compounds that are likely to leach from soil.

Its half life is 1.5 to 16 days.

Soil microbes are primarily responsible for its disappearance in soil.
A number of 2,4-D-degrading bacteria have been isolated and characterized from a variety of environmental habitats. Incomplete degradation can result in creation of dichlorophenol which is more toxic than 2,4-D.
In order to be able to elucidate the degradation pathways and kinetics of pesticides in the soil by selected microorganisms, it is necessary to **identify all the products** of degradation present in the soil sample.
Since not all the products can be predicted, this analytical task presents a **screening of unknown contaminants**.

Highly **accurate mass** and **high resolution** data acquisition are necessary requirements for this type of analysis.
CEFSER laboratory for Mass Spectrometry is equipped with The Exactive-Thermo Fisher Scientific new Orbitrap™ mass spectrometer coupled with Accela U-HPLC chromatograph.

It is capable of providing high mass accuracy at resolutions of up to 100 000.
Benefits of very high resolution are:

- resolving co-eluting, isobaric target compounds
- elemental composition determination
Resolution of Isobaric Pesticides

The pre-requisite for the analysis of low concentration compounds in the presence of higher abundant ones is resolution higher than 40,000.
Pesticide Mixture in Horse Feed Matrix

- Peak at m/z 239.15181
  - Formula: C_{11}H_{19}O_{2}N_{4}
  - Relative Abundance: 6.50 ppm
  - Resolution (R): 15,000
  - Error: 6.50 ppm

- Peak at m/z 239.15033
  - Formula: C_{11}H_{19}O_{2}N_{4}
  - Relative Abundance: 0.32 ppm
  - Resolution (R): 80,000
  - Error: 0.32 ppm

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• Thus, the new benchtop Orbitrap mass spectrometer we possess in the CEFSER Lab demonstrates high resolving power (up to 100,000), providing precise mass accuracy for complex sample analysis, and …
excellent sensitivity, linearity and selectivity in multi-residue screening of complex matrices
while a triple quadrupole mass spectrometer TSQ Vantage™ (Thermo Scientific) is capable of highly sensitive **full-scan** as well as **Selected Reaction Monitoring (SRM)** scan functions.
The **MS/MS fragmentation pattern** is a powerful tool for obtaining confidence in the identification of the studied compounds.
With these two instruments, CEFSER lab is equipped to

• identify possible unknown metabolites of biodegradation of pesticides by employing the Exactive high resolution MS

• quantify targeted compounds by TSQ Vantage
Quantification of the original pesticide before and after bioaugmentation will reveal the degree of its degradation, and give information about the degradation kinetics.
By untargeted high resolution screening of the samples, degradation products present in the samples will be identified and the degradation pathways of studied pesticides will be elucidated.
GOOD NEIGHBOURS CREATING COMMON FUTURE

THANK YOU

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