BIOMARKERS AND TOXICITY MECHANISMS

01 - INTRODUCTION

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1) Introduction
- Intro and overview of the mechanisms beyond the toxicity 
  \textit{(with special respect to environmental contaminants)}
- Intro and concept of biomarkers

2) Details on selected important toxicity mechanisms
- Membrane toxicity, enzyme inhibitions, oxidative stress, genotoxicity, Nuclear Receptors (AhR, ER, AR ....) etc.
- Methods to determine toxicity mechanism

3) Biomarkers
- What it is and how to find (identify) suitable biomarker(s)?
- The overview of the most important biomarker classes
- Methods of biomarker assessment
The importance of understanding to toxicity mechanisms
**In vivo:** shell thinning

**In situ:** bioaccumulation

-> bird population decline

Biochemistry discovered in 1970s: **Bird** carbonate dehydratase

Thalidomide

- Originally marketed in 1957 as sedative / hypnotic
  - also curing anxiety, gastritis, tension
  - against nausea and morning sickness of pregnant
  - TERATOGENICITY → Development of phocomelia = limb malformations (10,000 children worldwide / 40% survived)

- Currently still in use - completely different targets: anticancer (multiple myeloma), antileprosis, immunosuppression
Thalidomide
... mechanisms of action

(1) Sedative effects
... mechanism unknown

(2) Teratogenicity

(3) Anticancer
Basics and keywords from toxicology
Figure 1 The effective concentration of a pollutant in an organism (e.g. fish, daphnia, algae) or at the target site inside the organism is the link between the environmental fate of a pollutant and its toxic effect.

From mechanisms (or modes of action) to biomarkers

- Chemical enters organism + may be metabolized/detoxified, transported, released ...

- Chemical reacts with target (e.g. DNA) and changes a specific nucleotide (e.g. G → de-oxo-G)

- Elevated de-oxo-G in blood

→ Toxicokinetics

→ Toxicodynamics
  = toxicity mechanisms (MoA) and following toxic effects (e.g. mutation, cancer ...)

→ (Selective) biochemical marker (biomarker)
  = information about exposure and/or effect
Toxicity – the cause-effect paradigm

Paracelsus (1493 - 1541)

‘What is there which is not a poison?

- All things are poison and nothing without poison.
- Solely the dose determines that a thing is not a poison.

- Toxicology – the science of doses
What processes are beyond toxicokinetics?

Toxicokinetics ...

... EXPOSURE phase → Determines the final dose
Toxicokinetics in fish

Fig. 3.5 Uptake, accumulation and loss processes for a toxicant in the ambient water with fish.
ToxicoDYNAMICS

TARGETS = macromolecules (DNA/RNA, proteins, membrane lipids)

Exposure Assessment → Risk Assessment

Toxicokinetics
- Exposure
- Internal Dose
- Biologically Effective Dose
- Early Biological Effects
- Altered Structure/Function
- Disease

Toxicodynamics

MoA
... and measurable EFFECTS

Exposure → Delivery to target site → Toxic action at target site → Physiological response → Increased mortality
What is toxicity? What are the types of effects?

- **Toxicity**
  - degree to which a substance (at certain dose) can damage an organism

- **Exposure & toxicity**
  - *acute* (immediate, high doses, days)
  - *chronic* (sublethal / low doses, long-term)

- **Effect & toxicity**
  - *lethal* (acute)
    - mortality – definitive endpoint / high doses
    - easy to determine (single endpoint – death)
  - *nonlethal, sublethal* (chronic)
    - endocrine disruption, reproduction toxicity, immunotoxicity, tumor induction etc.
    - difficult to determine (multiple endpoints)
    - more specific – low concentrations / longer exposures
    - often reflected by specific biochemical changes (*biomarkers*)

- **Systems and organ & toxicity**
  - Systemic lethal toxicity
  - Organ-specific toxicity (neurotoxicity, hepatotoxicity, nefrotoxicity ...)
  - Developmental toxicity
  - Reproduction toxicity
MECHANISMS of chronic toxicity

• Various chronic effects have uniform biochemical basis

  – principle studies with mechanistically based *in vitro* techniques
  – estimation of *in vitro* effects of individual compounds

Understanding MoA ... may predict higher-level effects
Concept of “Adverse Outcome Pathway” (AOP)

Mechanisms or modes of action → Effects

5 ng/L (!) 7 years
Principles of toxicity testing

1) Define and know **biological target** (molecule, cell, organism, population) and its properties

2) Define and know **chemical** and its properties

3) **Define exposure** of biological system to a chemical
   - variable concentrations
   - defined or variable duration (time)
   - conditions (T, pH, life stage ....)

4) **Assess effects**, i.e. Changes in measurable parameter in relationship to variable doses

5) **Dose-response evaluation** & estimation of the toxicity value (i.e. concentration or dose):
   LDx, ICx, ECx, LOEC/LOEL, MIC ...
Effect assessment - procedure

Cu addition

Effect concentrations expressed in total/dissolved Cu

Concentration:

- Control
- 13 μg/L
- 25 μg/L
- 50 μg/L
- 100 μg/L
- 200 μg/L

96-hour LC50 = 50 μg/L
How to study (chronic) toxicity?

- **In vitro studies (biochemical mechanisms)**
  - easy to perform, short-term
  - highly controlled conditions
  - lower amounts of chemicals needed (new cmpnds screening)
  - ecotoxicological relevancy
  - mostly with vertebrate cells

- **In vivo biotest testing**
  - unique whole organisms
  - controlled conditions
  - better ecological interpretation
  - only few (ecologically nonrelevant) organisms used
  - mostly ACUTE assays
  - chronic: long exposures

- **Field and in situ observations, epidemiological studies**
Keywords to remember and understand

• What is meant by the “mechanism of action” (or “mode of action”) in toxicology?
• Why is it necessary to understand MoAs? What is the AOP concept?
• What is toxicokinetics? What is ADME?
• What is toxicodynamics?
• What is the relationship between the exposure and the effect?
• What are the different types of toxicity?
• How can the (toxic) effect be measured / assessed?
• What types of “bioassays” are available to study toxicity and/or MoA?
• How is the result (i.e. „toxicity“) described in numbers?