# Preclinical Safety Testing of Compounds Derived from Plants Grown in Polluted Environments

## Evaluating the Toxicological Implications of Medicinal Plants from Contaminated Ecosystems

INNO-SAFE-LIFE

Co-funded by the European Union

ERASMUS KA220-HED - Cooperation partnerships in higher education
Project no. 2023-1-RO01-KA220-HED-000164767
Title: Partnership for innovation on the exchange of best practices and the design of joint collaborative initiatives at European level related to the awareness of the effects of contamination on human health
Acronym: INNO-SAFE-LIFE

# Introduction

- Medicinal plants are a cornerstone of traditional and modern pharmacology.
- However, the environment in which plants grow greatly influences their chemical composition.
- Plants cultivated in polluted areas may absorb and accumulate harmful substances.
- These contaminants can compromise the safety of plant-derived compounds used in drug development.
- Hence, thorough preclinical safety testing is essential before advancing to clinical trials.

# Why Study This?

- With rising global pollution, many medicinal plants are now grown in contaminated environments.
- Soil, water, and air pollutants may be taken up and metabolized by plants.
- This could lead to the presence of **toxins**, **mutagens**, or **carcinogens** in therapeutic extracts.
- Preclinical studies help ensure that potential drug candidates are not only effective but also safe for human use.
- This research is crucial to avoid adverse effects, especially in herbal medicine consumers who assume “natural = safe.”

# Objectives

- **Screen** plant-derived compounds from polluted environments for contaminants.
- **Evaluate** their safety through **in vitro** and **in vivo** toxicological studies.
- **Determine** if environmental pollution alters therapeutic or harmful properties.
- **Support** regulatory frameworks for approving herbal and natural products.

# Common Pollutants Affecting Plants

## Heavy Metals:

- Lead (Pb), Cadmium (Cd), Arsenic (As), Mercury (Hg)
- Common sources: Industrial waste, mining, traffic emissions

## Persistent Organic Pollutants (POPs):

- Pesticides (DDT), Polychlorinated biphenyls (PCBs), Dioxins
- Can bioaccumulate and persist in plant tissues

## Air Pollutants:

- Sulfur dioxide ($SO_2$), Nitrogen oxides (NOx), Ground-level ozone ($O_3$)
- Affect plant growth and metabolite synthesis

Impact: Disruption in secondary metabolism, phytotoxicity, reduced therapeutic quality

# Preclinical Safety Testing Workflow

**Phytochemical Profiling:**
Identify and quantify active and inactive constituents, look for anomalies due to pollution.

**In Vitro Testing:**
Assess cellular toxicity, mutagenicity, oxidative stress responses.

**In Vivo Animal Testing:**
Determine whole-body toxicity, organ-specific effects, behavior changes.

**Toxicokinetic Studies:**
Track absorption, distribution, metabolism, and excretion (ADME).

**Histopathological Analysis:**
Microscopic examination of tissues for damage or abnormalities.

# Phytochemical Screening

## Qualitative Tests:

- Detection of alkaloids, saponins, tannins, flavonoids, etc.

## Quantitative Tests:

- HPLC, GC-MS, LC-MS for detailed profiling

## Comparative Analysis:

- Compare samples from polluted vs. unpolluted areas

## Contaminant Detection:

- ICP-MS or AAS for heavy metals
- ELISA or chromatography for pesticide residues

# In Vitro Toxicity Tests

| | |
|---|---|
| **Cytotoxicity Assays:** | MTT, Trypan Blue, LDH release<br>Measure cell viability and membrane integrity |
| **Genotoxicity Assays:** | Ames test for mutagenicity<br>Comet assay for DNA strand breaks |
| **Oxidative Stress Markers:** | Reactive oxygen species (ROS) generation<br>Glutathione levels, SOD and catalase activity |

Benefits: Ethical, fast, scalable screening before animal testing

# In Vivo Toxicity Studies

## Acute Toxicity ($LD_{50}$):

- Single-dose study to identify lethal dose

## Sub-Chronic and Chronic Toxicity:

- Repeated exposure over weeks/months
- Observe long-term effects on organ function

## Endpoints Monitored:

- Weight gain/loss, food intake, behavior
- Biochemical markers (liver enzymes, kidney function)

## Organ Histopathology:

- Liver, kidney, brain, spleen – checked for lesions or degeneration

# Case Studies / Research Examples

**Example 1:** *Ocimum sanctum* (Holy Basil) grown near highways

- Elevated Pb and Cd levels found in leaves
- In vivo tests showed hepatotoxicity at doses considered safe for non-polluted samples

**Example 2:** Medicinal herbs from industrial zones

- Contained pesticide residues above WHO permissible limits
- Genotoxic effects observed in mouse models

**Example 3:** Traditional Chinese herbs grown near mining areas

- Bioaccumulation of arsenic led to renal and neurological toxicity in rodents

# Regulatory Considerations

**WHO Guidelines:** Good Agricultural and Collection Practices (GACP)

**OECD Guidelines:** Standard toxicity testing protocols

**ICH Guidelines:** Preclinical safety pharmacology (S4), genotoxicity (S2)

**Good Laboratory Practices (GLP):** Ensure reproducibility, accountability, and integrity of data

# Challenges & Limitations

**Chemical Complexity:** Herbal extracts are mixtures, hard to isolate one compound

**Environmental Variability:** Pollution levels change by season, location, plant part

**Data Gaps:** Limited studies on long-term low-dose exposure

**Lack of Standardization:** Variation in testing protocols across labs and countries

# Conclusions

- Pollution can significantly alter the safety profile of medicinal plants.
- Standard preclinical safety testing is vital for identifying hidden toxic risks.
- Regulatory bodies and researchers must work together to enforce quality control.
- This is essential for both pharmaceutical development and public health protection.

# Future Directions

- **Use of Clean Cultivation Systems:** Hydroponics, greenhouses, vertical farming
- **Pollution-Monitoring Tools:** Real-time sensors, satellite imagery
- **Phytoremediation Awareness:** Plants used to clean soil should not be used medicinally
- **Development of Guidelines:** Clear thresholds for contaminant levels in herbal raw materials

# Short test

What is a major concern when using medicinal plants grown in polluted environments?

**A.** Increased nutritional value
**B.** Enhanced therapeutic properties
**C.** *Accumulation of toxic pollutants*
**D.** Faster growth rate

Which of the following is commonly used for detecting heavy metals in plant samples?

**A.** PCR
**B.** *ICP-MS*
**C.** ELISA
**D.** Western Blot

# Short test

What is the purpose of the MTT assay in preclinical safety testing?

**A.** Detect DNA mutations
**B.** Measure oxidative stress
**C.** *Determine cell viability*
**D.** Analyze protein content

Which organization provides Good Laboratory Practice (GLP) guidelines?

**A.** UNESCO
**B.** WHO
**C.** *OECD*
**D.** FDA

# Short test

Why is in vivo testing necessary in preclinical safety assessments?

**A.** It is cheaper than in vitro testing
**B.** It eliminates the need for ethics approval
**C.** *It allows observation of whole-body effects and organ-specific toxicity*
**D.** It focuses only on genetic mutations