



# Ecotoxicity and Herbal Health

A Symbiotic Approach

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## CHAPTER 7

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# Toxicity Assessment of Herbal Remedies: Methods and Tools

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### ABSTRACT

The increasing popularity of herbal remedies in healthcare has led to a growing concern regarding their safety and potential toxicity. This study aims to evaluate various methods and tools employed in the assessment of toxicity associated with herbal remedies. A comprehensive review of literature was conducted to identify pertinent studies published between 2000 and 2024. The review encompasses *in vitro*, *in vivo*, and computational methods utilized in toxicity assessment, including cytotoxicity assays, animal models, and predictive modelling approaches. Furthermore, factors influencing herbal toxicity, such as plant species variability, extraction methods, and formulation, are critically analyzed.

Several key findings emerged from the review. *In vitro* assays, such as cell viability assays (MTT, SRB), have been extensively employed

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for preliminary toxicity screening due to their cost-effectiveness and rapidity. Animal studies remain indispensable for assessing systemic toxicity, organ-specific effects, and long-term outcomes. Additionally, computational models, particularly quantitative structure-activity relationship (QSAR) and molecular docking, offer valuable insights into the molecular mechanisms underlying herbal toxicity, facilitating the prediction of adverse effects. The integration of multiple approaches, including omics technologies (genomics, transcriptomics, metabolomics), enhances the predictive power and mechanistic understanding of herbal toxicity. Moreover, the development of standardized protocols and databases for toxicity data compilation is imperative for ensuring reproducibility and comparability across studies. This review underscores the importance of employing a multi-faceted approach in toxicity assessment of herbal remedies to mitigate potential health risks and promote their safe utilization in healthcare. Future research directions should focus on advancing predictive modeling techniques and elucidating the complex interactions between herbal constituents and biological systems.

### 7.1 INTRODUCTION TO HERBAL REMEDIES

Herbal remedies, also known as botanical medicine or phytotherapy, have been exploited for periods as an essential outline of healthcare in various backgrounds around the world. Derived from plants and plant extracts, herbal remedies encompass a wide range of substances with medicinal properties. These remedies contain active compounds that interact with the body to promote healing and alleviate symptoms of various ailments. Unlike synthetic drugs, herbal remedies often offer a holistic approach to health, targeting not only specific symptoms but also addressing underlying imbalances within the body. The efficacy of herbal remedies is attributed to the diverse array of bioactive compounds present in plants, including alkaloids, flavonoids, terpenoids, and phenolic compounds. These compounds exert pharmacological effects by interacting with specific molecular targets in the body, such as enzymes, receptors, and signaling pathways [1, 2].

Furthermore, herbal remedies are often praised for their relatively low risk of adverse effects compared to synthetic drugs. However, it's essential to acknowledge that herbal remedies can still pose risks,

particularly when used improperly or in combination with other medications.

Modern scientific research has begun to validate the efficacy of many traditional herbal remedies, uncovering their mechanisms of action and potential therapeutic benefits. Additionally, advances in technology have enabled the standardization and quality control of herbal products, ensuring consistency and safety. Regardless of the growing body of scientific evidence supporting the use of herbal remedies, challenges remain in terms of standardization, regulation, and integration into mainstream healthcare practices. Nevertheless, interest in herbal medicine continues to rise, driven by a growing demand for natural and alternative healthcare options. The herbal remedies represent a rich source of therapeutic compounds with the potential to complement conventional medical treatments. As scientific research progresses, further exploration of herbal medicine holds imply for the extension of innovative therapies and the promotion of global health and well-being [3–6].

## 7.2 OVERVIEW OF HERBAL MEDICINE USAGE

Herbal medicine involves the use of plants or plant extracts for therapeutic purposes. This practice has been a part of human healthcare for thousands of years and remains prevalent worldwide. Herbal remedies are often utilized to alleviate symptoms, promote health, and prevent diseases [5–9].

1. **Historical Context:** Herbal medicine has been integral to various traditional healing systems globally, including “Traditional Chinese Medicine (TCM), Ayurveda, and Native American medicine.” Many modern pharmaceuticals have their roots in herbal remedies.
2. **Active Compounds:** Plants comprehend a plethora of bioactive complexes like alkaloids, flavonoids, terpenes, and phenolics, which contribute to their medicinal properties. These compounds interact with biological systems in the body to produce therapeutic effects.

3. **Evidence-Based Usage:** Scientific research has increasingly validated the efficacy of certain herbal remedies for specific health conditions. Clinical trials and studies aim to elucidate the mechanisms of action, safety profiles, and potential interactions of herbal medicines.
4. **Common Uses:** Herbal remedies are employed for various purposes, including pain relief, digestive disorders, immune support, stress reduction, skin conditions, and respiratory ailments. Examples include ginger for nausea, peppermint for indigestion, and echinacea for immune support.
5. **Safety Considerations:** Although herbal medicines are generally remarked as innate and safe, it's crucial to recognize potential risks. Adverse effects, herb-drug interactions, variability in potency, and contamination issues can occur. Proper dosage, quality control, and consultation with healthcare professionals are essential.
6. **Regulatory Framework:** Regulatory approaches for herbal medicines vary globally. Some countries have specific regulations governing their production, labeling, and sale to ensure quality, safety, and efficacy. Regulatory bodies may require evidence of traditional use, pharmacological data, and clinical trials for product approval.
7. **Integration with Conventional Medicine:** Herbal medicine is often used alongside conventional medical treatments, either as complementary or alternative therapy. Integrative approaches seek to leverage the benefits of both traditional and modern healthcare modalities for optimal patient outcomes.
8. **Research Challenges:** Despite growing interest and investment in herbal medicine research, several challenges persist. These include standardization of herbal preparations, funding limitations for large-scale clinical trials, cultural biases, and the need for interdisciplinary collaboration.

The herbal medicine represents a rich source of therapeutic agents with a long history of human use. Scientific investigation continues to uncover the potential of plant-based remedies while addressing safety concerns and integrating them into modern healthcare practices.

### **7.2.1 IMPORTANCE OF TOXICITY ASSESSMENT IN HERBAL REMEDIES**

Toxicity assessment in herbal remedies is of paramount importance due to several scientific reasons [8–12]:

1. **Safety:** Herbal remedies are often consumed for their perceived health benefits, but they may contain compounds that could be toxic or harmful to human health. Toxicity assessment helps ensure that these remedies are reliable for consumption and do not set any consequences for individuals.
2. **Quality Control:** Herbal products can vary widely in their composition and potency due to differences in plant species, growing conditions, and preparation methods. Toxicity assessment allows for quality control measures to be implemented to ensure that herbal remedies meet safety standards and contain the intended beneficial compounds.
3. **Regulatory Compliance:** Many countries have regulations governing the sale and use of herbal remedies. Toxicity assessment provides crucial data to regulatory authorities to evaluate the safety of these products and determine appropriate regulations to protect public health.
4. **Identification of Potential Risks:** Herbal remedies may interact with medications or exacerbate certain health conditions, leading to adverse effects. Toxicity assessment helps identify potential risks associated with herbal remedies, allowing healthcare providers to make informed decisions about their use and advise patients accordingly.
5. **Public Health Protection:** Ensuring the safety of herbal remedies through toxicity assessment protects public health by reducing the likelihood of adverse reactions and ensuring that individuals can safely access alternative treatment options.

Toxicity assessment in herbal remedies is essential for ensuring safety, quality control, regulatory compliance, identifying potential risks, and protecting public health. By conducting thorough toxicity assessments, researchers and regulatory authorities can promote the safe and responsible use of herbal remedies in healthcare practices (Table 7.1).

**TABLE 7.1** Methods for Toxicity Assessment of Herbal Remedies

Assessment Method or Tool	Significance	Applications/ Benefits
LC <sub>50</sub> (lethal concentration)	Determines the concentration of a substance that causes death in 50% of a test population over a specified time.	Assessing acute toxicity in aquatic organisms.
LD <sub>50</sub> (lethal dose)	Measures the dose of a substance that causes death in 50% of a test population.	Assessing acute toxicity in animals.
Ames test	Screens substances for mutagenic potential by examining their ability to cause mutations in bacterial strains.	Evaluating genotoxicity of herbal extracts.
Bioassays	Utilizes living organisms to measure the toxic effects of herbal remedies. Common organisms include daphnia, fish, algae, and bacteria. Provides insights into acute and chronic toxicity. Can be conducted in laboratory settings or natural environments. Allows for the assessment of individual compounds or complex mixtures.	Evaluating the toxicity of herbal remedies on plants.
MTT assay	Measures the viability of cells treated with herbal extracts by assessing mitochondrial activity.	Assessing cytotoxicity <i>in vitro</i> .
OECD guidelines	Developed by the Organization for Economic Co-operation and Development, these guidelines provide standardized protocols for various toxicity tests.	Assessing toxicity across different organisms and endpoints.
Microtox test	Utilizes bioluminescent bacteria to assess the toxicity of substances by measuring changes in light output.	Screening for acute toxicity in water samples.
Chemical analysis	Involves the identification and quantification of toxic constituents in herbal remedies. Utilizes techniques such as chromatography, spectroscopy, and mass spectrometry. Determines the presence of heavy metals, pesticides, and other contaminants. Allows for the establishment of safety thresholds for specific compounds.	Assessing the threshold for specific compounds.
Comet assay	Evaluates DNA damage by measuring the migration of fragmented DNA in an electric field.	Assessing genotoxicity of herbal compounds.

TABLE 7.1 (Continued)

Assessment Method or Tool	Significance	Applications/ Benefits
Cell culture assays	Uses cell lines to evaluate the cytotoxicity of herbal extracts or compounds. Measures various cellular parameters such as viability, proliferation, and apoptosis. Provides information on the mechanisms of toxicity at cellular levels. Enables high-throughput screening of multiple samples. Offers insights into potential therapeutic applications.	Evaluating the cytotoxicity of herbal extracts or compounds.
Daphnia magna immobilization test	Determines the toxicity of substances by observing the immobilization of water fleas ( <i>Daphnia magna</i> ) exposed to them.	Assessing acute toxicity in aquatic environments.
Zebrafish embryo toxicity test	Measures the toxicity of substances by observing developmental abnormalities and mortality in zebrafish embryos.	Assessing developmental toxicity of herbal extracts.
Phytotoxicity bioassays	Assesses the effects of substances on plant growth and development.	Evaluating the toxicity of herbal remedies on plants.
Acute oral toxicity	Measures the adverse effects of herbal remedies after oral administration in a short-term study.	Evaluate the adverse effects.

### 7.3 ECOTOXICITY OF HERBAL REMEDIES

#### 7.3.1 ECOLOGICAL IMPACT OF HERBAL REMEDIES

Herbal remedies, derived from plants, have been utilized for millennia across diverse cultures for their perceived medicinal properties. However, while they offer potential benefits for human health, the ecological impact of their utilization warrants attention [12–15].

1. **Biodiversity Conservation:** Many medicinal plants are harvested from natural habitats, leading to concerns about overharvesting and habitat destruction. Unsustainable harvesting practices can threaten the biodiversity of ecosystems, particularly in regions with high levels of plant endemism.

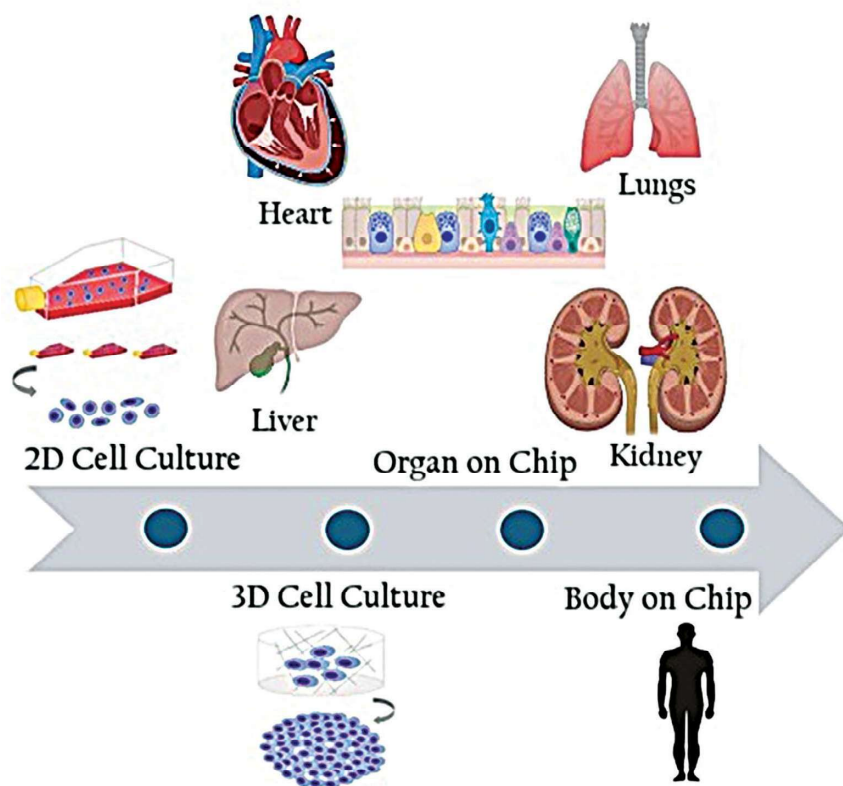
2. **Habitat Degradation:** Clearing land for the cultivation of medicinal plants can conclude in habitat deprivation and failure of crucial ecosystems such as forests, grasslands, and wetlands. This can disrupt ecological processes, decrease soil fertility, and reduce water quality.
3. **Invasive Species Introduction:** Introducing non-native medicinal plants for cultivation can lead to invasions into native ecosystems, outcompeting native species and altering community dynamics. This can result in the loss of native biodiversity and disrupt ecosystem functioning.
4. **Genetic Erosion:** Overharvesting of medicinal plants can lead to genetic erosion within wild populations, reducing their ability to adapt to changing environmental conditions. This diminishes the resilience of plant populations and can ultimately threaten their long-term survival.
5. **Impact on Pollinators and Wildlife:** Herbivores, pollinators, and other wildlife species may depend on medicinal plants for food, shelter, and habitat. Alterations in plant populations due to harvesting or cultivation can disrupt these ecological relationships, leading to declines in wildlife populations and ecosystem stability.
6. **Chemical Pollution:** Intensive cultivation of medicinal plants may involve the use of pesticides, fertilizers, and other agrochemicals, which can pollute soil, waterways, and adjacent habitats, impacting non-target species and ecosystem health.
7. **Climate Change Resilience:** Medicinal plants are vulnerable to “climate change-persuaded shifts in temperature, precipitation patterns, and extreme weather events.” Climate change can alter the distribution and abundance of medicinal plant species, affecting their availability for both ecological and medicinal purposes.
8. **Cultural and Traditional Knowledge Preservation:** Many indigenous cultures have deep-rooted relationships with medicinal plants, relying on them for cultural, spiritual, and medicinal

purposes. Preserving traditional knowledge and sustainable harvesting practices is essential for maintaining biodiversity and ecosystem health while respecting cultural diversity.

While herbal remedies offer valuable therapeutic benefits, their ecological impact must be carefully managed to ensure the conservation of biodiversity, ecosystem integrity, and the long-term sustainability of medicinal plant resources. Adopting sustainable harvesting practices, promoting cultivation in agroecological systems, and integrating traditional ecological knowledge are crucial steps toward mitigating the ecological footprint of herbal medicine (Figure 7.1; Tables 7.2 and 7.3).

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**FIGURE 7.1** Advancements in *in-vitro* models for evaluating toxicity.

**TABLE 7.2** Tools for Toxicity Assessment of Herbal Remedies

Tool for Analysis	Significance of the Study
High-throughput screening (HTS)	Automates the testing of numerous herbal extracts or compounds. Uses robotics and automation to conduct bioassays or cell culture assays rapidly. Screens large libraries of samples for toxicity or therapeutic effects. Facilitates the identification of lead compounds for drug discovery.
Computational toxicology	Applies computer models and algorithms to predict the toxicity of herbal remedies. Utilizes data on chemical structure, physicochemical properties, and biological activity. Employs methods such as QSAR, molecular docking, and machine learning. Provides rapid and cost-effective toxicity predictions. Requires validation with experimental data for reliability.
Omics technologies	Integrates genomics, transcriptomics, proteomics, and metabolomics to assess toxicity. Examines changes in gene expression, protein profiles, and metabolic pathways. Offers comprehensive insights into the mechanisms of toxicity. Requires sophisticated bioinformatics tools for data analysis.

**TABLE 7.3** Ecotoxicity Assessment Tools for Herbal Remedies

Tool	Description
Microtox assay	Determines the toxicity of herbal remedies to aquatic organisms by measuring bioluminescence inhibition.
<i>Daphnia magna</i> test	Evaluates the effects of herbal remedies on aquatic organisms' survival and reproduction using water fleas.
Fish toxicity testing	Assesses the impact of herbal remedies on fish health and behavior through exposure in controlled tanks.
Soil microbial assays	Measures the effects of herbal remedies on soil microbial communities and their ecological functions.
Plant bioassays	Examines the growth and development of plants exposed to herbal remedies to assess phytotoxicity.

#### 7.4 FACTORS INFLUENCING ECOTOXICITY

Ecotoxicity, within the context of herbal medicine, refers to the potential of herbal compounds to adversely impact the environment and its living

organisms. Understanding the factors influencing ecotoxicity in herbal medicine is crucial for mitigating environmental risks associated with their use. Several factors contribute to the ecotoxicity of herbal medicines [14–20]:

1. **Chemical Composition:** Herbal medicines consist of complex mixtures of bioactive compounds. Some of these compounds may possess inherent toxic properties to non-target organisms. For instance, alkaloids, flavonoids, and essential oils found in many herbs can demonstrate lethal impacts on marine organisms, soil microorganisms, and terrestrial fauna.
2. **Concentration and Dose:** The concentration of bioactive constituents in herbal preparations directly influences their ecotoxicological effects. Higher concentrations or doses of herbal extracts may result in greater toxicity to environmental organisms. Additionally, the cumulative effects of repeated or chronic exposure to sublethal doses can also impact ecosystems.
3. **Persistence and Bioaccumulation:** Some herbal compounds exhibit persistence in the environment, resisting degradation processes. Furthermore, certain compounds may bioaccumulate in organisms, leading to magnified toxic effects as they move up the food chain. Persistent and bioaccumulative herbal constituents pose significant ecotoxicological risks, especially in aquatic ecosystems.
4. **Interactions with Environmental Factors:** Environmental conditions such as pH, temperature, light intensity, and nutrient availability can influence the ecotoxicity of herbal medicines. These factors can affect the stability, bioavailability, and toxicity of herbal compounds in various environmental compartments.
5. **Mode of Administration and Disposal:** The route of administration and subsequent disposal of herbal medicines can impact their environmental fate and toxicity. Improper disposal methods, such as dumping unused herbal products down drains or into water bodies, can lead to direct exposure of ecosystems to toxic constituents.

6. **Ecological Sensitivity:** Different organisms exhibit varying degrees of sensitivity to herbal compounds based on their physiological and ecological characteristics. Endangered or keystone species may be particularly vulnerable to the ecotoxic effects of herbal medicines, leading to disruptions in ecosystem dynamics and biodiversity loss.
7. **Synergistic and Antagonistic Interactions:** Herbal compounds may interact synergistically or antagonistically with other environmental pollutants or stressors, amplifying or attenuating their ecotoxic effects. These interactions can complicate risk assessments and necessitate comprehensive evaluation of multiple stressors in ecological systems.

Understanding these factors is essential for conducting accurate risk assessments and implementing effective mitigation strategies to minimize the ecotoxicological impacts of herbal medicines on the environment. Incorporating eco-friendly practices in the production, usage, and disposal of herbal products can help mitigate environmental risks and promote sustainable use of medicinal plants.

### 7.5 CASE STUDIES ON ECOTOXICOLOGICAL EFFECTS

The widespread use of herbal remedies raises concerns about their potential ecotoxicological effects. Below are some case studies highlighting the ecotoxicological impacts associated with the use of herbal medicine [16, 18–24].

1. **Case Study 1: Neem (*Azadirachta indica*) Extracts:** Neem, a tropical tree native to the Indian subcontinent, is extensively used in traditional medicine. Its extracts are known for their pesticidal and medicinal properties. However, studies have shown that neem extracts can have adverse effects on non-target organisms. In one study, the application of neem-based pesticides led to a decline in beneficial insect populations such as bees and butterflies. Furthermore, neem extracts have been found to inhibit the growth of aquatic organisms, disrupting freshwater ecosystems. These findings emphasize the need for careful consideration of the ecological impacts of neem-based products.

2. **Case Study 2: Ginseng (*Panax spp.*):** Cultivation Ginseng is a popular herbal remedy used for its purported health benefits. However, the widespread cultivation of ginseng has raised concerns about its ecological impact, particularly in forest ecosystems. Clearing land for ginseng cultivation can result in habitat loss and fragmentation, leading to declines in native plant and animal species. Moreover, the use of agrochemicals such as pesticides and fertilizers in ginseng farming can contaminate soil and water resources, affecting ecosystem health. Sustainable cultivation practices and habitat conservation efforts are essential to mitigate the ecotoxicological effects of ginseng production.
3. **Case Study 3: Eucalyptus (*Eucalyptus spp.*):** Essential Oil Eucalyptus essential oil is widely used in aromatherapy and herbal medicine for its soothing properties. However, the cultivation of eucalyptus trees for oil production can have detrimental effects on soil and water quality. Studies have shown that eucalyptus plantations can alter soil microbial communities and deplete soil nutrients, leading to reduced fertility and productivity. Additionally, the leaching of eucalyptus oil compounds into water bodies can be toxic to aquatic organisms, posing a threat to freshwater ecosystems. Sustainable management practices, such as agroforestry techniques and proper waste disposal, are necessary to minimize the ecotoxicological impacts of eucalyptus cultivation.

The case studies presented above demonstrate that herbal medicine can have unintended ecotoxicological effects on the environment. From neem extracts to ginseng cultivation and eucalyptus essential oil production, the use of herbal remedies can impact biodiversity, soil health, and water quality. It is imperative to conduct thorough risk assessments and adopt sustainable practices to confirm the reliable and liable use of herbal medicine while minimizing environmental harm.

## 7.6 METHODS FOR ASSESSING TOXICITY IN HERBAL REMEDIES

### 7.6.1 *IN VITRO* TOXICITY ASSAYS

*In vitro* toxicity assays are vital tools used in assessing the potential harmful effects of herbal remedies. They involve conducting experiments

outside of a living organism, typically using cultured cells or subcellular components. These assays provide valuable insights into the safety profile of herbal products before they are introduced for human consumption. Below is the scientific overview of common *in vitro* toxicity assays used in evaluating herbal remedies:

1. **Cell Viability Assays:** These assays measure the viability of cells exposed to herbal extracts or compounds. Common techniques include the MTT “(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) assay, the resazurin assay, and the LDH (lactate dehydrogenase) release assay.” These assays assess cellular metabolic activity, mitochondrial function, and membrane integrity, providing insights into cytotoxic effects.
2. **Genotoxicity Assays:** These assays estimate the prospective of herbal remedies to cause harm to DNA, which can manage mutations and cancer. “The Ames test, comet assay, and micronucleus assay are commonly used to assess genotoxic effects.” These assays detect DNA damage, chromosomal aberrations, and micronuclei formation in exposed cells.
3. **Oxidative Stress Assays:** Herbal remedies may induce oxidative stress, leading to cellular damage and dysfunction. Assays like the measurement of reactive oxygen species (ROS) levels, lipid peroxidation assays (e.g., TBARS assay), and assessment of “antioxidant enzyme activities (e.g., superoxide dismutase, catalase) help in evaluating the oxidative stress-inducing potential of herbal extracts.”
4. **Cytokine Release Assays:** These assays assess the immunomodulatory effects of herbal remedies by measuring the release of cytokines from immune cells. ELISA (enzyme-linked immunosorbent assay) or multiplex cytokine assays can quantify the levels of pro-inflammatory or anti-inflammatory cytokines, providing insights into the immune response triggered by herbal products.
5. **Apoptosis and Necrosis Assays:** “Herbal remedies may induce programmed cell death (apoptosis) or unprogrammed cell death (necrosis) in exposed cells. Annexin V/propidium iodide staining, TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) assay, and flow cytometry are common techniques used