PENNSTATE 1855

eTOXIC
Environmental Toxicology Inquiry Curriculum

# Module 1: Everyday Toxicology
# Lesson 5: What Does the Body Do With Toxins?

## *Lesson Overview*

**Summary:**

In this lesson, students will begin by extending their knowledge of certain molecules persisting in fat stores to a quantitative understanding of toxicokinetics and pharmacokinetics, the description of the metabolism and elimination of exogenous molecules (like drugs and toxins).

Students will make further connections to a selection of enzymatic mechanisms that represent two important evolutionary strategies for metabolism: detoxification and promotion of elimination. Understanding that the liver is the central organ in metabolism of drugs and toxins, students will consider two categories of enzymes found in liver cells: cytochrome P450s and alcohol dehyrogenases.

Finally, students will consider quantitative time courses of absorption and elimination, including the important concept of half-life, the amount of time it takes for an exogenous molecule to be eliminated from the body to a level that is 50% of the peak level.

The homework assignment applies the idea of half-life to understanding the health effects of a beneficial molecule found in green tea (and certain supplement pills) called green tea catechins. Students will draw and compare time courses that demonstrate that small doses throughout the day (cups of tea) can be beneficial, while one large dose (an overdose of supplements) can be toxic. This activity thus connects concepts of dose and response, acute and chronic effects, and pharmacodynamics.

**Objectives:**

By the end of the lesson, students will be able to:

1. identify three routes of toxin exposure with 100% accuracy.
2. identify four routes of toxin elimination with 100% accuracy.
3. utilize quantitative measures of time course regarding physiological effects of substances.
4. differentiate harmful and beneficial effects of equal daily doses over different time courses.

**Grades:**

9th through 12th

## *Prepping the Lesson*

**Instructions:**

**Materials/Technical Resources:**

It is our recommendation that you walk through the teacher and student materials for this lesson to ensure that students will be able to receive the information through the modes of delivery that we intended prior to using the material in the classroom. If you or your school does not have the resources needed, you may need to make some modifications depending on the resources you have available.

The following materials/technical resources will be needed to complete the lesson. We recommend using Option #1 to provide the materials to your students in the manner in which they were intended to be delivered.

**Option #1** (Preferred Technology Requirement)
You will need to have access to a computer, the Internet, and a projection device during the entire lesson. Your students will need to have access to computers and the Internet. You and your students will simultaneously step through the module while using their given computers. You may have to make special arrangements for all of your students to have a computer. Be sure you and your students will not be blocked from Google Documents, YouTube, and your selected online collaborative tool. You may be currently using an online collaborative tool but if not, we recommend Facebook groups, Edmodo, or eChalk.

**Option #2** (Minimum Technology Requirement)
If you do not have a way for your students to access the Internet individually, then you will need to facilitate their access to the information. You will need access to a computer, the Internet, and a projection device during the entire lesson. You will step through the module as your students watch and complete the presented activities. There may be modifications to the delivery of the materials that you will need to make, depending upon the resources you have available. Be sure you have access to Google Documents, You Tube, and your selected online collaborative tool. You may be currently using an online collaborative tool but if not, we recommend Facebook groups, Edmodo, or eChalk.

**Lesson Time and Supply List:**

This document will provide you with information on prep time needed, a list of supplies, and total lesson time for this particular lesson. See appendix for the Lesson Time and Supply List document.

**Student Homework:**

Prior to starting this module, it is important to determine which online collaborative tool you and your students will be using. Once you have had an opportunity to review all of the lessons, decide how you will facilitate the homework discussions and submissions using your selected online collaborative tool. Be sure to give your students clear directions and objectives on your expectations of the use of this tool and their participation in their homework activities. We highly encourage you to participate with your students in their homework discussions to enhance the quality of the experience.

**Essential Vocabulary:**

absorption, acetaldehyde, alcohol dehydrogenases, cytochrome P450s or CYPs, dioxin, epoxide, ethanol, exogenous, functional group, formaldehyde, green tea catechins, half-life, hydrophilic, hydrophobic, hydroxyl (-OH), ingestion, inhalation, methanol, micronutrients, pharmacodynamics, pharmacokinetics, phenanthrene, TCDD, toxicant, toxicodynamics, toxicokinetics, toxin, xenobiotic (See Lesson 1, 2, 3, and 4 for additional vocabulary words.)

**Student Notebook:**

Laboratory notebooks are arguably the most useful tool at an experimenter's bench. Remind students of the critical importance of recording all experimental observations, and especially recording all experimental conditions (e.g., the type of chemical treatment, the range of concentrations, number and species of seeds, etc.) when starting an experiment so that they can maintain comprehensive, unambiguous control over those variables when they follow up with subsequent experiments.

## *Implementing the Lesson*

**Instructions:**

**1. Guided Discussion (5 Minutes)**

Elimination and Metabolism Rid the Body of Toxins and Drugs

Consider the concepts and vocabulary in this section as background, which depending on their preparation you may or may not share with students in this lesson. Previous topics in this module have addressed the issue of **toxicodynamics** (which we have framed as "biological response"). This lesson addresses the related issue of **toxicokinetics** (which we have previously raised in terms of the fast elimination of water-soluble vitamins versus the slow elimination of fat-soluble vitamins).

Benet (1984) provides two succinct definitions:

Toxicodynamics is **"what the poison does to the body."**
Toxicokinetics is **"what the body does to the poison."**

The terms pharmacodynamics ("what the drug does to the body") and pharmacokinetics ("what the body does to the drug") are close parallels.

In a broader context, we can replace the words "poison" and "drug" with the concept of "exogenous molecule," meaning simply a molecule that comes from outside the body. (An equivalent term is "xenobiotic.") This category includes all of the examples we have previously discussed:

- **pharmaceuticals** ("drugs") like aspirin, acetaminophen, digitalis, etc.
- environmental **toxicants** from industrial and other sources like TCDD.
- natural **toxins** from plants, animals, and other sources like caffeine, puffer fish toxin, etc.
- **micronutrients** like selenium and the essential vitamins.

*__Teacher Tip:__ Technically, a toxin is made by a living organism (e.g., snake venom, puffer fish toxin, plant alkaloids, etc.) while a toxicant is not (e.g., dioxins, polycyclic aromatic hydrocarbons, etc.) "Toxics" (as a noun) technically covers both categories. In order to avoid confusion, we use the common term "toxin" for both categories throughout this curriculum.*

Again, labels aside, "toxicology" and "pharmacology" overlap extensively with "nutrition."

Another way to connect the two concepts is to consider that **toxicokinetics provides a time frame in which to consider toxicodynamics** (which we have been discussing in terms of "biological response"). The case of Viktor Yushchenko, in which one exposure to TCDD has had and will continue to have health effects over decades, is an extreme example of exceptionally long toxicokinetics.

In short, toxicokinetics provides detailed answers to the question "How does the body get rid of toxins?" There are two related mechanisms, namely **metabolism and elimination**.

**2. Guided Discussion (5 Minutes)**

<u>Smoking and Mutagenesis: Primary Literature and Popular Press</u>
Begin the lesson with a review of the responses on the online collaborative tool to the first homework question, addressing the news article and the journal article about mutagens from cigarette smoke.

***Ideal answers might look like the following:***

*1. Since even one cigarette rapidly gives rise to elevated blood levels of certain mutagens (see below),*

*it is possible that even one exposure (depending on the dose) could cause mutations that are harmful over the long term—especially to a fetus or to germ cells.*

*2. However, one single dose of these mutagens would very likely not give rise to a level of mutations higher than most body tissues could recover from, through the mechanisms of DNA repair and immune clearance of mutant cells. Thus, again, it is the chronic use of cigarettes that is the true danger, especially in the context of nicotine's exceptionally potent ability to lead to chemical dependence.*

*3. Students ought to have noticed at least some of the following differences between popular press and journal articles:*

*a. funding: advertisements vs. high subscription and publication fees*
*b. authors: journalists vs. scientists*
*c. level of detail of explanation: summary for laypeople vs. sufficient detail to repeat the experiment in another laboratory*
*d. key facts missing in the version written for the public*

**3. Guided Discussion (5 Minutes)**

***Teacher Tip:*** *Note that most of the examples we have considered so far fall under the route of ingestion. Indeed, route can affect toxicity. Acrylamide, for example, is much more toxic when inhaled than when ingested orally. See appendix for website reference.*

Toxins In, Toxins Out

The second homework question called for students to answer the question "How does the body get rid of toxins?" Preface the discussion of the responses to this question by posing this related question to the class: **"How do toxins get into the body?"**

There are three answers, known as **routes of exposure**: (some sources also include injection as a route of exposure)

1. through the **mouth ("ingestion")** (e.g., pills, coffee)
2. through the **lungs ("inhalation")** (e.g., cigarette smoke, nuclear fallout)
3. through the **skin ("absorption")** (e.g., volatile or caustic solvents)

**4. Guided Discussion (10 Minutes)**

How Does the Body Get Rid of Toxins?

The discussion of routes of exposure frames the discussion of the analagous routes of **elimination** which students addressed in the second homework question. Elicit from the class a review of the group's answers and record them on the board.

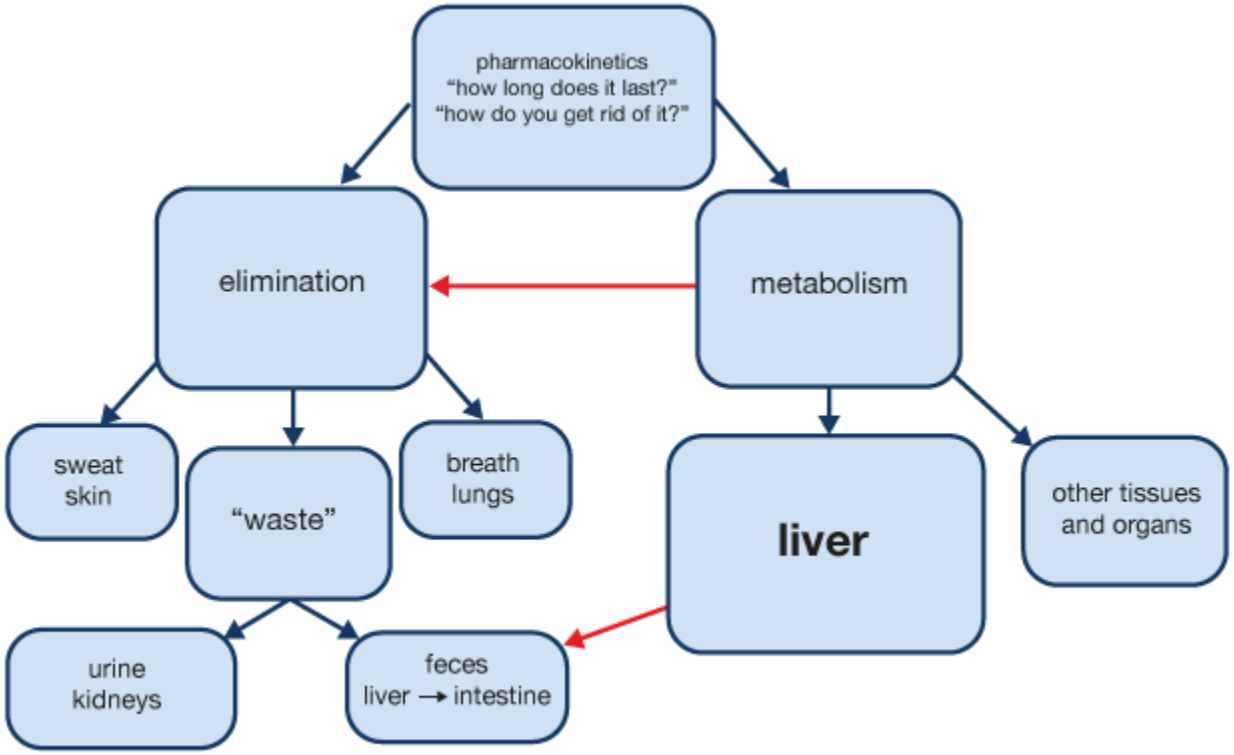
Exogenous molecules can exit the body through four main **routes of elimination**:

1. through the **kidneys** into **the urine**
2. through the **bile duct** from the liver into **the feces**
3. through the **lungs** into **the breath**
4. through the **skin** into **the sweat**

Students may express the insight that in **addition to elimination**, the body deals with toxins via **metabolism to less harmful, and/or more easily eliminated, molecules**. If not, prompt them with questions like "What is the function of the liver?"

> ***Answer:*** *Among other functions, the main role is to metabolize exogenous molecules.*

A concept map might be a useful method for recording and connecting the ideas that come from a discussion of how the body eliminates exogenous molecules. Use the following example as a guideline for the concept map the class constructs via discussion.




**5. Guided Discussion (15 Minutes)**

<u>Two Strategies for Metabolism</u>

A wide variety of mechanisms of metabolism of exogenous

***Teacher Tip:*** *Recall that we contrasted response to acetominophen between a mother and her baby in the first activity in Lesson 1: the baby received a much larger dose in both scenarios because its body is so much smaller. But note also that adults have the ability to both metabolize and eliminate acetominophen much faster than children can. This effect means that children also have a higher sensitivity to acetominophen than adults do.*

molecules have evolved, and they may be divided into two broad categories:

1. **detoxification**: catalyzing the conversion of toxic molecules into less toxic molecules

2. **promoting elimination**: catalyzing the conversion of hydrophobic ("fat soluble") molecules into more hydrophilic ("water soluble") molecules

The first strategy simply aims to produce metabolic derivatives that exhibit lower toxicity.

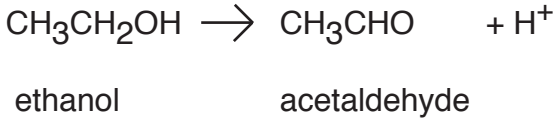
The second strategy is one of modifying hydrophobic molecules to be more hydrophilic. In some cases, however, the metabolic derivatives produced are indeed **easier to eliminate, but in fact exhibit higher toxicity**. Another way to think of this strategy, then, is to consider it a trade-off between higher acute toxicity with faster elimination versus lower chronic toxicity with slower elimination. In other words, it is (usually) better to eliminate a more toxic molecule more quickly than to prolong the exposure to a less toxic molecule for a longer time.

The liver is the central organ in the metabolism of toxins, drugs, and other exogenous molecules. The importance of this role is evident; the liver is by far the largest single organ in the body, typically accounting for 10% of body weight.

Note also that the liver produces bile, which can carry some relatively fat-soluble substances into the intestine for elimination in the feces. So the liver can be the site of both metabolism and elimination.

Consider two examples of enzymes expressed in cells in the liver, each reflecting one of these strategies:

1. A category of enzymes known collectively as **alcohol dehydrogenases** catalyze the reaction that detoxifies ethanol (the type of alcohol found in alcoholic beverages) into acetaldehyde. (Acetaldehyde is less toxic then ethanol and is further metabolized into molecules with very low toxicity.)



However, alcohol dehydrogenase will also catalyze the conversion of methanol (so-called "wood alcohol," used as a solvent) into formaldehyde, which is extremely toxic.

$$CH_3OH \longrightarrow CH_2O + H^+$$

methanol formaldehyde

Students may have seen an episode of *House* in which Dr. House saves the life of a death row inmate who attempts suicide by drinking methanol. The cure is to give the victim a large amount of ethanol to drink—a side effect of this treatment is to get the victim heavily drunk.

In a whole class discussion, invite students to **explain why this treatment works**.

***Hint:*** *the goal of the treatment is to provide a large stoichiometric excess of ethanol compared to methanol.*
***Answer:*** *this excess causes most of the binding sites of most of the alcohol dehydrogenase enzymes to be occupied with ethanol, so that very few interact with methanol. Thus most of the methanol is excreted before it can be converted to formaldehyde.*

2. A large category of related enzymes is known collectively as **cytochrome P450s or CYPs**. They commonly catalyze a reaction of the general form:

$$RH + O_2 + 2H^+ + 2e^- \longrightarrow ROH + H_2O$$

These enzymes catalyze this reaction quite nonspecifically, adding an oxygen atom (in the form of a **hydroxyl** functional group -OH) to a wide variety of hydrophobic molecules (symbolized as R). This addition makes the original exogenous molecule more easily eliminated, but also possibly more reactive, and thus possibly more toxic.

In some cases, moreover, the enzyme will add the highly reactive **epoxide** functional group instead of a hydroxyl. This addition renders the molecule more easily eliminated, but extremely more reactive, and thus significantly likely to be more toxic.

In the previous homework assignment, students read an article about a certain class of toxins from cigarette smoke, polycyclic aromatic hydrocarbons, in particular phenanthrene. In that article, scientists demonstrated that a (very much more toxic) epoxide

derivative of this molecule, formed by the activity of a CYP enzyme, rises to maximum levels in just minutes. What makes this epoxide derivative of phenanthrene so much more toxic than phenanthrene itself is that instead of merely associating between DNA base pairs (via intercalation), it covalently attaches to the DNA molecule. A covalently attached adduct is much more likely to cause a replication error.

**6. Video (13 Minutes)**

Half-Life is a Measure of Persistence

Prompt students to view the **"Half-Life is a Measure of Persistence"** video with their groups.

Guide the students through the act of sketching a time course given the following parameters for aspirin.

| | |
|---|---|
| peak concentration: | 1.2 mg/dl |
| time to peak concentration: | 0.2 h |
| half-life: | 3.1 h |
| time to complete elimination: | 12 h |

**7. Guided Discussion (2 Minutes)**

Conclusion

The homework assignment connects the concepts of toxicokinetics and pharmacokinetics to a real-world example in which a daily dose spread throughout the day is beneficial, while the same dose at one time is quite harmful.

There is a large body of good evidence that drinking green tea has health benefits, including helping to control weight. There are a number of molecules that appear to underlie these beneficial effects for human health. In particular, a class of molecules called green tea catechins appear to block the digestion (and thus, ultimately, the absorption) of triglycerides to fatty acids. However, since not everybody likes green tea, supplement companies sell pills that contain green tea extracts that contain green tea catechins.

The homework assignment calls for students to draw and compare time courses of absorption and elimination of green tea catechins taken under two circumstances:

1. in small doses throughout the day (as cups of tea)
2. in one large dose at the beginning of the day (as an overdose of supplements)

One helpful way to contextualize the homework assignment might be an open-ended question to prompt a quick in-class discussion,

such as “What is the difference between consuming green tea catechins from a cup of tea versus consuming them from a supplement pill?”

A number of answers to that question might be correct. One point to consider is that both tea and supplement pills are complex mixtures of a wide variety of molecules, and the composition of tea may or may not be maintained through the processes of extraction and pill production.

## *Homework*

**8. Activity (10 Minutes)**

Not Just How Much—How Much, How Often

Distribute the worksheet **“Not Just How Much—How Much, How Often”** to students, and post the reflective questions to your class’s online collaborative tool.

**9. Reflective Questions (10 Minutes)**

Not Just How Much—How Much, How Often

Answer the following questions on your online collaborative tool.

1. You completed the worksheet **“Not Just How Much—How Much, How Often.”** Please answer the following questions:

    a. What insights did you gain about how identical daily doses can lead to very different effects?

    b. Are “natural” sources better than supplements? Why or why not?

2. You have already exploited an experimental system (seeds, petri dishes, and salt solutions) to design an experiment that measures an acute response to salt in plants. Since then, you have learned a number of extensions and refinements to the concepts of dose and response, and median lethal dose (or, in this case, median germination failure concentration).

    a. What other experimental designs could you propose? For example, to measure the chronic effects of salt on plants? Or the effects of different kinds of contaminants?

# Appendix

## Prep Time, Supply List, and Total Lesson Time

### Prep Time:

We recommend 30-45 minutes depending on your expertise level with the content. Each module will vary depending on your previous experience with the content and technology.

### Materials:

Each student will need

- access to a computer to view the “Half-Life is a Measure of Persistence” video (unless you decide to show the video through the class projector unit).
- their worksheet “Not Just How Much—How Much, How Often” posted to their online collaborative tool or will need to recieve it in a printed copy prior to leaving.
- their homework added to the online collaborative tool.

You will need

- to identify two or three student examples from responses to the first homework question addressing the news article and the journal article about mutagens from cigarette smoke.
- to prepare an empty concept map for use during a discussion on how the body eliminates exogenous molecules.
- access to a computer to view the “Half-Life is a Measure of Persistence” video (unless you decide to show the video through the class projector unit).
- decide whether you will post the “Not Just How Much—How Much, How Often” worksheet or if you will print it and distribute it to student prior to them leaving.
- to post your students’ homework to your class’s online collaborative tool.

### Total Lesson Time:

| **Lesson Activity** | **Amount of Time in Class** |
|---|---|
| Guided Discussion: Elimination and Metabolism Rid the Body of Toxins and Drugs | 5 Minutes |
| Guided Discussion: Smoking and Mutagenesis: Primary Literature and Popular Press | 5 Minutes |
| Guided Discussion: Toxins In, Toxins Out | 5 Minutes |
| Guided Discussion: How Does the Body Get Rid of Toxins? | 10 Minutes |
| Guided Discussion: Two Strategies for Metabolism | 15 Minutes |
| Video: Half-Life is a Measure of Persistence | 13 Mintues |
| Guided Discussion: Conclusion | 2 Minutes |
| **Total Time** | **55 Minutes** |

| **Lesson Activity** | **Amount of Time out of Class** |
|---|---|
| Worksheet: Not Just How Much—How Much, How Often | 10 Minutes |
| Reflective Questions: Not Just How Much—How Much, How Often | 10 Minutes |
| **Total Time** | **20 Minutes** |

# Resources–Web Addresses

Toxins In, Toxins Out
Acrylamide (http://en.wikipedia.org/wiki/Acrylamide)

Addiction and Carcinogenesis from Smoking
“Smoking Causes Gene Damage in Minutes” (http://news.discovery.com/human/smoking-cigarettes-genetic-damage-110115.html)

“Immediate Consequences of Cigarette Smoking: Rapid Formation of Polycyclic Aromatic Hydrocarbon Diol Epoxides” article in the *Chemical Research in Toxicology* journal (http://pubs.acs.org/doi/full/10.1021/tx100345x)

## Not Just How Much—How Much, How Often

A class of molecules called green tea catechins appear to block the digestion of fatty acids. However, since not everybody likes green tea, supplement companies sell pills that contain green tea extracts that include green tea catechins (as well as a large number of other molecules).

Consider one individual: a 46-year-old female weighing 70 kg who has lived her whole life on the island of Taiwan. Throughout a typical day, she drinks 10 cups of green tea (one every hour between 7:00 a.m. and 4:00 p.m.), each containing 40 mg of green tea catechins.

**What is the dose of catechins in each exposure (i.e., each cup)?**

The half life of this dose of catechins is 15 minutes; blood levels of catechins return to zero after 45 minutes. The blood concentration of catechins from this dose reaches its peak of 6.9 mg/dl after 10 minutes.

**Draw the absorption/elimination curves of green tea catechins from each cup of tea throughout the day.**

Now, consider a 46-year-old female weighing 70 kg who has lived her whole life in Kansas. On a typical morning, she takes green tea catechin supplement pills. However, she (unwisely) **exceeds the recommended dosage** on the bottle and consumes 4 pills, each containing 100 mg of catechins at 7:00 a.m. (The recommended dose is 1 pill.)

**What is the dose of catechins in this exposure?**

The half life of this dose of catechins is 200 minutes; blood levels return to zero after 480 minutes. The blood concentration of catechins from this dose reaches its peak of 69 mg/dl after 20 minutes.

**Draw the absorption/elimination curve of green tea catechins from this large supplement dose taken at the beginning of the day.**

**What is the total daily dose for each woman?**

Now consider that the blood level at which catechins block digestion of fatty acids (possibly leading to weight loss benefits) is 5.0 mg/dl. **Estimate how much time** (i.e., how many total minutes throughout the day) **each woman gets this benefit from the green tea catechins.**

Next, consider that at blood concentrations above 40 mg/dl, green tea catechins are quite toxic, causing damage to the liver! **Estimate how much time** (i.e., how many total minutes throughout the day) **each woman suffers this harm from the green tea catechins.**

# Educator Key with Answers

### Not Just How Much—How Much, How Often

A class of molecules called green tea catechins appear to block the digestion of fatty acids. However, since not everybody likes green tea, supplement companies sell pills that contain green tea extracts that include green tea catechins (as well as large number of other molecules).

Consider one individual: a 46-year-old female weighing 70 kg who has lived her whole life on the island of Taiwan. Throughout a typical day, she drinks 10 cups of green tea (one every hour between 7:00 a.m. and 4:00 p.m.), each containing 40 mg of green tea catechins.

**What is the dose of catechins in each exposure (i.e., each cup)?** (answer: 0.57 mg/kg)

The half life of this dose of catechins is 15 minutes; blood levels of catechins return to zero after 45 minutes. The blood concentration of catechins from this dose reaches its peak of 6.9 mg/dl after 10 minutes.

**Draw the absorption/elimination curves of green tea catechins from each cup of tea throughout the day.**

Now, consider a 46-year-old female weighing 70 kg who has lived her whole life in Kansas. On a typical morning, she takes green tea catechin supplement pills. However, she (unwisely) **exceeds the recommended dosage** on the bottle and consumes 4 pills, each containing 100 mg of catechins at 7:00 a.m. (The recommended dose is 1 pill.)

**What is the dose of catechins in this exposure?** (answer: 5.7 mg/kg)

The half life of this dose of catechins is 200 minutes; blood levels return to zero after 480 minutes. The blood concentration of catechins from this dose reaches its peak of 69 mg/dl after 20 minutes.

**Draw the absorption/elimination curve of green tea catechins from this large supplement dose taken at the beginning of the day.**

**What is the total daily dose for each woman?** (answer: 5.7 mg/kg daily dose; 400 mg daily *exposure*)

Now consider that the blood level at which catechins block digestion of fatty acids (possibly leading to weight loss benefits) is 5.0 mg/dl. **Estimate how much time** (i.e., how many total minutes throughout the day) **each woman gets this benefit from the green tea catechins.** (answer: ten small doses: about 300 minutes; one large dose: about 420 minutes)

Next, consider that at blood concentrations above 40 mg/dl, green tea catechins are quite toxic, causing damage to the liver! **Estimate how much time** (i.e., how many total minutes

throughout the day) **each woman suffers this harm from the green tea catechins.**

(answer: ten small doses: exactly 0 minutes; one large dose: about 180 minutes)