BIOL 142
Case study: The Mystery of the Toxic Flea Dip
(This case study is based on an actual case of poisoning. DeWilde, 1986)

THE CASE:

You are interning at the medical examiner's office at San Francisco County Hospital. It has been a particularly light day, with only 1 homicide and a dead chipmunk that you checked over for rabies. The chipmunk didn't have rabies, and you're ready to go home. Just as you're flipping the switch, you get a call from your secretary. "We've got a dead kid up here that you'll want to look at right away. Might be foul play."

Thinking of your four-year old daughter waiting for you at home, you grimace. "OK, I'm heading to the morgue." Performing autopsies on kids is the least favorite part of your job. But you are paid to solve medical mysteries, and it looks like you've got one here.

In the morgue, you find the report from the hospital. Glancing over it, you notice a narrative of the girl's last hours and read it carefully:

At 10 AM, mother returns from the store to find girl vomiting, not feeling well, and sleepy. Mother put girl to bed. Ten minutes later, she noticed that the child's breathing became irregular and slow. She tried to wake her daughter but was not able to do so. The child became comatose. At noon, the girl was admitted to the hospital, with no heartbeat or spontaneous breathing.

A police report states the following:

The parents discovered that the girl had been giving her dog a bath using a flea dip called Fleacide. According to the label on the container, Fleacide is an insecticide made of plant material only and appropriate for external use on animals.
Part 1: The Flea Dip

FLEACIDE

all natural product
non-toxic

Instructions for use:  add 1/2 cup per
bathtub full of water. Dunk your dog. Rinse.
Repeat if necessary.
Active ingredient:  Rotenone.................6.1 mg
Inactive ingredients:
  ethereal oil of cinnamon..................18.5 mg
  ethereal oil of cloves....................27.5 mg
  ethereal oil of fir......................17.5 mg
  ethereal oil of rosemary...............1.0 mg
  Lecithin (an emulsifier)

Questions:
1) What could have been in the flea dip that killed the girl?

2) How could a product that is normally harmless to humans and pets have killed the girl?

3) How could you use bacteria to test your hypothesis?
Part 2:

**Autopsy Report**
- The girl died within two hours of first vomiting
- Immediate cause of death was hypoxia (lack of oxygen)
- Tissue sections from the kidneys, lungs, thymus, and heart show massive cell death
- Staining with cellular dyes indicates that the mitochondria within the affected tissues were damaged

**Bacterial Assay Report**
- Rotenone rapidly killed the Gram positive bacteria *Staphylococcus epidermidis* and Gram negative bacteria *E. coli*.
- Further investigation using electron microscopy and immunogold labeled antibody to rotenone revealed that rotenone localized at the cell membrane.
- Rotenone was not present in the cytoplasm of the cells.

**Question:**
1) Given the autopsy report, and recalling your knowledge about the function of mitochondria and their relationship to bacterial cells, what functions of the cell did the Fleacide affect?
Part 3: ATP Analysis

A more detailed analysis of the cells from the girl's heart showed that ATP levels were reduced in the mitochondria. ATP levels in the cytoplasm of the heart cells, however, were normal. In addition, pyruvate and acetyl-CoenzymeA levels were normal in the cytoplasm of the heart cells. ATP levels were depressed in the *Staphylococcus* cells.

**Question:**

1) What cellular process (or processes) was impaired by the Fleacide?
Part 4: Subcellular Analysis
Using a new molecular technique, you are able to determine the levels of various metabolites in *S. epidermidis* and the girl's the heart cells. Key highlights of the report are listed below:

### S. epidermidis

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Cellular Finding</th>
<th>Normal Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>218 µmol</td>
<td>200 µmol</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>46 µmol</td>
<td>50 µmol</td>
</tr>
<tr>
<td>NAD⁺</td>
<td>15 µmol</td>
<td>150 µmol</td>
</tr>
<tr>
<td>NADH</td>
<td>512 µmol</td>
<td>100 µmol</td>
</tr>
</tbody>
</table>

### Heart cells

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Cellular Finding</th>
<th>Normal Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>102 µmol</td>
<td>100 µmol</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>23 µmol</td>
<td>25 µmol</td>
</tr>
<tr>
<td>NAD⁺</td>
<td>6 µmol</td>
<td>75 µmol</td>
</tr>
<tr>
<td>NADH</td>
<td>383 µmol</td>
<td>50 µmol</td>
</tr>
</tbody>
</table>

**Questions:**

1) *Given this new information, what specific cellular process do you think was affected by the Fleacide? Why?*

2) *Some health food stores sell supplements containing NAD⁺. If you administered the supplement to the girl, could you save her? Why or why not?*

3) *Would artificial respiration or oxygenation save the girl? Why or why not?*