Mystery of the Toxic Flea Dip: An Interactive Approach to Teaching Aerobic Cellular Respiration

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The case

- 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

• 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?
There are many different Chemicals List and combinations of chemicals in different brands of flea dip. For example, some brands contain rotenone, which is known to be toxic in high levels and has a dermal LD50 of 200 mg/kg.

Several different Organophosphates and Carbamates are also listed as active ingredients, and are known to cause Cancer. The former can include any of the following Organophosphates: Chlorpyrifos, Parathion, Diazinon, famphur, Phorate, Terbufos, and malathion. Like other organophosphate Insecticides, tetrachlorvinphos, that is found in some flea dips, is a known cholinesterase inhibitor.
La rotenona se obtiene en forma industrial a partir de raíces desecadas y molidas de plantas del género Tephrosia, mediante solvente (tetracloruro de carbono).

Junto a la rotenona se obtienen otras sustancias como la deguelina, la trefosina, y el toxicarol. La deguelina es un compuesto isomérico de la rotenona ya que tiene la misma fórmula molecular pero diferente estructura, con lo cual podemos inferir que puede servir cuando los ácaros de Varroa generen resistencia.
Rotenone is classified by the World Health Organization as moderately hazardous. It is mildly toxic to humans and other mammals, but extremely toxic to insects and aquatic life including fish. This higher toxicity in fish and insects is due to the fact that the lipophilic rotenone is easily taken up through the gills or trachea, but not as easily through the skin or through the gastrointestinal tract.

The lowest lethal dose for a child is 143 mg/kg. Human deaths attributed to Rotenone are rare because its irritating action causes vomiting. Deliberate ingestion of rotenone can be fatal.

The compound breaks down when exposed to sunlight and usually has a short lifetime of six days in the environment. In water rotenone may last six months.
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
1) Given the autopsy report, and recalling your knowledge from your reading about the functions of cellular organelles, what functions of the cell did the Fleacide affect?
<table>
<thead>
<tr>
<th>Conversion</th>
<th>CO₂ Molecules Produced</th>
<th>NAD Molecules Reduced to NADH</th>
<th>FAD Molecules Reduced to FADH₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 glucose molecule to 2 pyruvate molecules</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2 pyruvates to 2 acetyl CoA molecules</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2 acetyl CoA to 4 CO₂ molecules</td>
<td>4</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>6</strong></td>
<td><strong>10</strong></td>
<td><strong>2</strong></td>
</tr>
</tbody>
</table>
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 these cells, however, were normal. In addition, acetyl-CoenzymeA levels were normal.

**Question:**
What cellular process (or processes - metabolism) was impaired by the Fleacide?
ATP Production Pathways

Glycolysis

Glucose → Pyruvate → Acetyl CoA → Citric Acid Cycle

High Energy Electrons and H+ → Electron Transport System

O2 + H2O → ATP

Mitochondria Inside a Cell

Fat & Sugar "Intermediates" → Beta Oxidation → Citric Acid Cycle

Inside the Mitochondrion
Part 4: Subcellular Analysis

- Using a new chromatographic technology developed late last year, you are able to determine the levels of various subcellular components in the heart cells. Key highlights of the report are listed below:

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Autopsy Finding</th>
<th>Normal Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>102 mmol</td>
<td>100 mmol</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>23 mmol</td>
<td>25 mmol</td>
</tr>
<tr>
<td>NAD⁺</td>
<td>6 mmol</td>
<td>75 mmol</td>
</tr>
<tr>
<td>NADH</td>
<td>383 mmol</td>
<td>50 mmol</td>
</tr>
</tbody>
</table>
Question 1:

• 1) Given this new information, what specific cellular process do you think was affected by the Fleacide? Why?
La rotenona inhibe al complejo I

Ubiquinol

Piericidin A

Rotenona
Questions:

• 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?