Part I – Demise of the Hives

Dad snapped shut his cell phone and his shoulders slumped. “Now we’ve got a real problem,” he sighed.

Life as an apple farmer in Wenatchee, Washington, could be challenging, but Dad was looking particularly depressed.

“What is it now?” Bruce asked.

“Seems that the last beekeeper in the area has lost almost all of his hives,” Bruce’s dad replied.

After a semester of introductory entomology at Washington State University and a childhood spent in an apple orchard, Bruce knew that this was bad. Bees were necessary to pollinate apple trees to produce the fruit. They pollinated over 130 different food crops, such as berries, beans, nuts, melons, and tree fruit. In fact, bees were responsible for over $15 billion in agricultural products in the United States alone. Without bees, there would be none of these foods. And that included apples—which were paying for Bruce’s college education!

Bruce thought for a moment. “Can you call anyone from outside the area?” he asked.

“If we can find someone—but, even if we could, it might be too late. We only have a window of a week for pollination,” Bruce’s dad answered. “With the loss of so many hives around here, beekeepers from outside the area might be reluctant to come up here.”

Bruce pondered this. Colony Collapse Disorder (CCD), the phrase used to describe the unexplained death or disappearance of a hive, could have over 60 different factors involved. Pesticides and herbicides used in the fields, fungal infections, viruses, and gut-eating mites all had been implicated.

Dad looked perplexed. “The thing is…this just happened over the last three weeks. Our local beekeepers’ hives have had a clean bill of health these last few years. I’ve got money invested with one keeper and there were no signs of infections or chemicals. We’ve been watching this very closely.”

“I’m going to run some errands,” said dad. “I think I’ll stop by the library and extension office and see if I can find anything out.”

Dad came back much later in the day. “Bruce, what do you know about high fructose corn syrup? I found a research article written by scientists at the U.S. Department of Agriculture that seems to say it could be involved with CCD. Can you help me figure this thing out? Recently we have been feeding high fructose corn syrup to our hives.”

Bruce had gone over a few scientific articles in school his senior year—hopefully that would help him analyze the data in the article his father handed him. “Well, let’s just give this a look,” he said. Sitting down, Bruce pulled out a note pad and began to read.
“So…what are your questions?” he asked his father.

Pulling out his own notes, Dad said, “Well, this could be pretty controversial and damaging stuff, so I really want to understand what is going on.”

Your Task

Read the following abstract of the scientific article* as well as the introduction section of the same article (which your instructor will provide you) and then answer the questions that follow. To help you with your reading, a list of terms and their definitions is included at the end of this section (next page).

Abstract

In the United States, high-fructose corn syrup (HFCS) has become a sucrose replacement for honey bees and has widespread use as a sweetener in many processed foods and beverages for human consumption. It is utilized by commercial beekeepers as a food for honey bees for several reasons: to promote brood production, after bees have been moved for commercial pollination, and when field-gathered nectar sources are scarce. Hydroxymethylfurfural (HMF) is a heat-formed contaminant and is the most noted toxin to honey bees. Currently, there are no rapid field tests that would alert beekeepers of dangerous levels of HMF in HFCS or honey. In this study, the initial levels and the rates of formation of HMF at four temperatures were evaluated in U.S.-available HFCS samples. Different HFCS brands were analyzed and compared for acidity and metal ions by inductively coupled plasma mass spectroscopy. Levels of HMF in eight HFCS products were evaluated over 35 days, and the data were fit to polynomial and exponential equations, with excellent correlations. The data can be used by beekeepers to predict HMF formation on storage. Caged bee studies were conducted to evaluate the HMF dose–response effect on bee mortality. Finally, commercial bases such as lime, potash, and caustic soda were added to neutralize hydronium ion in HMF samples, and the rates of HMF formation were compared at 45 °C.

Dad’s Questions

1. What are the major issues that the authors are addressing in the article?
2. Why is acid added to the syrups? Are there consequences to this?
3. What are the hypothesis and objectives of the authors in this article? Is this relevant to our problem?

Bruce had his own questions after quickly scanning the introduction of the article.

Bruce’s Questions

4. Who would be most interested in the results of this scientific study?
5. With what I know about the scientific method, what should the researchers’ next steps be to test their hypothesis and meet their objectives? How would I outline the experiments required to answer the researchers’ questions?
6. How long do honey bees live?

“Dad, I think I’ll get back to you in the morning.”

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* The scientific article excerpts and figures presented in this case study are reprinted with permission from Blaise W. LeBlanc et al. (2009), “Formation of Hydroxymethylfurfural in Domestic High-Fructose Corn Syrup and Its Toxicity to the Honey Bee (Apis mellifera),” Journal of Agricultural and Food Chemistry. Copyright 2009 American Chemical Society.
Definitions of Terms

**Codex Alimentarius Commission** – organization within the United Nations and Food and Agriculture Organization that develops international food standards to protect health and ensure fair trade.

**Dissolved solids** – material dissolved in water that can be recovered by drying. In HFCS these would primarily be sugars.  
\[
\% \text{ dissolved solids} = \frac{\text{g dissolved material}}{100 \text{ mL of solution}}.
\]

**Dysentery** – inflammation of the intestinal tract that can lead to diarrhea and death.

**Enzymes** – specialized proteins that speed up chemical reactions (catalysts).

**Fermentation** – conversion of sugar into alcohol and other substances by microorganisms such as yeast. These compounds can be toxic to bees.

**Heavy metals** – e.g., cadmium, mercury and lead.

**Hydrolyze** – to break a molecule apart chemically by the addition of water.

**Invertase** – enzyme that hydrolyzes sucrose into glucose and fructose (the mixture is called invert sugar).

**Kinetics** – the study of the rate of chemical reactions, commonly expressed as unit of product per time.

**First order kinetics** – the rate of a chemical reaction is proportional to the amount of the starting materials, i.e., the sugars.

**Mineral acids** – acids that do not contain carbon, e.g., HCl (hydrochloric acid) and H₂SO₄ (sulfuric acid); generally much stronger acids compared to organic acids.

**Organic acids** – acids that contain carbon, e.g., citric acid (lemons), tartaric acid (cream of tartar), and lactic acid (yogurt).

**Pasteurization** – a heat treatment given to foods to kill off pathogens (disease causing) microorganisms, but not all microorganisms; much more mild than sterilization.

**ppm** – parts per million, unit of concentration, i.e., microgram/gram or milligram/kilogram.

**Sucrose** – table sugar, composed of chemically bonded glucose (blood sugar) and fructose (fruit sugar).

**Thermal effect** – the results of heating. Increased temperatures generally result in faster chemical reactions.

**Transition metals** – e.g., cobalt, copper, iron and manganese.
Part II – Analysis of Commercial High Fructose Corn Syrup Samples

“Ok Dad, let’s go through this paper. What are your questions?”

Dad pulled out his notes again, “So I was looking at Table 1 and I don’t get it.”

Your Task

Examine Table 1 from the article below, and then answer Dad’s questions.

Table 1. Hydronium Ion Concentration, Hydroxymethylfurfural Concentration, and Elemental Analysis of Domestically Produced High-Fructose Corn Syrup. (The D-blend sample was a HFCS-55–sucrose syrup blend that is specifically formulated for beekeepers. The higher pH of this D-blend syrup is due to its high sucrose concentration. Sucrose is less stable at pH values <8.3 and particularly at even lower pH values (18).) Note: The HFCS samples were gifts from Roquette, Archer Daniels Midland, Mann Lake, Inc. (Cargill HFCS), and Tate & Lyle. All of the HFCS samples were received in quart containers, with the exception of Mann Lake, Inc. (Cargill HFCS), which were provided as 5 gal (18.5 L) samples.

<table>
<thead>
<tr>
<th>HFCS</th>
<th>pH</th>
<th>% HCl</th>
<th>HMFo (µg/g)</th>
<th>% Fructose</th>
<th>% C, H, N, S *</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-42</td>
<td>4.15 ± 0.04</td>
<td>0.0104 ± 0.0013</td>
<td>20.75 ± 0.004</td>
<td>42</td>
<td>29.30 ± 0.09, 5.55 ± 0.08, 0.0, 0.0</td>
</tr>
<tr>
<td>B-42</td>
<td>3.86 ± 0.02</td>
<td>0.0108 ± 0.0007</td>
<td>3.07 ± 0.002</td>
<td>42</td>
<td>29.50 ± 0.06, 7.65 ± 0.04, 0.0, 0.0</td>
</tr>
<tr>
<td>C-42</td>
<td>4.18 ± 0.04</td>
<td>0.0092 ± 0.0005</td>
<td>8.13 ± 0.000</td>
<td>42</td>
<td>29.53 ± 0.11, 7.55 ± 0.04, 0.0, 0.0</td>
</tr>
<tr>
<td>A-55</td>
<td>4.86 ± 0.17</td>
<td>0.0776 ± 0.0004</td>
<td>28.65 ± 0.005</td>
<td>55</td>
<td>31.72 ± 0.14, 7.39 ± 0.04, 0.0, 0.0</td>
</tr>
<tr>
<td>B-55</td>
<td>4.16 ± 0.04</td>
<td>0.0092 ± 0.0030</td>
<td>20.77 ± 0.006</td>
<td>56</td>
<td>31.56 ± 0.08, 7.42 ± 0.0, 0.0, 0.0</td>
</tr>
<tr>
<td>C-55</td>
<td>5.02 ± 0.02</td>
<td>0.0074 ± 0.0004</td>
<td>7.89 ± 0.004</td>
<td>56</td>
<td>31.69 ± 0.13, 7.43 ± 0.07, 0.0, 0.0</td>
</tr>
<tr>
<td>D-55</td>
<td>4.34 ± 0.06</td>
<td>0.0085 ± 0.0005</td>
<td>27.47 ± 0.003</td>
<td>55</td>
<td>31.70 ± 0.09, 7.41 ± 0.01, 0.0, 0.0</td>
</tr>
<tr>
<td>D-50 blend</td>
<td>6.09 ± 0.06</td>
<td>0.0062 ± 0.0008</td>
<td>4.05 ± 0.001</td>
<td>50</td>
<td>32.79 ± 0.05, 7.19 ± 0.03, 0.0, 0.0</td>
</tr>
</tbody>
</table>

* C – Carbon, H - Hydrogen, N - Nitrogen, and S - Sulfur

Dad’s Questions

1. What do 42, 50, and 55 stand for?
2. What do A, B, C, and D indicate?
3. What does Table 1 tell us about the relationships between HFCS samples, HFCS composition, acidity, and HMF content?

“The table doesn’t make much sense to me,” Dad continued. “Is there another way to present the data that is more meaningful?”

“Let’s get some graph paper and see if there is a pattern,” suggested Bruce.

Your Task

Graph the data in Table 1 using the graph paper that your instructor will give you. Each student should graph the data individually, then compare your results with your group, after which we will discuss the data as a class.

Bruce constructed a graph of the data in Table 1 and handed it to his father. Not surprisingly, Dad had some questions for Bruce about it.
Dad's Questions

4. Explain why you chose the particular axes on the graph.

5. What are the dependent and independent variables?

6. What conclusions can you draw from the resulting graph?

After answering Dad’s questions, Bruce moved on to the figures in the article. “Well it seems that the scientists looked at how heat affected HMF production in the syrups,” he noted.
Part III – Formation of Hydroxymethylfurfural in High Fructose Corn Syrup

Looking at Figure 2 from the article, Bruce began to consider the ways in which the specific graphs were similar and different.

Your Task

Examine the graphs below, and then answer Bruce’s and Dad’s questions.

Bruce’s Questions

1. What are the overall trends?
2. How are the graphs similar?
3. How are the graphs different?
4. How are the axes different and what are the implications?

“Ok Bruce, my turn to ask some questions.”

Figure 2. Rates of increase of HMF (ppm) with respect to time over 35 days at 31.5, 40.0, 49.0, and 68.8 °C. From LeBlanc et al. 2009. Copyright American Chemical Society.
Dad’s Questions

5. Why did the researchers select these times and temperatures?

6. What do the temperatures 40°C and 49°C equal in °F?

7. What can we conclude from these graphs?”

Bruce was really getting intrigued by the study.

Bruce’s Question

8. Given the hypothesis I developed earlier, what as a researcher would I want to test next to determine if HMF is an issue in HFCS?
Part IV – Caged Bee Studies

“Well, it seems that heat can really influence the rate of HMF formation,” Dad concluded. He started reading the next section of the article, titled “Materials and Methods,” which was associated with Figures 3 and 4 in the article.

Caged Honey Bee Experiments

As previously reported, a caged bee method was used (17). Approximately 100 freshly emerged Italian honey bees were placed into the cage for each caged bee trial (conducted in triplicate). Current research laws use committee approval for honey bee research. The caged trials were recorded in multiples of four, so that average and standard deviation counts can be reported. For all trials, the bees were fed water, ad libitum, and a plug of pollen–sugar. For the HFCS syrup formulation, we used A-55, which was determined to have 57 ppm HMF. For the higher HMF concentration solutions (100, 150, 200, and 250 ppm), pure HMF was added to the 57 ppm HFCS to obtain the desired concentrations.

“What does ad libitum mean?” asked Dad.

“I think it means that they could eat as much as they like,” Bruce suggested.

“Okay, but I have some other questions about these figures,” Dad continued.

Your Task

Examine the figures below, and then answer Dad’s questions.

![Figure 3](https://example.com/figure3.png)

Figure 3. Consumption of HFMS in milligrams of HFCS per bee at 3 days (A) and over 27 days (B). Different letters in the bar graph indicate significant differences in mortality between different HMF dosages, ANOVA, Dunnet two-sided (P < 0.005). From LeBlanc et al. 2009. Copyright American Chemical Society.
Figure 4. Mortality data from caged bee studies for bees dosed with 57, 100, 150, 200, and 250 ppm HMF. Different letters indicate significant differences in mortality between different HMF dosages, ANOVA, Dunnet two-sided ($P < 0.005$).

From LeBlanc et al. 2009. Copyright American Chemical Society.

**Dad’s Questions**

1. What is the purpose of the sucrose (table sugar) in Figure 4A?
2. Why did the researchers choose % survival at 26 days?
3. Why were these levels of HMF selected?
4. What do these graphs tell us?!!

“Whoa, slow down,” said Bruce.
Part V – Industry Response

“So Bruce, do we have a problem here?” asked Dad.

Bruce thought to himself:

1. Given all of the results presented, what overall conclusions can I draw from this study?
2. Did the researchers meet their objectives and demonstrate enough evidence to support their hypothesis?
   a. If not, what other studies may need to be done?
   b. Would these assist in answering the most important questions facing Dad, or just be of interest?

Dad came back into the room after being on the internet. “Seems the high fructose corn syrup producers were not that happy with this article. I found a response by the HFCS manufacturers.”

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Humans, bees not at risk from heated HFCS, says CRA

by Jess Halliday, 01-Sep-2009

Storage standards and temperature control for HFCS mean human health is not at risk from the formation of hydroxymethylfurural (HMF), the Corn Refiners Association asserts, which also refutes suggestions that the toxin could be a factor in honeybee colony collapse disorder.

In a new study published in the Journal of Agriculture and Food Chemistry, USDA researchers measured HMF levels in samples of HFCS over a 35 day time frame, at temperatures of 31.5, 40.0, 49.0 and 68.8ºC.

Study leader LeBlanc and team saw that HMF levels increased steadily with temperature, and that there was a dramatic jump at 49 ºC—a finding they said is important for commercial beekeepers, for manufacturers of HFCS, and for purposes of food storage.

But the CRA has called the study “flawed”, and emphasized that its members have safety measures and best practices in place.

Dr John White of White Technical Research, a consultant whose clients include the CRA, told FoodNavigator.com that there are well-established and widely-available industry storage standards for HFCS: for HFCS 55 the temperature standard is between 75 ºF and 86 ºF (23.9 ºC to 30 ºC), and for HFCS 42 between 95 ºF and 106 ºF (35 ºC to 41.1 ºC).

Moreover, the standards specify use of containers made with stainless steel or mild steel coated with stainless steel material.

“Clearly LeBlanc used extreme conditions aimed at maximizing HMF formation which contradicted both temperature and vessel composition specifications. It should be noted that any syrup source subjected to such harsh treatment would produce elevated levels of HMF,” White said, on behalf of the Corn Refiners Association.

No danger to bees or humans

The CRA and White say the risk of HMF to humans presented by the new study are also over-egged. They say that a 2000 study by Janzowski et al. discounts HMF as posing a serious health risk to humans.
The new study also suggested that the formation of HMF could be a factor in the decline in honey bee populations, known as colony collapse disorder (CCD). It leaned on a study published in 1966 by Bailey to support claims that the toxin that causes gut ulceration and dysentery-like symptoms in bees.

HFCS is given to bees to stimulate brood rearing and boost honey production. But according to White, properly stored HFCS would not pose a risk for honeybees.

He cites a study by Jachimowicz et al, published in 1975, which saw that concentrations of up to 3mg HMF per 100g of solution was harmless for bees. This would mean that the base HMF level established by LeBlanc, of 30 parts per million (ppm) is also harmless.

“Honeybee producers clearly violate published storage recommendations when they expose HFCS to excessive temperatures and store it for prolonged periods in unapproved containers.”

Nor was HFCS cited as a potential cause of CCD published this year in the Proceedings of the National Academy of Sciences; rather, ribosomal RNA degradation was seen to be the likely cause.

References
Apidologie 1975; 6:121-143. “Problems of invert sugar as food for honeybees.” Authors: Jachimowicz, T; El Sherbiny, G.

Journal of Agriculture and Food Science 2009, 57, 736907376. DOI: 10.1021/jf9014526. “Formation of hydroxymethylfurfural in domestic high fructose corn syrup and its toxicity to the honey bee (Apis mellifera).” Authors: LeBlanc, B; Eggleston, G; Sammataro, D; Cornett, C; Dufault, R; Deeby, T; St Cyr, E.

Food Chemical Toxicology 2000; 38:801-809. “5-Hydroxymethylfurfural: assessment of mutagenicity, DNA-damaging potential and reactivity toward cellular glutathione.” Janzowski, C; Glaab, B; Samimi, E; Schlatter, J; Eisenbrand, G.

After reading the article, Bruce asked: “Dad, how long did the beekeepers have the HFCS, and where did they get the HFCS from?”

Final Questions
3. Given all of the information presented, what overall conclusions can you draw from the study and response by the HFCS manufacturers?

4. What is the probability that the beekeepers may have killed off their own bees?

Homework Assignment
The local Washington State apple growers association has asked Bruce’s father to draft a position paper on the potential problems of continued use of HFCS as bee food. Your job is to write the paper for him, summarizing the major issues and findings of the articles in order to provide a fair and balanced assessment of the threat of the use of HFCS to apple growers. You should address the concerns of the various stakeholders in your decision (apple growers, bee keepers, HFCS manufacturers, and the general public). It is important to consider what is known, what is speculation, and what needs to be determined.

“Is High Fructose Corn Syrup Bad for the Apple Industry?” by Jeffri C. Bohlscheid
References Cited in the Scientific Research Article

**Original Article**

**Cited References**


