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| **A close up of a logo  Description automatically generated**  **UNIVERSITY OF TORONTO MISSISSAUGA**  **TERM TEST 3**  **BIO360H5, Winter 2023**  **Celia Hein**  **Duration – 100 min**  **Aids: Non-Programmable Calculator, Crib Sheet (3 sheets, double sided)**  *The University of Toronto Mississauga and you, as a student, share a commitment to academic integrity. You are reminded that you may be charged with an academic offence for possessing any unauthorized aids during the writing of an exam, including but not limited to any electronic devices with storage, such as cell phones, pagers, personal digital assistants (PDAs), iPods, and MP3 players. Unauthorized calculators and notes are also not permitted. Do not have any of these items in your possession in the area of your desk. Please turn the electronics off and put all unauthorized aids with your belongings at the front of the room before the examination begins. If any of these items are kept with you during the writing of your exam, you may be charged with an academic offence. A typical penalty may cause you to fail the course.*  *Please note, you* ***CANNOT*** *petition to* ***re-write*** *an examination once the exam has begun.* |

**TEST QUESTIONS**

1. This exam contains 18 problems with a total of **39 questions on pages 2 - 9**.

2.Pages 11 – 17 contain the description of a case study with three parts.

3. There is additional space for notes on pages 10 and 18.

4. Question point values add to a total of 50 points:

- Matching questions are worth 1 point each (28 points)

- Multiple choice questions are worth 2 points each (22 points).

5. Choose the single best answer for each question.

6. No marks are deducted for incorrect answers, so answer all questions.

7. Transfer all of your answers to the Scantron computer sheet. No answers on the test paper will be marked.

8. You must hand in both your Scantron computer sheet AND your test paper.

9. No questions will be answered during the test.

**SCANTRON computer sheet**

1. Follow the instructions on the sheet.

2. Use PENCIL and erase any changes completely.

3. Bubble your Form (A or B).

4. Write your student number in the boxes and bubble in the numbers in the correct columns.

5. Write your name, date, and course in the upper right in the spaces provided.

6. Do NOT write anything along the top or side of the Scantron sheet.

7. For each question bubble your answer during the time allowed.

**REFER TO CASE STUDY**

Refer to pages 14 - 15 to answer the questions in this section. On average, the larvae reared on inbred plants grew faster. This difference translated to the adult stage, where inbred-fed moths had a larger body mass, on average, than outbred-fed moths. Inbred-fed moths also had higher

**Study Design**

Familiarize yourself with the topic (page 11), then refer to the case study description on pages 12 - 13 to answer the questions in this section.

**Part 1: LN Particulate Accumulation and Age**

Refer to pages 14 - 15 to answer the questions in this section. On average, the larvae reared on inbred plants grew faster. This difference translated to the adult stage, where inbred-fed moths had a larger body mass, on average, than outbred-fed moths. Inbred-fed moths also had higher flight muscle mass, on average. Can this difference in flight muscle mass be explained by the difference in body mass alone?

**Topic: Study Design**

*2 marks* **1 Multiple choice:** What best describes the study type of Part 1? Select one answer and enter it under question 1 of the Scantron form.

(A) Laboratory experiment.

(B) Field experiment.

(C) Case-control study.

(D) Cross-sectional survey.

*2 marks* **2 Multiple choice:** Which of the following best describes the researchers’ sampling design in Part 1 of this study? Select one answer and enter it under question 2 of the Scantron form.

(A) Randomization.

(B) Blocking.

(C) Stratification.

(D) Simple Random Sample.

**Matching:** Refer to Fig. XX. For each of the statements listed below, indicate whether it is true (A) or false (B). Use questions 3 – 6 on the Scantron form, one question per statement.

*1 mark* **3** A The age of the donor is an observed, random variable.

*1 mark* **4** B The type of tissue is an observed, random variable.

*1 mark* **5** A percent particulate accumulation is an observed random variable

*1 mark* **6** B The level of replication of the analysis in Fig X was 17.

*2 marks* **32 Multiple choice:** Refer to Fig. X. Assume the authors sampled lung and mesenteric LN tissue from each individual. Which of the following statements most accurate? Select the best answer and enter it under question 32 of the Scantron form.

(A) This is paired sampling, so the conditions of simple linear regression are not met.

(B) This is paired sampling, which reduces variance of the model.

(C) This is not paired sampling because LLNs and MLNs are modelled separately.

(D) This is not paired sampling because it was not a “before and after” study.

**Topic: Hypothesis Testing**

*2 marks* **32 Multiple choice:** Refer to Fig. X. The authors noticed that particulate accumulation in in LLNs was noticeably higher for individuals with age > 40 years. If the authors were to perform a test using age categories ( < 40, 40 – 64, and ≥ 65 years) to explain mean particulate accumulation, what test should they use? Select the best answer and enter it under question 32 of the Scantron form.

(A) A Wilcoxon rank sum test.

(B) A Chi-square test.

(C) A *t*-test assuming unequal variances (two-sample *t*-test as taught in this course).

(D) A one-way ANOVA.

**Topic: One-way ANOVA**

As we have not yet learned how to perform ANOVA with two predictors, your instructor re-analyzed the data using a one-way ANOVA with the four groups shown in Fig. 9, where each group represents a combination of gender and breeding type. The R output is shown in Fig. 11.

*2 marks* **35 Multiple choice:** Refer to Fig. XX. Are the assumptions and conditions for performing an ANOVA on these data met? Select the best answer and enter it under question 35 of the Scantron form.

(A) Yes, there is no major issue with the data.

(B) No, because the equal means assumption is not met.

(C) No, because the equal variances assumption is not met.

(D) No, because the data are not independent, they are paired.

*2 marks* **35 Multiple choice:** Refer to Fig. XX. Are the assumptions and conditions for performing an ANOVA on these data met? Select the best answer and enter it under question 35 of the Scantron form.

(A) Yes, there is no major issue with the data.

(B) No, because the equal means assumption is not met.

(C) No, because the equal variances assumption is not met.

(D) No, because the data are not independent, they are paired.

**Matching:** Refer to Fig. XXX and the summary statistics in Fig. XXX. Assume the conditions for the ANOVA are met. For each of the statements listed below, indicate whether it is true (A) or false (B). Use questions 18 – 21 on the Scantron form, one question per statement.

*1 mark* **18** A The total sum of squares (SS) is 7403699.

*1 mark* **19** B CD68+CD169- was NOT statistically different from the other two groups.

*1 mark* **20** A The total df is 53.

*1 mark* **21** B The overall effect size was medium.

**Topic: Influential Points**

**Matching:** Refer to Fig. XXX and the summary statistics in Fig. XXX. Consider the two data points labeled "P1" (a male moth) and "P2" (a female moth) in Fig. 4, and their respective regression models (blue line for point P1, red line for point P2). For each of the statements listed below, indicate whether it is true (A) or false (B). Use questions 18 – 21 on the Scantron form, one question per statement.

*1 mark* **18** A Point P1 has a larger predicted value than point P2.

*1 mark* **19** A Point P2 has a larger squared residual than point P1.

*1 mark* **20** B Point P3 has a large squared residual and high leverage.

*1 mark* **21** A Point P4 has a large squared residual and high leverage.

*2 marks* **2 Multiple choice:** Which of the following is the best option for dealing with outliers or influential points. Select one answer and enter it under question 2 of the Scantron form.

(A) If removing the point reduces the p-value, you should remove it.

(B) If removing the point increases the R2, you should remove it.

(C) You should present the analysis with and without the point.

(D) If the point has a Cook’s D score > 1, you should remove it.

**Topic: Residual Analysis**

The researchers fitted a simple regression model of each marker against age. Box 1 shows how they described the regression results in the published paper. They did not comment on residual analysis, hence your instructor re-analyzed the data in R to check the residuals.

**Box 1:** " expression of the key activation markers CD80 and CD86 [CD80/86] and the phagocytic marker CD36 decreased with age specifically in CD68+CD169− macrophages in LLNs but not in MLNs. In contrast, CD209 expression was not altered significantly with age in any macrophage subset at either site. These results show that […] the expression of functional markers specifically in the CD68+CD169− subset within LLNs decreases with age, suggesting that particulates may have specific effects on macrophage function.”

*2 marks* **22 Multiple choice:** Refer to Box 1 and the residual plots in Fig. 6. What is the appropriate interpretation of the regression results of marker CD209 for the lungs and the mesentery? Select one answer and enter it under question 22 of the Scantron form.

(A) Marker CD209 was *not* statistically significant in the lungs nor the mesentery, but the effect in both sites was large enough to be relevant.

(B) Marker CD209 was *not* statistically significant in the lungs nor the mesentery, and the effect was too small to be practically relevant in the mesentery only.

(C) Marker CD209 was *not* statistically significant in the lungs nor the mesentery, but there may be a considerable risk of a type II error, more data are needed to be sure.

(D) Marker CD209 was *not* statistically significant, and the effect in both sites was too small to be biologically relevant.

(E) The model should not be interpreted; it is not valid because at least one condition has been violated.

**Matching:** Refer to Figures 6 and 7. Your instructor fitted a new, more complex model. Compare the residual plot of the new model (Fig. 7) with the residual plot of the model reported by the researchers (Fig. 6). For each of the statements listed below, indicate whether it is true (A) or false (B). Use questions 23 – 26 on the Scantron form, one question per statement.

*1 mark* **23** The distribution of the residuals is less skewed in Fig X than in Fig X.

*1 mark* **24** The model in Fig. 7 fits better in terms of the thickening of the plot.

*1 mark* **25** The model in Fig. 6 fits better in terms of the relationship being linear.

*1 mark* **26** The model in Fig. 6 fits better in terms of influential points.

*1 mark* **27 A** Both models may benefit from a log-transformation of the data.

**Topic: Regression Interpretation**

**Matching:** Refer to Box 3 and Fig. 8. Assume all models met the conditions for linear regression. For each of the statements listed below, indicate whether it is true (A) or false (B). Use questions 27 – 30 on the Scantron form, one question per statement.

*1 mark* **27** A No models showed a statistically significant, positive association with age.

*1 mark* **28** Dropping B1 increased the slope for inbred-fed moths.

*1 mark* **29** Dropping B1 increased the effect size for inbred-fed moths.

*1 mark* **30** Dropping B1 increased the p-value for inbred-fed moths.

**Case study: air pollution reduces lung immune function**

People become more susceptible to various diseases as they age, particularly to diseases in the lungs. Ural et al. (2022) investigated the effects of lifelong exposure to air pollution on lung-lymph nodes and lung immune function.

Lymph nodes are small structures found throughout the body that contain important immune cells of various types. They are vital to the body’s immune response against cancers and infectious diseases. The researchers saw that some lymph nodes were black in color and others were beige. The lungs, unlike other organ systems, are continuously exposed to the environment and carbon-based air pollutants, such as those found in vehicle exhaust, heating emissions, and fires. The researchers found that lymph nodes in the lungs (LLNs) turned black with age, unlike intestinal, mesenteric lymph nodes (MLNs). They found that long-term exposure to air pollution resulted in the accumulation of inhaled particulates in CD68+CD169- macrophages (cells with the ability to “eat” foreign bodies through phagocytosis) in LLNs. They also found that macrophages with accumulated particulates had impaired immune function.

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| Who: | * 84 deceased organ donors aged 11 – 93 years, with no history of smoking |
| What: | * **Tissue samples** (LLN, MLN): from lymph nodes in the lungs (LLN) and mesentery (MLN). * **Age** (years): of each organ donor, ranging from 11 - 93 * **Gender** (M, F): biological sex of the donor (M: male, F: female). * **Percent of LN containing particulates**: Particulate content in LNs, quantified by measuring the area (μm2) of each LN that contained black particulates * **Percent expression of immunity markers** (CD80/86, CD36, and CD209): associated with activation, phagocytosis, and phagocytic capacity |
| Why | * **Part 1:** To test the association of particulates to lymphatic region (LLN and MLN) and donor age * **Part 2:** To assess which types of macrophages accumulate inhaled particulates * **Part 3**: To test if macrophages with particulates have impaired immune function |

**Note:** The description, analyses and results presented here may differ from the original paper.

**Reference:**

* Ural, B.B., Caron, D.P., Dogra, P. *et al.* Inhaled particulate accumulation with age impairs immune function and architecture in human lung lymph nodes. *Nat Med* **28**, 2622–2632 (2022). https://doi.org/10.1038/s41591-022-02073-x

**Part 1: Lymph Nodes and Age**

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**Part 2: Macrophage Particulate Accumulation**

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**Part 3: Particulate Accumulation and Immune Function**

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| LN Type | Marker | Intercept | Slope | P-value for slope | R2 |
| LLN | CD36 | 62.02 | -0.63 | < 0.001 | 0.55 |
| CD209 | 4.89 | 0.25 | 0.140 | 0.11 |
| CD80/86 | 26.95 | -0.30 | 0.021 | 0.25 |
| MLN | CD36 | 47.63 | -0.05 | 0.822 | < 0.01 |
| CD209 | 30.78 | -0.16 | 0.38 | 0.04 |
| CD80/86 | 36.28 | 0.10 | 0.64 | 0.01 |

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