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Example Ethics Application Template for Insect Research

Project Title: Assessment of Acute Oral Pesticide Effects and Welfare Outcomes in Adult *Bombus terrestris*

Proposed Start and End Dates: 1 April 2027 – 30 September 2027

Funding Source: University internal research funding.

Project Description

1. Aims and Objectives of the Research:

To determine the effects of a test pesticide on adult worker bumblebees (Bombus terrestris) under controlled laboratory conditions. The study will: (1) quantify pesticide-related mortality and sublethal effects at predefined exposure concentrations; (2) compare exposed groups with an appropriate untreated/vehicle control; (3) apply prospective welfare monitoring and humane endpoints throughout the exposure period; and (4) generate robust data while using the minimum number of insects required to answer the research question.

2. Summary in Lay Terms (≤300 words):

Pesticides intended to control agricultural pests may also affect beneficial insects such as bumblebees. This study will investigate the effects of a pesticide on adult worker bumblebees under controlled laboratory conditions.

A total of 120 commercially reared buff-tailed bumblebees (*Bombus terrestris*) will be divided between one control group and three pesticide-exposure groups. Bees will receive pesticide orally in a sucrose solution and will then be monitored for 48 hours.

In addition to recording survival, researchers will monitor predefined indicators of welfare and functional impairment, including activity, coordination, ability to remain upright and ability to access food. Bees showing severe or sustained impairment will not be left to deteriorate until spontaneous death. Instead, predefined humane endpoints will be applied and affected individuals will be removed from the experiment and humanely dispatched.

All remaining bees will be humanely dispatched at the end of the 48-hour study because they will not be returned to their source colonies or released into the environment.

The study will provide information relevant to understanding and reducing pesticide risks to pollinating insects while incorporating measures to reduce insect use and minimise avoidable harm.

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3. Methodology Overview:

A total of 120 adult worker Bombus terrestris will be obtained from commercially reared colonies supplied by an approved supplier. Bees will be acclimatised to laboratory conditions before experimentation and individuals that are visibly injured, abnormal or otherwise unsuitable at baseline will not be enrolled in the study.

Bees will be allocated randomly to four experimental groups of 30 individuals:

- untreated/vehicle control;
- low pesticide exposure;
- intermediate pesticide exposure; and
- high pesticide exposure.

The three pesticide concentrations will be selected on the basis of existing toxicity data and preliminary/range-finding information to provide a biologically informative dose range while avoiding unnecessarily extreme exposure.

Exposure will occur orally using pesticide incorporated into a 50% (w/v) aqueous sucrose solution. The control group will receive an equivalent sucrose solution without the pesticide. Oral exposure and a minimum 48-hour observation period are consistent with the established approach for acute oral toxicity assessment in adult worker bumblebees.

Treatment allocation will be recorded before exposure and experimental observations will, where practicable, be undertaken without reference to treatment group.

Following exposure, bees will be maintained under controlled environmental conditions with appropriate ventilation and access to uncontaminated food after the defined exposure period. Environmental conditions will be monitored throughout the experiment.

Survival and welfare observations will be recorded at baseline and at predefined intervals following exposure, including more frequent observations during the period in which acute pesticide effects are most likely to occur. At a minimum, formal assessments will occur at 1, 4, 8, 24 and 48 hours following exposure, with additional checks where adverse effects are observed.

Recorded outcomes will include:

- survival;
- normal activity and locomotion;
- coordination;
- ability to remain upright and right following displacement;

- responsiveness to appropriate external stimuli;
- ability to access and consume food; and
- occurrence of abnormal behaviours or other signs of marked functional impairment.

Individuals meeting a predefined humane endpoint will be removed immediately and humanely dispatched. The time and reason for endpoint intervention will be recorded as an experimental outcome.

All surviving bees will be humanely dispatched at completion of the 48-hour observation period and death will be confirmed before subsequent disposal or tissue collection.

4. Type(s) and Number of Insects Involved:

Species: *Bombus terrestris* (buff-tailed bumblebee). Life stage: adult workers. Origin: commercially reared colonies obtained from an approved supplier; no wild-caught insects will be used.

Number of insects: 120 adult workers will be used. Bees will be allocated across four experimental groups, comprising one control group and three pesticide-exposure groups, with 30 individuals per group.

The sample size has been determined prospectively to provide approximately 80% statistical power at a significance level of $\alpha = 0.05$ to detect a moderate-to-large treatment effect between the four experimental groups. Based on a one-way four-group comparison and an anticipated effect size of approximately Cohen's $f = 0.3–0.32$, a total sample size of approximately 120 individuals is appropriate.

Thirty bees per group therefore provides sufficient replication to address the study objectives while limiting the number of insects used. No additional animals will be included to compensate for anticipated pesticide-related mortality or removal at predefined humane endpoints, as these constitute experimental outcomes.

Ethical Considerations

5. Ethical Justification:

Live bumblebees are required because the research question concerns whole-organism responses to pesticide exposure, including survival, behaviour, coordination and functional

capacity. These interacting outcomes cannot currently be reproduced adequately using cell culture, isolated tissues or computational models alone.

The potential harms include behavioural impairment, reduced mobility or feeding, loss of coordination and death following pesticide exposure. These harms are balanced against the potential benefit of generating evidence relevant to pesticide risk assessment and protection of pollinating insects.

The principles of Replacement, Reduction and Refinement have been applied as follows.

Replacement: Existing published toxicity data and available non-animal information will be reviewed before experimentation and used to inform dose selection. Live bumblebees will only be used for endpoints requiring assessment of an intact organism.

Reduction: The total number of 120 bees has been prospectively justified statistically. The study uses the minimum number of treatment groups necessary to establish an exposure-response relationship, with 30 individuals per group providing adequate power. Multiple compatible outcomes will be collected from each individual, including survival and behavioural/welfare measures, thereby maximising the information obtained from each bee. No additional animals will be included as replacements for individuals reaching humane endpoints.

Refinement: Handling will be minimised, housing and environmental conditions will be appropriate for *B. terrestris*, and the least invasive exposure method compatible with the scientific objective will be used. Welfare will be monitored prospectively using predefined criteria. Monitoring frequency will increase during periods in which adverse effects are most likely. Humane endpoints will be applied before severe or prolonged deterioration wherever this can be identified prospectively.

Animals meeting humane endpoint criteria will not be retained solely to obtain spontaneous mortality data.

6. Welfare Considerations:

Pesticide exposure may cause stress, behavioural impairment, reduced feeding or activity, loss of coordination and death. These effects are therefore anticipated and will be actively monitored.

Bees will be acclimatised before experimentation and maintained under controlled, species-appropriate environmental conditions. They will have access to suitable food and hydration except where temporary restriction is specifically required for

standardisation of oral exposure. Any such restriction will be kept to the minimum duration necessary.
Handling will be restricted to that required for allocation, dosing, welfare assessment and terminal procedures. Staff undertaking the work will be trained in handling bumblebees and recognising normal and abnormal behaviour.

Welfare monitoring
Each individual will be assessed for:

- normal posture and ability to remain upright;
- coordinated locomotion;
- ability to right itself when placed in an abnormal position;
- responsiveness;
- ability to access and consume food;
- markedly reduced or absent activity;
- tremors, uncontrolled movement or other abnormal motor behaviour; and
- evidence of moribundity or severe functional impairment.

Observations will occur at baseline and at least 1, 4, 8, 24 and 48 hours after exposure. Additional observations will be conducted where signs of deterioration are identified.

Humane endpoints
A bee will be removed from the study and humanely dispatched where any of the following occur:

- persistent inability to remain upright or right itself;
- severe loss of coordinated locomotion preventing normal movement;
- inability to reach or access food as a consequence of pesticide-related impairment;
- persistent near-complete unresponsiveness to appropriate stimulation;
- severe, uncontrolled neurological signs; or
- a combination of abnormalities indicating that meaningful recovery is unlikely and continued observation would result in unnecessary deterioration.

Where an abnormality is initially mild or equivocal, the bee will be reassessed within a shortened monitoring interval. Severe impairment will trigger immediate intervention rather than waiting until the next scheduled observation point.
The time at which a humane endpoint is reached and the reason for intervention will be recorded.

Humane dispatch
Bees reaching a humane endpoint will be dispatched immediately using the institutionally approved euthanasia method for *Bombus terrestris*. All remaining experimental bees will be dispatched following the final 48-hour assessment.

The approved SOP will specify the method, exposure duration, confirmation of loss of responsiveness and confirmation of death. Death will be confirmed before insects are disposed of or used for terminal tissue collection.

7. **Risks**

The principal risks associated with the work are occupational pesticide exposure, accidental escape of bumblebees, bumblebee stings and accidental environmental release of pesticide or contaminated material.

A formal laboratory risk assessment and pesticide COSHH assessment have been completed before commencement of the study.

Control measures include:

- appropriate PPE and chemical handling procedures;
- preparation and administration of pesticide within designated laboratory areas;
- secure storage and labelling of pesticide stocks and working solutions;
- trained personnel handling bumblebees;
- procedures for managing stings and known allergy risks;
- escape-resistant experimental containment;
- procedures for recovery of escaped insects;
- segregation of exposed insects and contaminated material; and
- disposal of pesticide waste, contaminated consumables and insect remains through approved waste streams.

No pesticide-exposed experimental bees will be released into the environment.

- Has a formal risk assessment been completed? Y

Yes. A project-specific laboratory risk assessment and pesticide/COSHH assessment has been uploaded onto OSHENS.

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Regulatory Compliance

9. Is the study regulated under the Animals (Scientific Procedures) Act (ASPA)?

- If yes, provide licence numbers and summary.
- If no, explain why not.

No

The study involves bumblebees, which are invertebrate insects and are not within the animal taxa currently protected under ASPA for scientific procedures. No vertebrates or protected cephalopods will be used.

Institutional ethical review is nevertheless being undertaken because the study deliberately exposes insects to a potentially harmful substance and includes mortality and terminal endpoints.

10. Will the project involve field collection in protected areas or of protected species?

- If yes, attach appropriate permits or explain exemption.

No.

All *Bombus terrestris* workers will originate from commercially reared colonies obtained from an approved supplier. No insects will be collected from the wild and no fieldwork will take place in protected areas.

Data and Consent

11. Data Management Plan:

Each experimental unit will be assigned a unique study identifier. Treatment allocation, pesticide concentration, exposure records, welfare observations, humane-endpoint interventions, mortality and final experimental outcomes will be recorded contemporaneously.

The reason and time of removal will be recorded for every individual reaching a humane endpoint. Humane-endpoint removals will not simply be recorded as missing data.

Data will be entered into an approved electronic data-management system and stored on secure institutional servers with appropriate access controls and backup arrangements.

Raw data, processed datasets and analysis code will be retained for a minimum of 10 years following completion of the study, in accordance with institutional research-data requirements.

Data will be quality checked prior to analysis and any exclusions or missing observations will be documented with reasons.

Non-identifiable research datasets and accompanying analysis code will be made publicly available through an appropriate research repository where contractual, intellectual-property and publication restrictions permit.

Additional Declarations

13. Potential Conflicts of Interest:

No conflicts of interest are declared.

The pesticide manufacturer has no role in study design, data collection, analysis, interpretation or decisions concerning publication.

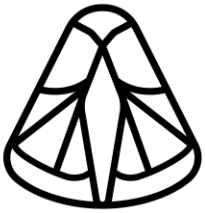
Any conflict arising during the study, including financial or professional relationships with the manufacturer or supplier of the pesticide, will be declared to the institution and disclosed in subsequent publications.

14. Third-party or External Ethical Approval (if applicable):

- State name of organisation and attach documentation.

No external ethical approval is required.

The project will proceed only following approval through the institution's internal insect research ethics/welfare review process and completion of the associated laboratory and chemical risk assessments.



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15. Incentives Offered (if any):

None.

16. Summary of Dissemination Plans:

Study findings will be submitted for publication in a peer-reviewed scientific journal and presented at relevant scientific conferences.

Methods will be reported in sufficient detail to allow the study to be interpreted and reproduced, including insect origin, husbandry conditions, pesticide exposure, numbers used, welfare monitoring procedures, humane endpoints and terminal procedures.

The numbers of insects surviving, dying and reaching humane endpoints will be reported transparently by experimental group. Animals removed at humane endpoints will be incorporated appropriately into the analysis rather than being omitted without explanation.

Results will be disseminated regardless of whether statistically significant pesticide effects are identified. Reporting negative or inconclusive findings will help reduce unnecessary repetition of comparable pesticide studies.

Where possible, underlying data and analysis code will be deposited in an appropriate public research repository following publication.