New substances: risk assessment summary, new substances notifications 22022 and 22023

Official title: New substances: risk assessment summary, New Substances Notifications 22022 and 22023 – Schedule 5 of the *New Substances Notification Regulations (Organisms)* [NSNR(Organisms)]

Notified organisms:

- A genetically modified *Drosophila melanogaster* strain with a single insert of a construct containing a gene expressing human IL-10 and a marker gene balanced over two chromosome balancers (hereafter referred to as strain 10)
- A genetically modified *Drosophila melanogaster* strain with a single insert of a construct containing a gene expressing human FGF-7 and a marker gene balanced over a chromosome balancer (hereafter referred to as strain 11)

Schedule of the NSNR(Organisms): Schedule 5 - Information Required in Respect of Organisms Other than Micro-organisms

Organism type: Insect

Use: Strains 10 and 11 are to be used as a living insect bioreactor to produce human IL-10 and human FGF-7, respectively, to be sold commercially for research and development only.

Anticipated quantity: confidential and not for disclosure.

Assessment level of concern:

- Human health hazard: Low
- Human exposure: Low
- Environmental hazard: Low
- Environmental exposure: Low

Assessment conclusion under section 64 of the *Canadian Environmental Protection Act, 1999* (CEPA): Low risk, not suspected to be toxic

Recommended action: None.

Waiver: Requested for information element 5(a) of Schedule 5 of the NSNR(Organisms), under paragraph 106(8)(a) of CEPA.

Synopsis

Drosophila melanogaster strains 10 and 11 are genetically modified common fruit flies that were notified to produce human interleukin-10 (IL-10) and human fibroblast growth factor 7 (FGF-7) for use in research and development only.

A waiver to submit data from a test to determine the pathogenicity, toxicity, or invasiveness of the notified strain under paragraph 5(a) of the *New Substances Notification Regulations (Organisms)* (NSNR(Organisms)) was recommended by the program under paragraph 106(8)(a) of the *Canadian Environmental Protection Act, 1999* (CEPA) as the information on toxicity (not capable of producing toxins) is not needed in order to determine whether the living organism is toxic or capable of becoming toxic.

Based on the evaluation of information submitted by the notifier and information from literature reviews, the risk assessment concluded that the **hazard potential** and the **exposure potential** to human health and the environment from the manufacture of the notified organisms is **low**.

The notified strains are **not suspected to be toxic** under section 64 of CEPA as there is **no evidence** to suggest that the notified organisms:

- would have an immediate or long-term effect on the environment or its biological diversity,
- may constitute a danger to the environment on which life depends, or
- may constitute a danger in Canada to human life or health.

No risk management is recommended.

Background information

As a species, *D. melanogaster* has an extensive history of safe use in research and development. It is not known to have adverse effects on other species present in the natural environment nor is it known to be a vector for disease. *D. melanogaster* strain 10 is a genetically modified common fruit fly to be used to produce human IL-10, which plays a role in immune response, cell growth and survival. *D. melanogaster* strain 11 is a genetically modified common fruit fly to be used to produce human FGF-7, which plays a role in immune response, cell growth and differentiation. Strains 10 and 11 are derived from a commercially available laboratory strain of *D. melanogaster* and will be manufactured under controlled conditions in a single contained facility in Edmonton, Alberta. The produced recombinant protein will be used for research and development purposes only.

Hazard

The environmental hazard potential of *D. melanogaster* strains 10 and 11 is assessed to be **low** because:

1) As a species, *D. melanogaster* has an extensive history of safe use in research and development. *D. melanogaster* is not known to have adverse effects on other species present in the natural

environment nor is it known to be a vector for disease. Additionally, there are several modified strains of *D. melanogaster* that are now on the Domestic Substances List (DSL) with no reports of adverse effects.

2) *D. melanogaster* strains 10 and 11 are derived from a commonly used inbred laboratory strain. Although the inserted phenotypic marker partially rescues some of the drawbacks associated with the parental strain (i.e. retinal degeneration and male copulation success), it may not be enough to offset the genetic disadvantages these strains exhibit due to inbreeding depression including progressive loss of mobility, reduced reproductive fitness, as well as impaired resistance to various forms of stress. Therefore, they are not expected to propagate effectively in the natural environment in the event of accidental release.

3) The inserted genetic material, the expressed human IL-10 in *D. melanogaster* strain 10 and the expressed human FGF-7 in *D. melanogaster* strain 11 are not known to have pathogenic or toxic adverse effects on other species present in the natural environment.

4) A waiver requested for toxicity testing was granted under paragraph 106(8)(a) of CEPA for *D. melanogaster* strains 10 and 11 as, after extensive review of the literature and information submitted by the notifier, it was concluded that additional data on toxicity was not required to determine whether the living organisms are toxic or capable of becoming toxic.

The human hazard potential of *D. melanogaster* strains 10 and 11 is assessed to be **low** because:

1) *D. melanogaster* strains 10 and 11 are a genetically modified fruit flies expressing human IL-10 and human FGF-7 under the control of a heat-shock protein such that it expresses the recombinant protein only when temperature requirements are met. The notified organism is derived from a commonly used laboratory strain,which is not known to be associated with any hazards to humans.

2) The genetic modifications used to produce strains 10 and 11 are well defined, stable and do not raise any human health concerns. None of the source (donor) organisms from which the inserted genetic material was derived are known to produce toxins. Furthermore, neither the genetic material nor the human IL-10 or human FGF-2 are associated with any pathogenicity or toxicity in humans.

3) While wild *D. melanogaster* have been reported to harbour opportunistic human pathogens, there are no reported cases of zoonotic infections attributed to the species.

4) The expressed human IL-10 and human FGF-7 are not expected to be toxic or allergenic as they are not structurally similar to any known allergens or toxins.

5) Strains 10 and 11 retain the antibiotic resistance gene for ampicillin, which is ubiquitous in the environment and inserted in the chromosome that doesn't contain genetic elements known to facilitate the horizontal transfer of a transgene. Therefore, in the unlikely event of release from the facility, no adverse human health effects are expected.

Hazards related to organisms used in the workplace should be classified accordingly under the Workplace Hazardous Materials Information System (WHMIS)[1].

Exposure

The environmental exposure potential of *D. melanogaster* strains 10 and 11 is assessed to be **low** because:

1. *D. melanogaster* strains 10 and 11 are intended for use as a living insect bioreactor in a contained facility and are not expected for release into the environment. There are no other foreseeable potential uses for the notified strains other than the production of the proteins for use in a specific commercial product to be sold for use in research and development.
2. The organism has been genetically modified to result in flies having a curly wing phenotype, however, it is indicated that this trait will be rapidly lost over a few generations. However, the containment and standard operating procedures (SOPs) used in the facility are sufficient to ensure containment of all life stages of the notified strain of *D. melanogaster*.
3. The genetic mutations of the parent strain and modifications to the notified organism result in poor stress responses, less effective reproductive behaviour, and impacted locomotion. In the unlikely event of escape into the environment, the notified organism and its genes are unlikely to propagate in external *D. melanogaster* populations.
4. Protein is harvested only from the larval life stage (which are flightless and have limited mobility), and adult flies representing only 1-2% of the total population are used for strain maintenance and propagation. The notified organism will be euthanized before extracting expressed proteins of interest.

The human exposure potential of *D. melanogaster* strains 10 and 11 is assessed to be **low** because:

1) The main source of human exposure to strains 10 and 11 is expected to be from the manufacture of *D. melanogaster* strains 10 and 11 in a Plant Pest Containment Level 1 (PPC-1) level contained facility located in Edmonton, Alberta.
2) Strains 10 and 11 are not intended for release into the environment from the facility. Containment measures in place are expected to prevent releases, and larvae will be harvested and euthanized to extract the human IL-10 from strain 10 and the human FGF-7 from strain 11. The general population is therefore not expected to be exposed to any of the live strain 10 or 11 insects.
3) In the unlikely event of an inadvertent release of straight-wing adult flies of strain 10 or 11, or if the containment measures change leading to the release of the notified organisms, environmental conditions may be favourable, since potential escapees can find warm refuges during cooler weather in human controlled environments, like all urban populations do. Nevertheless, it is noted

[1] A determination of whether one or more of the criteria of section 64 of CEPA are met is based upon an assessment of potential risks to the environment and/or to human health associated with exposure in the general environment. For humans, this includes, but is not limited to, exposure from air, water and the use of products containing the substances. A conclusion under CEPA is not relevant to, nor does it preclude, an assessment against the criteria in the *Hazardous Products Regulations,* which is part of the regulatory framework for the Workplace Hazardous Materials Information System (WHMIS) for products intended for workplace use.

that wild type fruit flies already live in close association with humans and, thus the general population are routinely exposed to the species without reported cases of adverse effects.

Risk characterization

Owing to the low potential hazard and the low potential exposure, the environmental and human health risks associated with the use of *D. melanogaster* strains 10 and 11 for production of human IL-10 and human FGF-7, respectively, is low.

Risk assessment conclusion

There is no evidence to suggest a potential risk of adverse environmental effects or propagation in external, wild *D. melanogaster* populations at the exposure levels predicted in the environment from the use of *D. melanogaster* strains 10 and 11 for production of human IL-10 and human FGF-7, respectively. The risk to the environment associated with *D. melanogaster* strains 10 and 11 is not suspected to meet criteria in paragraphs 64 (a) and (b) of CEPA.

There is no evidence to suggest a potential risk of adverse human health effects at the exposure levels predicted in the environment from the use of *D. melanogaster* strains 10 and 11 for production of human IL-10 and human FGF-7, respectively. The risk to human health associated with *D. melanogaster* strains 10 and 11 is not suspected to meet criteria in paragraph 64 (c) of CEPA. No further action is recommended.