New substances: risk assessment summary, new substances notifications 21725, 21726, 21727, and 21728

Official title: New substances: risk assessment summary, New Substances Notifications 21725, 21726, 21727, and 21728 – Schedule 5 of the *New Substances Notification Regulations (Organisms)*

Notified organisms:

- A genetically modified *Drosophila melanogaster* strain with a single insert of a construct containing a gene expressing human Prolactin and a marker gene balanced over a chromosome balancer (NSN-21725, hereinafter referred to as strain 3)
- A genetically modified *Drosophila melanogaster* strain with a single insert of a construct containing a gene expressing bovine Activin and a marker gene balanced over a chromosome balancer (NSN-21726, hereinafter referred to as strain 4)
- A genetically modified *Drosophila melanogaster* strain with a single insert of a construct containing a gene expressing TGF-beta 1 and a marker gene balanced over a chromosome balancer (NSN-21727, hereinafter referred to as strain 5)
- A genetically modified *Drosophila melanogaster* strain with a single insert of a construct containing a gene expressing bovine Albumin and a marker gene balanced over a chromosome balancer (NSN-21728, hereinafter referred to as strain 6)

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

Organism type: Insects

Use: Manufacture of strain 3 for production of the proteins human Prolactin.Fc, strain 4 for production bovine Activin-A, strain 5 for production of bovine transforming growth factor beta1 (TGF-beta1), and strain 6 for production of bovine Albumin, for use as a supplement for growing cells *in vitro* for research and development use 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*: Low risk, not suspected to be toxic

Recommended action: None

Waiver: A waiver to submit the data from a test conducted to determine its pathogenicity, toxicity, or invasiveness under paragraph 5(a) of Schedule 5 of the *New Substances Notification Regulations (Organisms)* was granted under paragraph 106(8)(a) of the *Canadian Environmental Protection Act, 1999* (CEPA) because the notified organisms are not expected to have any adverse effects on plants, invertebrates or vertebrates in the environment based on the long history of safe use of *D. melanogaster* and the lack of any identified toxin or virulence genes in the organisms or their specific genetic modifications.

Synopsis

The new genetically modified lines of *D. melanogaster* (fruit flies) were notified to the New Substances program for their manufacture and subsequent production of the recombinant proteins, namely, human Prolactin.Fc, bovine Activin-A, bovine TGF-beta1, and bovine Albumin. No other potential uses were identified for the notified organisms.

There is no evidence to suggest a potential risk of adverse environmental and indirect human health effects at the exposure levels predicted to the environment and general population in Canada from the production of the notified organisms.

It is determined that the notified organisms are not toxic or capable of becoming toxic according to the criteria under section 64 of the Act as there is no evidence to suggest that the notified organism may enter the environment in a quantity or concentration or under conditions that:

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

No risk management is recommended.

This assessment was subject to a scientific expert peer review external to the New Substances program.

Background information

The notified organisms are lines of fruit flies that were genetically modified to produce the proteins human Prolactin.Fc, bovine Activin-A, bovine TGF-beta1, and bovine Albumin. The notified organisms are derived from a common and commercially available laboratory line of *D. melanogaster* and will be manufactured under controlled conditions in a contained facility in Edmonton, Alberta. The produced recombinant proteins will be used as growth medium supplements in cell culture for research and development only.

Hazard

The environmental and human hazard potential of the notified organisms is determined to be low because:

1) The notified organisms are derived from a commonly used laboratory line known to have progressive loss of climbing ability, shortened life span, as well as impaired resistance to various forms of stress. Therefore, they are not expected to propagate in the natural environment in the event of accidental release. Additionally, the parental line is not known to be associated with any hazards to humans.
2) 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. It is not known to carry and transmit infectious pathogens from infected organisms to other organisms.
3) The inserted genetic material and the expressed proteins are not known to have pathogenic or toxic effects on other species present in the natural environment or humans. None of the source (donor) organisms from which the inserted genetic material was derived are known to produce toxins. Additionally, the methods used to produce the notified strains do not raise any human health concerns.
4) While wild *D. melanogaster* has been reported to harbour opportunistic human pathogens, there are no reported cases of zoonotic infections attributed to the species.
5) The expressed proteins in each of the notified organisms are not expected to be toxic or allergenic as they are not structurally similar to any known allergens or toxins.
6) While strains 3 and 4 retain the antibiotic resistance gene for ampicillin, in the unlikely event of release from the facility, no adverse human health effects are expected. Additionally, analysis of the amino acid sequence of strain 6 indicates the potential for allergenicity. There are reported cases in the literature of allergenicity or sensitization from exposure to the expressed protein, bovine Albumin. However, as the protein is not constitutively expressed, it is unlikely strain 6 would lead to allergic reactions in humans should exposure occur.

Exposure

The environmental and human exposure potential of the notified organisms is determined to be low because:

1) The notified organisms are intended for use as living insect bioreactors in a contained facility and are not intended for release into the environment. There are no other foreseeable potential uses for the notified strains outside of the intended use, as the genetic modification leads to the expression of a single protein in each line for use in a specific commercial product for research and development use.
2) The main source of human exposure to the notified organisms is expected to be occupational exposure from their manufacture in the contained facility. However, the contained facility and operating procedures that the notifier has put in place are sufficient to prevent escape of the

notified organisms into the environment. In addition, the genetic modifications introduced into the notified organisms negatively impacts their ability to fly. Therefore, the general population is not expected to be exposed to any of the notified organisms.

3) The genetic mutations of the parent line and modifications to the notified organisms result in reduced locomotion and reproductive fitness. Therefore, in the unlikely event of escape into the environment, the introduction and dispersal of the genes from the notified organisms the natural population is not expected.
4) In each of the notified organisms, the expressed protein of interest is harvested only from the larval life stage (which are flightless and have limited mobility), by euthanizing of the organisms following a standard protocol of protein extraction and purification. The proteins will be used in research and development only.

Risk characterization

Owing to the low potential hazard and the low potential exposure, the environmental and human health risks associated with the use of the notified organisms for the production of recombinant proteins are both low.

Risk assessment conclusion

There is no evidence to suggest a potential risk of adverse environmental and human health effects at the exposure levels predicted to the environment and general population from the use of the notified organisms for the production of recombinant proteins. The risks to the environment and human health associated with the notified organisms are not suspected to meet the criteria in paragraphs 64(a), (b), or (c) of the *Canadian Environmental Protection Act, 1999*. No further action is recommended.