

Center for Cellular and Molecular Therapeutics at
The Children's Hospital of Philadelphia (CCMT/CHOP)

AAV8-hFIX19

**Conclusions on the potential environmental impact from the
release or the placing on the market of genetically modified
organisms**

(In accordance with Part D of Schedule II, SI 500/2003)

Final – January 2013

Conclusions on the potential environmental impact from the release or the placing on the market of genetically modified organisms

On the basis of an environmental risk assessment carried out in accordance with the principles and methodology outlined in parts B and C of Schedule II SI 500/2003, the information on the points listed in parts D1 of this schedule is provided with a view to assisting in drawing conclusions on the potential environmental impact from the release of the genetically modified organism AAV8-hFIX19 for use in the following clinical trial:

A Phase 1 safety study in subjects with severe Hemophilia B (Factor IX deficiency) using a single-stranded, adeno-associated pseudotype 8 viral vector to deliver the gene for human Factor IX [Protocol # AAV8-hFIX19-101].

D.1. In the case of genetically modified organisms other than higher plants:

1. Likelihood of the genetically modified organism to become persistent and invasive in natural habitats under the conditions of the proposed release(s).

AAV8-hFIX19 is unable to replicate independently, even in the presence of a helper virus, since it lacks the *rep* and *cap* genes required for rescue/packaging.

Homologous recombination between AAV8-hFIX19 and a wild type AAV could occur if both were present in the same cell in the presence of a helper virus (triple infection). However, such recombination could only result in the exchange of the hFIX expression cassette with the *rep* and *cap* genes of the wild type virus. It is not possible for the AAV genome to contain both *rep/cap* genes and the transgene, as this is beyond the packaging limit of the virion.

The likelihood of the GMO to become persistent or invasive is therefore negligible.

2. Any selective advantage or disadvantage conferred to the genetically modified organism and the likelihood of this becoming realised under the conditions of the proposed release(s).

The selective pressure on AAV8-hFIX19 will be towards reversion to wild-type, since both gene deletions (*rep* and *cap*) are required for rescue and replication of the organism in its host species.

The transgene (human coagulation Factor IX) is not expected to confer any advantage to the GMO in terms of survival and selective pressure.

3. Potential for gene transfer to other species under conditions of the proposed release of the genetically modified organism and any selective advantage or disadvantage conferred to those species.

AAV8-hFIX19 is a replication-incompetent virus derived from AAV2/8. The genetic modifications do not affect its natural host (primate) and tissue tropism.

Primate (human) AAV serotypes are not known to actively transfer genetic material to organisms other than primates under natural conditions, although an absence of zoonosis is not documented. The potential for gene transfer to other organisms is considered negligible.

DNA derived from the plasmids used to manufacture AAV8-hFIX19 may be present at low level in the medicinal product and could theoretically be taken up and integrated into microbial genomes, although infection of microbes by AAV is not described to our knowledge. Such an event could result in transient expression of a plasmid gene, but the genes present are unlikely to confer any selective advantage and consequently will be quickly lost. The possible exception is the transfer of the gene for Kanamycin resistance, which would confer a selective advantage in certain environments, though likely not in sewerage and water systems.

4. Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the genetically modified organism and target organisms (if applicable).

The potential for environmental impact of the interactions between the GMO and humans is negligible.

The GMO will be administered intravenously to eligible patients by medical professionals in a medical facility and is unable to replicate independently, even in the presence of a helper virus. Shedding of infective virus from treated individuals is expected to be low level and transient.

5. Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the genetically modified organism with non-target organisms, including impact on population levels of competitors, prey, hosts, symbionts, predators, parasites and pathogens.

There are no known natural predators, preys, parasites, competitors or symbionts associated with AAV, although it does require helper functions of co-infecting viruses for replication in nature. The potential for environmental interactions with non-target organisms is considered negligible.

6. Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the genetically modified organism and persons working with, coming into contact with or in the vicinity of the genetically modified organism release(s).

The potential effects in humans are limited to the transmission of AAV8-hFIX19 to an unintended human recipient.

The potential magnitude of unintended spread within the human population is considered low. Cases of the transmission of AAV8-hFIX19 to an unintended human recipient are likely to be isolated, and transmission of AAV vectors to any unintended human recipient has not been reported. The medicinal product will be administered to (and handled by) a limited number of individuals. It is estimated that 4-8 patients will receive the treatment at the medical facility in Ireland. Any inadvertent exposure will be self-limiting, since AAV8-hFIX19 is unable to replicate independently, even in the presence of a helper virus, as it lacks the *rep* and *cap* genes required for rescue/packaging.

For those unintended individuals that may be exposed to AAV8-hFIX19, the potential adverse effects are expected to be of a lower severity than those expected in patients receiving considerably higher doses (immune response, potential for insertional mutagenesis and potential for germline transmission), and of no greater severity than wild type AAV, which is not known to be pathogenic.

The presence of wild-type AAV, either as a contaminant of AAV8-hFIX19 derived during manufacture, or as an indirect consequence of homologous recombination with an existing wild type AAV in an intended human recipient, is not considered to constitute an increased risk to the environment, since it is already globally endemic and is not associated with pathogenesis.

7. Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the genetically modified organism and any product derived from it, if it is intended to be used as animal feed.

Not applicable. The product will be administered to humans for the treatment of Haemophilia B.

8. Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the genetically modified organism and target and non-target organisms in the vicinity of the genetically modified organism release(s).

AAV is not known to be involved in environmental processes. It does not respire and does not contribute to primary production or decomposition processes. In its virion form, it does not display any metabolic activity.

9. Possible immediate and/or delayed, direct, and indirect environmental impacts of the specified techniques used for the management of the genetically modified organism where these are different from those used for non-genetically modified organisms.

The handling and disposal procedures used in the management of the GMO are no different to those used in medical facilities for the management of potentially infective material.