Novel Oral Poliomyelitis Type 2 Vaccine - nOPV2

GMO Deliberate Release Notification

Part 3 - Environmental Risk Assessment

April 2018

"A Phase 2, double-blind, randomized, placebo-controlled, multicenter study to evaluate the safety and immunogenicity of two novel live attenuated serotype 2 oral poliovirus vaccines candidates, in healthy adults and adolescents previously vaccinated with oral polio vaccine (OPV) or inactivated polio vaccine (IPV), compared with historical controls given Sabin OPV2 or placebo."

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List of Abbreviations

5' UTR	5' Untranslated Region
cDNA	complementary DNA
$CCID_{50}$	Cell Culture Infectious Dose 50
	i.e. dose that leads to infection in 50% of the cells
CRE	Cis-acting replication element
CU	Contained Use
cVDPV	Circulating Vaccine-Derived Poliovirus
DR	Deliberate Release
ECDC	European Centre of Disease Control and prevention
ERA	Environmental Risk Assessment
EU	European Union
FIH	First-in-Human
GMO	Genetically Modified Organism
GSK	GlaxoSmithKline
IMP	Investigational Medicinal Product
IPV	Inactivated Poliovirus Vaccine
IRES	Internal Ribosome Entry Site
ISP-WIV	Institut de Santé Publique- Wetenschappelijk Instituut Volksgezondheid
LLOQ	Lower Limit of Quantification
nOPV2	novel Oral Poliovirus Vaccine Type 2
OPV	Oral Poliovirus Vaccine
OPV2	Oral Poliovirus Vaccine Type 2
PCR	Polymerase Chain Reaction
PD_{50}	Paralysis Dose 50
	i.e. dose at which 50% of the animals show paralysis
RNA	Ribonucleic Acid
SAE's	Serious Adverse Events
SAGE	Strategic Advisory Group of Experts on Immunization
SmPC	Summary of Product Characteristics
TgPVR mice	transgenic mice carrying the human poliovirus receptor
tOPV	Trivalent Oral Poliovirus vaccine
ts	Temperature sensitive
VAPP	Vaccine Associated Paralytic Poliomyelitis
WHO	World Health Organization

Introduction

The global effort to eradicate polio through vaccination (with either the attenuated oral poliovirus vaccine (OPV) or the inactivated poliovirus vaccine (IPV)) has made significant progress with only three countries remaining with endemic wild-type poliovirus transmission—Nigeria, Afghanistan and Pakistan. This progress has been substantially impacted by the initial use of the attenuated oral poliovirus vaccines, which have as benefit compared to the inactivated vaccines, that the attenuated polioviruses present in the vaccine replicate in the gut, thereby not only providing a more "natural like" immune response but also resulting in shedding of these attenuated viruses from the gut which through the fecal-oral transmission route can lead to immunization of close contacts thereby also providing herd immunity. However, despite these advantages, most industrialized countries have transitioned to IPV, primarily because OPV has the major disadvantage of causing paralytic disease in rare cases which is due to the reversion of the attenuated virus to a more virulent form thereby causing vaccine-associated paralytic poliomyelitis (VAPP) in vaccine recipients and close contacts. Additionally, the shed vaccine virus can circulate in the community and become more virulent over time. Those circulating vaccine-derived poliovirus (cVDPV) strains also can rarely cause disease, especially in undervaccinated populations. So while OPV is more effective than IPV in interrupting transmission in settings of poor sanitation and hygiene, as long as OPV is in use, the risk for cVDPV exists and polio cannot be entirely eradicated from susceptible populations. The type 2 component of OPV, Sabin OPV2, is of particular concern as the majority of reported cases following cVDPV outbreaks that have occurred in recent years have been associated with type 2. E.g. 95 cVDPV cases have been reported in 2017 – all due to Sabin OPV2.

A (clinical) development program that aims to develop a novel oral poliovirus type 2 (nOPV2) vaccine to be used in the effort to eliminate polio as a deadly disease from the globe and prevent reappearance of it is currently underway. This nOPV2 vaccine should combine the advantages of Sabin OPV2 with increased genetic stability and reduced reversion to virulence, thereby reducing the risk of causing vaccine-related disease.

The two nOPV2 candidate vaccines that are currently under investigation are attenuated serotype 2 polioviruses derived from a modified Sabin 2 infectious cDNA clone, intended for use as oral live-attenuated polio vaccines. nOPV2 Candidate 1 (S2/cre5/S15domV/rec1/hifi3) and nOPV2 Candidate 2 (S2/S15domV/CpG40) were generated by modifying the Sabin 2 RNA sequence to improve the genetic stability compared to the Sabin 2 virus and thereby produce a more stable vaccine, i.e. less likely to revert to virulence.

In 2017, the two nOPV2 candidate vaccine strains were for the first time clinically evaluated in a first-in-human (FIH) clinical trial conducted under contained used (CU) conditions in Antwerp, Belgium (EudraCT number 2017-000908-21). The trial assessed the safety, immunogenicity, shedding and genetic stability of both candidate vaccines. Building on the

successful data of this FIH trial, both candidate vaccines are moving to a Phase 2, double-blind, randomized, placebo-controlled, multicenter study of the approximately 10^6 50% cell culture infectious dose ($CCID_{50}$) dose-level of both candidate vaccines in healthy OPV-vaccinated adults and IPV-vaccinated older adolescents and adults (15-50 years old) in Belgium to evaluate their general safety and immunogenicity. As an exploratory endpoint, virus shedding of both of the nOPV2 candidate vaccine strains will also be assessed.

In this Phase-2 trial, the nOPV2 candidate vaccine strains are administered orally by a trained medical professional in an authorized medical study site facility. After administration of the study vaccine, subjects leave the study facility and through shedding the candidate vaccines may enter the environment. In consequence, the study is considered to be a deliberate release (DR) of genetically modified organisms (GMO's) in accordance with European Directive 2001/18/EC .

Objective of the Environmental Risk Assessment

In the context of the conduct of clinical trials in the European Union with the nOPV2 candidate vaccines, this Environmental Risk Assessment (ERA) has been conducted in accordance with Annex IIA of European Directive 2001/18/EC and Commission Decision 2002/623/EC to identify and evaluate potential adverse effects of the two nOPV2 candidate vaccines, either direct or indirect, immediate or delayed, on human health and the environment which the conduct of a clinical trial with these genetically modified organisms (GMO's) may exert. It is conducted with a view of identifying if there is a need for risk management and if so, the most appropriate methods to be used.

General Principles of the Environmental Risk Assessment

This ERA is performed according to the methodology laid out in Annex IIC of directive 2001/18/EC, supplemented by the guidance notes in Commission Decision 2002/623/EC and taking into account the precautionary principle:

- Characteristics of the GMOs and its use which have the potential to cause adverse effects are identified and compared to those presented by the non-modified organism from which they are derived and its use under corresponding situations;
- The ERA is carried out in a scientifically sound and transparent manner based on the available scientific and technical data;
- The ERA is carried out on a case by case basis, taking into account the GMO's concerned, their intended use and the potential receiving environment.

1 Step 1: Identification of nOPV2 characteristics which may cause adverse effects (i.e. hazard identification)

1.1 CHARACTERISTICS OF THE RECIPIENT VIRUS, MODIFIED VIRUS AND RECEIVING ENVIRONMENT

1.1.1 Characteristics of the recipient virus Sabin 2 (Sabin OPV2)

Live attenuated Sabin 2 poliovirus

Polioviruses are small, non-enveloped viruses with a single positive strand RNA genome of approximately 7400 nucleotides. They cause poliomyelitis (polio) disease, which in a small proportion of cases can result in paralysis, which is often permanent. There is no cure for polio, it can only be prevented by immunization.

A type 2 strain (P2/712) isolated from the stools of a healthy child from New Orleans, and therefore naturally attenuated, was the basis of the recipient type 2 vaccine strain, P2/P712,Ch,2ab (Sabin OPV2) (Minor, 2015). Sabin OPV2 was derived from the isolated poliovirus type 2 strain by passaging in nonhuman cells. Attenuation of the virus in culture greatly reduces its neurovirulence and transmissibility (WHO, 2016).

Sabin OPV2 has been used as part of the trivalent live attenuated oral poliovirus vaccine (tOPV) throughout the world for many years to protect against wild-type polio type 2 and is also registered as a monovalent vaccine expected to be used primarily for outbreak response (see WHO list of prequalified vaccines at https://extranet.who.int/gavi/PQ_Web/).

In 2012, the Strategic Advisory Group of Experts on Immunization (SAGE) recommended the withdrawal of the type 2 component (OPV2) of tOPV from routine use globally by April 2016. This withdrawal, which was motivated by the eradication of wild-type 2 polioviruses in 1999, occurred according to plans.

In Belgium, tOPV was used as the mandatory polio vaccination until the end of 2000. Since January 2001 tOPV was replaced with the inactivated polio vaccine (IPV) in the mandatory routine vaccination schedule. OPV vaccines are still recommended to be used in case of exceptional epidemiological circumstances (Ministerial decree of 18 September 2000).

Genotypic and phenotypic determinants of attenuation

Reduced neurovirulence of the Sabin OPV2 strain compared to the wild-type poliovirus type 2 strain is attributed to a large extent to a mutation in the 5' untranslated region (UTR) of the Sabin OPV2 RNA at nucleotide position 481. The attenuating mutation G481A results in reduced thermodynamic stability of a particular portion of the structure, designated domain V which forms part of the Internal Ribosome Entry Site (IRES). Mutations in other regions also

play a role in attenuation e.g. C2909U, resulting in a threonine for isoleucine at position 143 in the capsid protein VP1 (Ren *et al.*, 1991; Macadam *et al.*, 1993).

Sabin OPV2 also exhibits a temperature-sensitive phenotype, which is a marker of attenuation and maps to the 5' UTR mutation although other changes are also involved (Macadam *et al.*, 1991).

Life cycle

The life cycle of poliovirus occurs within the confines of the cytoplasm of infected cells. After entry into the host cell cytoplasm, the RNA genome is translated by the host cell translation machinery as a single polyprotein, which is cleaved during translation by genome-encoded proteases into three regions referred to as P1, P2, and P3. The P1 region is further processed into viral structural (capsid) proteins, while P2 and P3 encode the proteins involved in protein processing and viral replication. Viral replication takes place in the host cell cytoplasm and the final step is the release of poliovirus virions through lysis of the infected cell.

As the lifecycle is entirely cytoplasmic, poliovirus does not have the ability to integrate into the host cell genome and even if such an event could occur, the infected cells would be destroyed by the virus.

Host range and transmission (spread)

Poliovirus, from which Sabin OPV2 is derived, has a highly restricted host range. When Sabin OPV2 is administered orally, the attenuated poliovirus attaches to the poliovirus receptor (CD155) on the cytoplasmic membrane of the cells within the host's gastrointestinal system. Non-primates and their cell cultures lack the human poliovirus receptors and are refractory to natural infection. There is no evidence that non-primates are infected by poliovirus in nature or could serve as reservoirs. While filter-feeding shellfish can concentrate poliovirus from polluted waters, poliovirus does not replicate in these organisms and is purged when the source of contamination is removed (Dowdle & Birmingham, 1997).

Some lower primates are susceptible to poliovirus infection. However, even in the cynomolgus monkey, considered to be one of the species more susceptible to oral infection, poliovirus excretion is low and of short duration with very limited transmission. There are reports that suggest that chimpanzee, and possibly other higher nonhuman primates, may acquire poliovirus in nature. However, they are less susceptible than humans and it is unlikely that populations are sufficient to sustain poliovirus transmission in the absence of human infection (Dowdle & Birmingham, 1997).

Poliovirus is transmitted by infected humans directly or indirectly by droplets or aerosols from the oropharynx and by fecal contamination of hands, eating utensils, food and water. Epidemiologically, at least 80% of transmission appears to be person-to-person (fecal-oral or oral-oral) (Dowdle & Birmingham, 1997).

The recipient strain, Sabin OPV2, does not occur naturally. Sabin OPV2 is typically cultured in cells, such as Vero cells derived from African green monkey epithelial cells, and is used for vaccine production. Once administrated to a human subject, the vaccine virus can replicate and can be shed in nasopharyngeal secretions and feces.

Data from non-immune subjects given mOPV1, mOPV2, or tOPV demonstrate oropharyngeal virus shedding for up to 20 days (median of 6-10 days), and more than 75% of primary OPV recipients shed virus in the feces for a period that is highly variable (mean 20-28 days, range of up to 8-10 weeks in less than 10% of subjects at low and intermittently detectable levels) (Gelfand *et al.*, 1959; Ghendon & Sanakoyeva, 1961; Ginter *et al.*, 1961; Henry *et al.*, 1966; Asturias *et al.*, 2016). The virus is eventually eliminated by normal immune defenses.

Shedding is reduced when the vaccine is administered to individuals who had previously received OPV or IPV (Fine & Carneiro, 1999). Fewer than 5% of children who have received 3 or more doses of OPV shed virus from the oropharynx following a challenge OPV dose. Fecal shedding is also reduced to 22-37% of recipients who shed the challenge virus for a mean of 5-7 days at titers that are approximately 3 $\log_{10}$ lower than non-immune OPV recipients (Horstmann *et al.*, 1959; Ghendon & Sanakoyeva, 1961; Henry *et al.*, 1966; Onorato *et al.*, 1991). Fine and Carneiro (1999) describe that after challenge with Sabin type 1 37% of OPV-vaccinated subjects will experience fecal shedding of an average of 2.2 log $CCID_{50}$ per gram of feces for a duration of on average 4.6 days, resulting in a total excretion of 4.05×10^4 $CCID_{50}$ shed on average in the faeces per person challenged (Fine & Carneiro, 1999). Recent data suggest even lower post-challenge fecal shedding in OPV-vaccinated subjects. Data collected on fecal shedding in OPV-vaccinated children after a challenge with Sabin type 2 indicate that only 8.1% experience shedding on day 7 post challenge with a median titer of 3.1 log $CCID_{50}$ in shedders. This dropped to 4.8% and 2.9 log $CCID_{50}$ resp. by day 28 post challenge (Asturias *et al.*, 2016). These reports are consistent with results of a trial conducted in Belgium by the University of Antwerp—"A Phase 4 study to evaluate the safety and immunogenicity of monovalent oral polio vaccine type 2 in healthy OPV-vaccinated adults" (EudraCT 2015-003325-33)—where 13% of OPV-vaccinated adults had detectable Sabin-2 viral RNA in stools collected day 7 post-administration. No viral RNA was detected in stools beyond Day 14 (i.e. samples tested at Day 21 and Day 28).

While IPV shows a similar effect to OPV in inducing pharyngeal immunity, and thus may reduce oral-oral transmission, the effect of IPV on reducing shedding in the stool, and thereby the impact on reducing fecal-oral transmission, is limited (Bandyopadhyay *et al.*, 2015). Across studies, 63-100% of IPV vaccinated children demonstrate fecal excretion at 7-10 days after the OPV challenge (Horstmann *et al.*, 1959; Ghendon & Sanakoyeva, 1961; Henry *et al.*, 1966; Onorato *et al.*, 1991; Mohammed *et al.*, 2008). The effect of IPV vaccination on the duration and titer of virus shed is greater (mean 12 days, 50% shorter and $10^{4,1}$ $tcid_{50}$/gram, 1 $\log_{10}$ lower than non-immune OPV recipients, respectively) (Ghendon & Sanakoyeva, 1961; Hird & Grassly, 2012). Fine and Carneiro (1999) describe that after challenge with Sabin type 1 in previously IPV-only vaccinated subjects, 74% will experience fecal shedding of an average of 4.1 log $CCID_{50}$ per gram of feces for a duration of on average 12.3 days, resulting

in a total excretion of 1.72 x 10^7 $CCID_{50}$ shed on average per person vaccinated (Fine and Carneiro, 1999). Specifically for Sabin OPV2, fecal shedding data gathered in a study in Panama after challenge of previously IPV-vaccinated children with Sabin OPV2 indicates that one week after challenge 78.3% experience shedding with a median fecal titre of 4.45 log $CCID_{50}$, which dropped to 46% and 2.75 log $CCID_{50}$ resp. after 3 weeks (Sáez-Llorens *et al.*, 2016). A study in Chile found slightly higher percentage of fecal shedders (up to 92.4%) and median log $CCID_{50}$ in shedders (up to 6.7) after Sabin 2 challenge of IPV-vaccinated children (O'Ryan *et al.*, 2015).

Pathogenicity and adverse effects due to genetic instability

Sabin OPV vaccination is generally well tolerated; however, fever, vomiting, diarrhea and allergic/anaphylactic reactions (latter presumably due to hypersensitivity to vaccine components or manufacturing process residues, e.g. neomycin, polymyxin) have been described after vaccination with GSK's oral trivalent poliomyelitis vaccine (Polio Sabin mono two SmPC).

On rare occasions, particularly in immunodeficient infants, aseptic meningitis and encephalitis have been reported after OPV (WHO, 2014).

The main reasons for discontinuation of Sabin OPV2 in tOPV are the risks for vaccine-associated paralytic polio (VAPP) in vaccinees or their close contacts and the emergence of circulating vaccine-derived polioviruses (cVDPVs) that have acquired transmissibility and neurovirulence. As Sabin strains can replicate in the gut of vaccine recipients, there is a possibility that the attenuating mutations in the vaccine strains revert and that virulence of the vaccine strain is restored. This reversion of attenuating mutations during OPV replication in humans is the underlying cause of VAPP and VDPVs. This is further discussed below.

Genetic instability leading to reversion to neurovirulence

Sabin OPV2 has inherent genetic instability at the attenuating positions which leads to reversion to virulence. After oral administration of the Sabin OPV2 vaccine, reversion at the 481 site occurs quickly in the human gut, leading to (shedding of) a virus with increased neurovirulence. For example, virus shed only one week after trivalent OPV (tOPV) vaccination in children was shown to contain 33-96% reverted (481G) type 2 virus (Laasri *et al.*, 2005). This reversion results in a domain V structure which is more thermodynamically stable than the attenuated sequence, and rare but serious cases of vaccine-associated disease are caused by strains that have reverted this attenuation determinant.

The other mutation which accounts for some attenuation of the Sabin OPV2 vaccine is in amino acid 143 in VP1, resulting in a threonine to isoleucine substitution. Selective pressure in the human gut against this mutation appears to be lower than for nucleotide 481, with about half of the VP1-143 codons exhibiting changes in samples isolated 3 weeks after vaccination (Macadam *et al.*, 1993).

Also recombination between polioviruses and other type C enteroviruses has been described and is one of the mechanisms that can lead to the reversion of the attenuated vaccine strain to a neurovirulent phenotype in cVDPVs (e.g. Rakoto-Andrianarivelo *et al.* 2007; Holmblat *et al.*, 2014).

Vaccine-associated paralytic polio (VAPP)
In extremely rare cases, OPV vaccination has led to paralysis in vaccinees or in their unimmunized or immunodeficient close contacts. Onset of VAPP usually occurs 4-30 days following receipt of OPV or within 4-75 days after contact with a recipient of OPV.

In industrialized countries, VAPP occurs mainly in early infancy associated with the first dose of OPV and decreases sharply with subsequent OPV doses. A review of VAPP cases in the United States from 1990-1999 was performed by Alexander *et al.* (2004). The rate of VAPP for vaccine recipients is estimated as 1 case per 6.4 million doses (1 case per 1.4 million first doses and 1 case per 35.4 million subsequent doses). For contacts of vaccine recipients the rate is estimated at 1 case per 13.3 million doses OPV given (1 case per 4.5 million first doses and 1 case per 23.6 million subsequent doses) (Alexander *et al.*, 2004). A review of VAPP cases in Hungary, which used monovalent OPVs almost exclusively from 1961-1981, estimates the VAPP risk for Sabin OPV2 at 0.56 per million doses given (Estívariz *et al.*, 2011).

Persons with primary immunodeficiency disorders are at much higher risk of VAPP (approximately 3000-fold) than the general population, but VAPP is rare even in this group.

The Sabin OPV2 strain was found to be the cause of 30% of cases of VAPP following OPV vaccination (Platt *et al.*, 2014).

Vaccine-derived polioviruses (VDPVs)
Through prolonged replication in either individuals with primary immunodeficiency disorders or in a community with low OPV coverage, vaccine-derived polioviruses (VDPVs) can emerge. These are characterized by a VP1 sequence divergence of greater than 1% from the parental strain for type 1 and 3 and greater than 0.6% for type 2, indicating prolonged replication (or transmission) of the vaccine virus. Though the definition of VDPVs is based on the estimated duration of replication, it is likely that many of these have re-acquired the neurovirulence and transmissibility characteristics of wild-type poliovirus. Especially among isolates of type 2 VDPVs, the mutations controlling neurovirulence are frequently found to have reverted (Minor, 2009; Macadam *et al.*, 1991; Macadam *et al.*, 1993).

In regions with low vaccination coverage rates, where competing wild-type poliovirus has been eliminated and where epidemiologic conditions (e.g. low socioeconomic status, poor hygiene/sanitation and crowding) favor poliovirus transmission, VDPVs have the potential for sustained circulation and when there is evidence of person-to-person transmission in the community these are called circulating VDPVs (cVDPVs). Strikingly, the majority of reported cases following cVDPV outbreaks that have occurred since 2000 have been

associated with type 2 (Burns *et al.*, 2014). While wild-type poliovirus type 2 has been eradicated since 1999, type 2 Sabin virus accounts for more than 95% of cVDPV outbreaks detected in recent years (Bandyopadhyay *et al.*, 2015).

In a small number of individuals with primary immunodeficiency, OPV immunization can lead to infections which persist for prolonged periods, resulting in chronic shedding of VDPVs that show increased neurovirulence, the iVDPVs. No iVDPV is known to have generated secondary cases with paralysis. (WHO, 2016). The occurrence of iVDPVs is very rare. Since the introduction of OPV in 1961 to March 2015, approximately 100 persons with primary immunodeficiencies worldwide have been found to be excreting iVDPVs. Like cVDPVs, type 2 iVDPVs are the most prevalent (65%) (Diop *et al.*, 2015).

Natural habitat and geographic distribution

Sabin OPV2 vaccine virus does not occur naturally. Sabin OPV2 for vaccine production is typically cultured in cells, such as Vero cells derived from African green monkey epithelial cells.

When used for vaccination, the virus can replicate in the human host and shedding of viral particles in feces or oropharyngeal secretions can occur. This is in most cases of short duration and usually does not result in circulation of vaccine virus in the population. However, in countries where OPV is used, cVDPV outbreaks have been described in settings with poor immunization coverage. In 2015, 12 cases of cVDPV of type 2 were reported in Guinea, Myanmar, Nigeria and Pakistan. In 2016, 2 cases of cVDPV of type 2 were reported in Nigeria and Pakistan. In 2017, 95 cases of cVDPV of type 2 were reported in the Democratic Republic of the Congo and the Syrian Arab Republic *(http://polioeradication.org/polio-today/polio-now/this-week/circulating-vaccine-derived-poliovirus/).* In 2018, up to March 2018, three cases of cVDPV type 2 have been reported, all in the Democratic Republic of the Congo *(http://polioeradication.org/polio-today/polio-now/this-week/).*

Interaction with, and effects on, other organisms in the environment

As poliovirus has a very narrow host range, effects on organisms besides humans are expected to be limited. There are no reports of transmission of the Sabin OPV2 vaccine strain to organisms other than humans outside of laboratory settings (experimental infection of transgenic mice expressing the human poliovirus receptor).

As mentioned above, recombination with type C enteroviruses is possible.

Ability to survive in the environment

Polioviruses are resistant to inactivation by many common detergents and disinfectants, including soaps, but are rapidly inactivated by exposure to ultraviolet light (WHO, 2016). Also dilute solutions of formaldehyde or free residual chlorine can inactivate polioviruses (Dowdle & Birmingham, 1997).

Poliovirus from infected stool has been reported to have survived in fresh water for 188 days at 4°C under laboratory conditions. However, in nature survival will depend on physical, chemical and biological factors in the environment. The estimation as used by WHO is that at ambient temperatures a 90% decrease in infectivity is expected every 5.5 days in fresh water and every 2.5 days in seawater (Dowdle & Birmingham, 1997). Duizer *et al.* (2016) use a more conservative estimate of 90% decrease in infectivity every 17.5 days in fresh water and every 7 days in seawater at 18.5°C.

At ambient temperatures, a 90% decrease in infectivity occurs in sewage every 26 days, (WHO, 2003). Also, sewage treatment as commonly practiced will substantially reduce virus concentrations. A reduction by 0.7-2 $\log_{10}$ (5 to 100 times) is assumed (Duizer *et al.*, 2016).

Poliovirus may survive in soil for weeks or months, often longer than in water. In temperate climates, poliovirus infectivity in soil was found to decrease by 90% every 20 days in winter and every 1.5 days in summer (Dowdle & Birmingham, 1997).

Polioviruses do not form biological survival structures. There is no indication that the survival and the ability to form survival structures would be different for Sabin OPV2.

Involvement in environmental processes
Sabin OPV 2 is not involved in any environmental process.

Availability of appropriate therapies and prophylactic measures
No specific anti-viral drugs are available for poliomyelitis. Treatment consists of supportive, symptomatic care during the acute phase, including respiratory support in cases with respiratory muscle paralysis. Neuromuscular sequelae are mitigated by physiotherapy and orthopedic treatment (WHO, 2016).

OPV and IPV effectively prevent disease from poliovirus, including paralytic poliomyelitis. The risk of asymptomatic infection is likely to be higher among IPV-vaccinated individuals than among OPV-vaccinated, but both vaccines significantly reduce the risk of infection and the overall quantity of viruses shed in the event of infection (ECDC, 2013).

1.1.2 Characteristics of the Genetically Modified nOPV2 candidate vaccines

Genetic modifications compared to the recipient live attenuated Sabin OPV2 poliovirus strain
The two nOPV2 candidate vaccines were constructed from cDNA matching the Sabin OPV2 sequence. Genetic modifications were introduced to improve genetic stability and make the strains less prone to reversion to virulence. With the exception of the polymerase modifications in candidate 1 (resulting in two amino acid substitutions), all purposeful modifications are silent modifications of the RNA sequence designed to improve the stability of the attenuated phenotype. There are no heterologous or new proteins expressed by these candidates as a result of the modifications.

The modifications of the nOPV2 candidate vaccine strains aimed at stabilizing the genetic sequence against reversion compared to the Sabin OPV2 strain are summarized below:

- *5' UTR domain V modifications (S15 dom V)*
  In both nOPV2 candidate vaccines, the sequence of RNA structural domain V in the 5' untranslated region (5' UTR) (nucleotides 468-535) has been replaced with the equivalent region of the virus S15 (nucleotides 471-538) (Macadam *et al.,* 2006). In Sabin OPV2 a single attenuating mutation (nucleotide 481A) results in thermodynamic instability in domain V, which can quickly revert during replication in the gut following administration of the Sabin OPV2 vaccine. The modifications in the S15 domain V eliminate the potential for further stabilization of the structure by a single nucleotide change. As the mechanism of attenuation in strains with the S15 domain V is the same as in Sabin strains, a similar level of attenuation is expected, however with increased stability of the attenuated phenotype as multiple mutations would be required to result in a more thermodynamically stabilized domain V RNA stem-loop structure and increased neurovirulence.

- *CRE relocation*
  In nOPV2 Candidate 1 (S2/cre5/S15domV/rec1/hifi3), an additional modification has been made to drastically reduce the chances of loss of the S15 dom V by recombination events.

  The cis-acting replication element (CRE) which is located in the 2C gene (nucleotides 4447-4499) is a highly conserved structural RNA element; a 14-nucleotide unpaired terminal loop, presented on a suitably stable stem and is essential for genome replication (it templates the uridylylation of VPg, the protein primer for negative-strand RNA synthesis) (Goodfellow *et al.,* 2003). Without it the virus is not viable.

  In order to prevent loss of the S15 dom V by a single recombination event, the CRE in its normal location in the 2C gene (nucleotides 4447-4499) of nOPV2 Candidate 1 has been disrupted by a number of point-mutations without altering the protein coding sequence and a new CRE was inserted between nucleotides 120 & 121 in the 5'UTR. In addition, two UAG triplets were engineered into the sequence of the stem of the CRE so that in the event that this CRE were to somehow relocate by recombination back to its normal position in 2C, it would encode two STOP codons which would prevent translation of 2C and all of P3, rendering the virus non-viable. This strategy further increases the genetic stability of the modifications.

  The result of this additional modification is that the attenuation determinant in domain V cannot be removed by a single recombination event with another enterovirus without also removing the CRE which would render the virus non-viable. Restoration of domain V would require multiple recombination events with another enterovirus; either two simultaneous recombination events on either side of domain V or a sequential process where first the CRE in the 2C gene would be restored followed by a

separate recombination event to replace the domain V. The attenuated phenotype is therefore expected to be significantly more genetically stable.

- *Polymerase modifications for enhancing stability and reducing recombination*
  In nOPV2 Candidate 1 (S2/cre5/S15domV/rec1/hifi3), the polymerase gene 3D has been mutated so that amino acids at positions 38 (rec1 modification) and 53 (hifi3 modification) have been replaced. The rec1 modification is made to further reduce the chances of recombination occurring at any position in the genome and the hifi3 modification is expected to improve genetic stability by increasing the fidelity of the viral RNA polymerase. As mutation and recombination have been found to be important for poliovirus spread and virulence (Xiao *et al.*, 2016, Vignuzzi *et al.*, 2006), these modifications are expected to result in a more stable attenuated phenotype.

  The amino acid changes to the polymerase are not expected to cause any additional toxicity as poliovirus replication is cytopathic. As the poliovirus polymerase does not have a human counterpart and the mutations do not increase likeness to human proteins, also no altered immune response to this protein is expected.

- *P1 codon de-optimization*
  In nOPV2 Candidate 2 (S2/S15domV/CpG40), synonymous codon usage in the capsid protein coding region (P1) has been de-optimized by increasing the proportion of CpG dinucleotides to 40%. A total of 95 codons were changed resulting in an increase of 94 $CpG_{2\text{-}3}$ and a loss of 7 $CpG_{3\text{-}1}$, yielding a net increase of 87 CpG over Sabin OPV2. All substitutions are synonymous (i.e., not resulting in an amino acid change) so that surface antigens remain unaltered. (Burns *et al.*, 2006; Burns *et al.*, 2009) This CpG40 modification results in improved attenuation of the virus and the phenotype is expected to be genetically stable since it would require multiple mutations to produce significant reversion.

To summarize:

- nOPV2 Candidate 1 (S2/cre5/S15domV/rec1/hifi3) carries the 5’ UTR domain V modification (S15 dom V), the CRE relocation and the polymerase modifications rec1 and hifi3.
- nOPV2 Candidate 2 (S2/S15domV/CpG40) carries the 5’ UTR domain V modification (S15 dom V) and the P1 codon de-optimization.

Evidence of genotypic and phenotypic attenuation and stability of the nOPV2 candidate vaccine strains

Presence of determinants of attenuation of the recipient strain
The major determinant of attenuation of the recipient Sabin OPV2 strain is located in the 5' UTR of the Sabin OPV2 RNA at nucleotide position 481. Because in both nOPV2 candidate vaccines the sequence of RNA structural domain V in the 5' UTR has been replaced with the equivalent region of the virus S15, this site is not present in the candidate vaccine strains. As a result, the Mutant Analysis by PCR and Restriction Enzyme Cleavage (MAPREC) assay which is recommended by WHO TRS 980 Annex 2 to monitor the molecular characteristics of the virus seeds is not meaningful. To monitor consistency in the dom V region with the S15 sequence, the results from deep sequencing conducted on the candidate nOPV2 vaccines are used. Testing on research seeds, the master viral seed and working viral seed was carried out and showed that all introduced mutations were present.

To test whether the nOPV2 candidate vaccines exhibit the temperature-sensitive (ts) phenotype of Sabin OPV2 which is a marker of attenuation, the two nOPV2 candidate vaccines are cultured in Vero cells and the overall ts phenotype compared with Sabin OPV2. Candidate 1 shows similar productivity to Sabin OPV2 at lower temperatures and a similar susceptibility to culture at evaluated temperatures. Candidate 2 shows similar productivity to Sabin OPV2 at lower temperatures but increased susceptibility to culture at evaluated temperatures.

Both these tests are carried out during the release testing of the virus to be used in this clinical trial.

Non-clinical evidence of attenuation
Neurovirulence is tested during manufacture of the nOPV2 vaccine strains using the standard WHO Monkey Neurovirulence Test. The attenuated phenotype of the candidate vaccine strains has been confirmed by monkey neurovirulence test on the working seed lots, 3 lots of final bulk drug substance for each candidate strain as well as the clinical trial drug product lots.

In addition, to further investigate the attenuation level of the nOPV2 candidate vaccine strains, non-clinical neurovirulence testing was carried out on the research seeds in a TgPVR mice model (transgenic mice expressing the human poliovirus receptor (Tg66-CBA)). The dose which causes a 50% paralysis rate in the mice (PD_{50}) for Sabin OPV2 in this model was 5.6 $\log_{10}$ $CCID_{50}$. Candidate 1 induced no paralysis in any mouse at dose levels up to 8.3 $\log_{10}$ $CCID_{50}$, indicating a significant decrease in neurovirulence as compared to Sabin OPV2. Whereas the PD_{50} for Candidate 2 was 6.3 $\log_{10}$ $CCID_{50}$, indicating that Candidate 2 may be slightly less neurovirulent than Sabin OPV2. Both candidates are therefore expected to be at least as attenuated in humans as the recipient strain Sabin-2, with Candidate 1 likely even more attenuated.

Stability of the attenuated phenotype in cell culture conditions
The absence of a relevant animal model that includes replication and shedding of polioviruses following oral dosing makes a definitive assessment of genetic stability in vivo difficult prior to human clinical trials. However, culture of Sabin OPV2 in animal cells at physiological temperatures has been shown to result in reversion in a manner similar to that seen in human shedding samples (Macadam *et al.*, 2006). As discussed earlier, most notable among these changes is the reversion of the domain V attenuating mutation (481A) to the wild-type nucleotide (481G). Because of the temperature sensitive phenotype, selective pressure towards reversion is high at physiological temperatures.
Therefore, to test the potential for reversion to a more neurovirulent phenotype of the candidate vaccine strains, an experiment was set up where after 10 passages in mammalian (Vero) cells under conditions which promote genetic drift, neurovirulence testing in TgPVR mice was carried out. Both nOPV2 candidates are much more phenotypically stable than Sabin OPV2 following the ten passages in Vero cells. Specifically, Sabin OPV2 increased in virulence more than 1000-fold while Candidate 2 showed a marginal (less than 10-fold) loss of attenuation and Candidate 1 showed no apparent loss of attenuation. Deep sequencing confirmed that both candidates retained all the deliberately introduced modifications and no changes known to be associated with reversion to neurovirulence were observed.

The candidates are therefore expected to be more resistant to reversion in the human gut than Sabin OPV2.

Stability of the attenuated phenotype after human administration
Preliminary data were obtained with the candidate nOPV2 vaccines in a FIH Phase 1 clinical study performed in Belgium in 2017, evaluating the general safety, immunogenicity, viral shedding and genetic stability of two candidate nOPV2 vaccines in IPV-only vaccinated adults (18-50 years old) (EudraCT number 2017-000908-21). As an exploratory analysis, samples from subjects that shed the candidate vaccines in stools were tested for neurovirulence in the above mentioned mice model as well as by deep sequencing. Data collected to date support the phenotypic stability (lack of significant reversion) of the nOPV2 candidates. No meaningful increases in neurovirulence could be detected and sequencing confirmed that there were no reverting mutations in the main site of attenuation which are analogous to the domain V A481G reversion in Sabin OPV2. Annex 3 of the technical dossier provides a summary of the results of the Phase 1 study conducted with the nOPV2 candidate vaccines.

<u>Life cycle</u>
The life cycle of the nOPV2 candidates is expected to be the same as for Sabin OPV2. As the lifecycle is entirely cytoplasmic, poliovirus does not have the ability to integrate into the host cell genome and even if such an event could occur, the infected cells would be destroyed by the virus.

Host range and transmission

As there are no meaningful modifications of the structural proteins compared to Sabin OPV2, no changes in tropism should occur and the nOPV2 candidate vaccines are not expected to have a different host range or a different mode of transmission.

The Phase 1 study confirmed that the nOPV2 candidate vaccines are shed following administration to IPV vaccinated human subjects. Shedding was detected in fecal samples with a median duration of approximately 4 and 2 weeks for Candidate 1 and 2 respectively (longest period of shedding detectable by PCR was 89 days). Nasopharyngeal swabs taken at all timepoints were all negative for all participants. These results are generally in line with expectations for individuals with an IPV-only vaccination history who are given Sabin OPV2 although fecal shedding seemed to be somewhat longer than described in literature for Candidate 1. Further details on shedding in the Phase 1 study is provided in Annex 3 of the technical dossier.

Pathogenicity and adverse effects due to genetic instability

The modifications aim to obtain a vaccine which is at least as immunogenic as the widely-used Sabin OPV2, combined with a strong reduction -if not elimination- of the risk of reversion to neurovirulence.

As the non-clinical safety profile shows that the candidate nOPV2 strains are at least as attenuated as the recipient Sabin OPV2 strain the immediate pathogenicity of the nOPV2 candidates is expected to be at worst similar to that of the recipient Sabin OPV2 virus. The production in Vero cells of the candidate vaccine strains is common for OPV vaccines and the excipients used match those of BioFarma's WHO Pre-Qualified OPV vaccines. In the FIH trial, immune responses to both candidates were clearly evident and safety results showed that the candidate vaccines were well tolerated with no serious adverse events (SAEs). None of the solicited adverse events were severe, and most were mild. Results of the Phase 1 study are summarized in Annex 3 of the technical dossier.

As discussed in Section 1.1.1, the most serious adverse event associated with the use of Sabin OPV2 is paralytic disease due to reversion to neurovirulence whereby the attenuating mutations are lost during replication in the human gut after administration. In case of reversion of Sabin OPV2, the pathogenicity approaches that of wild type poliovirus with prolonged circulation. Non-clinical evidence mentioned above suggests that the candidate nOPV2 strains are more resistant to reversion in the human gut than Sabin OPV2. Also preliminary data from the Phase 1 clinical study with the two candidate nOPV2 vaccines support the phenotypic stability of the nOPV2 candidates. Therefore, the risk of paralytic disease due to reversion to a more neurovirulent phenotype is considered to to be lower with the nOPV2 strains compared to Sabin OPV2.

Natural habitat and geographic distribution

The two nOPV2 candidate vaccines do not occur naturally. When used for vaccination, some replication in the human host similar to Sabin OPV2 is expected. As mentioned above, the

data from the FIH study have shown that fecal shedding of both candidate vaccine viruses occurs following administration with median durations about 2 and 4 weeks for respectively Candidate 1 and Candidate 2. However, no oropharyngeal shedding has been observed. If used in countries with poor immunization, further circulation in the population could occur, as observed for Sabin OPV2 (type 2 cVDPVs, see Section 1.1.1).

Interaction with, and effects on, other organisms in the environment
Theoretically, the same possibilities for recombination with other C enteroviruses may exist for the candidate nOPV2 vaccines as for the recipient Sabin OPV2 virus (see Section 1.1.1). There is however no evidence for circulation of Group C enteroviruses in the local environment.

Ability to survive in the environment
In principle no difference is expected compared to Sabin OPV2 as none of the genetic modifications result in changes of the viral structural proteins so survival and sensitivity to inactivating agents is expected to be the same.

Involvement in environmental processes
As for wild type poliovirus and Sabin OPV2, the candidate nOPV2 vaccines are not expected to have an impact on environmental processes.

Effectivity of prophylactic measures
As the genetic modifications do not change the structural proteins compared to Sabin OPV2, prior vaccination with OPV or IPV is expected to provide the same level of protection against possible adverse events associated with the nOPV2 candidate strains or genetic variants thereof as it would for those caused by Sabin OPV2 or Sabin OPV2-derived viruses.

1.1.3 Characteristics of the receiving environment

The purpose of the release is to conduct a Phase 2, multicenter trial to evaluate safety, immunogenicity and viral shedding of the two nOPV2 candidate vaccine strains in subjects who have been previously vaccinated with OPV or IPV. Study site facilities are planned to be located in Antwerp and Ghent and participants will be recruited in Belgium.

The vaccine will be administrated orally by a trained medical professional in an approved study site facility. After administration of the study vaccine at the clinical study site, subjects will leave the study facility. As observed in the FIH study, following the administration of the nOPV2 candidate vaccines, fecal shedding is expected to occur.

Humans are the only host naturally present in the receiving environment.

Poliovirus vaccination has been mandatory in Belgium since 1966 and the rate of vaccination of the inhabitants is extremely high. From the first mass vaccination campaigns starting in 1958 and in mandatory routine vaccination from 1966 up to 2001, OPV has been used for

vaccination. Starting in 2001, OPV was replaced by IPV, still under mandatory routine vaccination policy. The latest studies from 2012 show coverage with the third dose of IPV of 98.7% in Brussels, 98.9% in Flanders and 99.2% in Wallonia (Sabbe et al., 2015). Polio immunization coverage for Belgium in 2016 was estimated to be 98%. Polio immunization coverage in nearby countries is similar, with estimates for 2016 as follows: Netherlands-95%, Germany-94%, France-97%, Luxembourg-99%, United Kingdom-94% (WHO Global Health Observatory data repository, consulted December 9, 2017). The likelihood of a vaccinated person developing poliomyelitis is very low, regardless of whether the person was vaccinated with OPV or IPV.

As the rate of polio vaccination coverage in Belgium and in the European Union (EU) (ECDC 2014) is high due to mandatory routine vaccination, there are also no large groups of susceptible individuals that could support circulation of poliovirus. ECDC highlights specific vulnerable groups that are under-vaccinated: the groups of highest concern for propagated outbreaks are orthodox Christian groups in the Netherlands and the large Roma populations in the south-eastern parts of the EU. For risk groups such as refugees and asylum seekers from polio endemic countries and travelers to such countries, guidelines have been formulated to ensure appropriate vaccination.

In view of the possibility that Group C enteroviruses, including polioviruses and the derived Sabin OPV2 and candidate nOPV2 vaccines, can recombine in humans if co-infection occurs, it is of interest that there is no evidence for circulation of type C enteroviruses in the Belgian population in recent years (Personal communication from Prof. M. Van Ranst, Rega Institute, KULeuven, national reference laboratory on enterovirus typing for the period 2011-2016/2017).

1.1.4 Conclusion on possibly relevant characteristics of the candidate nOPV2 GMO vaccines

Based on the information provided above, genetic instability that defines the reversion to virulence which can lead to VAPP and cVDPV, shedding leading to possible transmission, and the recombination properties with C group Enteroviruses are the relevant characteristics to consider in assessing possible adverse effects the candidate vaccines may cause. This also considering the environment of release, in which humans are the only relevant species to which the candidate vaccines can be transmitted, yet a human population which has high coverage of OPV or IPV vaccination. Apart from that, the biological degradation in nature and the sensitivity to selected physicochemical agents are other relevant characteristics to assess the possible adverse effect on the environment.
Data from the FIH study conducted with the candidate nOPV2 vaccines provide preliminary evidence of the characteristics of the candidate vaccines especially with respect to shedding and genetic stability (See Annex 3 of Technical dossier).

1.2 N-OPV2 CHARACTERISTICS WHICH MAY CAUSE ADVERSE EFFECTS

The genetic modifications that were made to the two nOPV2 candidate vaccines compared to the Sabin OPV2 RNA sequence are mutations that were introduced to improve phenotypic stability and make the strains less prone to reversion to virulence. With the exception of the polymerase modifications in Candidate 1 (resulting in two amino acid changes), all modifications are silent modifications of the RNA sequence designed to improve the stability of the attenuated phenotype. No heterologous proteins are expressed. As such, the genetic modifications are not expected to cause any additional adverse effects compared to Sabin OPV2. While the FIH trial had a limited sample size and no comparator was included, the nOPV2 candidates were well-tolerated and no SAEs were reported.

However, as the recipient organism Sabin OPV2 is known to have characteristics which may cause adverse effects – briefly summarized above - and taking into consideration that Sabin OPV2 is being withdrawn from the tOPV vaccine used worldwide in April 2016 and has not been used in Belgium since 2000, these characteristics of the recipient organism are also being considered for the ERA of the GMO's based on Sabin OPV2. Where appropriate, available data on how the modifications made to the nOPV2 candidate vaccine strains influence these characteristics of the recipient organism is discussed.

1.2.1 Characteristics which may cause adverse effects on human health

1.2.1.1 Potential pathogenicity of nOPV2

Sabin OPV2 has been used as part of tOPV throughout the world for many years to protect against wild-type polio type 2 and is also registered as a monovalent vaccine expected to be used primarily for outbreak response. It is generally well tolerated by vaccine recipients. However, side effects including extremely rare cases of VAPP have been reported after OPV use, which in 30% of cases of VAPP following OPV vaccination were attributed to the Sabin OPV2 strain (see Section 1.1.1).

As the nOPV2 candidate vaccines are expected to replicate in the human gut and to elicit a similar immune response as the current Sabin OPV2, adverse events that are associated with OPV2 use may potentially also occur with nOPV2.

1.2.1.2 Potential altered pathogenicity of genetic variants of nOPV2

As described in Section 1.1.1, reversion of the attenuating mutations in Sabin vaccine strains is well-known. Back mutations, site suppression mutations, recombination and a steady drift in molecular sequence can occur and this reversion of attenuating mutations is the underlying cause of the rare cases of VAPP observed in OPV recipients and their close contacts (WHO, 2014). In addition, reversion of attenuating mutations is also found in virtually all cVDPVs (Burns *et al.*, 2014).

Manufacture of the nOPV2 vaccine strains is strictly controlled to prevent the presence of any adventitious viruses and reversion is strictly monitored by testing each batch for neurovirulence and identity. However, upon administration to human subjects, the nOPV2 candidate vaccines are expected to replicate in the gut, which could result in the accumulation of mutations and possibly also recombination with related viruses if a subject would be infected with such a virus at the time of vaccination. Therefore the possibility that genetic variants of the nOPV2 strains could arise and exert indirect adverse effects on human health needs to be considered.

1.2.2 Characteristics which may cause adverse effects on effects on the environment

Poliovirus has a very narrow host range. Humans are the only natural host that is present in the environment. It does not infect plants or animals with the exception of very rare cases of infection of higher non-human primates. Infections of higher non-human primates with wild-type polioviruses have been observed in the past. However, these are very rare events and it is unlikely that poliovirus transmission can be sustained in these populations in the absence of human infections (Dowdle and Birmingham, 1997). There are no reports that the Sabin OPV2 vaccine strain has caused infections in organisms other than humans outside of a laboratory setting using transgenic mice expressing the human poliovirus receptor. While filter-feeding shellfish can concentrate poliovirus from polluted waters, poliovirus does not replicate in these organisms and is purged when the source of contamination is removed (Dowdle and Birmingham, 1997). The modifications made to the nOPV2 candidate vaccine strains are not expected to change host range or tropism of the virus, therefore no adverse effects on non-human organisms in the environment are expected.

Recombination between polioviruses and other type C enteroviruses is possible. However, this is dependent on co-infection in humans and as these viruses are human pathogens, no adverse effects on non-human organisms in the environment are expected if such a recombination event would result in a new virus.

Poliovirus does not respire and does not contribute to any primary production or decomposition process. In its virion form, it does not have any metabolic activity. None of the modifications made to the nOPV2 candidate vaccine strains would be expected to alter these traits and therefore the effect on environmental processes.

Survival in the environment is finite and depends on physical, chemical and biological factors. None of the modifications made to the nOPV2 candidate vaccine strains would be expected to alter the sensitivity to inactivating agents or the survivability in the environment. nOPV2 candidate vaccine in the environment outside of a human host would naturally degrade.

Taken together, no potential adverse effects of the nOPV2 candidate vaccine strains on non-human organisms in the environment, ecosystems or environmental processes could be identified.

1.3 MECHANISM THROUGH WHICH ADVERSE EFFECTS MAY OCCUR

It is known that once administrated to a non-immune subject as part of tOPV, the recipient organism Sabin OPV2 from which the candidate vaccines are derived, can replicate and can be shed in nasopharyngeal secretions and feces. Poliovirus is transmitted by infected humans directly or indirectly by droplets or aerosols from the oropharynx and by fecal contamination of hands, eating utensils, food and water. Epidemiologically, at least 80% of poliovirus transmission appears to be person-to-person (fecal-oral or oral-oral) (Dowdle & Birmingham, 1997). In an unvaccinated population, vaccine virus can easily be transmitted within and to a lesser extent outside the household (WHO, 2016).

None of the modifications made to the nOPV2 candidate vaccine strains would be expected to eliminate these risks and there is currently no data available on shedding of the nOPV2 candidate vaccines upon administration to non-immune recipients. However, shedding (in particular nasopharyngeal shedding) is reduced when the vaccine is administered to individuals who had previously received OPV or IPV (see Section 1.1.1). Preliminary data obtained with the candidate nOPV2 vaccines in a FIH Phase 1 clinical study, confirm that in IPV-primed subjects fecal shedding occurs following administration of the nOPV2 candidate vaccines, but no nasopharyngeal shedding could be detected. Therefore, shedding of the nOPV2 candidate vaccine strains is expected and transmission of nOPV2 must be considered as a potential mechanism by which adverse effects potentially associated with nOPV2 or genetic variants thereof could be realized. This is further discussed in Section 3.2.3.

1.4 CONCLUSIONS

Taking into account the nature of the recipient organism Sabin OPV2, the genetic modifications resulting in the nOPV2 candidate vaccine strains and the receiving environment (in which humans are the only relevant species to which the candidate vaccines can be transmitted), the potential adverse effects that the nOPV2 candidate vaccine strains may exert by conducting the clinical trial are:

- Direct effects due to pathogenicity of the nOPV2 candidate vaccine strains on people not participating in the trial.
- Indirect effects due to altered pathogenicity of a genetic variant of the nOPV2 candidate vaccine strains on people not participating in the trial.

Potential indirect effects could be either immediate (upon transmission of a variant from a study subject to an unintended human) or delayed (in case a genetic variant would be able to circulate in the population for a prolonged period of time after the study).

No potential adverse effects of the nOPV2 candidate vaccine strains on non-human organisms in the environment, ecosystems or environmental processes could be identified.

2 Step 2: Evaluation of the potential consequences of each adverse effect should it occur and the magnitude of the consequences, i.e. hazard characterization

2.1 DIRECT ADVERSE EFFECTS OF NOPV2

As the non-clinical safety profile shows that the nOPV2 candidate vaccine strains are at least as attenuated as the Sabin OPV2 strain and safety results from the FIH study showed that the candidate vaccines were well tolerated (see Section 1.1.2), consequences of shedding and subsequent possible transmission of a nOPV2 candidate vaccine strain are expected to be at worst similar to those associated with use of Sabin OPV2.

While OPV vaccination is generally well tolerated by vaccine recipients, fever, vomiting, diarrhea and allergic/anaphylactic reactions (latter presumably due to hypersensitivity to vaccine components or manufacturing process residues, e.g. neomycin, polymyxin) have been described after vaccination with the GSK oral trivalent poliomyelitis vaccine (Polio Sabin mono two SmPC) containing the recipient Sabin OPV2 vaccine used to construct the candidate nOPV2 vaccines. On rare occasions, particularly in immunodeficient infants, aseptic meningitis and encephalitis have been reported after OPV (WHO information sheet).

As discussed before, the main reason for discontinuation of Sabin OPV2 are however the risks for vaccine-associated paralytic polio (VAPP) in vaccinees or their unimmunized or immunodeficient close contacts, where following administration of Sabin OPV2, reversion of attenuating mutations has occurred upon replication of the vaccine virus in the gut. A review of VAPP cases in Hungary, which used monovalent OPVs almost exclusively from 1961-1981, estimates the VAPP risk for Sabin OPV2 at 0.56 per million doses given (Estívariz *et al.*, 2011).

This is however less likely with the nOPV2 candidate vaccine strains compared to Sabin OPV2 as the modifications that were made are intended to stabilize the attenuated phenotype (see Section 1.1.2).

nOPV2 Candidate 1 (S2/cre5/S15domV/rec1/hifi3) carries the 5' UTR domain V modification (S15 dom V), the CRE relocation and the polymerase modifications rec1 and hifi3. As multiple mutations to the S15 dom V would be required to result in a more thermodynamically stabilized domain V RNA stem-loop structure and increased neurovirulence, this domain can only be restored by multiple recombination events with related enteroviruses because of the CRE relocation, and the polymerase modifications further reduce the chance of mutation and recombination occurring, the genetic variants that could arise from the nOPV2 Candidate 1 are much less likely to have reverted to neurovirulence than those arising from Sabin OPV2.

Also in nOPV2 Candidate 2 (S2/S15domV/CpG40) which carries the 5' UTR domain V modification (S15 dom V) and the codon de-optimization in the capsid protein coding region (P1), multiple mutations to the S15 dom V would be required to result in a more thermodynamically stabilized domain V RNA stem-loop structure and increased neurovirulence, and also multiple mutations would be required to produce significant reversion of the P1 codon de-optimization. Possible variants resulting from recombination between nOPV2 Candidate 2 and related type C enteroviruses could swap the 5'UTR of the nOPV2 strain with the corresponding domain of the related enterovirus. However, the attenuating mutations in the capsid region would be retained, which by themselves have a modest effect on attenuation. Therefore, genetic variants that could arise from the nOPV2 Candidate 2 are less likely to have reverted to neurovirulence than those arising from Sabin OPV2.

While there is no specific anti-viral treatment for VAPP (treatment consists of supportive, symptomatic care), as the genetic modifications do not change the structural proteins compared to Sabin OPV2, prior vaccination with OPV or IPV is expected to provide the same level of protection against possible adverse events associated with the nOPV2 candidate strains or genetic variants thereof as it would for those caused by Sabin OPV2 or Sabin OPV2 derived viruses. The vast majority of the Belgian population (> 98 %) has been vaccinated as a result of mandatory poliovirus vaccination since 1966 (see Section 1.1.3).

Therefore, consequences of shedding and potential subsequent transmission of a nOPV2 vaccine strain to an unintended human subject could be:

- In the case of transmission to an immune-competent individual who has received OPV or IPV vaccinations in the past, consequences are expected to be minimal as prior immunization would protect the individual from the possibility of developing paralysis, and also reduce the amount and duration of shedding the vaccine virus, minimizing chances of person-to-person spread. Therefore, the magnitude of the potential consequences of transmission of an nOPV2 strain to these individuals is considered negligible.
- In the case of transmission to an immune-competent individual who has not received polio vaccination in the past, the consequences would be at worst similar to those of vaccination with Sabin OPV2. OPV is generally well tolerated, however in rare cases adverse events such as fever, vomiting, diarrhea, aseptic meningitis and encephalitis and VAPP have been reported. Meaning that neuroparalytic events may be possible, and these individuals are also likely to shed more virus than previously vaccinated individuals. Although VAPP is considered a serious negative effect, the very low incidence of VAPP that has been reported, leads to the conclusion that the magnitude of the potential consequences of transmission of a nOPV2 strain to these individuals can be considered low.
- Persons with primary immunodeficiency disorders are at much higher risk of VAPP (approximately 3000-fold) than the general population, but VAPP is rare even in this group. In this group there is also a possibility of prolonged shedding.

Therefore, also for people with immunodeficiency the magnitude of the potential consequences of transmission of a nOPV2 strain to these individuals is considered low.

Taking into account the manner of the release in the setting of a clinical trial, the individuals who may be at risk of unintended exposure to the nOPV2 candidate vaccine strains are:

- Study site personnel involved in the administration of the nOPV2 candidate vaccine strains or sample preparation
- Close contacts of the study participants (partners and family members directly in contact with the study participants, sharing facilities, consuming food handled by the study participants)
- Secondary contacts of the study participants

However, widespread dissemination of the nOPV2 vaccine strains is expected to be severely limited due to:

- Administration of the candidate nOPV2 vaccines to study participants with full OPV or IPV vaccination in the past. Shedding is expected to be reduced and of shorter duration in these subjects compared to subjects who have never been vaccinated before. No nasopharyngeal shedding was seen in the Phase I trial in IPV-primed subjects with these candidates. Therefore, it is expected that fecal shedding will be the most important potential route of transmission and that the likelihood of oral-oral transmission is minimal.
- Very high vaccination coverage rate and existing immunity to polio in the vast majority of the population as a result of mandatory vaccination (OPV from 1966-2000, IPV from 2001 on) in Belgium. Therefore there are no large groups of susceptible individuals that could support circulation.
- Limited persistence in the environment outside of a human host, any virus shed from study participants and released in the environment will eventually be degraded.

2.2 INDIRECT ADVERSE EFFECTS OF GENETIC VARIANTS OF NOPV2

Mutations and recombination events are common in polioviruses. Even though the candidate nOPV2 vaccine strains were designed to be less prone to reversion to virulence compared to Sabin OPV2, until the nature of shed virus has been fully characterized in clinical trials, one must take into account that genetic variants of the nOPV2 strains could potentially that have increased virulence. Immediate adverse effects caused by genetic variants of the nOPV2 strains could potentially include VAPP in unimmunized or immunodeficient persons and could also happen in a delayed manner if any genetic variant would be able to circulate for a prolonged period in the human population (thereby becoming a cVDPV).

Preliminary data from the Phase 1 clinical study with the two candidate nOPV2 vaccines show that in shed virus isolated from stool samples, variations in genetic sequence do occur, but no reverting mutations in the main site of attenuation could be detected and there was no meaningful increase in neurovirulence of these viruses compared to the original nOPV2 strains. Therefore, consequences of transmission of a genetic variant of a nOPV2 vaccine strain to an unintended human subject would likely be less serious and at worst similar to those of exposure to genetic variants of the recipient Sabin OPV2 strain. As reversion of attenuating mutations upon replication of the vaccine virus in the gut is the underlying cause of paralytic disease following administration of OPV, the magnitude of the immediate potential consequences of shedding and possible subsequent transmission of genetic variants of nOPV2 is considered the same as those described for exposure to nOPV2 described in the section above, i.e. negligible for immune-competent fully vaccinated individuals, low for unvaccinated immune-competent individuals and low for people with immunodeficiency.

The individuals who may be at risk of unintended exposure to genetic variants of the nOPV2 candidate vaccine strains during the study and could experiencing immediate consequences are:

- Study site personnel that comes in contact with the study participants during return visits or involved in the handling of clinical samples
- Close contacts of the study participants (partners and family members directly in contact with the study participants, sharing facilities, consuming food handled by the study participants)
- Secondary contacts of the study participants

Widespread dissemination of genetic variants of the nOPV2 vaccine strains, and thereby also the potential for delayed consequences in the population following circulation of such a strain as a cVDPV, is expected to be extremely limited due to the factors mentioned in the section above. If it were to occur, the magnitudes of the potential consequences would be negligible for immune-competent fully vaccinated individuals, low for unvaccinated immune-competent individuals and low for people with immunodeficiency.

2.3 CONCLUSIONS

The magnitude of the potential consequences of shedding and potential subsequent transmission of the nOPV2 candidate vaccines or their genetic variants to unintended human subjects is considered negligible for immune-competent fully vaccinated individuals as prior vaccination with OPV or IPV protects them from adverse events such as VAPP. In view of the very high vaccination coverage rate (98%) and existing immunity to polio in the vast majority of the population as a result of mandatory vaccination in Belgium, most of the subjects in the potential area of the release will be part of this group. Therefore there are also no large groups of susceptible individuals that could support circulation and thus lead to delayed consequences.

For people who are immune-competent but who have not received polio vaccination in the past or who are immunocompromised, the magnitude of the consequences of shedding and potential subsequent transmission of the nOPV2 candidate vaccines or their genetic variants rated as low, as VAPP could potentially occur in very rare cases, and this risk is most likely very much reduced compared to the recipient Sabin OPV2 strain in view of the available data supporting the phenotypic stability of the nOPV2 strains.

3 Step 3: Evaluation of likelihood of occurrence of each identified potential adverse effect

3.1 MANNER, ENVIRONMENT AND SCALE OF RELEASE

The nOPV2 candidate vaccine strains are intended to be used as investigational medicinal product (IMP) in a proposed Phase 2 trial of a 10^6 $CCID_{50}$ dose-level of both candidate vaccines in healthy OPV-primed adults (age range 18 to 50 years) and healthy IPV-only primed adults and adolescents (15 to 50 years) to be conducted in Belgium to evaluate the safety, immunogenicity and virus shedding of the nOPV2 candidate vaccine strains.

One dose will be approximately 10^6 $CCID_{50}$ in 0,3 mL (6 drops) which will be delivered via a spoon.

A total of 332 study subjects is foreseen (200 prior OPV-vaccinated and 132 prior IPV-only vaccinated).

- 50 OPV-vaccinated adults to receive 1 dose of nOPV2 candidate 1 (Group 1);
- 50 OPV-vaccinated adults to receive 2 doses of nOPV2 candidate 1 (Group 2), administered 28 days apart;
- 50 OPV-vaccinated adults to receive 1 dose of nOPV2 candidate 2 (Group 3);
- 50 OPV-vaccinated adults to receive 2 doses of nOPV2 candidate 2 (Group 4), administered 28 days apart;
- 44 IPV-only vaccinated subjects of which approximately 24 adults and approximately 20 adolescents will each receive 2 doses of nOPV2 candidate 1 (Group 5);
- 44 IPV-only vaccinated subjects of which approximately 24 adults and approximately 20 adolescents will each receive 2 doses of nOPV2 candidate 2 (Group 6);
- 44 IPV-only vaccinated subjects of which approximately 24 adults and approximately 20 adolescents will each receive each 2 doses of placebo (Group 7).

Taken together, a total of 238 doses (238 x 10^6 $CCID_{50}$) of Candidate 1 and 238 doses (238 x 10^6 $CCID_{50}$) of Candidate 2 is foreseen to be administered during the study.

The nOPV2 candidate vaccines will be administered orally as a single dose administered at a single timepoint (Day 0) or administered twice (Days 0 and 28). Participants will remain at the clinical trial site for at least 30 min following the administration of vaccine after which the participants will leave the study facility.

Following the administration of the nOPV2 candidate vaccines, shedding is expected to occur and the location of the participants during shedding events is not known. The national territory is considered the wider potential release area covered by under this GMO Deliberate Release application, which complements the Clinical Trial Application submitted to the Belgian FAMHP's for the trial to be conducted exclusively in Belgium.

3.2 POTENTIAL MECHANISMS AND LIKELIHOOD OF EXPOSURE

3.2.1 Potential for exposure during administration

The nOPV2 candidate vaccine strains will be administered orally (6 drops of study vaccine delivered via a spoon). Administration will be performed by trained health care professionals. Administration procedures and precautions will be put in place to minimize risk of exposure during administration.

Considering the minimal amount of manipulation needed to administer the study vaccine solution and the lack of risk for aerosol formation, the potential for exposure during administration is considered to be low.

3.2.2 Potential for release into the environment from the study site

The study will be conducted at approved clinical trial facilities. Instructions for disposal of waste and for the handling and cleaning of accidental spills and breakages will be put in place. The potential for exposure of the environment at the study site is considered negligible.

3.2.3 Potential for exposure following administration

As nOPV2 candidate strains are also expected to replicate in the gut following administration, some shedding is expected. However, study subjects participating to the planned study will have been previously vaccinated with either OPV or IPV, which is known to reduce shedding (see Section 1.1.1).

The available literature discussed in Section 1.1.1 is mainly investigating the effects of prior vaccination with OPV or IPV on shedding after a challenge with OPV in children. This may not be fully predictive of shedding in the planned study as the fraction of subjects shedding and the titer and duration of shedding after an OPV challenge dose is not only determined by the number of OPV or IPV doses that have been received in the past but also by the titer of neutralizing antibodies in the serum that is present at the time of the challenge dose (Behrend *et al.*, 2014; Alexander *et al.*, 1997). For this reason, data from studies conducted by the

University of Antwerp in Belgium in which OPV- and IPV-primed adults (matching the population of participants for the planned Phase 2 study) who received either Sabin 2 or the nOPV2 candidate vaccines, is also considered when evaluating the potential for shedding in the planned study.

As shown in a Phase 4 study conducted by the University of Antwerp (EudraCT 2015-003325-33) with monovalent oral polio vaccine type 2 in healthy OPV-primed adults, shedding occurred in about 13% of OPV-vaccinated adults at Day 7 post administration and less than 3% at Day 14 post administration and no shedding was observed beyond Day 14. Titers in positive samples were very low (under or at the 2.75 LLOQ (lower limit of quantification) for $CCID_{50}$ determination). This is overall in line with the published literature.

Preliminary data obtained with the candidate nOPV2 vaccines in the first-in-human Phase 1 clinical study performed in Belgium, confirm that in IPV primed subjects, fecal shedding occurs following administration of the nOPV2 candidate vaccines, with median durations of fecal shedding of about 4 and 2 weeks for Candidate 1 and 2 respectively. However, no nasopharyngeal shedding was observed in this study. Observed titers during peak shedding and duration of fecal shedding were overall in line with the published literature for individuals with an IPV-only vaccination history who are given Sabin OPV2. Fecal shedding was longer for Candidate 1 compared to Candidate 2. The shedding period for Candidate 1 also seemed to be somewhat longer than described in literature with Sabin OPV2 although most studies have not examined shedding beyond 28 days.

Therefore, taking into consideration the study population for the planned Phase 2 study (OPV- or IPV-vaccinated), some fecal shedding is to be expected in the planned study and the potential for exposure of people not participating in the study to virus shed from the study participants needs to be considered. However, the vaccination status of the trial volunteers should limit nasopharyngeal shedding (as shown in the Phase 1 study) and therefore minimize the possibility of oral-oral transmission of the GMO.

Material shed via feces may enter septic tanks and/or the sewage system. Shed vaccine virus could also be released to the direct environment of the subject if good hygiene practices are not followed.

Potential for exposure through direct contact with shed material

The potential for exposure of close contacts of the study subjects or study site staff handling samples is considered highest during the peak of shedding, which is typically around a week after oral dose. If good hygiene is not practiced during the period that shedding occurs, contacts of the subjects may be exposed by faecally shed virus particles.

Whether or not such an exposure to shed virus would lead to infection of the close contacts of the study subjects or site staff (who will have been previously vaccinated with OPV and IPV) is uncertain. OPV virus transmission studies have shown that under conditions of high

preexisting immunity, transmission is limited and reinfection is dose-dependent. Contacts also often show abortive excretion and while previous IPV-vaccination is not fully protective against reinfection, duration of excretion is reduced and also pharyngeal excretion is reduced (Fine and Carneiro, 1999).

The likelihood that site staff or close contacts of study participants would be infected if exposed to virus shed from the study participants is therefore considered low. Also a low potential for exposure of other unintended recipients who are secondary contacts of the study participants cannot be excluded. However the likelihood of further dissemination into the population is considered negligible. This is due to the very high degree of vaccination for polio in the population (>98 %). As a result in almost all cases these people will have been fully vaccinated against polio and therefore will experience limited shedding. Also, their own contacts, who they could potentially transmit the virus to, will also in almost all cases have been fully vaccinated and therefore the are less likely to become infected by a exposure to a small dose of shed virus. Research into VDPVs (see Section 3.4 for further detail), supports the notion that in highly immunized populations circulation of polioviruses cannot be sustained.

Potential for exposure through contaminated wastewater

Unintended human subjects who are not close contacts of the study participants or part of the study staff could in theory also be exposed by contact with wastewater that is contaminated with fecal material containing virus particles shed by study participants.

Material shed via feces may enter septic tanks and/or the sewage system. Any shed virus entering these systems will immediately be diluted and eventually degrade naturally and/or be removed during sewage treatment. This was also observed in a recent wild type poliovirus type 2 shedding event following an accidental exposure in the Netherlands. Stools of the infected person were released into the sewage system for 10 days after the exposure. Sewage monitoring downstream of the residence of the infected operator showed that virus could be detected up to 20 days after the last discharge of positive stool into the sewage system, after which no further virus could be detected (Duizer *et al.*, 2017). As these are environments that are generally only accessed by trained professionals who take precautions to avoid exposure to the various pathogens that are expected to be present in these environment, it is unlikely that unintended human subjects would be exposed to shed virus that may be temporarily present in septic tanks or the sewage system.

As most wastewater will eventually be discharged in rivers following wastewater treatment, the possibility that unintended human subjects could be exposed to residual amounts of shed virus present in the water that is discharged from wastewater treatment plants into rivers needs to be considered. Activities such as swimming or fishing in contaminated rivers could potentially be a source of exposure. Assuming a hypothetical worst case scenario where all subjects who participate in the planned study would shed the full amount of expected shed virus after each dose at the same time and at the same place, the total amount of virus shed by

all study participants would amount to 1.5 x 10^9 $CCID_{50.}$ This takes into account the total viral output per person as described by Fine and Carneiro (1999), who provide an estimate of total shedding for previously IPV- and OPV-vaccinated subjects. It is further based on 88 doses given to previously IPV-only vaccinated subjects who are expected to shed 1.72 x 10^7 $CCID_{50}$ per person dosed and 388 doses given to previously OPV-vaccinated subjects expected to shed 4.05 x 10^4 $CCID_{50}$ after each dose. It includes IPV-only vaccinated subjects who receive their second dose of nOPV2 in the study.

1.5 x10^9 $CCID_{50}$ is far below the recently reported accidental release of 10^{13} $CCID_{50}$ infectious wild poliovirus type 3 particles by a vaccine production plant in Belgium which resulted in the discharge of water from the nearby sewage plant with an estimated concentration of 1.7 x 10^5 $CCID_{50}$ wild poliovirus type 3 per liter and after which no poliovirus could be detected in the waters of the rivers into which the virus was discharged (Duizer *et al.*, 2016).

This is a hypothetical scenario which would not happen in reality as shedding by study participants would be spread both geographically and in time as subjects leave the study site following study vaccine administration and dosing will be spread over time. Experience gained during Phase 4 trials with Sabin mOPV2 and tOPV that were initiated to provide control data for the clinical studies with the nOPV2 candidate vaccines on safety, immunogenicity and virus shedding, shows that although a concentration of study participants living close to the study center can be expected, there is considerable geographical spread, especially if multiple study centers are involved (see map below).

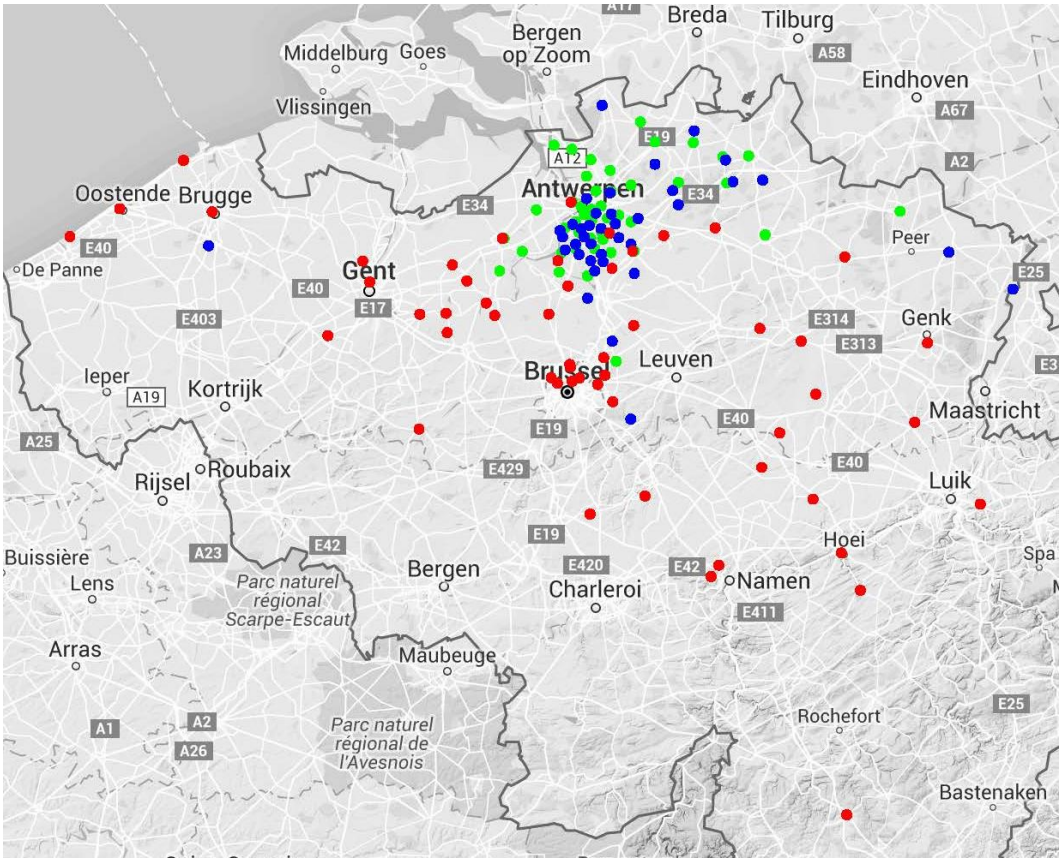

Figure 1: ***Overview of geographical spread of volunteers participating in the previous Phase 4 trials with Sabin mOPV2 and tOPV.*** (Red and green dots: Volunteers participating in a Phase 4 study to evaluate the safety and immunogenicity of trivalent oral polio vaccine in adults previously vaccinated with oral polio vaccine (EudraCT Number: 2015-003324-32). Red dots are volunteers participating at the Brussels site, green dots are volunteers participating at the Antwerp site. Blue dots are volunteers participating in a Phase 4 study to evaluate the safety and immunogenicity of monovalent oral polio vaccine type 2 in healthy OPV-vaccinated adults (EudraCT Number: 2015-003325-33), study site is located in Antwerp.)

This further supports the conclusion that the possibility that the general population could be exposed by contact with wastewater contaminated with virus particles shed by study participants (for example by swimming or fishing in rivers) is negligible.

Taken together, the likelihood that an unintended human subject would be infected from exposure to nOPV2 candidate vaccine virus through wastewater is considered negligible.

3.3 LIKELIHOOD OF TRANSMISSION OF AN NOPV2 CANDIDATE VACCINE STRAIN TO AN UNINTENDED HUMAN SUBJECT

Based on the shedding data of the FIH study, where fecal shedding but no nasopharyngeal shedding was observed, it is expected that study participants will experience some fecal shedding of the study vaccine virus. Therefore, and even if shedding from a subject does not necessarily result in transmission to another subject, a low potential for transmission of the nOPV2 candidate vaccine strains to close contacts of the study participants is considered.

Also a low potential for transmission to unintended human subjects who are secondary contacts of the study participants cannot be excluded.

For study site personnel who are involved in vaccine administration or processing of samples that may contain shed virus collected from participants a low potential for transmission is taken into account.

For other unintended human subjects the likelihood that they would be exposed to virus shed from the study participants either directly or indirectly by contaminated wastewater is negligible.

3.4 LIKELIHOOD OF TRANSMISSION OF A GENETIC VARIANT OF NOPV2 TO AN UNINTENDED HUMAN SUBJECT

Spontaneous mutations are common in polioviruses and are known to already occur within the duration of a single infection. Also recombination between Sabin strains and related enterovirus type C strains is common (Famulare *et al.*, 2016). As such, also the likelihood of transmission of genetic variants of the nOPV2 strains used in the study to unintended humans needs to be considered. As discussed in Section 2.2.1 consequences from transmission of a genetic variant of a nOPV2 strain could be either immediate (upon transmission of a variant from a study subject to an unintended human) or delayed (in case a genetic variant would be able to start circulating in the population and become a cVDPV).

Likelihood of immediate effects

In view of **immediate** effects, the mechanisms for exposure of close contacts of study participants and other unintended human subjects to a genetic variant of nOPV2 are similar as for exposure to the nOPV2 candidate strains themselves as discussed above. However, an additional mutation or recombination event would need to take place before genetic variants of nOPV2 candidate strains can be excreted and exposure to these could take place.

While mutations are known to occur frequently, even within the duration of a single infection, variants resulting from recombination between the nOPV2 candidate strains and related type C enteroviruses would require that the study vaccine and a related type C enterovirus are replicating at the same time in the same cell. As there is no evidence for circulation of type C enteroviruses in the Belgian population in recent years (Personal communication from Prof. M. Van Ranst, Rega Institute, KULeuven, national reference laboratory on enterovirus typing for the period 2011-2016/2017), simultaneous infection in a study participant is expected to be a very rare event and therefore the frequency of genetic variants arising from recombination of nOPV2 candidate strains with circulating type C enteroviruses is expected to be extremely low.

Taking both mutation (likely to occur due to the inherent genetic instability of poliovirus) and recombination (very unlikely to occur as there are no suitable recombination partners present

in the environment of the release) into account as factors in the emergence of genetic variants of nOPV2 candidate strains, the likelihood of exposure to such a genetic variant shed from a study participant is at worst the same as that of exposure to nOPV2 candidate vaccine virus.

It is expected that study participants will experience some fecal shedding of genetic variants of the study vaccine virus. The likelihood of transmission of genetic variants of the nOPV2 strains to close contacts of the study subjects is considered low. For study site personnel who are involved in processing of samples from the study subjects, the likelihood of transmission is considered low. Also a low potential for transmission to unintended human subjects who are secondary contacts of the study participants should be taken into account. For other unintended recipients the likelihood that they would be exposed to virus shed from the study participants either directly or indirectly by contaminated wastewater is negligible.

Likelihood of delayed effects

In view of **delayed** effects, if the virus could circulate in the population for an extended amount of time, additional mutations and recombinants could arise that could potentially result in a virus strain that could cause outbreaks of poliomyelitis indistinguishable from those caused by wild-type poliovirus.

However, these outbreaks are usually associated with OPV use in regions with low OPV vaccination coverage rates, where competing wild-type poliovirus has been eliminated and where epidemiologic conditions (e.g. low socioeconomic status, poor hygiene/sanitation and crowding) favor poliovirus transmission. There is no evidence for the sustained circulation of VDPVs in a highly vaccinated population. Data from environmental surveillance in the Yogyakarta Province in Indonesia, which switched from OPV-use to IPV-use in September 2007, while maintaining vaccination coverage rates of >95%, showed that vaccine poliovirus disappeared from sewage samples within 3 weeks of cessation of OPV use. As the province is surrounded by OPV-using provinces, occasional detections of OPV virus in sewage were made due to importation but no VDPVs could be isolated in the sewage samples or from acute flaccid paralysis surveillance at any point of the project (last samples taken in October 2012). This indicates that under circumstances of good hygiene, nearly universal vaccination coverage and high population immunity, the immunity induced by IPV is sufficiently robust to prevent the emergence and circulation of VDPVs (even in a setting where OPV viruses are frequently introduced from travelers, visitors, or tourists from the neighboring OPV-using provinces)(Wahjuhono *et al.*, 2014).

Similarly, in Cuba where OPV is solely administered through twice yearly National Immunization Days and no OPV is given through routine childhood immunization, results from stool and environmental sampling have shown that after 15 weeks following the vaccination campaign no virus could be detected anymore. No sustained circulation could be detected (Más Lago *et al.*, 2003).

Also when OPV was used to control an outbreak of wild-type poliovirus type 3 in 1992-1993 in a religious community opposed to vaccinations in the Netherlands, no widespread transmission of wild polioviruses or vaccine-derived polioviruses (VDPVs) was found (Kimman *et al.*, 2001). As the Netherlands have always offered IPV in routine immunization programs and OPV was only used for outbreak control, this is supportive for the notion that high levels of IPV vaccination prevent the emergence and circulation of VDPVs after use of OPV in an IPV-vaccinated community.

Given that the high rate of polio vaccination coverage in the environment of the release of the planned clinical trial due to mandatory routine vaccination in Belgium (OPV from 1966-2000, IPV from 2001 on) means that there are no large groups of susceptible individuals that could support circulation of VDPVs, the likelihood that a genetic variant of a nOPV2 strain could become a circulating VDPV strain and be transmitted to an unintended recipient in a delayed manner is considered negligible. OPV has been used in nearly 120 countries in the world for primary polio immunization, and even without any restrictions of the population movement from these countries, no incident of circulating VDPV has ever been reported from a highly immunized setting such as Belgium.

4 Step 4: Estimation of risk posed by each identified characteristic of nOPV2 which has the potential to cause adverse effects

4.1 RISK POSED BY POTENTIAL PATHOGENICITY OF NOPV2 (DIRECT EFFECTS)

As discussed in Section 2.1, the magnitude of the potential consequences of transmission of the nOPV2 candidate vaccines to unintended humans are considered negligible for immune-competent fully vaccinated individuals, low for unvaccinated immune-competent individuals and low for people with immunodeficiency, the latter considering the seriousness of VAPP, even if occurring extremely rarely.

As the study participants are expected to experience some fecal shedding, **close contacts** of the study participants could be exposed to shed virus and a low likelihood of transmission of nOPV2 to close contacts of the study subjects is taken into account. In view of the very high vaccination coverage rate in the population, this low likelihood of transmission combined with the negligible magnitude of the potential consequences results in a negligible risk posed by potential transmission of nOPV2 to most close contacts of the study subjects. For close contacts of the study participants who would not be fully vaccinated or have immunodeficiency, the combination of the low magnitude of the potential consequences results in a low risk posed by potential transmission.

The magnitude of the potential consequences of exposure of unintended humans to the nOPV2 candidate vaccines is considered negligible for **study site personnel** as they will be

required to be fully vaccinated and will have received a recent booster immunization with an IPV vaccine. Therefore prior vaccination with OPV or IPV protects them from adverse events possibly associated with nOPV2 exposure. Study site personnel is considered to have a low likelihood of exposure to and hence transmission of the nOPV2 virus either by accidental contact during administration or when samples from study participants are processed. Combining the negligible magnitude of the potential consequences with the low likelihood that transmission of nOPV2 to study site personnel will occur, the overall risk posed by nOPV2 transmission is considered negligible for study site personnel.

For **other unintended recipients** who are secondary contacts of the study participants there is a low likelihood that they could be exposed to virus shed from close contacts of the study participants in case transmission would have occurred. Similarly to the close contacts of the study participants, most of these people will have been vaccinated and the magnitude of the potential consequences for these recipients is negligible. Combined with the low likelihood of transmission, the overall risk posed by nOPV2 transmission is considered negligible for this group.

However, some could be unvaccinated or immunocompromised and low magnitude of consequences, combined with the low likelihood of transmission would result in a low risk posed by nOPV2 transmission for these groups.

For the **general population**, who do not have direct contact with the study participants or their close contacts, the likelihood that they would be exposed to shed virus is negligible and that nOPV2 could be transmitted to them is negligible. Therefore, the overall risk posed by transmission of nOPV2 is negligible for the general population.

4.2 RISK POSED BY POTENTIAL PATHOGENICITY OF A GENETIC VARIANT OF NOPV2 (INDIRECT EFFECTS)

As discussed in Section 2.2, the magnitude of the immediate potential consequences of transmission of genetic variants of nOPV2 is considered the same as those described for exposure to nOPV2, i.e. negligible for immune-competent fully vaccinated individuals, low for unvaccinated immune-competent individuals and low for people with immunodeficiency.

As spontaneous mutations are common in polioviruses and are known to already occur within the duration of a single infection, the likelihood of transmission of genetic variants of the nOPV2 strains is at worst the same as that of exposure to nOPV2 candidate vaccine virus. This means that as the study participants are expected to experience some shedding, **close contacts** of the study participants and **study site personnel** could be exposed to genetic variants of the nOPV2 candidates and a low likelihood of transmission to these groups is taken into account. For **other unintended recipients** who are secondary contacts of the study participants there is a low likelihood that they could be exposed to genetic variants of the nOPV2 strains shed from close contacts of the study participants in case transmission would

have occurred. For the **general population**, who do not have direct contact with the study participants or their close contacts, the likelihood that they would be exposed to shed virus is negligible and therefore also the likelihood that genetic variants of nOPV2 could be transmitted to them is negligible.

As the magnitude of the immediate potential consequences of transmission of genetic variants of nOPV2 to unintended recipients and also the likelihood of transmission of these variants is similar to those of nOPV2, the same overall risks specified in Section 4.2 also apply to genetic variants of nOPV2.

As the likelihood that a genetic variant of a nOPV2 strain could become a circulating VDPV strain in the environment of the release and be transmitted to an unintended recipient in a delayed manner is negligible, also the risk associated with possible delayed transmission of a genetic variant of a nOPV2 vaccine strain to an unintended human is considered negligible, even if the magnitude of the consequences is low for unvaccinated or immunocompromised individuals who are present in the general population.

4.3 OVERALL CONCLUSIONS

Overall the risks related to potential transmission of nOPV2 or genetic variants of nOPV2 to unintended recipients are very limited. The manner of the release (study participants that have been OPV- or IPV-primed will shed less) and the environment of the release (population with high vaccination coverage which does not support circulation of the virus), provide a setting in which potential transmission of nOPV2 or genetic variants is expected to be very limited.

The very high vaccination coverage rate of the population in the area of the release also means that transmission of nOPV2 or genetic variants of nOPV2 to unintended recipients, if it would occur, would in most cases pose a negligible risk as the potential consequences would be negligible.

However, there are a limited number of individuals for whom potential transmission of nOPV2 or genetic variants of nOPV2 could pose a low risk: close contacts of the study participants, study site personnel, secondary contacts of the study participants who are not vaccinated or who suffer from immunodeficiency. (Of note, health care staff conducting the trial will as per Belgian occupational law be required to be vaccinated and not to be immunocompromised.) In these groups there may be low magnitude of consequences and a low likelihood of transmission of nOPV2 or genetic variants of nOPV2; leading to a low estimated risk under worse case conditions..

5 Step 5: Application of management strategies for risks from deliberate release

5.1 DESIGN OF CONSTRUCTS

As described in Section 1.1.2, multiple modifications have been incorporated into the nOPV2 candidate vaccine viruses to make the strains more attenuated and less prone to reversion to virulence than Sabin OPV2 which is associated with VAPP and emergence of VDPVs:

- Replacement of the sequence of RNA structural domain V in the 5' untranslated region (5' UTR) with the equivalent region of the virus S15 in both nOPV2 candidates, stabilizing the attenuated phenotype as multiple mutations would be required to thermodynamically strengthen the domain V and reduce attenuation. In Candidate 1, an additional modification relocating the essential cre element has been made to prevent loss of S15 5' UTR by recombination events.
- Candidate 1 also carries modifications to the polymerase gene 3D, made to further reduce the chances of recombination occurring at any position in the genome and improve genetic stability by increasing the fidelity of the viral RNA polymerase.
- In Candidate 2 codon usage in the capsid protein coding region (P1) has been de-optimised by increasing the proportion of CpG dinucleotides. This modification results in improved attenuation of the virus and the phenotype is expected to be genetically stable since it would require multiple mutations to produce significant reversion.

5.2 CONTROL OF RELEASE

Following approval of the Clinical Trial Application for the proposed Phase 2, multicenter trial to evaluate safety, immunogenicity and viral shedding of the 2 nOPV2 candidate vaccine strains in subjects who have been previously vaccinated with OPV or IPV, the study product will only be supplied to the approved study sites and administered to subjects enrolled in the study by a trained medical professional, in accordance with the clinical trial protocol (Protocol UAM4) .

The manufacture, supply and traceability of the nOPV2 candidate vaccines will be carried out and controlled in accordance with the European legislation laying down the requirements for investigational medicinal products for human use.

Prior to administration, the product will be stored in a locked, secure storage facility with access limited to those individuals authorized to dispense the study vaccine. All study vaccine must be stored as specified at delivery and in the original packaging.

5.3 HANDLING AND ADMINISTRATION PRECAUTIONS

Administration will only be performed by trained fully OPV-primed/recently IPV-vaccinated medical professionals (who cannot be immunocompromised) in an approved study facility.

Guidelines for handling, cleaning of accidental spills and waste disposal will be put in place and should be followed during product preparation and administration.

Staff, (recently vaccinated with IPV vaccine) will wear a lab coat and disposable gloves. Disposable wipes should be used when handling samples.

5.4 CLEANING AND WASTE MANAGEMENT

Appropriate procedures for cleaning of accidental spills and breakages and disposal of contaminated waste will be put in place. All waste material should be handled as hazardous medical waste and either chemical inactivation or collection as hazardous medical waste for heat inactivation/incineration will be used.

After the last visit of the last subject in the study, any used and unused study vaccine will be returned to the Sponsor, or destroyed at the clinical site with the Sponsor's written permission.

5.5 PRECAUTIONS TO BE TAKEN BY THE STUDY SUBJECTS

To be eligible for inclusion in the study, subjects will need to have received at least 3 doses of OPV or 3 doses of IPV in the past. To further reduce the likelihood that nOPV2 candidate vaccine strains or their genetic variants could be transmitted to people who may not be fully vaccinated for polio or who may be immunocompromised, the following participant exclusion criteria are put in place:

- Professional handling of food, catering or food production activities during the total duration of the study;
- Having Crohn's disease or ulcerative colitis or having had major surgery of the gastrointestinal tract involving significant loss or resection of the bowel;
- Having any confirmed or suspected immunosuppressive or immunodeficiency condition (including human immunodeficiency virus infection, hepatitis B and C infections or total serum IgA level below lab lower limit of normal);
- Having household or professional contact with known immunosuppressed people or people/children without full polio vaccination (i.e. complete primary infant immunization series), e.g. babysitting during the total duration of the study;
- Neonatal nurses or others having professional contact with children under 6 months old during the total duration of the study.

To minimize potential spread of shed virus to their close contacts, study participants will be asked to observe good hygienic practices (i.e. flushing the toilet with the toilet lid closed, hand washing after toilet use, hand washing before handling food, and no sharing of cutlery).

5.6 MONITORING ACTIVITIES

Monitoring of the direct and indirect effects of the nOPV2 candidate vaccines in subjects will be achieved by the clinical assessments defined in the clinical trial protocol (Protocol UAM4).

Study subjects will be followed for adverse events throughout the study and at specific times, blood samples will be taken for the determination of neutralizing type 2 poliovirus antibodies.

Exploratory endpoints will include assessment of the extent and time course of shedding of vaccine virus following administration. Also assessment of the genetic sequence heterogeneity and potential for neurovirulence of shed virus may be investigated in a subset of stool samples.

5.7 CONCLUSIONS

Appropriate measures for risk prevention and management will be in place to minimize the risk and the possible consequences of exposure to unintended individuals. Monitoring activities will be in place and will detect possible adverse events caused by the nOPV2 candidates in the study participants as well as provide information that will reduce uncertainty on the environmental risk assessment for future uses.

6 Step 6: Determination of overall risk of the GMO

As presented in previous sections of this ERA, taking into account the nature of the recipient organism, the potential adverse effects that the nOPV2 candidate vaccine strains may exert on human health by conducting the clinical trial identified in Step 1 are:

- Direct effects due to potential pathogenicity of the nOPV2 candidate vaccine strains
- Indirect effects due to potential altered pathogenicity of genetic variants of the nOPV2 candidate vaccine strains. These indirect effects could be immediate (upon transmission of a variant from a study subject to an unintended human) or delayed (in case a genetic variant would be able to able to circulate in the population for a prolonged period of time after the study).

As reversion of attenuating mutations upon replication of the vaccine virus in the gut is the underlying cause of paralytic disease following administration of OPV, and taking into account that mutations are common in polioviruses and are known to already occur within the duration of a single infection, the magnitude of the immediate potential consequences and likelihood of transmission discussed in Step 2 and Step 3 are considered similar for nOPV2 and genetic variants of nOPV2.

The risk of potential delayed consequences related to genetic variants of nOPV2 is considered negligible as the likelihood that a genetic variant of a nOPV2 strain could become a circulating VDPV strain in the environment of the release and be transmitted to an unintended recipient in a delayed manner is negligible.

An overview of the magnitude of the consequences infection with the candidate GMO’s or genetic revertants to virulence assigned in Step 2, taking into account groups who may be at increased risk, the likelihood of occurrence of transmission of shedding would result in transmission assigned in Step 3, the estimate of risk for VAPP determined in Step 4, and the estimate of the remaining risk for VAPP after application of the management strategies proposed in Step 5 is provided in the table below.

Direct and immediate indirect effects of nOPV2 on an unintended recipient				
Individuals who may be at risk of exposure	**Magnitude of consequences of infection with the candidate vaccine or genetic revertants to virulence**	**Likelihood of occurrence of transmission assuming worst case that shedding always leads to transmission**	**Estimate of risk of VAPP**	**Estimate of risk after application of management strategies**
Close contacts of study participants *(partners and family members directly in contact with the study participants, people sharing facilities, people consuming food handled by the study participants)*	Immunocompetent and vaccinated: **negligible**	**Low** *(potential exposure following shedding by study participants)*	**Negligible**	**Negligible**
	Immunocompetent but unvaccinated: **low**		**Low**	**Negligible through:** - *exclusion of subjects with household/ professional contact with unvaccinated people* - *hygiene measures to reduce likelihood of exposure* - *exclusion of subjects professionally involved in food production or catering during the study*
	Persons with immunodeficiency: **low**		**Low**	**Negligible through:** - *exclusion of subjects with household/ professional contact with people with immunodeficiency* - *hygiene measures to reduce likelihood of exposure* - *exclusion of subjects professionally involved in food production or catering during the study*
Study site personnel	Immunocompetent and vaccinated: **negligible**	**Low** *(potential exposure from spills of study vaccine or samples from participants that contain shed virus)*	**Negligible**	**Negligible**
	Immunocompetent but unvaccinated: ***Not existing due to occupational law requirements in Belgium***		**Low**	**Negligible as:** - *study site personnel required to be fully vaccinated with OPV or IPV* - *recent IPV-booster*
	Persons with immunodeficiency: **low** ***Not existing due to occupational law requirements in Belgium***		**Low**	Negligible as - *study site personnel with immunodeficiency disorders or taking immunosuppressants*

				will not be allowed to work on this study
Secondary contacts of study participants	Immunocompetent and vaccinated: **negligible**	**Very low** *(as this would require close contacts of the subjects to be infected and in turn transmit the virus, so an additional event would be needed compared to direct transmission from a participant)*	**Negligible**	**Negligible**
	Immunocompetent but unvaccinated: **low**		**Low**	**Very low to negligible** *(in the overall risk to be taken into account that this is a very small group of people and that the exclusion criteria significantly reduce the chances that close contacts of the participants could be infected and in turn transmit the virus to their contacts)*
	Persons with immunodeficiency: **low**		**Low**	
General population	Immunocompetent and vaccinated: **negligible**	**Negligible** (*circulation cannot be sustained in population and risk of direct exposure to shed material is negligible)*	**Negligible**	**Negligible**
	Immunocompetent but unvaccinated: **low**			
	Persons with immunodeficiency: **low**			

In summary:

- The **magnitude of the potential consequences** of immediate or delayed transmission of nOPV2 candidate vaccine strains or their possible genetic variants to unintended humans is considered negligible for **most of the population** given the high degree of previous polio vaccination which protects against adverse events associated with Sabin OPV2 use and limits the possibility for widespread dissemination of nOPV2 candidate strains or their genetic variants.
-
- The **magnitude of the potential consequences** of immediate or delayed transmission of nOPV2 candidate vaccine strains or their possible genetic variants to unintended humans who are **unvaccinated or immunocompromised** is considered to be low, taking into account that VAPP could occur even though it is extremely rare even with Sabin OPV2 use and the nOPV2 strains are expected to have a reduced risk compared to Sabin OPV2 given their attenuated nature and increased resistance to reversion to neurovirulence.

Taking into consideration the manner, scale and environment of the release, the potential mechanisms for exposure and the risk management measures in place, the **likelihood that transmission of nOPV2 candidate vaccine strains** to unintended humans could occur is low for close contacts of the study subjects, study site personnel and secondary contacts of the study subjects. The likelihood of further dissemination either directly or through contaminated

wastewater is expected to be negligible therefore the likelihood of exposure for the general population is considered negligible.

The **likelihood that immediate transmission of a genetic variant of a nOPV2 candidate vaccine strain** to unintended humans could occur is slightly lower since an additional mutation or recombination event would be required to create the genetic variant. However, taken into consideration that spontaneous mutations are common in polioviruses and are known to already occur within the duration of a single infection, the same likelihood as for nOPV2 is taken into account.

The **likelihood that a genetic variant of a nOPV2 strain could become a circulating VDPV strain** in the environment of the release and be transmitted to an unintended recipient **in a delayed manner** is considered negligible.

The **potential risk posed by transmission of the nOPV2 candidate vaccine strains or genetic variants thereof** to an unintended human is estimated by combining the likelihood of occurrence (i.e. transmission under worst case assumption that all shedding will result in transmission) with the magnitude of the consequences if it occurs. Thus by combining negligible consequences for the groups in which transmission of nOPV2 candidate vaccine virus or a genetic variant thereof has a low likelihood to occur and possible low level consequences in a very small group which is at negligible to low likelihood of being exposed to nOPV2 candidate vaccine virus or a genetic variant thereof, **the overall risk posed by transmission of nOPV2 candidate vaccine virus or a genetic variant thereof to an unintended human is considered to be negligible.**

Appropriate measures for risk prevention and management will be in place to minimize the risk further and the possible consequences of exposure to unintended individuals. Monitoring activities will be in place to detect possible adverse events caused by the nOPV2 candidates in clinical trial participants as well as provide information to address uncertainties in the available data on the possible environmental risk.

To date the most remaining uncertainty is on the genetic stability of the nOPV2 candidate vaccines when administered to human subjects and the risk for reversion to virulence. The neurovirulence observed with shed virus from M4a trial participants confirm results observed previously with cell culture passaged nOPV2 candidate 1 and candidate 2 research materials. No specimens from the trial showed a meaningful increase in neurovirulence as compared to the matched input clinical trial lot.

Therefore, at this stage it is of negligible risk to conduct clinical trials with the candidate vaccines under deliberate release conditions. Clinical trials still are more controlled than eventual release for commercial purposes or at large scale when needed and conducting further clinical trials under contained use conditions is no longer warranted, also considering the logistic complexities of such trials. The proposed trial will allow to further reduce the remaining uncertainty on the risk around the impact of shedding and possible genetic

instability which to date is not expected to be higher than with the recipient Sabin OPV2 vaccine which was used for decades for routine immunization globally.

In conclusion, considering the overall potential environmental impact from the deliberate release of the nOPV2 candidate vaccines as investigational products in the frame of the proposed clinical study, the scale and conditions of release, the precautions in place and the proposed monitoring activities, the risk on human health and the environment is considered negligible.

7 Conclusions on the potential environmental impact from the release or the placing on the market of GMOs

A Likelihood of the GMO to become persistent and invasive in natural habitats under the conditions of the proposed release(s).

This likelihood is negligible as the nOPV2 candidate vaccines are RNA vaccines with a cytoplasmic life-cycle and hence there is no risk for integration of the modified genes in recipient (human) cells. In addition, once the nOPV2 candidate vaccine strains are released through the study participants that will leave the clinical trials sites, there may be shedding yet the shed virus will be diluted in waste water and be naturally biologically degraded or physicochemically inactivated through waste water treatment. Furthermore, the environment of the release with high rate of polio vaccination coverage does not support sustained circulation of polioviruses.

B. Any selective advantage or disadvantage conferred to the GMO and the likelihood of this becoming realised under the conditions of the proposed release(s).

There is no selective advantage or candidate nOPV2 vaccines. The fact that they are designed to be genetically more stable that the recipient Sabin nOPV2 vaccine strain rather reduces the likelihood of reversion to virulence and hence a competitive advantage in natural selection.

C. Potential for gene transfer to other species under conditions of the proposed release of the GMO and any selective advantage or disadvantage conferred to those species.

There is no potential for gene transfer to other species as polioviruses under natural conditions only have a human tropism and they are RNA viruses with a cytoplasmic life cycle.

D. Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO and target organisms (if applicable).

The recipient Sabin OPV2 vaccine has been used for decades for routine immunization globally. The main reasons for discontinuation of Sabin OPV2 in tOPV are extremely rare cases of VAPP in vaccinees or their close contacts and the emergence of cVDPVs that have

acquired transmissibility and neurovirulence. As the candidate nOPV2 vaccines used in this study are designed to be genetically more stable than the recipient Sabin nOPV2 vaccine strain which reduces the likelihood of reversion to virulence, they are expected to be at least as safe as the recipient organism. However, even in the event that VAPP could occur it would be extremely rare and because the vast majority of the Belgian population has been vaccinated as a result of mandatory poliovirus vaccination, they would be protected from experiencing the disease. In addition, because highly immunized populations cannot support sustained circulation of polioviruses, the likelihood of prolonged circulation of the candidate vaccine virus or genetic variants thereof is negligible.

Considering the overall potential environmental impact from the deliberate release of the nOPV2 candidate vaccines as investigational products in the frame of the proposed clinical study, the scale and conditions of release, the precautions in place and the proposed monitoring activities, the risk on human health and the environment is considered negligible.

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

Not applicable given the human only tropism of polioviruses.

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

The risk for immediate effects from potential direct and indirect interactions of the candidate nOPV2 vaccines and study personnel, waste water treatment plant personnel or close contacts of the study participants is negligible especially considering that all these subjects are expected to be vaccinated against polio.

No delayed effects are expected as the risk that cVDPV’s would circulate in the general population as a result of conducting the study is negligible given the high coverage of polio vaccination.

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

Not applicable and/or negligible risk as polioviruses have a human tropism only.

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

Not applicable.

I. Possible immediate and/or delayed, direct and indirect environmental impacts of the specific techniques used for the management of the GMO where these are different from those used for non-GMOs.

Not applicable.

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