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to be used in the national breeding program of the endangered species Arctic fox

Espen Rimstad (Chair), Jan Engelsen Brinchmann, Åsa Helena Frostegård, Ville Erling Sipinen, Ragnhild Tønnessen, Hege Salvesen Blix, Tor Gjøen, Toril Lindbäck and Kjetil Klaveness Melby

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Preparation of the opinion

The Norwegian Scientific Committee for Food and Environment (Vitenskapskomiteen for mat og miljø, VKM) appointed a project group to draft the opinion. The project group consisted of 4 VKM members and one from the VKM staff. The VKM Panel on Genetically Modified Organisms - Medicinal Products, assessed, and approved the final opinion.

Authors of the opinion

The authors have contributed to the opinion in a way that fulfils the authorship principles of VKM (VKM, 2023). The principles reflect the collaborative nature of the work, and the authors have contributed as members of the project group and/or The VKM Panel on Genetically Modified Organisms - Medicinal Products.

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Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third-party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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Summary

The Norwegian Environment Agency (NEA) asked the Scientific Committee for Food and Environment (VKM) to carry out an environmental risk assessment of the vaccine Recombitek®C4, which contains a genetically modified virus vector component. The vaccine is intended for use in the national breeding program of the endangered species Arctic fox (Vulpes lagopus), administered by the Norwegian Institute for Nature Research (NINA). To date the Recombitek®C4 vaccine is not approved in Norway or in the EU but has been used regularly for vaccination of dogs for two decades in the USA, Canada and several Asian countries. So far, no negative environmental impacts have been reported.

The rationale for submitting an application to use the vaccine originates from a need to handle an outbreak of infectious canine hepatitis (ICH) (hepatitis contagiosa canis (HCC)) at the breeding station in Oppdal, where four pups succumbed to the disease. HCC is caused by canine adenovirus 1 (CAV-1). There is cross-immunity between CAV-1 and CAV-2, which means that a vaccine containing CAV-2 will protect against infection with CAV-1.

The Recombitek®C4 vaccine contains four components of which three are live modified viruses: CAV-2, canine parvovirus and canine parainfluenza virus. In addition, the vaccine includes a canarypox virus vector (ALVAC) that carries, and expresses, the genes for canine distemper virus (CDV) glycoproteins. While alternative vaccines to Recombitek®C4 are available (for dogs) which do not contain genetically modified virus vector components, they are undesirable because Arctic foxes are susceptible to disease caused by the live, attenuated (for dogs) CDV component in these vaccines.

When the vaccine is administered to the host, the modified live viruses will replicate and induce protection against HCC, parvovirus infection (PVI) and parainfluenza. The ALVAC vector will enter the mammalian cells but will not complete a full replication cycle. However, the CDV genes inserted into the ALVAC vector will be expressed, leading to immunity against CDV. The main topic addressed in this report is the possible release of the ALVAC vector and its subsequent spread to the environment, with a specific emphasis on its impact on wildlife, especially birds. If the proposed vaccine and vaccination program receive approval, the intention is to vaccinate all Arctic foxes at the station on an annual basis.

Canarypox virus (CNPV), from which the ALVAC vector was developed, belongs to genus *Avipoxvirus* of the *Poxviridae* family. The natural host range of avipoxviruses is birds, and a broad range of bird species can be infected depending on the type of virus. The CNPV primarily causes disease in passeriform birds, such as canaries, characterised by cutaneous and diphtheritic disease which may be followed by high mortality. The ALVAC vector is a genetically engineered, attenuated derivative of CNPV designed for vaccine delivery. CNPV is, like ALVAC, unable to complete a full replication cycle in mammalian cells. Consequently, neither the vector nor the parent virus can produce or spread new viruses between mammalian hosts. Supporting this, tests have confirmed that ALVAC does not cause disease in canaries, domesticated bird species, or mammals, including humans. However, testing of ALVAC-based vaccines against West Nile fever, which were developed for horses, has been shown to cause adverse effects in the muscle at injection site in some species of wild birds. No vector shedding was observed from these birds, indicating that transmission of the vector to other birds is highly unlikely.

Several vaccines containing the ALVAC vector are approved for animal use in Norway, e.g. in vaccines for cats and horses.

Since the ALVAC vector does not reproduce in mammalian cells, the most plausible route to the environment would be transfer to wild birds. The breeding station does report some interaction between the captive Arctic foxes and birds like eagles, ravens and crows, e.g., around feed-emplacements. A possible (hypothetical) way for birds to be exposed to vaccine virions could be through contact with spilled vaccine or transfer through immediate contact with the inoculation site on vaccinated animals, e.g., if a bird of prey, such as an eagle, snaps a pup shortly after vaccination. To mitigate this risk, the possible interaction between birds and Arctic foxes at the breeding station has been minimized. This has been achieved by using specially designed feeding boxes in the enclosures, which decrease the attraction of birds, such as ravens and crows. Additionally, particular "eagle structures" have been constructed to discourage eagle presence around the station. Additionally, the eagles are normally not common in the area at the time of the year when vaccination of pups will take place. Even if eagles or other birds were to come into contact with the vaccine vector, the exposure would be oral, not through injection. The quantity would be minor compared to a standard vaccine dose, and for any effect, the vector would have to enter the tissues through uptake and replication in the bird's gut mucosa. For this to pose an environmental risk, the virus would have to be shed from the exposed bird and transmitted to other birds. This is a highly unlikely event.

VKM concludes that use of Recombitek®C4 in the national breeding program of endangered Arctic foxes, does not represent an increased environmental risk compared to other vaccines containing the ALVAC vector already in use for other species in Norway.

Sammendrag på norsk

Miljødirektoratet har bedt Vitenskapskomiteen for mat og miljø (VKM) om å utføre en miljørisikovurdering av vaksinen Recombitek®C4, som inneholder en genmodifisert virusvektor komponent. Vaksinen er tenkt brukt i det nasjonale avlsprogrammet for den rødlistede arten fjellrev (*Vulpes lagopus*). Avlsprogrammet administreres av Norsk institutt for naturforskning (NINA). Recombitek®C4 er pr i dag ikke godkjent i Norge eller i EU, men har blitt regelmessig brukt til vaksinasjon av hunder i ~20 år i både USA og Canada, samt i flere asiatiske land. Det har så langt ikke blitt rapportert om negative miljøpåvirkninger koblet til vaksinen.

Årsaken til at man ønsker å ta i bruk vaksinen er for å håndtere et utbrudd av smittsom leverbetennelse (hepatitis contagiosa canis (HCC)) ved avlsstasjonen i Oppdal, hvor fire fjellrevalper har dødd av sykdommen. HCC forårsakes av hundeadenovirus 1 (CAV-1). Det er kryssimmunitet mellom CAV-1 og CAV-2. Det betyr at en vaksine som inneholder CAV-2 vil beskytte mot infeksjon med CAV-1. Recombitek®C4-vaksinen inneholder fire komponenter, hvorav tre er levende modifiserte virus: CAV-2, hundeparvovirus og hundeparainfluensavirus. I tillegg inneholder vaksinen en kanarikoppevirusvektor (ALVAC) som uttrykker genene for glykoproteiner fra valpesykevirus (CDV). Det finnes godkjente alternative vaksiner til Recombitek®C4 (til hund) som ikke inneholder en genmodifisert virusvektor, men disse ønsker man ikke å bruke ettersom vaksinene inneholder et svekket (for hund) men levende CDV, som kan føre til sykdom hos fjellrev.

Når vaksinen gis til dyret, vil de modifiserte levende virusene reproduseres (replikere) og indusere immunologisk beskyttelse mot HCC, parvovirusinfeksjon (PVI) og parainfluensa. ALVAC-vektoren kommer seg inn i pattedyrcellene, men vil ikke kunne fullføre en full replikasjonssyklus, dvs., kan ikke lage nye virus. Imidlertid vil genene for CDV-glykoproteiner som er satt inn i ALVAC bli uttrykt, noe som vil gi immunitet mot CDV. Hovedtemaet i denne rapporten omhandler muligheten for spredning av ALVAC-vektoren til miljøet, og om vektoren vil kunne ha en innvirkning på dyrelivet, og da spesielt på fugler. Dersom vaksinen og foreslått vaksinasjonsprogram blir godkjent, er intensjonen å innføre en årlig vaksinering av alle fjellrever på stasjonen.

Kanarikoppeviruset (CNPV), brukt til å utvikle ALVAC-vektoren, tilhører slekten *Avipoxvirus* i *Poxviridae*-familien. De naturlige vertene for avipoxvirus er fugler, og en lang rekke fuglearter kan smittes, avhengig av typen virus. CNPV forårsaker først og fremst sykdom hos spurvefugler (Passeriformes), som kanarifugler. Forløpet kjennetegnes ved sykdom i hud og difteriliknende symptomer, med mulig høy dødelighet. ALVAC-vektoren er en genetisk konstruert, svekket variant av CNPV designet for bruk i vaksiner. CNPV er, i likhet med ALVAC, ikke i stand til å fullføre en full replikasjonssyklus i pattedyrceller. Følgelig kan verken vektoren, eller viruset den er basert på, produsere og spre nye virus mellom pattedyr. Tester har vist at ALVAC ikke forårsaker sykdom hos kanarifugl, tamme fuglearter eller pattedyr, inkludert mennesker. Imidlertid har tester gjort på ville fugler med ALVAC-baserte vaksiner utviklet mot Vestnilfeber hos hest vist alvorlige reaksjoner i muskelen på injeksjonsstedet hos noen fuglearter. Ingen utskillelse av virusvektor ble observert fra disse fuglene, som indikerer at overføring av vektoren til andre fugler er svært usannsynlig. Flere vaksiner som inneholder ALVAC-vektoren er godkjente til bruk på dyr i Norge, for eksempel i vaksiner til katt og hest.

Ettersom ALVAC-vektoren ikke reproduserer i pattedyrceller, vil den mest sannsynlige veien til miljøet være overføring til ville fugler. Avlsstasjonen rapporterer om en viss interaksjon mellom fjellrevene og fugler, som ørn, ravn og kråke, for eksempel rundt fôrplasseringer. En

mulig (hypotetisk) måte for fugler å bli eksponert for vaksine-virioner kan være via kontakt med sølt vaksine eller overføring via umiddelbar kontakt med inokulasjonsstedet på vaksinerte dyr, for eksempel hvis en rovfugl som ørn snapper en valp kort tid etter vaksinasjon. For å minimere denne risikoen har mulighetene for interaksjonen mellom fugler og fjellrev blitt redusert ved avlsstasjonen. Dette har man oppnådd ved bruk av spesialdesignete fôringsbokser i innhegningene, noe som reduserer tiltrekningen til fugler, som ravn og kråke. Det er også konstruert spesielle "ørnestrukturer" for å motvirke tilstedeværelsen av ørn ved avlsstasjonen. I tillegg oppholder ørn seg normalt ikke i området på den tiden av året da vaksinasjonen av fjellrevvalpene er planlagt. Om ørn eller andre fugler likevel skulle komme i kontakt med vaksinevektoren, vil eksponeringen være oral, og ikke gjennom injeksjon. Mengden vektor vil også være minimal sammenlignet med en standard vaksinedose. I tillegg må vektoren kunne komme inn i vev via opptak og replikasjon i fuglens tarmslimhinne. For at dette skal kunne utgjøre en miljørisiko, må viruset kunne skilles ut fra den eksponerte fuglen og overføres til andre fugler. Dette er et høyst usannsynlig scenario.

VKM konkluderer at bruk av Recombitek®C4 i det nasjonale avlsprogrammet for den rødlistede arten fjellrev, ikke utgjør en økt miljørisiko sammenlignet med tilsvarende vaksiner med ALVAC-vektoren allerede i bruk for andre dyrearter i Norge.

Abbreviations and glossary

Abbreviations

ALVAC	Canarypox virus vector
CAV-1	Canine adenovirus 1
CAV-2	Canine adenovirus 2
CD	Canine distemper
CDV	Canine distemper virus
CNPV	Canarypox virus
DNA	Deoxyribonucleic acid
dsDNA	Double-stranded DNA
EEA	European Economic Area
ERA	Environmental risk assessment
EU	European union
GM	Genetically modified
HCC	Hepatitis contagiosa canis = Infectious canine hepatitis (ICV)
ICV	Infectious canine hepatitis = Hepatitis contagiosa canis (HCC)
NEA	Norwegian Environment Agency/Miljødirektoratet
NINA	Norsk institutt for naturforskning/Norwegian Institute for Nature Research
PV	Parvovirus
PVI	Parvovirus infection
SNIF	Summary Notification Information Format
VKM	Vitenskapskomiteen for mat og miljø/Norwegian Scientific Committee for Food and Environment

Glossary

Attenuated virus	Weakened strain of disease-causing virus. Attenuated viruses are often used as vaccines because they stimulate a protective immune response while causing no disease or only mild disease.
Genetic modification	The process of inserting novel DNA/genes from the same or foreign species or deleting genes. Common to all is the use of recombinant DNA technology.
Fennoscandia	Or the Fennoscandian Peninsula, includes the Scandinavian (Sweden and Norway) and Kola peninsulas, mainland Finland, and Karelia.
Gene flow	The introduction of genetic material from one population of a species to another.
Inoculation	The action of immunising someone against a disease by introducing infective material, microorganisms, or vaccine into the body.
Recombinant DNA	Denotes sequences of DNA formed by laboratory methods that bring together genetic material from multiple sources, creating sequences that wouldn't normally occur.
Shedding (virus)	The release of virus from an infected animal.
Tropism	The specificity of a virus for a particular host, tissue, or cell type. It is determined by the interaction between viral surface and host cell receptors, and by intracellular factors that support or restrict viral replication.
Virus vector	A modified form of a virus used to deliver genetic material into a cell. The virus is attenuated to not cause disease in the recipient organism. Examples of viruses used as vectors are adenoviruses, adeno-associated viruses, retroviruses, lentiviruses, and herpes simplex viruses.
Virion	The complete infective form of a virus when outside a host cell.

Background as provided by the Norwegian Environment Agency

Assignment to VKM on risk assessment of genetically modified viral vaccine for deliberate release in arctic fox

The Norwegian Environment Agency has received an application for deliberate release of genetically modified viral vaccine for use in arctic fox and has commissioned The Norwegian Scientific Committee for Food and Environment (VKM) to assess risks for the deliberate release of GMO into the environment in accordance with the Gene Technology Act. With reference to a formal assignment letter in Norwegian sent VKM 06.11.2024, here is an English translation of the assignment.

Background

The Norwegian Environment Agency is the decision-making authority for the deliberate release of genetically modified organisms for several purposes according to the Gene Technology Act and received on 05.11.2024 an application for the deliberate release of genetically modified viral vaccine for use in arctic fox from the Norwegian Institute for Nature Research (NINA). The application was shared with VKM on the same day. VKM was notified about the application in advance.

The Norwegian Environment Agency refers to dialogue about the application and the notification of assignment letter of 25.10.2024. Furthermore, reference is made to the collaboration agreement between the Norwegian Environment Agency and VKM on 14.02.2024, and the authorization for assignments to VKM on risk assessment in 2024.

Briefly about the application

The background for the application is an outbreak of infectious canine hepatitis at the breeding station on Sæterfjellet in Oppdal, where four arctic fox pups have died as a result of the disease. The breeding station is run by NINA, and the responsible veterinarian has applied to vaccinate arctic foxes with a genetically modified virus vaccine, Recombitek C4. The purpose of using the GMO vaccine is to prevent disease and potential mortality in arctic foxes. The applicant plans to vaccinate the pups at the breeding station, and then give annual booster vaccines to the arctic foxes that will be used as breeding pairs. The pups are intended to be released into the wild, but not the adult individuals that will be used as breeding pairs, which will be kept in enclosures at the breeding station. The vaccine does not have a marketing authorization in the EU/EEA but has been risk-assessed and approved in the USA and Canada. The GMO vaccine consists of four virus components, three live modified virus vaccines and a distemper component in the form of a recombinant canarypox vector vaccine. The inserted genetic material encodes for two glycoproteins – hemagglutinin (HA) and fusion protein (F) genes isolated from canine distemper virus.

Elaboration of assignment

VKM has been given access to the application's part I (information) and part II environmental risk assessment, for assessment. The applicant has not yet submitted a SNIF (summary of the application). This will be requested from the applicant and shared with VKM.

The use of the GMO vaccine regarding animal welfare falls under animal welfare regulations and is not part of this assignment. However, any unintended adverse effects on the health of animals and humans in contact with the GMO must be assessed (see below).

Terms of reference as provided by the Norwegian Environment Agency

The Norwegian Environment Agency asks VKM to assess:

1. Whether the information in the present application is sufficient to be able to assess environmental risk, or whether there is a need for more information:

VKM must assess the submitted information and documentation from the applicant, form questions and point out any deficiencies/needs for additional information in order to carry out a risk assessment of the GMO. Questions and any deficiencies/needs are to be summarized in a document that is sent to the Norwegian Environment Agency, which will forward this to the applicant. If information is required, joint meetings can be held with the applicant. Exchange of documents with any deficiencies/need for additional information takes place for each round in which new information will be obtained from the applicant.

2. Risk to the environment, including any effects on human and animal health, which may arise as a result of the deliberate release:

The risk assessment is based on the information in the application, the applicant's own risk assessment and attached documentation, as well as other relevant scientific literature. The risk assessment must consist of an assessment of, among other things, but not limited to:

- Molecular characterization of the modification and the genetically modified organism
- Effects on the environment, including but not limited to:
    - Risk of spread, survival and persistence of the GMO in the environment, and
    - further assessment of possible consequences
- Risk of gene flow
- Risk of affecting the animal health of other wild animals
- Effects on target organisms
- Effects on non-target organisms
- Potential adverse effects on human health, as a result of changes in the genetic modification, which are in contact with the GMO
- Potential adverse effects on animal health, as a result of changes in the genetic modification, which are in contact with the GMO
- Assessment of the effect of risk reduction measures proposed in the application

1 Introduction

The Arctic fox (*Vulpes lagopus*) is an endangered species in mainland Norway (Norwegian Red List for Species, 2021). To prevent extinction, captive breeding and release of Arctic foxes has been ongoing since 2005 at Oppdal breeding station (Fjellrev - miljodirektoratet.no). This station is operated by the Norwegian Institute for Nature Research (NINA). The breeding program is important for the conservation of this species in Fennoscandia. Since 2006, 465 Arctic foxes have been released into nine mountain areas in Norway: Varangerhalvøya, Reisa Sør, Saltfjellet, Junkern, Kjølifjellet/Sylane, Knutshø, Snøhetta, Finse and Hardangervidda (NINA, 2024).

In 2024, an outbreak of infectious canine hepatitis (ICH) (hepatitis contagiosa canis (HCC)) occurred among the foxes at Sæterfjellet breeding station, Oppdal. Four pups died from the disease. HCC is caused by canine adenovirus 1 (CAV-1). Serological investigations conducted by NINA have revealed that other infectious agents such as parvovirus (PV) have also been present in some Arctic foxes at the breeding station. NINA assesses that HCC, PV infection (PVI) and canine distemper (CD) represent health risks to Arctic foxes at the breeding station (NINA, 2024). To prevent these diseases, NINA would like to vaccinate against these diseases.

In dogs, routine vaccination for HCC, CD, PVI and parainfluenza is widely used as a preventive measure. However, Arctic foxes are susceptible to the modified live CD virus (CDV) component in the vaccines currently sold in EU/EEA. That is, the live CDV component is attenuated in dogs, but not in Arctic foxes, and thus cannot be used in Arctic foxes. The veterinarian at the breeding station in Oppdal therefore proposes to use the genetically modified (GM) vaccine Recombitek®C4. As the vaccine includes a GM-component, the veterinarian has applied to the Norwegian Environment Agency (NEA) for approval of deliberate release of a GMO according to the Norwegian Gene Technology Act.

Recombitek®C4 contains modified live canine adenovirus 2 (CAV-2), PV and canine parainfluenza virus (CPIV) components, and a recombinant canarypox virus vector (named ALVAC) carrying two genes from the CDV. The CAV-2 component induces immunity against both CAV-1 and CAV-2.

All the Arctic foxes at the station will be vaccinated. The pups will receive the first dose at 8 weeks of age, followed by revaccination 2-3 weeks later. Vaccination of the pups will take place in the period July to August, and most of the pups will be released into the wild in January or February the following year. Breeding animals will remain at the station and receive annual booster vaccinations in January/February prior to the breeding season. Breeding animals caught in the wild will first receive two doses 2-3 weeks apart, and then an annual booster dose. The breeding animals are not released.

Recombitek®C4 is not approved in EU/EEA but is used in the USA, Canada and several Asian countries. In addition, the ALVAC has long been approved and in use in several other vaccines in Norway and the EU.

2 Methodology and data

2.1 Data and information gathering

VKM has assessed the application and other relevant documentation provided by the applicant (Oppdal breeding station) via the Norwegian Environment Agency (NEA). Key data includes a confidential safety dossier provided by the developer of Recombitek®C4 (Boehringer Ingelheim), a publicly available environmental risk assessment of live canarypox vector by the Canadian Food Inspection Agency (CFIA, 1998), as well as the filled-out form Summary Notification Information Format (SNIF) for the release of genetically modified organisms other than higher plants in accordance with article 11 of Directive 2001/18/EC).

Additional scientific literature referred to in the report were acquired by individual searches made by members of the project group.

3 Assessment

3.1 Molecular characterization of the modification and the genetically modified organism

Recombitek®C4 is a GM virus vaccine containing four components of which three are live modified viruses: canine adenovirus 2, canine parvovirus and canine parainfluenza virus. In addition, the vaccine contains ALVAC, based on Rentschler strain, where the two genes encoding hemagglutinin (HA) and fusion (F) glycoproteins from CDV from the Onderstepoort strain have been inserted. An H6 promotor from vaccinia virus was inserted upstream of the HA and F genes to enable their transcription. When the vaccine is administered to the host, the modified live viruses will replicate and induce protection against HCC, PVI and parainfluenza. The ALVAC vector will enter the mammalian cells but will not complete a full replication cycle. However, the HA and F genes will be expressed and induce humoral and cellular immune responses against CDV.

3.1.1 Canarypox virus

Canarypox virus (CNPV), together with other avian poxviruses, belongs to genus *Avipoxvirus* of the *Poxviridae* family (Tulman et al 2004). Poxviruses are large viruses with a linear dsDNA genome and replicate in the cytoplasm of the infected cells.

The natural host range of avipoxviruses is birds, and a large number of species can be infected (Tulman et al., 2014). The host specificity varies from broad to narrow between the different avipoxviruses. CNPV primarily causes disease in passeriform birds such as canaries characterised by cutaneous and diphtheritic disease, which may be followed by high mortality, especially in wild birds and commercial aviaries. Pigeons can also be infected and get generalised or local lesions. The virus can also infect ducks, chickens and turkeys, causing mainly local lesions in these species. The virus is transmitted directly between birds, or indirectly by vectors such as mites or mosquitos. Poxviruses can remain infectious for long periods of time (months to years) outside the host in organic material such as dry scabs (Tripathy and Reed, 1997).

3.1.2 Canarypox vector (ALVAC)

The ALVAC technology is used in vaccines for companion animals and horses in the European union and the USA (Poulet et al., 2007). The ALVAC vector will enter the mammalian cells but will not complete a full replication cycle, which means that new infectious virus particles are not being produced. The vector is known to be genetically and physically stable and is useful since it can express antigens which induce humoral and cellular immune responses in the vaccinated host (Poulet et al., 2007). The large genome of the CNPV allows insertion of several genes.

The Rentschler strain used to produce ALVAC is attenuated compared to the wild type (wt) virus. Due to the natural host restriction of the CNPV and its attenuation of the Rentschler strain, the ALVAC is not pathogenic for canaries, domesticated bird species or mammals. However, testing of ALVAC based vaccines against West Nile fever developed for horses in wild birds have caused adverse effects in the muscle at injection site in some species. Mammalian cells were demonstrated not to be permissive for the replication of CNPV already in the 1990s

(Somogyi et al., 1993). The exact molecular events responsible for this block in viral replication have not yet been identified.

At the recommended commercial dose, the reactogenicity of recombinant CNPV vaccines is low (De Bruyn et al., 2004). Intramuscular injection of human volunteers with ALVAC containing a recombinant rabies virus insert only caused mild, transient local reactions (Cadoz et al., 1992). Attempts to cultivate recombinant CNPVs on feline, canine or equine cells have failed, and no change of tropism has been observed.

The safety of different recombinant CNPVs has been tested in canaries by inoculation of large doses and comparing it to the parental strain (Poulet et al., 2007). The recombinant canarypox virus only induces mild local lesions at the inoculation site. The virus can be isolated from the skin and various organs, but the titer decreases over time until it is no longer detected, i.e. the vector does not cause persistent infection. No change in tropism has been seen in canaries or other species compared to the parental strain. Since domestic birds may also come into contact with vaccinated animals, ALVAC based vaccines have also been tested in chickens. Inoculation in mice showed absence of replication.

ALVAC based vaccines, e.g. vaccines against West Nile fever developed for horses, have also been tested in some species of wild birds (Wheeler et al., 2011, Angenvoort et al., 2014). In these studies, the vaccine was shown to cause adverse effects in scrub-jays and large falcons. The vaccine was injected into the pectoral muscle of these birds. After vaccination it was shown that the vector likely replicated at the injection site and caused local inflammation or necrotic lesions. In the falcons, no shedding of vector was detected from the oropharynx or cloaca, i.e. there was no indication that the vector was shed into the environment after vaccination (Angenvoort et al., 2014).

3.1.2.1 Use outside EU and Norway

The Recombitek®C4 vaccine is approved and used for vaccination of dogs in USA, Canada and several Asian countries.

3.1.2.2 Approved vaccines in Norway with ALVAC

In Norway, several vaccines containing ALVAC are approved for animal use. These include influenza vaccines for horses, ProteqFlu / ProteqFlu-T, both utilizing the ALVAC to express the hemagglutinin (HA) of equine influenza viruses (A/H3N8 strains). Additionally, vaccines for leukaemia in cats, Purevax FeLV / Purevax RCP FeLV /Purevax RCPCh FeLV, containing a FeLV recombinant CNPV.

3.2 Environmental effects, including but not limited to: Risk of spread and survival of the GMO in the environment and potential consequences of this

3.2.1 Risk of gene flow

Interaction between different viruses in co-infected cells has been shown for several viruses, including poxviruses. The risk for recombination events between the different live viruses in the C4 combination vaccine can therefore not be ruled out. The risk is, however, considered to be low. Firstly, the attenuated canarypox vaccine strain does not complete a full replication

cycle in non-avian cells. In addition, the compartment location of the viruses in the cells restricts the possibility for recombination since poxviruses replicates in the cytoplasm while CAV-2 and CPV replicate in the nucleus. There is no risk that Arctic foxes are infected with other viruses in the genus avipoxviruses as these do not replicate in mammals. Although it is not described in literature, we should assume that Arctic fox, as any mammal, may be infected with poxviruses of other genera. However, such occurrences are considered rare, and the likelihood of recombination between different genera of poxviruses is similarly low. Nevertheless, in the unlikely event that the attenuated CNPV encounters other poxviruses, recombination resulting in increased adaptability could theoretically occur.

3.2.2 Risk of impact on the health of other wild animals

The health of other wild animals could only be affected if the GMO entered their body. The source of such spread could theoretically be the vaccinated animal, or a source outside the vaccinated animal.

It has been well documented that avipoxviruses do not replicate in mammalian cells. In addition, the ALVAC is known to be an attenuated strain (Rentschler strain). Thus, no viral material from ALVAC will spread from the site of inoculation. This means that no other part of the animal will contain viral material, and no viral material will be shed from the vaccinated animal through droplets, mucous, urine, faeces or by any other means**.**

At the site of inoculation some of the virions will enter cells, while others will remain in the extracellular space. In the cells, the vector will undergo one round of translation to produce the desired antigen proteins. No new viral particles will be produced. The components of the vector will then be broken down by enzymatic digestion. In a few days no viral material from ALVAC will likely remain. In the extracellular space, enzymatic digestion will also take place, presumably by similar kinetics.

The only conceivable way by which other wild animals might be affected by the vector would be if they ingested tissue from the site of inoculation within a few days of vaccination. The vaccinated animals will be protected by tall fences at the breeding station. Even if a wild mammal were to be able to enter the breeding station and were to bite and ingest tissue from the site of vaccination, the tissue would enter the alimentary canal and be fully digested. Also, as the vector is replication incompetent in mammals, no spread of the ALVAC vector is conceivable through this route.

Another possibility for spread of vaccine material would theoretically be if a large bird of prey such as an eagle were to catch and subsequently ingest the vaccinated animal within a few days of vaccination. An eagle may catch a pup, and possibly even a full-grown Arctic fox. The pups will be vaccinated in July and August. This also applies to adult foxes recently caught from the wild to serve as breeding animals. At this time of the year there are very few eagles observed in the vicinity of the breeding station, and as other sources of food are readily available for the eagles, this scenario would be highly unlikely. The adult foxes established as breeding animals and already exposed to the initial vaccination programme will be revaccinated in January or February. At this time eagles are occasionally observed near the breeding station. Still, the likelihood of an eagle attacking an adult Arctic fox is very low. However, even if a vaccinated animal were to be caught by an eagle, and the eagle were to ingest some of the vaccinated tissue, this would contain replication attenuated viral particles.

So, even if whole virions were to enter eagle tissues, this would be very unlikely to cause viral replication. The consequence of ALVAC vector replication in eagles is not known.

In conclusion, the likelihood of canarypox disease or spread of the ALVAC vector in wild birds through spread from vaccinated animals must be considered highly unlikely.

It is known that poxviruses may survive for long periods outside the host, particularly if they are embedded in scabs. As no bleeding is expected to occur from the site of vaccination, spread of virions from a source outside the vaccinated animal could only occur following spill of vaccine material. The probability of this happening is very low, and if it does happen, measures will be taken to minimize the risk. Still, even if some spill was to remain, and if it were to be licked by an animal, the dominating animal inside the fences would be Arctic foxes. So, even if the licked material were to enter Arctic fox tissues, the only consequence would be that the animal would get a booster vaccine dose.

In conclusion, the risk of spread to wild animals by this route is considered highly unlikely.

3.2.3 Effects on target organisms

The intended outcome of vaccination on the target organism, the Arctic fox, is to stimulate an immune response against the components of the vaccine. As the ALVAC vector will not replicate in Arctic fox cells, no spread of the virus vector will occur, neither through the vaccinated fox's body nor to the surroundings.

3.2.4 Effects on non-target organisms

The effect on non-target organisms has been described in 3.2.2. As the ALVAC vector does not replicate in mammalian cells, the only possible effect if live vector virions were to enter mammalian tissues would be that the animal would get an immune response to the vaccine components.

If live vector virions were to enter avian tissues, this would most likely not lead to virus replication, because the ALVAC used here is replication attenuated. In the extremely unlikely event that the ALVAC were to replicate in eagle tissue, the effect on that eagle is not known.

3.2.5 Effects on human health

The only humans who could possibly get in contact with this vaccine would be breeding station personnel. Such contact could theoretically occur through:

- Spill of the vaccine. If vaccine material was to be spilt on clothing, this would represent no risk of contamination. The clothing should undergo ordinary washing procedures. If the vaccine material was to be spilt on intact skin, the material should be washed away using soap and water.
- Entry of vaccine material into human tissues. This might occur through spill onto damaged skin, or through accidental inoculation. As ALVAC does not replicate in human cells, there is no risk of systemic spread of the vector. Most likely the person involved would develop an immune response to the vaccine material. In a phase-1 study, side effects associated with ALVAC expressing rabies virus antigen were mild

and of short duration (Cadoz et al., 1992; Taylor et al., 1995; Plotkin et al., 1995; Paoletti et al., 1996).

- Personnel is bitten by an Arctic fox. This will represent no risk of contamination, as the Arctic fox will not have vaccine material in its saliva.

3.2.6 Any adverse effects on animal health due to changes from the genetic modification, in contact with the GMO

Not applicable, however:

The ALVAC vector containing the distemper insert (Recombitek®C4 vaccine) has been in regular use for vaccination of dogs in USA and Canada for at least two decades. This use has not been reported to cause adverse effects in the vaccinated animals nor has any environmental adverse effects been revealed.

The safety of ALVAC-based vaccines has been extensively evaluated in cats, dogs, ferrets or horses where very high doses (10 X) were administered subcutaneously giving only transient hyperthermia, mild and transient local pain and swelling at the injection site (Poulet et al., 2007). In regular doses the safety, i.e. lack of adverse effects ALVAC- based vaccines has been demonstrated in cat, dog, horse, ferret, sheep, pig, monkey, rabbits, guineapigs, mice, canary, chicken, duck, goose, crow and human (Poulet et al., 2007). However, testing of ALVAC based vaccines against West Nile fever developed for horses in wild birds have caused adverse effects in the muscle at injection site in some species.

3.3 Assessment of the effectiveness of risk mitigation measures proposed in the application

At the breeding station, the newly vaccinated Arctic foxes may come into contact with birds such as ravens, crows and eagles. To reduce this risk, specially designed feeding boxes for foxes are used in the enclosures to reduce attraction of birds. The applicant reports that these feeding boxes have significantly reduced the number of birds at the station.

Vaccination of pups is scheduled for late July, with a second dose administered three weeks later in August or September. During this period, eagles do not pose a problem at the station.

To ensure that the breeding females produce milk rich in antibodies for their pups, they will be revaccinated each January. This period occasionally sees increased eagle activity near the breeding station. To address this, "eagle structures" have been constructed to discourage eagle presence around the station.

Waste from the vaccination process will be managed in line with general biosecurity for treatment of infectious material.

VKM considers the proposed risk mitigation measures as satisfactory.

4 Uncertainties and data gaps

The effects of ALVAC on wild bird species are largely unknown. Although the possibility of an infection with the ALVAC strain of local wild bird species, such as eagles, ravens and crows at the Arctic fox breeding station, is considered highly unlikely, it cannot be completely ruled out.

5 Conclusion

VKM concludes that use of Recombitek®C4 in the national breeding program of endangered Arctic foxes, does not represent an increased environmental risk compared to other vaccines containing the ALVAC vector already in use for other species in Norway.

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