Annex 2

Scientific principles for regulatory risk evaluation on finding an adventitious agent in a marketed vaccine

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Guidance documents published by WHO are intended to be scientific and advisory in nature. Each of the following sections constitutes guidance for national regulatory authorities (NRAs) and for manufacturers of biological products.
1. Introduction

The finding of an adventitious agent in a biological medicinal product has been of concern to regulatory agencies, manufacturers and public health officials since the early 1900s when the issue first arose (1). Since then, there have been several instances of a signal being detected for a potential adventitious agent as a contaminant of a marketed product, for example in measles-mumps-rubella vaccine (2). The most recent examples are the finding of porcine circovirus (PCV) nucleic acid sequences or infectious circovirus in rotavirus vaccines in 2010 (3, 4).

In response to such developments, and recognizing the scientific advances made in the detection of adventitious agents in biological medicinal products, the 2010 WHO Expert Committee on Biological Standardization and the International Conference of Drug Regulatory Authorities (ICDRA) (5) recommended that WHO take the lead in providing guidance to its Member States on carrying out national regulatory risk evaluation strategies when an adventitious agent is detected in a vaccine that has already been licensed.

This WHO document is intended to provide guidance to regulators on the scientific principles of risk evaluation when a signal for a potential adventitious agent or novel endogenous agent is detected in an already licensed or registered vaccine. Risk evaluation may support potential regulatory actions by the national regulatory authority (NRA) and/or national control laboratory (NCL), the relevant inspectorate (for example, the Good Manufacturing Practice (GMP) inspector) and/or relevant public health officials such as the National Immunization Technical Advisory Group (NITAG). However, specific guidance on the national decision-making process for regulatory actions is beyond the scope of this document.

Manufacturers routinely manage risk to assure the quality of their products in the manufacturing procedures and environment as part of their compliance with GMP. In some countries, quality-by-design principles have also been applied. Public health officials make decisions on the basis of risk–benefit assessments and, often, on the basis of cost–benefit balances. These established practices are assumed to remain in place when a potential new adventitious agent is found, and are also beyond the scope of this document.

It is important to note that, in the context of this guidance document, it is understood that regulatory risk evaluation is an independent evaluation process performed by regulatory authorities on the basis of data provided by the manufacturer and that it differs from quality risk management principles, as outlined for example in guidelines published by WHO (6) and by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (7). Depending on the capability and capacity of the NRA and/or NCL, the independent evaluation process may include its own laboratory investigations.
2. Background

Although the principles of drug regulation are generally consistent internationally, the legislation, duties, responsibilities and structure of the institutions responsible for translating the principles into laws, regulations and guidance may vary substantially from country to country. Nevertheless, a number of functions are generally considered essential for an acceptable regulatory system in a producing country, namely: (a) marketing authorization and licensing; (b) post-marketing surveillance (including for adverse events); (c) lot release; (d) access to laboratory facilities; (e) GMP inspections of manufacturing sites and distribution channels; and (f) authorization and monitoring of clinical trials. Countries take these elements into consideration and adapt the principles to their structure. In addition to these regulatory functions, some countries have a NITAG (8) that helps to guide national immunization policies and that usually works closely with the relevant regulatory agencies when safety concerns arise.

To help assure that biological products and the biological starting materials from which they are manufactured are free of adventitious agents, NRAs and/or NCLs require a variety of tests to be performed by manufacturers at relevant stages of the production process. Further, it is important to ensure that the risk of potential contamination with adventitious agents, including those that cause transmissible spongiform encephalopathies (TSEs), is reduced by ensuring the quality of starting materials and/or of the production process for a biological medicinal product (for example, aseptic processing, viral clearance during purification processes and TSE risk assessment). In addition to traditional tests, new technologies for detecting adventitious agents are being developed and are coming into use. These new detection technologies may have higher sensitivity than methods used previously and may detect agents that earlier methods were not capable of detecting, or may detect nucleic acid sequences attributed to such agents. Until now (2014), screening for adventitious agents has relied upon the use of transmission electron microscopy, in vitro infectivity or biochemical assays, in vivo assays and specific polymerase chain reaction (PCR) tests. New methods and technologies, such as next-generation sequencing or microarrays, are powerful tools for the detection and identification of sequences from viruses and other adventitious agents without prior knowledge of the nature of the agent. In the future such new technologies may uncover the presence of other, as yet unrecognized, adventitious agents. Furthermore, new agents are emerging and being discovered. Therefore, the situation may arise where, subsequent to marketing authorization of a product, it is discovered that the cell substrate from which it was produced or the raw materials used in its production are contaminated with a previously undetected or unknown adventitious agent (9).
Adventitious agents and the viral safety of biological medicinal products are governed by a broad pre-licensure regulatory framework that includes: (a) evaluation by regulators of the manufacturer’s control of the manufacturing environment; (b) compliance with current GMP; (c) testing of starting materials, intermediates and the final product; and (d) requirements for the validation of viral testing and for inactivation and/or removal procedures. Detailed WHO recommendations are available in relation to the use of animal cell substrates for the manufacture of biological medicinal products (10). The risk associated with TSEs in general, and bovine spongiform encephalopathy (BSE) in particular, is addressed primarily through precautionary measures set out in WHO Guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (11). The latest version of WHO tables on tissue infectivity distribution in transmissible spongiform encephalopathies – which is periodically updated as new data become available – should also be consulted (12).

Nevertheless, the discovery of a signal for a potential adventitious agent in a product subsequent to marketing authorization raises concerns that are not well addressed in existing guidance in terms of regulatory actions and decision-making. For example, a clear evaluation strategy was not in place to support regulatory decision-making when nucleic acid sequences for PCVs or infectious circovirus were reported in rotavirus vaccines (3, 4). Similar situations have occurred in the past, including findings in the 1960s of SV40 in poliomyelitis vaccines (13) and of avian leukemia virus in yellow fever vaccines (14, 15). In the 1970s, the discovery of bacteriophages in commercial sera and live viral vaccines led to the need for regulatory actions (16, 17). The development of product-enhanced reverse transcriptase (PERT) and related PCR-based reverse transcriptase (RT) assays led to the discovery of RT activity at levels not detectable by the conventional assay used in the control of avian cell-derived vaccines in the mid-1990s, thus suggesting the possible presence of a contaminating retrovirus (18–22).

These examples illustrate that both conventional and new methods have led to the discovery of infectious agents or the marker of a viral agent in vaccines. Recent advances in technology have the potential for other types of findings to be made that are suggestive of contamination with an adventitious agent. Such findings could include the discovery of a structure suggestive of a viral particle by visualization technologies such as enhanced electron microscopy, or the discovery of a nucleic acid sequence suggestive of an adventitious agent by modern amplification or sequencing technologies. These sequencing technologies may involve assessing genomes (free or encapsidated) or RNA transcripts. Further, the technologies may entail either positive selection against a curated database of known sequences of adventitious agents or negative selection.
to eliminate host cell sequences followed by analysis of what remains. In either case, regulators may be faced with making risk evaluations and decisions about the safety of licensed vaccines on the market in their country on the basis of incomplete data on whether an adventitious agent is present or not.

3. Scope

This WHO document provides guidance to regulators on the scientific principles of risk evaluation when evidence (a “signal”) for a potential adventitious agent is detected in a vaccine that is already licensed or registered. The regulatory implications of such a finding are also considered.

While the same principles may apply to all biological products, this document focuses on vaccines, for several reasons. Vaccines are used globally in national immunization programmes and are given to whole populations of healthy individuals who are often children. As a result, there could be a major global impact if a serious safety issue were to arise involving the finding of a signal for a potential adventitious agent in a childhood vaccine. On the other hand, the premature withdrawal of a vaccine due to suspected contamination by an adventitious agent could lead to major outbreaks of vaccine-preventable disease in both immunized and non-immunized populations. An understanding of regulatory risk evaluation principles is therefore critically important in the context of vaccines. Nevertheless, if an adventitious agent were to be identified in a biological product other than a vaccine (including biotherapeutics prepared by recombinant DNA technology) the scientific principles described here could apply in the regulatory risk evaluation. However, it is beyond the scope of this document to provide further specific guidance since each case will have unique characteristics that will require judgements to be made by NRAs and manufacturers.

The regulatory risk evaluation process can, and often does, lead to other regulatory actions and considerations. For example, as a result of a regulatory risk evaluation, safety information may need to be revised. While such actions are to be expected when appropriate, they are beyond the scope of this document. In addition, a regulatory risk evaluation may indicate that a change in the production process could improve the ability to remove the agent from (or inactivate it in) future lots of the product, thus reducing the risks. However, changes to the manufacturing process may have impacts on the established quality, purity, potency, safety and efficacy of a licensed or registered product. These considerations are also beyond the scope of this document.

The document also does not cover any aspect of risk management or steps to be taken by manufacturers, nor does it provide guidance on decisions that may be taken by public health officials such as NITAGs. In the context of
complaints about pharmaceuticals and other incident-based risks to health, GMP inspectorates usually have their own risk-assessment or risk-management procedures, including a risk classification. Such risk-assessment or risk-management procedures are also not included in the scope of this document.

4. Terminology

The definitions given below apply to the terms as used in this WHO guidance document. They may have different meanings in other contexts.

Adventitious agent: contaminating microorganism of the cell culture or starting/raw materials, including bacteria, fungi, mollicutes (mycoplasmas or spiroplasmas), mycobacteria, rickettsia, protozoa, parasites, agents causing TSEs and viruses that have been unintentionally introduced into the manufacturing process of a biological product. The source of the contaminant may be the legacy of the cell line, the raw materials used in the culture medium to propagate the cells (in banking, in production or in their legacy), the environment, personnel, equipment or elsewhere.

Cell substrate: cells used to manufacture a biological product. The cells may be primary or cell lines, and may be grown in monolayer or suspension culture conditions. Examples of cell substrates include primary monkey kidney, MRC-5, CHO, and Vero cells. Cells used to generate essential components that will be used to make a final product, such as Vero cells used for “reverse genetics” of an influenza virus to seed vaccine production, are considered to be “pre-production” cell substrates. Cells used to manufacture the bulk product (for example, packaging cell lines for gene therapy vectors, Vero cells for vaccine production, and CHO cells for recombinant protein expression) are considered to be “production” cell substrates.

Dedicated facility: a manufacturing establishment or suite within the establishment that is used solely for the production of one product and is not used to manufacture any other product at any time. In contrast, a multi-use facility is one in which multiple products may be made either on a campaign basis (one at a time, in series) or simultaneously.

Environmental risk: the risk to public health and the environment. It does not include the risk to the intended recipient of the vaccine, which is assessed through clinical studies of the vaccine. It also does not include the risk to laboratory workers.

Inspectorate: a civil agency charged with inspecting and reporting on manufacturing facilities to ensure compliance with regulatory requirements.

Intermediate: partly processed product that must undergo further manufacturing steps before it becomes a bulk product.
Investigational and action plan: a documented approach to undertaking a risk-reduction and risk-management strategy through root-cause investigations and application of corrective and/or preventive actions.

Marketing authorization: an official document issued by the competent NRA for the purpose of marketing or free distribution of a product after evaluation for safety, efficacy and quality. In some countries, the term “licensing” or “registration” is used.

Microarrays: a collection of spots of nucleic acids attached to a solid surface. Each spot contains picomoles of a specific nucleic acid sequence that serves as a probe. A sample may be hybridized to the spots and detected by fluorophore, silver or chemiluminescence labelling. The solid support may be in the form of a chip or beads made from silicon or glass.

National Immunization Technical Advisory Group (NITAG): a technical resource providing guidance to national policy-makers and programme managers to enable them to make evidence-based immunization-related policy and programme decisions.

Next-generation sequencing: high-throughput sequencing technology that processes sequences in parallel, producing thousands or millions of sequences at once from a sample. Examples of methods and technologies include 454 pyrosequencing, Illumina and Ion Torrent. Each method has different attributes, such as length of a typical sequence read, accuracy, number of reads per run, time for a run and costs. As a result, the choice of method should take into account the purpose for which the data are to be generated. Significant bioinformatics using curated (trusted) databases are needed to analyse the considerable amount of data generated in each sequencing run.

Quality by design: a systematic approach to product development or manufacturing that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management. A means to build into a product or process the inherent set of characteristics needed to fulfil quality requirements.

Regulatory risk evaluation: a systematic process of evaluating information to support a risk–benefit decision within a regulatory review and evaluation framework. It consists of an independent evaluation of the risk assessment performed by the manufacturer, taking into consideration all relevant and available information and data.

Risk: the combination of the probability of occurrence of harm and the severity of that harm. In the context of pharmaceutical quality it is the probability and severity of any kind of negative impact (hazard) on the quality of the product.

Risk assessment: a systematic process of organizing information to support a risk decision to be made within a risk-management process. It consists
of the identification of hazards, and the analysis and evaluation of risks associated
with exposure to those hazards.

**Risk evaluation:** the comparison of the estimated risk to given risk
criteria, using a quantitative or qualitative scale to determine the significance of
the risk.

**Risk-reduction strategy:** a plan or method for achieving a decrease in
the probability of occurrence of harm and/or the severity of that harm.

**Risk management:** a systematic process for the assessment, control,
communication and review of risks. Risk management in the context of
pharmaceutical quality is often referred to as quality risk management – a
systematic process for the assessment, control, communication and review of
risks to the quality of the medicinal product across the product’s life-cycle. A
model for quality risk management is outlined in the relevant WHO and ICH
guidelines (6, 7).

**Root-cause investigation:** a problem-solving method that involves
systematic investigation of deviations or out-of-specification results in order to
identify an underlying root cause of the faults or problems that caused them.
Generally, the analysis aims to identify the factors that resulted in the nature,
magnitude, location and timing of hazards or adverse outcomes. In this way,
necessary behaviours, actions, inactions or conditions that require changing to
prevent the deviation or out-of-specification results from recurring in future may
be detected, corrected and/or prevented.

**Sensitivity:** the lower limit of quantification (LLOQ) or, for a non-
quantitative assay, the limit of detection (LOD) of an assay. The LLOQ is the
lowest amount of an analyte in a sample that can be quantitatively determined
with suitable precision and accuracy. The LOD is the lowest amount of the
analyte in a sample that can be reliably detected, but not necessarily quantified as
an exact value.

**Specificity:** the ability of a method to detect a specific microorganism or
range of microorganisms that might be present in the test sample.

**Starting material:** biological starting materials include the cell substrate
or cell banks, a cell seed in the case of bacterial vaccines, or a viral seed in the
case of viral vaccines. In the case of primary cell substrates, starting material
considerations should also include the source, such as species, tissue or organ,
from which the cell substrate was derived.

**Test or assay:** an analytical procedure or method – used for example
for the identification of an analyte, for measuring the content or presence of
impurities, or for the quantification of active ingredients.

**Upstream:** relating to activities that occur at or near the beginning of a
process or manufacturing flow – such as cell culture and harvest, or establishment
of seeds or cell banks – as distinct from downstream activities such as purification, concentration, formulation and filling.

**Validity:** an expression of the degree to which a measurement performed actually measures the characteristic which the investigator wishes to measure. This degree may be ascertained by a combination of analytical validation of the measurement method (23) and scientific validation that the method being used for a given purpose actually measures the intended characteristic and that the characteristic is scientifically meaningful.

## 5. Roles and responsibilities

Regulatory oversight is the responsibility of the NRA and/or NCL. Whenever there are new findings concerning adventitious agents with the potential to have a negative impact on the quality, safety or efficacy of a marketed vaccine, it is the responsibility of the manufacturer to provide the NRA and/or NCL with all relevant data and information, plus, when requested, currently available materials (samples or specimens). All this information is critical for regulatory investigation and decision-making, which should include: (a) confirmation and evaluation of the findings; (b) the manufacturer’s own risk assessment, and risk-reduction and risk-management strategy; and (c) an investigational and action plan in order to facilitate any regulatory action that might be necessary.

On the basis of the manufacturer’s data and any other reliable and credible data that are available, the NRA and/or NCL will evaluate the risk of the potential adventitious agent. If a regulatory agency is in a position to perform its own independent investigations on available biological material, this may help in assessing the risk.

The main areas to consider in a risk evaluation performed by a regulatory agency relate to the following questions:

- How was the signal detected?
- What is already known about the product concerned?
- Where was the signal detected?
- What exactly was detected?

These areas are described in detail in section 6 below. The sequence of the questions is of no significance. Moreover, regardless of whether sufficient data are already available to answer the questions, each of these areas should be considered prior to a new risk–benefit assessment. Each time new data emerge, a new risk–benefit assessment may be necessary.

Regulatory risk evaluation should be carried out on the basis of current science and technology. Regulators should conduct an independent evaluation
of the manufacturer’s new data in the context of the risk–benefit assessment of the licensed or marketed vaccine. The potential impact of regulatory decisions on public health should be discussed with public health officials. The evaluation should take into account the country-specific risk–benefit assessment of public health officials, if available. The whole evaluation process should lead to an updated risk–benefit assessment to be used as the basis for any regulatory action that may be necessary. In addition, the updated assessment will be important for helping public health officials to decide on current recommendations regarding the use of the product in their country.

Since the process is likely to be dynamic and new data for evaluation will continue to emerge during the process, the assessment should include feedback loops at each step. There is a need for transparent communication between the NRA and/or NCL and the manufacturer, and potentially between NRAs, as well as between NRAs and other groups such as the NITAG, GMP inspectorate and relevant experts from the scientific community.

Depending on national practice or due to the characteristics of the finding, it may also be necessary to include and/or facilitate direct communication between the manufacturer and public health officials such as the NITAG. If the marketed product is still being evaluated in ongoing clinical trials (for example, Phase IV studies) then ethics review committees will need to be informed and involved in the decision-making process.

Importantly, the role of WHO in the global coordination of responses and communication between the NRAs of different countries and regulatory regions has been, and continues to be, pivotal in such situations – as for example in the case of the discovery of RT activity in chicken-cell derived measles vaccine (19). This is especially the case for licensed vaccines that may be in use and are marketed in many countries. Coordinated efforts will be required in such situations to avoid public confusion. When a company discovers – and/or an NRA receives a report of – a signal of a potential adventitious agent in a marketed vaccine, communication with WHO should be undertaken immediately. For vaccines that are prequalified by WHO, communication with the unit responsible for prequalification would be an appropriate entry point (24, 25). Different regulators may have different risk–benefit considerations for their country on the basis of, among other factors, vaccine supply, disease prevalence and severity, and the specific epidemiological situation. Nonetheless, for the purposes of risk communication and public transparency, a globally coordinated response is warranted whenever a potential adventitious agent is found in a vaccine that is marketed in countries across the world.

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6. Regulatory risk evaluation

Fig. 1 outlines the process for conducting a regulatory risk evaluation.

Fig. 1
Regulatory risk evaluation process

Any finding of a potential adventitious agent previously unrecognized

Risk management performed by the manufacturer: confirmation/evaluation/risk assessment

Information to be provided to NRAs for an independent regulatory risk evaluation: Investigational and action plan

Points to consider for regulatory risk evaluation

How was the signal detected?
- Sensitivity, specificity and validity of the assay used to identify the agent including appropriate control materials
- Laboratory investigations performed by NRA/NCL (if applicable)

What is already known about the product concerned?
- Data provided in the original file for licensing (if appropriate)
- Post-marketing data, pharmacovigilance (if available)

Where was the signal detected?
(Risk associated with the product)
- Type of vaccine
- Starting material, intermediate, final product
- Purification/inactivation
- Route of administration
- Environmental risk
- Others

What exactly was detected?
(Risk associated with the agent)
- Kind of signal (e.g. DNA, virus, ...)
- Origin (e.g. animal, ...)
- Infectious/pathogenic
- Potential long-term effects
- Environmental risk
- Others

New risk–benefit assessment
(consider already vaccinated persons and those to be vaccinated)
6.1 **How was the signal detected?**

This question addresses all the issues related to sensitivity, specificity and validity of the methodology used to detect and/or identify the potential agent. The question indicates the need for reliable confirmatory data provided by the manufacturer and, if applicable, by laboratory investigations performed by the NRA and/or NCL.

6.2 **What is already known about the product concerned?**

Manufacturer data relevant to the finding, and all other reliable data available, should be evaluated together with relevant information from the initial marketing authorization. This is of particular interest if the newly detected agent or potential agent was likely to have been present at the time of marketing authorization. Manufacturing and quality-control data, including control over raw-material suppliers, should be assessed in close cooperation and communication with the responsible GMP inspectorate. It is especially important to assess potential GMP failures, strategies to avoid or mitigate the newly detected agent, and the root-cause investigation.

In cases where the detected potential agent is a previously unrecognized adventitious agent, nonclinical and clinical data, including post-marketing and/or pharmacovigilance data if available, will be vital in evaluating the safety of the contaminated vaccine and any potential risks associated with the contaminating agent. The assessment of these data should consider risks that may be unique to a specific patient population – such as immunocompromised individuals, infants or the elderly – if they are included in the clinical indication for the product or often receive the product off-label. Moreover, the epidemiology of the agent may inform the assessment of the risk. The evaluation of potential long-term effects will depend upon the type and amount of data available.

6.3 **Where was the signal detected?**

The type of vaccine concerned has an important impact on the potential risk. Other factors that are linked to the type of product should also be considered. For example: Was the signal found in the starting materials, reagents, intermediates or the final product? Do any of the manufacturing steps, including purification and/or inactivation processes on the starting materials or on the product, have a positive or negative impact on the signal? What is the route of administration of the product, and how does this influence the risk to the product recipient from the potential agent?

Other parameters, such as the dose, the schedule of administration and characteristics of the recipient population, may be usefully considered in the risk evaluation. It may be vitally important for the manufacturer to investigate...
retained materials (raw or starting materials, intermediates or bulks, and final containers) in order to define a root cause, and for regulators to review and evaluate such investigations. This may be especially significant if an adventitious agent of an unexpected species is found (such as an equine virus being found when no equine materials were thought to have been used in manufacturing). Furthermore, the potential risk to the environment is influenced by features of the product concerned (for example, by the way in which it is excreted by the product recipients, or the way manufacturing wastes are handled) as well as by the type of agent found.

Within this context, it is appropriate to distinguish the characteristics of the three main stages in vaccine production – namely, starting materials, intermediates and final product. The following questions are applicable once an adventitious agent has been detected or the finding is suggestive of the presence of an agent:

- How was the agent introduced (that is, what were the results of the root-cause investigation)? If the agent was introduced by starting materials then most (or all) lots could be implicated, including clinical lots used in product development. This means that the agent could have been consistently present during clinical investigations of the vaccine. If the agent was introduced by the environment, by personnel or by specific batches of raw materials then a more limited number of lots may be implicated. Assessment of whether those specific lots were used in humans, and clinical follow-up of humans exposed to the implicated lots, could provide valuable data on the actual risk to humans. In terms of consistency, it is crucial to characterize the genetic sequence and load of the agent between different lots.

- Are other products affected? If the agent was introduced in the cell substrate or raw materials that are used by the manufacturer in multiple products then products other than just the implicated product may be affected. If the agent was introduced during production of a specific lot (or lots) then other products manufactured concurrently in the same facility as the affected lot could be implicated. A thorough investigation should be undertaken to evaluate whether other products or lots could be contaminated and, if so, these should be treated in the same way as the vaccine lot that was originally implicated. However, if the affected cell substrate or raw material batches are used for the sole purpose of producing one product, and/or if the facility is a dedicated facility, the risk to other products from that manufacturer may be limited. If product lots are infected by an upstream contamination, the frequency of the contamination
should be addressed. In this case, clinical lots used in product development may provide particularly useful information in terms of the potential impact of the contamination on the health of human subjects in clinical trials, as available in the clinical safety databases. A retrospective analysis of the clinical lots may be beneficial to show whether the integrity and loads of the agent are comparable between the clinical lots and the marketed product (commercial lots).

- Especially for a live viral vaccine that may be given by normal routes of infection, what is the impact of the route of administration of the product? Viruses vary in their routes of normal infection and may establish productive infections only when the host is exposed by a particular route. However, a product may be administered by a route other than the normal routes of viral exposure. In the case of parenteral products, which bypass the normal defence mechanisms of the host (such as skin, saliva and stomach acids), infection may occur more readily. Of course, it may also be the case that exposure through an abnormal route may preclude exposure of susceptible target cells, thus reducing risk. Alternatively, if a product such as a live viral vaccine is delivered by a normal viral infection route (such as orally or intranasally) then information about normal virus exposure (such as epidemiology data) may be more relevant to the situation.

- Is there a risk to the environment? It may be determined that the agent does not pose a significant risk to the recipients of the product. However, the agent to which they were exposed may be shed by recipients through normal excretory processes. For example, polioviruses can replicate in the human gut and be shed in faeces, thereby exposing the close contacts of recipients of oral poliomyelitis vaccine through the faecal-to-oral route. If the adventitious agent is shed by the product recipients, then human and animal contacts of these recipients could be placed at risk of exposure to the agent. In the case of shedding of the agent from product recipients, it will be valuable to evaluate whether any variation can be detected in the genetic sequence of the agent isolated from excreta.

- Is there a risk of dissemination at the manufacturing plant? Even if the product itself is not contaminated, but an upstream material such as a cell substrate is found to be contaminated, then there may be a risk to the environment from the manufacturing process and/or disposal of manufacturing waste products, particularly if the agent found is not already endemic to the geographical region in which the facility is located. It would be expected, in line with current GMP, that waste is decontaminated before release into the
environment, but procedures should be reviewed regarding their effectiveness in relation to the agent concerned.

6.4 **What exactly was detected?**

The risk associated with the agent detected depends primarily on its physical nature (for example, whether what was found was nucleic acid or an intact virus). In addition, thorough consideration should be given to the normal host species of the agent (for example, whether it is animal-, plant- or human-derived) and to whether or not it has the potential to be infectious or even pathogenic to humans or animals. Any potential long-term effects, or other effects that can be linked to the agent, will also need to be evaluated. As noted above, the potential risk to the environment will depend upon the characteristics of the agent and the product concerned.

The following series of questions and potential answers is intended to help decision-makers to evaluate the risks to humans associated with the discovery, through the use of new detection technology, of the nucleic acid of an adventitious agent in the starting materials, intermediates or final product of a licensed vaccine. The series may be modified for other types of findings, such as structures suggestive of viral particles, microbial agents or enzymatic activities suggestive of enzymes encoded by viruses.

Reasonable questions following a finding based upon nucleic acid detection might include, but would not necessarily be limited to:

- Is the agent a known agent, a member of a known family or a novel agent?
- Are the nucleic acids that were found simply fragments or are they full-length intact genomes?
- Are the nucleic acids that were found free or particle-associated?
- If the nucleic acids are associated with particles, have these particles the potential to infect cells of the suspected normal host species?
- Are these particles infectious in the suspected normal host species?
- Are the particles infectious in cell cultures, including human cells?
- Is the agent known to be infectious in humans?
- Is the agent pathogenic or an opportunistic pathogen in humans?
- Is the agent transmissible from human to human, from animal to human or from human to animal?

It should be borne in mind that, as in all scientific investigations, the evaluation will be complex and likely to be more complicated than answering a
series of questions. Each situation will likely be unique, and addressing all steps in the algorithm may not be possible. Any of the individual elements of such an algorithm may also not apply in some cases, or may need to be adapted depending on each particular situation. Where nucleic acids are found that might indicate the presence of an adventitious agent, the following illustrative general approach may be taken to address the above questions.

If the viral nucleic acids are full-length and intact but free (that is, not particle-associated), then they have the potential to be infectious if they are taken up by susceptible cells; thus they may still represent a risk, depending upon whether or not the conditions favour or disfavour infectivity. These conditions include, but are not limited to, those described here. For example, if the route of inoculation exposes the free nucleic acids to nucleases (for example, by oral administration), they would be eliminated or fragmented before they could be taken up by cells. The route of administration could also influence the availability of susceptible target cells to take up the free nucleic acids.

Similarly, if the nucleic acids are particle-associated but fragmented, consideration should be given to whether or not they infect cells in the vaccine recipient. Also, when the nucleic acids are uncoated in the cell, the possibility must be considered that they could be repaired by natural cellular repair mechanisms, thus leading to a productive infection in the recipient despite the nucleic acids being fragmented inside the viral particle.

In either of these cases, or in the case of infectious particles that lead only to an abortive infection in human cells, concern may still exist if the agent is one that is known to result in pathology following abortive infection. In particular, oncogenic viruses could still represent a risk even if they result only in abortive infections.

For some viruses it has been shown that, while the host species does not display disease, infection of humans (or another non-host species) by the virus may result in significant morbidity or mortality. A significant example of this is simian herpes B virus which usually does not cause disease in monkeys, which are the natural hosts, but can cause fatal disease in humans.

If it is unknown whether an adventitious agent causes disease in humans but it causes disease in the host species, this may also represent a potential risk. In such a situation, if the adventitious agent was found in a starting material (lot) that was used in clinical trials a re-evaluation of the existing data should be considered. The existing databases generated from post-marketing safety studies or surveillance, as well as from clinical trials, should be searched for signals from the clinical data reflective of the known pathology in the host species in order to determine if a similar disease syndrome might be occurring in product recipients. However, symptoms and pathology may be quite different in humans, and this must be kept in mind during the evaluation of clinical safety databases. As part
of assessing the potential limitation of existing information, consideration should also be given to the possibility that existing databases may not have captured the relevant signal (symptom or pathology).

One indicator that the agent may be able to infect humans, even in the absence of a well described disease syndrome or recognized zoonosis, would be the occurrence of antibodies in product recipients and naturally exposed individuals. In addressing the issue of whether the agent infects humans, one question that should be asked is whether there is evidence of immunity in humans – such as the presence of antibodies in veterinarians or individuals involved in the husbandry of the animal species associated with the agent (for example, pig farmers in the case of PCVs). In addition, if serum samples had been saved from human subjects from the clinical trials of the product, these could be screened for antibodies to the suspected agent. This may require a review of the informed consent forms from the original trial to determine if this additional use of the sera is covered by the consent given by the subjects. If not, subjects may need to be requested to give informed consent for their sera to be used for this purpose. When clinical trials are being designed, the potential need for such future uses of stored sera should be considered, along with the required storage conditions, in order to facilitate the rapid and ethical future use of existing samples.

### 6.5 New risk–benefit assessment

In principle, a new risk–benefit assessment is needed whenever the magnitude and scope of benefits, or of previously unrecognized risks, are elucidated and confirmed. The risk–benefit assessment should therefore be updated periodically, especially after process changes, in accordance with the principles of risk assessment. Methods and principles for a systematic approach for risk–benefit assessments are provided elsewhere (26) and are not within the scope of this document.

Within the health care community there may be different perspectives on benefit and risk. In the case of GMP and/or pharmaceutical technical issues, the risk–benefit assessment falls within the responsibility of the GMP inspectorate, or the NRA and/or NCL. Each (new) risk–benefit assessment of a vaccine may also have public health implications. The risk–benefit assessment of public health officials such as NITAGs is usually performed separately and may differ from the regulatory assessment due to additional considerations, such as population versus individual health considerations and the need for cost–benefit analyses. As with the GMP inspectorate, close collaboration and communication between the licensing authority and public health officials is considered to be crucial. In addition, communication with ethics committees should be considered if the product is still being investigated in clinical studies (for example, Phase IV studies).
Risk–benefit assessments of vaccines depend not only on scientific and biological considerations but also on regional considerations, and on the particular circumstances (for example, epidemiology, availability of alternative vaccines, and regulatory or legal framework) in those areas. Nevertheless, in an increasingly global environment, communication and the exchange of information on a global level is of utmost importance. Furthermore, there is a need for public transparency, and for risk communication to be clear, credible and consistent. As a result of these and other factors, WHO has a key role to play in the global coordination of communication efforts in this area.

7. Summary and conclusions

Regulatory risk evaluation is a dynamic process both in terms of how it has evolved over the past 60 years and in the way in which information is accumulated and evaluated in any given instance. Much has been learnt since the discovery of SV40 as a contaminant of poliomyelitis vaccines in the 1960s, and it is hoped that the lessons of past cases of finding an adventitious agent in vaccines will provide useful guidance for the future.

The four case studies discussed by Petricciani et al. (22) cover a broad range of possible contaminants of viral vaccines. In each case the initial response was to determine whether the finding posed an unacceptable risk to public health in light of the proven or expected benefits of the vaccine in question, and whether the signal really was indicative of a live infectious virus contaminant with serious adverse effects on recipients. In all four cases, and after consideration of the scientific advice, the vaccines concerned were not removed from the market, or were only temporarily suspended, since the benefits of immunization were believed to significantly outweigh the risk of any potential adverse effects. After further evaluation, that initial assessment proved to be correct.

In each case, the response to the initial findings benefited from knowledge gained from past experiences. It is important to make adjustments based on lessons learnt from experience. These events highlighted that in order to respond effectively, it is essential to have access to expert scientific advice, good communication, public transparency, international collaboration and effective global coordination.

The manner in which the international scientific community dealt with the case of RT in measles and mumps vaccines should serve as a model for dealing with similar issues in the future. During the scientific investigation and response to the RT finding, excellent coordination and collaboration allowed for the reaching of a global consensus on what regulatory actions were appropriate, which in turn facilitated clear communication of the issues.
Despite similar good collaboration in addressing the PCV issue, this incident also highlighted the complexities involved in arriving at a global consensus when local and/or regional considerations have to be taken into account. Different NRAs and NCLs may have different risk–benefit considerations for their country based on factors such as vaccine supply, disease prevalence and severity, and their specific epidemiological situation. All of these factors must be taken into consideration during the decision-making process, which should always be based on sound science. In all cases, the potential impact of regulatory decisions on public health should be discussed with public health officials, with a clear need for transparency in the decision-making process.

The PCV case also highlighted the same issue previously identified during the bacteriophage case, namely that the quality of reagents such as sera and trypsin used in vaccine production, and in the preparation of important biological starting materials such as cell banks and viral seeds, must be well controlled in order to have a final product that is free from contamination. Indeed, concern about the quality of starting materials has recurred periodically (for example, RT in eggs and BSE in bovines). The risk of such microbial contamination can be minimized by limiting the use of animal-derived materials in manufacturing, applying GMP, and conducting stringent testing and control of raw materials, manufacturing intermediates and final product.

The issue of the safety of vaccines for global use is an area where WHO collaborating centres and other expert advisors may, when requested, provide advice to WHO on cell substrate viral safety. Such expert advice, based on state-of-the-art technologies developed by manufacturers, NRAs, NCLs and academic institutions, would provide valuable support to WHO in facilitating timely responses to unexpected findings of adventitious agents in vaccines.

The final general observation from the four case studies relates to the key role that WHO has played in coordinating a global response to the finding of an adventitious agent (or signal of an agent). The role of WHO in coordinating the global actions to be taken and facilitating communication among the NRAs of various countries and regulatory regions continues to be pivotal in such situations, as for example in the case of the discovery of RT activity in chicken cell-derived measles vaccine. This is especially the case for licensed vaccines that may be in use in many countries. Although WHO coordinates efforts directed towards achieving international consensus, it is the NRA that will ultimately make decisions based upon the risk–benefit assessment for their own populations. As a result, there may be different decisions reached in different countries or regions. In order to avoid confusion, it is important that NRAs clearly communicate the rationale and basis for their decision. In addition, in the interest of global public health, any signal of a potential adventitious agent in a marketed vaccine should be reported promptly to WHO. NRAs and manufacturers have a responsibility
to immediately inform WHO of such a finding and to take appropriate follow-up actions, particularly in the case of prequalified vaccines.

Since the finding of SV40 in poliomyelitis vaccines there have been numerous scientific and technological advances, along with the routine use of more efficient methods of communication and information exchange. Such developments have led to an increasingly comprehensive and transparent response to the finding of an adventitious agent (or signal) in a marketed vaccine. Manufacturers and regulators continue to investigate new technologies, and when new methods prove to be superior to existing ones then updated practices should be introduced.

A central element of the regulatory risk evaluation process is that the assessment needs to be updated each time new significant data emerge; thus, it is an iterative process. Nevertheless, it is often the case that there is a need for immediate decisions at an early stage of the evaluation when many of the answers to the questions outlined in this guidance document will not be available. This presents particularly challenging situations for all interested and affected parties. Due to the potential complexity of future events, simple universally applicable guidance or a fully comprehensive list of priorities to consider cannot be provided. Among the most important lessons from the past is the desirability of transparency and open communication. When all parties with a vested interest in the outcome of a regulatory risk evaluation are aware of – and understand – the basis upon which decisions are made, the probability of miscommunication and error are minimized. WHO will therefore continue to play a vital role not only in the global coordination of responses to the discovery of a signal for a potential adventitious agent but also in the public communication of the outcomes of regulatory decision-making.

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