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

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This guidance document published by WHO is 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. If an NRA so desires, this document may be adopted as definitive national requirements, or modifications may be justified and made by the NRA. It is recommended that modifications to this document be made only on condition that the modifications ensure that the product is at least as safe and efficacious as that prepared in accordance with the principles set out below.
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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 (e.g. 2). The most recent examples are the findings of porcine circovirus sequences or infectious circovirus in rotavirus vaccines in 2010 (3, 4). In response to this development, and recognizing the scientific advances for the detection of adventitious agents in biological medicinal products, the WHO Expert Committee on Biological Standardization (in 2010) 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 document is intended to provide guidance to regulators regarding the 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 (e.g. the Good Manufacturing Practice [GMP] inspector), and/or the 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 guidance.

Manufacturers routinely manage risk to assure the quality of their products in the manufacturing procedures and environment as part of their compliance with GMP (e.g. 6, 7). In some countries, quality-by-design principles have also applied. Public health officials make decisions on the basis of benefit–risk 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 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 the guidelines of WHO and the International Conference on Harmonisation (6, 7). Depending on the capability and capacity of the NRA/NCL, the independent evaluation process may include its own laboratory investigations.

2. **Background information**

Although the principles of drug regulation are generally consistent internationally, the legislation, duties, responsibilities and structure of institutions that are 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: marketing authorization and licensing, post-marketing surveillance (including for adverse events), lot release, access to laboratory facilities, GMP inspections of manufacturing sites and distribution channels, and
authorization and monitoring of clinical trials. Countries take these elements into consideration and adapt the principles to their structure. In addition to those 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.

NRAs/NCLs require a variety of tests to be performed by manufacturers at relevant stages of the production process of biologicals which are intended to help assure that biological products and the biological starting materials from which they are manufactured are free of adventitious agents (i.e. viruses, bacteria, fungi, mollicutes, etc.). Further, it is important to ensure that the risk of potential contamination with adventitious agents, including transmissible spongiform encephalopathy (TSE) agents, is reduced by ensuring the quality of starting materials and/or by the production process of a biological medicinal product (e.g. aseptic processing, viral clearance during purification processes, 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. Up to now (2014), screening for adventitious agents has relied on 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 (NGS) 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 the product was produced or 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: evaluation by regulators of the manufacturer’s control of the manufacturing environment; compliance with current GMP; testing of starting materials, intermediates and the final product; and requirements for the validation of viral testing and for inactivation/removal procedures. Detailed WHO recommendations and guidance are available on 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’s Guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (11). The latest version of the WHO Guidelines on tissue infectivity distribution in transmissible spongiform encephalopathies should also be consulted (12). That document is periodically updated as new data become available.

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 sequences of porcine circoviruses or infectious circovirus were reported in rotavirus vaccines (3, 4). Similar
situations have occurred in the past, including finding SV40 in poliovirus vaccines in the 1960s (13) and avian leukosis virus in yellow fever vaccines (14, 15). In the 1970s, findings of bacteriophage 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 assays led to the discovery of reverse transcriptase activity at levels not detectable by the conventional assay used in 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 discovery of a structure suggestive of a viral particle by visualization technologies such as enhanced electron microscopy, or discovery of a nucleic acid sequence suggestive of an adventitious agent by modern amplification or sequencing technologies. The sequencing technologies may involve assessing genomes (free or encapsidated) or RNA transcripts. Further, the technologies may entail positive selection against a curated database of known sequences of adventitious agents or negative selection to eliminate host cell sequences and 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 document provides guidance to regulators on the 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 is focused 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 concerning finding the signal of 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, regulatory risk evaluation may suggest 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 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. This risk assessment/management is also not included in the scope of this document.

4. Roles and responsibility

Regulatory oversight is the responsibility of NRAs and/or NCLs. 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 NRAs/NCLs with all relevant data and information, plus, when requested, materials (i.e. samples or specimens) that are currently available. All this information is critical for the regulatory investigation and decision-making which should include: confirmation and evaluation of the findings; the manufacturer’s own risk assessment, risk reduction and management strategy; and 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/NCL will evaluate the risk of the potential adventitious agent. If an 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 the sections 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 benefit-risk assessment, and each time new data emerge a new benefit-risk 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 benefit-risk 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 benefit-risk assessment of public health officials, if available. The whole evaluation process should lead to an updated benefit-risk assessment as the basis for any regulatory action that may be
necessary. In addition, the updated assessment is important for helping public health officials to decide on current recommendations regarding 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 includes feedback loops at each step. There is a need for transparent communication between the NRA/NCL and the manufacturer, and potentially between NRAs as well as other groups, such as the NITAG and the GMP inspectorate, and with 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. Also, if the marketed product is still being evaluated in ongoing clinical trials (e.g. phase IV studies), communication with and inclusion in decision-making of ethics review committees will also be needed.

Importantly, the role of WHO in the global coordination of responses and the communication between 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 reverse transcriptase 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. Some coordinated efforts are required or there will be 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\(^1\) would be an appropriate entry point (23, 24). Different regulators may have different benefit-risk 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 reaction is warranted if a potential adventitious agent is found in a vaccine that is marketed in countries across the globe.

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Regulatory risk evaluation

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

**Figure 1. Regulatory risk evaluation process**

**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 of the NRA/NCL.

**What is already known about the product concerned?**
The manufacturer’s data relevant to the finding and all other reliable data that are 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/pharmacovigilance data if available, are important for the evaluation of the safety and potential risk of the agent in the contaminated vaccine. The assessment of these data should consider risks that may be unique to a specific patient population – such as immunocompromised persons, infants or 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 on the type and amount of data available.

**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 instance: 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 it 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 useful to consider in the risk evaluation. It may be critical for the manufacturer to investigate retained materials (raw or starting materials, intermediates or bulks, 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 of agent is found (e.g. an equine virus is found when no equine materials were thought to be used in manufacturing). Further, the potential risk to the environment is influenced by the product concerned (e.g. the way it is excreted by the product recipients, or the way the manufacturing wastes are handled) as well as by the type of agent that was found.

Within this context, it is appropriate to distinguish the characteristics of three main stages in the production of a vaccine – i.e. starting materials, intermediates and the 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 (i.e. what are the results of the root-cause investigation)?**
  If the agent was introduced by starting materials, 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, personnel, or 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 critical 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, more products than just the implicated product may be affected. If the agent was introduced during production of a specific lot (or lots), 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. For parenteral products, which bypass the normal defence mechanisms of the host (skin, saliva, stomach acids, etc.), infection may occur more readily through this route of exposure. 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 the product, such as a live viral vaccine, is delivered by a normal viral infection route (e.g. 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 the product recipients through normal excretory processes. For instance, poliovirus can replicate in the human gut and be shed in faeces, thereby exposing close contacts of recipients of oral polio vaccine through the faecal-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 disposal of manufacturing waste products, particularly if the agent found is not already endemic to the geographical region where the manufacturer’s 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.
**What exactly was detected?**

The risk associated with the agent depends primarily on the physical nature of the agent (e.g. whether what was found was nucleic acid or an intact virus). In addition, the normal host species of the agent – whether it is animal-, plant- or human-derived, as well as whether the agent has potential to be infectious or even pathogenic to humans or animals – needs to be considered thoroughly. Thus, potential long-term effects, or other effects that can be linked to the agent, need to be evaluated. As noted above, the potential risk to the environment depends on the characteristics of the agent and the product concerned.

The text below leads decision-makers through a series of questions and potential answers that should be considered with regard to the risks to humans associated with an adventitious agent, the nucleic acid of which was discovered, through the use of new detection technology, in starting material, intermediates or final product of a licensed vaccine. The series of questions may be modified for other types of findings, such as structures suggestive of viral particles, microbial agents, or enzymatic activities suggestive of the enzymes encoded by viruses.

Reasonable questions for a finding based on nucleic acid might include, but would not necessarily be limited to, the following:

- 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 opportunist 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 is complex and is likely to be more complicated than answering a series of questions. Each situation will likely be unique, and not all steps in the algorithm may be possible. All elements in the algorithm may not apply in some cases, or may need to be adapted depending on each particular situation. The finding of nucleic acids that might indicate the presence of an adventitious agent is used to illustrate the general approach that may be taken, as follows:

If the viral nucleic acids are full-length and intact but free (i.e. not particle-associated), they have the potential to be infectious if they are taken up by susceptible cells; thus they may still represent a risk, depending on whether the conditions favor or disfavor infectivity. These conditions include, but are not limited to, those described here.

For instance, if the route of inoculation exposes the free nucleic acids to nucleases (e.g. by oral administration), they would be eliminated or fragmented before they can be taken up by cells. The route of administration could also influence the availability of susceptible target cells to take up free nucleic acids.

Similarly, if the nucleic acids are particle-associated but fragmented, one must consider whether they could infect cells in the recipient of the vaccine. Also, when the nucleic acids...
are uncoated in the cell, one should ask whether 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.

Finally, if it is unknown whether an adventitious agent causes disease in humans but it causes disease in the host species, this may 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 existing data should be considered. The existing databases from post-marketing safety studies or surveillance, as well as 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. It is also possible that existing databases may not have captured the relevant signal (symptom or pathology) and consideration of this should also be given, in regards to the potential limitations in existing information.

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 exposed humans. 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 (e.g. pig farmers in the case of porcine circoviruses). Further, if sera were saved from human subjects from the clinical trials of the product, they 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 additional studies such as these were not covered by the original informed consent, subjects may need to be requested to give informed consent for their sera to be used for this purpose. Therefore, when clinical trials are being designed, this potential future use of stored sera and conditions of storage should be considered in order to facilitate the most rapid and ethical future use of the specimens.

**New benefit-risk assessment**

In principle, a new benefit-risk assessment is needed when the magnitude and scope of benefits, or previously unrecognized risks, are elucidated and confirmed. The benefit-risk assessment should be updated periodically, especially after process changes, consistent with the principles of risk assessment. Methods or principles for a systematic approach for benefit-risk assessments are given elsewhere and are not within the scope of this document (e.g. 25).
Within the health-care community, there may be different perspectives of benefit and risk. In the case of GMP and/or pharmaceutical technical issues, the benefit-risk assessment falls within the responsibility of the GMP inspectorate or the NRA/NCL. Each (new) benefit-risk assessment of a vaccine may also have public health implications. The benefit-risk assessment of public health officials (NITAGs) is usually separate and may differ from the regulatory assessment due to additional considerations (e.g. population vs. individual health considerations, 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 (e.g. phase IV studies).

Benefit-risk assessments of vaccines depend not only on scientific and biological considerations but also on regional considerations and the particular circumstances (e.g. epidemiology, availability of alternative vaccines, regulatory or legal framework) in those areas. Nevertheless, in a growing global environment, communication and the exchange of information on a global level is of utmost importance. Furthermore, public transparency and risk communication need to be clear, credible and consistent. As a consequence, WHO has an important role in global coordination of communication efforts.

5. 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 learned since the discovery of SV40 as a contaminant of polio 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 scientific advice, the vaccines concerned were not removed from the market, or were only temporarily suspended, since the benefits of immunization were believed to be much more beneficial than the risk of any potential adverse effects. After further evaluation, that initial assessment proved to be correct.

The response to the initial findings in each case 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 interactions and effective global coordination.

The manner in which the international scientific community dealt with the case of reverse transcriptase (RT) in measles and mumps vaccines should serve as a model for dealing with similar issues in the future because of the excellent coordination and collaboration during the scientific investigation. In responding to the RT case, there was a global consensus on what regulatory actions were appropriate, which facilitated clear communication of the issues.
There was good collaboration in addressing the PCV issue also, but the PCV incident highlighted the complexity of being able to arrive at a global consensus when local/regional considerations are taken into account. Different NRAs/NCLs may have different benefit/risk considerations for their country based on vaccine supply, disease prevalence and severity, and their specific epidemiological situation, among other factors. All of these factors must be taken into consideration when making a decision and the decision making should be based on sound science.

The potential impact of regulatory decisions on public health should be discussed with public health officials. The importance of transparency in the decision-making process is clear. The PCV case also highlighted the same issue that was identified by the bacteriophage case: 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 (e.g., RT in eggs, bovine spongiform encephalopathy in bovines). Such risks of microbial contamination can be minimized by limiting the use of animal-derived materials in manufacturing, applying good manufacturing practices, 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 to WHO may provide advice on cell substrates viral safety upon request from WHO. This expert advice, based on state-of-the-art technologies developed by manufacturers, NRAs/NCLs, and academic institutions, would serve as valuable support to WHO to facilitate responding in a timely manner to unexpected findings of adventitious agents in vaccines.

The final general observation from the four case studies is the key role that WHO has played in the coordination of a global response to the finding of an adventitious agent (or signal of an agent). Importantly, the role of WHO in the global coordination of actions to be taken and communication among NRAs of various countries and regulatory regions has been and continues to be pivotal in such situations, as it was, 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 globally. WHO coordinates efforts directed towards achieving international consensus, but NRAs ultimately make decisions based on benefit/risk assessment for their own populations. That may result in different decisions 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 the interest of global public health, any signal of a potential adventitious agent in a marketed vaccine should be reported promptly to WHO. In particular, the NRAs and manufacturers of prequalified vaccines have a responsibility to immediately inform WHO and to take appropriate follow up actions.

Since the finding of SV40 in polio vaccines, there have been many advances in science and technology as well as routine use of more efficient methods for communication and exchange of information. All of these changes 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 have investigated new technologies, and when new methods have proved to be superior to existing ones, 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 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 guidance or a 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 on which decisions are made, the probability of miscommunication and error are minimized. WHO has a critical role in global coordination of responses to the discovery of a signal for a potential adventitious agent and of public communication on regulatory decision-making.

6. Glossary (alphabetical order)

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

**Adventitious agent**
Contaminating microorganism of the cell culture or starting/raw materials, including bacteria, fungi, mycoplasmas/spiroplasmas, mycobacteria, rickettsia, protozoa, parasites, transmissible spongiform encephalopathies 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 (e.g. packaging cell lines for gene therapy vectors, Vero cells for vaccine production, 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 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 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 silicon or glass chip or as beads.

**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 (NGS)**
High-through-put sequencing technology that processes sequences in parallel, producing thousands or millions of sequences at once from a sample. Methods and technologies include 454 pyrosequencing, Illumina, Ion Torrent, and several others. Each method has different attributes, such as length of a typical sequence read, accuracy, number of reads per run, time for a run, costs, etc. In consequence, 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 benefit-risk 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 attaining 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 WHO and International Conference on Harmonisation 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 manner, 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 quantitation (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 quantitated as an exact value.
Specificity
The ability of a method to detect the required 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/assay
An analytical procedure or method – e.g. for identification of an analyte, for measuring the content or presence of impurities, or for quantitation of active ingredients.

Upstream
Activities that occur at or near the beginning of a process or manufacturing flow – e.g. cell culture and harvest or establishment of seeds or cell banks – as distinguished 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 (26) and scientific validation that the method being used for a given purpose actually measures the intended characteristic and that the characteristic is scientifically meaningful.

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References

2. Unexpected protein found in measles-mumps vaccine-entity said to be from chicken, harmless to humans. Washington Post, 9 December 1995.
10. Recommendations for the evaluation of animal cell cultures as substrates for the manufacturer of biological medicinal products and for the characterization of cell banks.


