Risk assessment: What is the risk to UK consumers of exposure to viable SARS-CoV-2 via the consumption of farmed produce that have become contaminated via exposure to infectious virus via wastewater systems, specifically, bivalve molluscs originating in UK waters, and crops (particularly ready to eat fresh fruits and vegetables) grown on land treated with sludge in the UK?

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Risk question

What is the risk to UK consumers of exposure to viable SARS-CoV-2 via the consumption of farmed produce that has become contaminated via exposure to viable virus via wastewater systems, specifically, bivalve molluscs originating in UK waters, and crops (particularly ready to eat fresh fruits and vegetables) grown on land treated with sludge in the UK?

Background

Since late 2019 the disease named COVID-19 (WHO, 2020a) caused by the novel coronavirus SARS-CoV-2 (Gorbalenya et al., 2020) has spread to most parts of the world, resulting in over 3.5M infections and over 240,000 deaths (correct as of 6th May 2020; (WHO, 2020b)). Some reports (cited later in this assessment) have suggested the possibility that viable virus might be excreted by infected individuals, and diarrhoea has been observed in a proportion of confirmed cases. In order to better understand the possible risk to UK consumers of shellfish originating in UK waters, and the risk posed by use of sewage sludge on arable land, the Microbiological Risk Assessment team of the UK Food Standards Agency was asked to assess the risk that consumers might be exposed to viable SARS-CoV-2 by consuming bivalve shellfish, or ready-to-eat and frozen fresh fruits and vegetables as a result of contamination during production.

The current risk assessment represents a conservative estimate of risk whilst acknowledging and reflecting current knowledge gaps, and the general potential for foodborne transmission of SARS-CoV-2 is explored in more detail in the overarching FSA risk assessment "What is the risk of food or food contact materials being a source or transmission route of SARS-CoV-2 for UK consumers?".

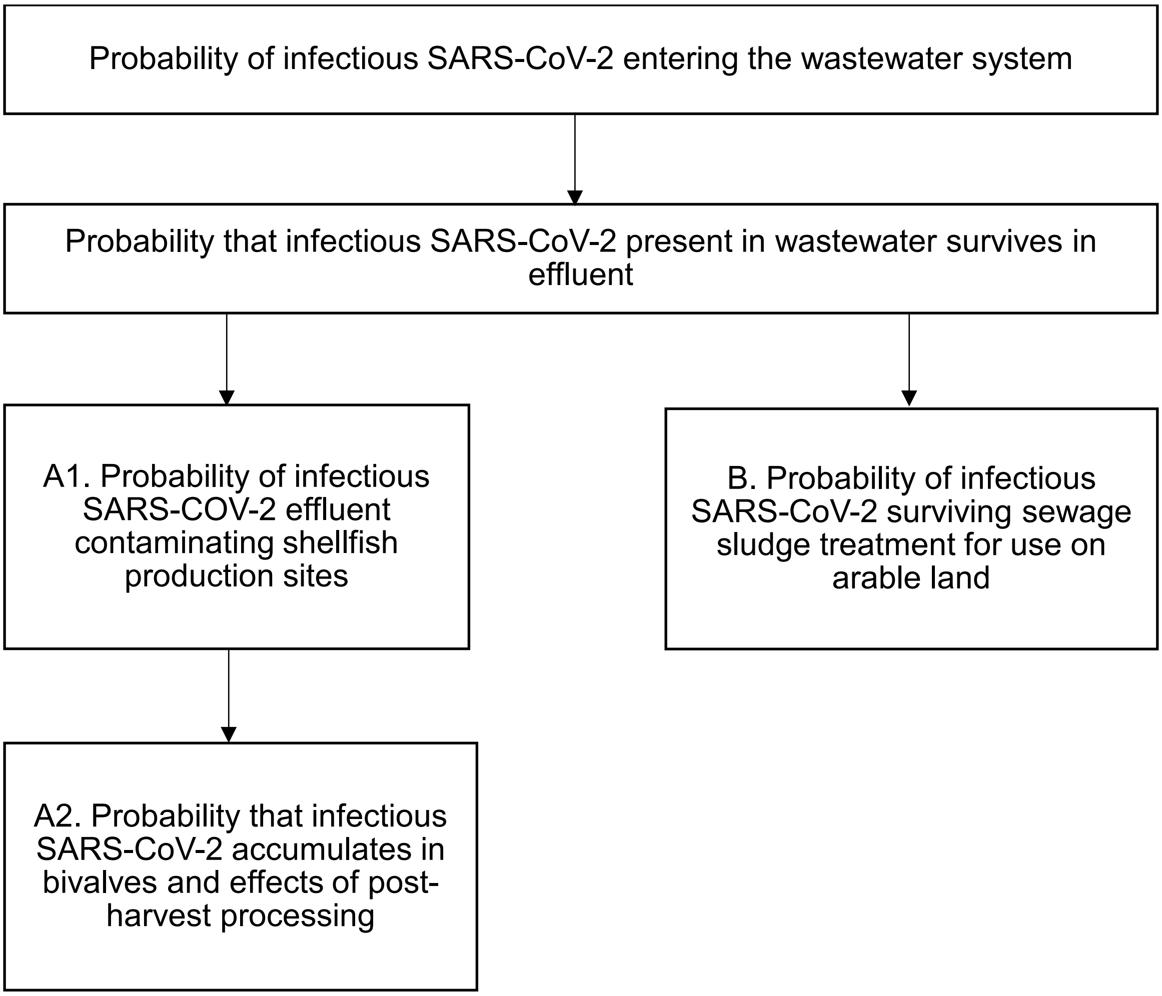
Hazard Identification

SARS-CoV-2 is located in the subgenus *Sarbecovirus*, genus *Betacoronavirus*, family *Coronaviridae* (Gorbalenya et al., 2020). Beta-coronaviruses are enveloped RNA viruses with a large (27-32kb), positive-sense single-strand genome. Phylogenetic analysis suggests that, like many other coronaviruses, it may have originally circulated within a bat reservoir, which moved via an as-yet unknown intermediate host into the human population via a single crossover event which has since been followed by human-human transmission. Phylogenetic data alone is unable to indicate whether human-human transmission has been direct or indirect.

Like the closely-related SARS-CoV and MERS-CoV, SARS-CoV-2 is a respiratory virus which spreads primarily via droplets spread when an infected person coughs or sneezes (WHO, n.d.). The potential for transmission via contaminated food or food contact materials is not known but not believed to be high, and this pathway was not implicated during outbreaks of either SARS-CoV (WHO, 2003) or MERS-CoV (WHO, 2019).

Exposure assessment

Risk pathway



Probability of viable SARS-CoV-2 entering the wastewater system

Several studies have reported the detection of SARS-CoV-2 RNA in faeces. In each of these cases, detection was via RT-PCR, which detects short sequences of the genome of the virus rather than indicating the presence of viable virus, and the quantity of viral RNA detected was not stated. A study of SARS-CoV-2 showed that faecal samples from 41 (55%) of 74 confirmed COVID-19 patients were positive for SARS-CoV-2 RNA (Wu et al., 2020). ECDC reports that 30% of cases have stool samples which test positive for SARS-CoV-2 from day 5 of symptom onset (ECDC, 2020). Studies have also reported the detection of SARS-CoV-2 viral RNA in faeces, (Xiao et al., 2020); (Holshue et al., 2020). A number of studies have shown that SARS-CoV-2 RNA can be detected in faecal swabs, these remained positive for a mean of 27·9 days after first symptom onset (Wu et al., 2020). Faecal samples can also remain positive for 6 to 20 days after respiratory samples are negative ((Wu et al., 2020);(Xing et al., 2020),(Chen et al., 2020)).

The extent to which these results represent viable virus is **uncertain**. As noted, the studies reported above used RT-PCR, which detects viral genome and does not necessarily reflect the presence of viable virus. Although viable SARS-CoV-2 can be detected via assays based on cell infection, some substrates (including sewage) are themselves typically toxic to cells, complicating detection. Based on reports from WHO (WHO, 2020c) there is some evidence that Covid-19 infection may lead to intestinal infection and be present in faeces, two studies have shown viable SARS-CoV-2 viral particles isolated from 1 stool sample (Zhang et al., 2020) and 2 stool samples (Wang et al., 2020), although the number of samples tested was not reported. There have been no reports of faecal−oral transmission of the COVID-19 virus to date. Additionally, studies in Australia and the Netherlands have detected SARS-CoV-2 RNA in untreated sewage ((Lodder and Husman, 2020), (Ahmed et al., 2020)).

The prevalence of infection in the UK varies by region and shedding of SARS-CoV-2 into wastewater systems is likely to occur at a higher rate in regions with a high prevalence of infection. However, any virus would be significantly diluted with the sewage from those not infected, and those infected but not shedding virus into faecal material, and the sewage will be diluted by wastewater from other sources (e.g. dishwater, bathwater).

Collectively, these studies suggest that although SARS-CoV-2 RNA is likely to be present in the faeces of a significant fraction of people infected with the virus, the probability of viable virus entering the UK wastewater system is **Medium** with **High** uncertainty.

Probability that viable SARS-CoV-2 present in wastewater survives in the wastewater and in effluent

Like other viruses, SARS-CoV-2 cannot replicate outside of suitable host cells. Any viable virus in the environment will be inactivated over time, but the rate of inactivation will depend on environmental conditions, such as temperature, pH, salinity, exposure to UV light, for example via sunlight and exposure to chemical like detergents. The most important of these is normally accepted to be temperature, with higher temperatures resulting in more rapid inactivation ((Pinon and Vialette, 2018);(Gerba, 2005)). The presence of populations of other microbes also accelerates viral inactivation compared to sterilised water (Pinon and Vialette, 2018). Non-viable virus may still be detectable by PCR and therefore PCR-positive results alone cannot be used to infer the presence of viable virus.

So far, no studies on SARS-CoV-2 have identified how long the virus can survive in raw sewage (**uncertainty**). A study by Gundy et al. (Gundy et al., 2008) using other coronaviruses showed that inactivation in water and wastewater was highly dependent on temperature, level of organic matter, and the presence of antagonistic bacteria, but a related coronavirus (human coronavirus 229E) showed a 3-log (99.9%) reduction in 3.5 days in primary effluent and 2.8 days in secondary effluent at 23°C. No other temperatures were included in the study and effluent from secondary biological filtration and from activated sludge treatment can cycle from 20ºC in summer down to about 8ºC in winter (Environment Agency, pers. comm. 2020). The reported rate of inactivation, of human coronavirus 229E, is similar to that reported for SARS-CoV which persisted for 2 days in hospital wastewater at 20°C and with a longer persistence (at least 14 days) at 4°C (Wang et al., 2005), although the latter study reported RT-PCR results which as previously noted may not have represented viable viral particles. Casanova et. al showed that the time to get 4 $\log_{10}$ reduction in infectivity titre in pasteurized settled sewage at 25°C was 14 days (mouse hepatitis virus) to19 days (transmissible gastroenteritis virus) and more than 4 weeks at 4°C for both surrogate viruses (Casanova et al., 2009).

The time spent in treatment is highly variable but is typically around 4-8 hours in secondary treatment (Environment Agency, pers. comm. 2020), plus additional time travelling to the wastewater treatment plant and undergoing primary treatment.

Collectively, the evidence available suggests that any viable virus present in wastewater can be inactivated by sewage, although because no published studies have studied SARS-CoV-2 specifically there remains medium **uncertainty** around this assumption.

Wastewater from about 96% of the UK population is treated in wastewater treatment works (DEFRA, 2012); most of the remainder is served by small private treatment works, cesspits or septic tanks, which are mostly in rural areas of lower population density. Wastewater is typically treated in the UK via a series of stages, depending on the size of the population of the area it covers (Environment Agency, 2019). Following pre-treatment to remove large solids, primary treatment occurs in a settlement tank where the solids sink to the bottom. Secondary treatment involves aeration to encourage the growth of non-pathogenic bacteria; the wastewater is then passed to another settlement tank (Southern Water, n.d.).

These processes are likely to reduce the levels of viral particles remaining in wastewater. (Ye et al., 2016) suggest that enveloped viruses adsorb effectively to the solid fraction of wastewater (greater than 75% absorption), meaning that levels in wastewater are reduced during settlement, although this will have a relatively minor effect compared to the rate of inactivation. The microbial growth promoted by aeration during secondary treatment is also likely to accelerate viral inactivation (Pinon and Vialette, 2018).

Several studies using PCR to compare influent and effluent levels of human norovirus following a variety of treatment methods suggest that, over the whole wastewater treatment process, a reduction of around one $\log_{10}$ in viral RNA is seen (Silva et al., 2007). Human norovirus is a relatively resistant, non-enveloped virus whereas coronaviruses possess an envelope, making them easier to inactivate; this reduction is therefore likely to represent a conservative estimate of the reduction in the level of coronaviruses as a result of wastewater processing. However, this conclusion has not been measured specifically for SARS-CoV-2 and relies upon PCR detection of viral RNA (**uncertainty**).

Although beyond the scope of this assessment, we note that untreated (consented or unconsented) sewage discharges into waterways may occur and will not undergo this step.

Overall, we consider that the net effects of inactivation in microbially active wastewater, combined with the effects of treatment, will result in a **Low** probability that viable virus entering the wastewater system will remain viable upon exit at a level which will result in exposure to those consuming shellfish, but with a **High** uncertainty.

A1. Probability of viable SARS-CoV-2 from effluent contaminating production sites

During normal operation, a proportion of wastewater that has been adequately treated may be released into the sea environment.

In the absence of more specific data, we would assume the rate of SARS-CoV-2 inactivation in raw (non-sterilised) seawater to be similar to the inactivation rate in wastewater at equivalent temperatures, although with high **uncertainty**. Given the temperature of seawater around the UK, the inactivation of virus is likely to be sufficiently slow that this will not substantially alter the probability of virus entering seawater remaining viable to contaminate production sites.

However, the significant dilution effect of effluent entering the marine environment after release is likely to result in a **Low** probability that viable virus present will contaminate shellfish with a **High** uncertainty.

A2. Probability that virus accumulates in bivalves and effects of post-harvest processing

In order for bivalves to become contaminated with SARS-CoV-2 via wastewater, they would need to be growing in sufficient proximity to a wastewater outflow location.

Shellfish production and relay areas are classified according to the levels of E. coli detected in shellfish flesh: shellfish from class A beds are considered fit for human consumption without further treatment, shellfish from class B beds require purification/depuration for 48 hours or relaying for 1 month in class A waters, and shellfish from class C beds must either undergo relaying for at least two months in an approved class B relaying area followed by purification, or relaying for at least two months in an approved class A relaying area. Alternatively, shellfish from class C beds may undergo one of a variety of approved heat treatment methods set out in EC regulation 853/2004, any of which are extremely likely to inactivate any viable virus present (FSA, 2020a). These processes aim to prevent faecal contamination of shellfish beds, a study by Cefas showed a statistically significant and predictive correlation between *E. coli* and norovirus levels when data was analysed by site rather than by sample but it is also worth noting all 39 sites tested positive for norovirus at least once (Lowther, 2011).

However, no studies of the potential for oysters to accumulate SARS-CoV-2, SARS-CoV or other coronaviruses were located, and expert opinion received from CEFAS via Defra[1] affirms that molluscs are not capable of being infected with betacoronaviruses. Norovirus binds to a specific receptor within oysters (Le Guyader et al., 2012) which facilitates its accumulation; it is unknown if SARS-CoV-2 would also bind within the oysters and no ACE2 receptor has been reported in shellfish (**uncertainty**). It is assumed that, as a worst-case scenario, SARS-CoV-2 would be able to accumulate in a similar way to human norovirus; however, we have no evidence that accumulation of this kind will occur.

During a study of human norovirus, oysters were suspended adjacent to a wastewater treatment plant outflow for one month; these oysters were then found to contain human norovirus at approximately ten times the concentration detected in the wastewater outflow (Flannery et al., 2012). Assuming a more reasonable distance between the outflow and the production area, the level of contamination present in oysters harvested from production areas approved for human consumption is likely to be several orders of magnitude lower, although as we do not have a dose response level for SARS-CoV-2 it is hard to equate this with infection.

The effect of depuration or relaying on the inactivation of SARS-CoV-2 is not known (**uncertainty**), but if similar to the rate of inactivation in wastewater and seawater, any relaying period of one month or greater is likely to eliminate any virus present.

We therefore consider that there is not sufficient evidence showing SARS-Cov-2 will accumulate in bivalve molluscs at sufficient levels to increase the probability of consumers exposure, and that most treatments needed for shellfish produced in Class B or Class C production areas (which are those most likely to become contaminated) are likely to result in the inactivation of viable virus present in those bivalve molluscs. Overall, the low probability for accumulation of viable virus by bivalve molluscs and the potential for inactivation of any contaminating virus during subsequent processing for product harvested from Class B and C production areas is considered likely to result in a **Low** probability that viable virus present will remain

[1] “Aquatic *Nidovirales* briefing note: (with respect to *Coronavirus*)” authored by R. Paley, 18th Feb 2020.

in shellfish at a level sufficient to result in exposure to consumers, with **High** uncertainty.

B. Agricultural practices: application of sludge to arable land

For the purpose of this risk assessment the term sludge encompasses both sewage sludge derived from urban sewerage systems and sludge derived from septic tank. The probability of cross contamination of fresh produce via contaminated sewage sludge depends on the frequency of viral shedding from infected individuals, the survival of any resultant SARS-CoV-2 in wastewater, the potential for SARS-CoV-2 to survive and accumulate in subsequent waste treatment processes and then the potential for SARS-CoV-2 to survive in agricultural soils and on fresh fruits and vegetables.

To date few studies have (WHO, 2020d) isolated viable SARS-CoV-2 from a stool sample (Zhang et al., 2020) (Wang et al., 2020) and any subsequent survival in wastewater is unknown (**uncertainty**). A number of other studies have detected SARS-CoV-2 RNA from stool samples however, the presence of viral RNA in the samples does not determine whether the virus is intact or the viability of any virus present (**uncertainty**). As described above sewage undergoes a number of treatments including settlement, where a percentage of the virus may not survive A study from (Ye et al., 2016) measured survival of enveloped viruses in water waters and partitioned sewage, temperature was shown to have a large effect on survival, however both enveloped viruses survived for a maximum of 72 hours depending on the virus and temperature and fraction.

The UK recycles a large proportion of sewage sludge on arable land (Milieu Ltd et al., 2010), The majority of treatments for processing sewage sludge from anaerobic digesters for application to farmland in the UK use increased pH or temperatures for prolonged periods (such as 30 minutes at 70°C or minimum of 4 hours at 55°C, or to above pH12 for at least 2 hours (DEFRA, 2018)) either of which should be sufficient to inactivate any viable SARS-CoV-2 from the sludge. The thermal tolerance of SARS-CoV-2 is described in detail in (Chin et al., 2020). Another treatment method is to store liquid sludge for 3 months before use. Sewage sludge that has not been treated must be injected below the surface of agricultural soils and must be injected before growing crops. For soft fruit, vegetables and potatoes this must be carried out at least 10 months (“The Sludge (Use in Agriculture) Regulations 1989,” n.d.) before planting, particularly if the edible part of the crop is directly in contact with soil and may be eaten raw. Although the soil survivability of SARS-CoV-2 has not been tested (**uncertainty**) it has poor environmental survival on exposed surfaces. Given the number of stages of processing any virus present would need to tolerate and the extremely long delay between sludge application and harvesting, the risk of transmission of SARS-COV-2 via food as a result of sewage sludge use on UK agricultural soils is **Negligible** with **Medium uncertainty** due to the lack of associated publications.

Hazard characterisation

Illness caused by coronavirus species vary and range from cold like symptoms to more severe illness in humans including gastroenteritis and respiratory tract diseases. SARS-CoV-2 has been associated with cases of viral pneumonia and respiratory tract disease ((WHO 2020), (GOV.UK 2020)10th January 2020).

An analysis of the clinical presentation of 41 patients (median age of 49) with lab-confirmed COVID-19 in China published online on 24th January 2020 (Huang et al. 2020) suggests that common symptoms at onset of illness are fever (98%), cough (76%) and myalgia/fatigue (44%); less common were sputum production (28%), headache (8%), coughing blood (5%), and diarrhoea (3%). Laboured breathing developed in 55% of patients after a median time from onset of 8 days. 63% of patients had lymphopenia and all patients had pneumonia with ground glass opacity. More severe cases progress to acute respiratory distress syndrome (ARDS), acute cardiac injury, acute kidney injury, and shock. (Jiang et al. 2020)

Current evidence suggests that a high proportion of patients developing severe clinical disease had pre-existing health conditions, and this group is likely to represent most of the individuals at risk of severe disease.

Further information on the potential for infection via ingesting virus can be found in the overarching FSA risk assessment. (FSA, 2020b)

Risk characterisation

We are not aware of any known or suspected cases of SARS-CoV-2 linked to consumption of seafood, including bivalve molluscs, globally, or of any cases where foodborne transmission has been implicated in any cases of infection with SARS-CoV-2 or in cases of the closely-related SARS-CoV. The World Health Organization (WHO) has stated that there is no evidence that the SARS-CoV-2 virus has been transmitted via sewerage systems with or without wastewater treatment (WHO, 2020d). WHO also notes that while persistence in drinking water is possible, there is no evidence from surrogate human coronaviruses that viable virus is present in surface or groundwater sources or transmitted through contaminated potable water.

Based on the evidence presented above we consider that:

- The probability of viable SARS-CoV-2 entering the wastewater system is between **Medium** with a **High** level of uncertainty;
- The probability of viable virus surviving to exit the wastewater system is **Low** with a **High** level of uncertainty;
- The probability of viable virus remaining in effluent reaching production areas, including dilution effects, is **Low** with **High** uncertainty;
- The potential for virus to accumulate in exposed shellfish, combined with the effects of post-harvest processing, is **Low** with **High** uncertainty;
- The severity of consequence if viable SARS-CoV-2 is capable of infecting consumers exposed through the consumption of bivalve molluscs contaminated with viable virus is **High** with **Low** uncertainty.

- The potential for SARS-CoV-2 to be present on crops grown on arable land treated with sludge to be **Negligible** with **Medium** uncertainty.

In arriving at an overall probability for a pathway, individual step probabilities were first combined using a matrix rule; a probability of “Low” lowered the overall qualitative probability by one category; “very low” by two categories, and “negligible” by three categories; “Medium” resulted in no change and “high” increased the probability category by one. An independent assessment was then made of the resulting overall risk level, following independent internal review, in view of the sum total of evidence to ensure it was within the definitions considered. This helps to ensure transparency in approach while addressing acknowledged inaccuracies that can result in some cases from unsupervised matrix combinations of qualitative probabilities, for example when combining high and low qualitative probabilities (WHO/FAO 2008).

For this assessment, both the matrix rule approach and independent final risk estimate arrived at the same qualitative measure of risk for both pathways (A and B).

We consider that the overall risk to UK consumers via the consumption of bivalve molluscs originating in UK waters is **very low**, with a **high level of uncertainty** due to the current lack of data specific to SARS-CoV-2, and that the risk to UK consumers via the consumption of food grown on arable land treated with sewage sludge is **Negligible** with **Low** uncertainty.

Uncertainties

Key uncertainties include:

- The incidence of infection in the population;
- The percentage of infected individuals that are shedding viable viral particles;
- The infectivity of viral particles detected in stool;
- The rate of inactivation of SARS-CoV-2 in sewage/wastewater and seawater;
- The effectiveness of wastewater treatment in removing potentially viable virus;
- The relative dilution between virus present in wastewater outflow and virus coming into contact with shellfish production areas;
- The potential for SARS-CoV-2 to bind and accumulate within the digestive tracts of bivalve molluscs;
- The rate of inactivation of SARS-CoV-2 within bivalve mollusc digestive tracts;
- Whether consumption of virus is a route of infection for SARS-CoV-2 and the relationship, if any, between ingested dose and infection.

Not considered in this document

- Consumption behaviours
- Cooking
- Changes to behaviours during the lockdown period

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