NASA does NOT establish that life from Mars can get to Earth faster and better protected in a meteorite – indeed they don't establish that ANY life on Mars has the capability to get to Earth in a meteorite – and their work does NOT overturn previous warnings of the potential for large scale effects from life returned from Mars, which has major legal implications

Author: Robert Walker (contact email <u>robert@robertinventor.com</u>). Do please contact me if you read this and happen to spot any mistakes, omissions or anything to fix however small, thanks!!

I am currently working on this document, so you can find the latest version here <u>https://osf.io/2jfnv</u>, doi 10.31219/osf.io/2jfnv

This version dated 26th December 2022.

For a fast overview, read the headings, and drill down into sections of interest for more details.

Section titles are written like mini-abstracts so the title tells you not just what the section is about but also the basic argument and conclusion, again to make it easier to navigate.

I hope to get this paper accepted for publication before completion of the NEPA process in spring / summer 2023 although it's unlikely to be enough time to complete peer review.

I was able to write this analysis quickly because I've been working for two years on another paper about planetary protection issues for NASA's Mars sample return mission: <u>NASA and ESA are likely to be legally required to sterilize Mars samples to protect the environment until proven safe – technology doesn't yet exist to comply with ESF study's requirement to contain viable starved ultramicrobacteria that are proven to pass through 0.1 micron nanopores - proposal to study samples remotely in a safe high orbit above GEO with miniature life detection</u>

<u>instruments – and immediately return sterilized subsamples to Earth</u>, Preprint DOI <u>10.31219/osf.io/rk2gd</u> For latest version of the main paper preprint please visit: (url <u>https://osf.io/rk2gd</u>)

I often refer to sections of that paper for additional details for anyone who wants to follow something up further. I refer to it as: (Walker, 2022b) (section title).

Colour coding. I use pale blue text for titles of sections in my main paper – I can't link to as they are in a separate document,

I also use this colour for quotes from my previous submissions for the NASA EIS comments process.

I use orange text for quotes from the NASA draft EIS and associated documents

All other quotes are black. This colour distinction should work for all forms of colourblindness except monochromats who will see both types of text as a pale gray according to <u>this simulator</u> – but they aren't easily confused with each other.

This is an extract from my longer study of NASA's draft EIS which I plan to submit for publication as series of smaller papers. The hope is that this evidence can help persuade NASA to find a different course of action. I hope this doesn't have to get to the point of a legal challenge but if it is these, papers would help support it and also hopefully help to direct the justice to find an equitable solution that would require pre-sterilization of samples that NASA returns to Earth rather than just telling them to abort the mission altogether.

The longer draft EIS is here.

For NASA's draft EIS see my preprint: <u>Such serious flaws in NASA's Environmental Impact</u> <u>Statement for a Mars Sample Return - omits major impacts – uses old science later overturned –</u> <u>statements cited to sources that say the opposite – no response to significant public concerns -</u> <u>and haven't done the update for size limits recommended by the ESF in 2012 after they reduced</u> <u>it from 0.2 to 0.05 microns in just 3 years</u>

N.B. this section of it currently includes cites that aren't needed for this section of it as a simple way to get it ready for publication quickly. If accepted for publication the citation list can be filtered down to the cites actually used here with a few hours of work.

[Early draft – work in progress]

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Introduction – this paper focuses on transfer from Mars to Earth and potential for large scale effects, public involvement and legal process – and brief summary of why there is potential for extant life in Jezero crater

This paper focuses on the transfer of life from Mars to Earth on meteorites, the potential for large scale effects, need for public involvement in decisions on acceptable risk – and the effects of all this on the legal process.

If this paper is considered too long, it can be divided into sub-papers, for instance on:

- That the meteorite argument doesn't work that it is not true that all or even any life can get back to Earth faster and more easily in a meteorite.
- Potential for large scale effects
- Legal implications

The EIS also argues that there is no risk of life in Jezero crater saying that it's a consensus view that Mars has been uninhabitable for terrestrial life for millions of years and saying that if there is present day life on Mars that it isn't present in Jezero crater. Their cites don't support this. This will be a topic for a separate paper but a brief summary is included here.

Future papers planned for this series on serious errors in NASA's draft EIS

I plan other papers on:

- 1. on the impossibility of containing 0.05 / 0.01 microns in a BSL-4, need for review of the size limit and survey of the literature on air filters showing none of them currently can meet the requirements set by the ESF in 2012.
- a refutation of NASA'S arguments that there is a consensus view that Mars is not currently habitable – their own source is about a search for currently habitable regions on Mars, and that if it has life it isn't in Jezero crater based on correct use of their own cites plus using MEPAG2 which overturned the results in MEPAG that habitable regions

can be delineated using maps because of microhabitats, biofilms and transport of microbes in dust (briefly summarized in this paper)

- 3. showing that it is impossible to do safety testing of Mars samples we can't distribute unsterilized samples to terrestrial laboratories until we have done Sagan's "vigorous program of unmanned Martian exobiology and terrestrial epidemiology" as well as review of effects more generally on the terrestrial biosphere, and other lifeforms of any life that we may find on Mars
- 4. Showing that Perseverance's permitted level of organics makes this a mission mainly of interest to geology and recommending sterilization as the simplest way to keep Earth safe
- 5. on the impossibility of protecting Earth with human quarantine in a human operated space station
- 6. Building on previous papers a request for NASA to include a sterilized return as a reasonable alternative in the EIS,
- 7. A request to NASA to include as a reasonable alternative a proposal to add a bonus sample for astrobiology collected in a sterile container to return for remote study to a satellite similar to other Geostationary satellites in size with a centrifuge inside to simulate Martian gravity, in a safe orbit for Earth, well above GEO this would let astrobiologists study martian dust and dirt in a similar way to studies in situ on Mars which would be a first start on Sagan's vigorous program of unmanned exobiology and preparation to send those instruments for in situ studies on Mars later

Motivation for this paper - why we need to consider the potential for life returned from Mars – NASA's summary of the literature on the potential for viable present day life in Jezero crater is mistaken – to be expanded on in other papers in this series

To reduce the length of this paper I will look just briefly at these central arguments in the EIS as we need to see that they are invalid in order to motivate the rest of this paper.

The details are for separate papers

Draft EIS says (MISTAKENLY) existing credible evidence suggests Mars hasn't been habitable for life as we know it for millions of years - their cite says that we need to search for current habitats in a seemingly uninhabitable Mars

One central argument in the draft EIS is that Mars is lifeless anyway and that they are doing the sample return precautions just out of an "abundance of caution". The draft EIS says that "conditions on Mars have not been amenable to supporting life as we know it for millions of years" (<u>NASA, 2022eis</u>: 1-6):

Existing credible evidence suggests that conditions on Mars have not been amenable to supporting life as we know it for millions of years (iMARS Working Group 2008, National Research Council 2011, Beaty et al. 2019, National Research Council 2022).

But their most recent 2022 source for this "existing credible evidence" says the opposite from their summary. It says that exploration of Mars will help establish whether localised habitable regions currently exist.

Their source refers to Mars as "seemingly uninhabitable", not "uninhabitable. See: (<u>Smith et al.</u> <u>2022</u>: <u>393</u>) (click on X button on banner to go straight to the page)

Section:

"Are There Chemical, Morphological and / or Physiologic / Metabolic or Other Biosignatures in **Currently Habitable Environments** in the Solar System

The exploration of ... Mars (Curiosity, Perseverance) will help establish whether localised habitable regions **currently exist** within these seemingly uninhabitable worlds.

[Emphasis on "currently" mine]

Here is a screenshot.



Chapter A Page 393 of 761 Q mars life HOW did NASA miss all these errors in its EIS?

Q11.3a Are There Chemical, Morphological and/or Physiologic/Metabolic or Other Biosignatures in Currently Habitable Environments in the Solar System?

The continued exploration of planetary bodies of the solar system is revealing a broader range of potentially habitable solar system environments than previously anticipated (Question 10). Data gathered by the Cassini spacecraft suggests that the subsurface ocean of Enceladus currently meets the requirements to sustain life (Cable et al. 2020). The Europa Clipper and Dragonfly missions will help constrain the biological potential of Europa's and Titan's subsurface oceans, respectively. The exploration of Venus (VERITAS; DAVINCI) and Mars (Curiosity; Perseverance) will help establish whether localized habitable regions currently exist within these seemingly uninhabitable worlds. Once habitable environments are identified, the search for evidence of life represents the logical next step, and also the greatest challenge.

The search needs to be conducted thoughtfully and with an open mind concerning potential outcomes, balancing the *stringency* and *inclusivity* of the observational strategy applied to a given environment. Stringency sets criteria for the quality and robustness of a biosignature detection, amidst potentially confounding conditions or background signals from the planetary environment, and thus seeks to minimize potential false positive results such as a "life-like" abiotic pattern or response. Inclusivity emphasizes consideration of a wide range of possible alien biosignatures (chemical, morphological and/or physiologic/metabolic), not relying solely on Earth life as a guide, as well as their prevalence and detectability in the given environment. As such, inclusivity seeks to minimize potential false negative results, where life could be "missed" for lack of the ability to detect or recognize it. These concepts apply equally to cases where life may have gone extinct, detectable through its imprint preserved over time (Q11.3b).

Source: "exploration ... will help establish whether localized habitable regions CURRENTLY exist within these seemingly uninhabitable worlds."

NASA: "Existing credible evidence suggests that conditions on Mars have not been amenable to supporting life as we know it for millions of years"

HOW did NASA miss all these errors in its EIS? Source: "exploration ... will help establish whether localized habitable regions CURRENTLY exist within these seemingly uninhabitable worlds."

NASA: "Existing credible evidence suggests that conditions on Mars have not been amenable to supporting life as we know it for millions of years.

Screenshot from: (Smith et al, 2022: 393)

Their source continues by saying that once habitable environments are identified, the greatest challenge is the search for evidence of life and it warns about the need for inclusivity, not relying solely on what life on Earth can do as a guide. (Smith et al, 2022: 393):

Once habitable environments are identified, the search for evidence of life represents the logical next step, and also the greatest challenge.

. . .

Inclusivity emphasizes consideration of a wide range of possible alien biosignatures (chemical, morphological and / or physiologic/ metabolic), not relying solely on Earth life as a guide, as well as their prevalence and detectability in the given environment. As such, inclusivity seeks to minimize potential false negative results where life could be "missed" for lack of the ability to detect or recognize it.

The details here are for a separate paper based on these sections of my preprint:

Draft EIS says MISTAKENLY that the 2014 MEPAG study represents a consensus opinion within the astrobiology scientific community – it was not a consensus even for forwards contamination as it was overturned by the 2015 MEPAG2 review, commissioned by ESA and NASA which emphasized potential for microhabitats within apparently uninhabitable regions, and transport of life on dust

Another central part of the reasoning is they claim that there is no life in Jezero crater where Perseverance is collecting samples even if there is life elsewhere. Again they falsely claim a consensus on this. (<u>NASA, 2022eis</u>: S-4)

Consensus opinion within the astrobiology scientific community supports a conclusion that the Martian surface is too inhospitable for life to survive there today, particularly at the location and shallow depth (6.4 centimeters [2.5 inches]) being sampled by the Perseverance rover in Jezero Crater, which was chosen as the sampling area because it could have had the right conditions to support life in the ancient past, billions of years ago (Rummel et al. 2014, Grant et al. 2018).

Their first cite here is <u>(Rummel et al , 2014)</u> which is a study of Mars special regions. But Rummel et al looked at forward contamination, to try to delineate areas where missions TO Mars risk introducing terrestrial life that might be able to replicate on Mars. Rummel et al is NOT an attempt to explore possible locations for extant native martian life returned FROM Mars.

Rummel et al say this explicitly that they are not going to discuss habitats for extant Martian life. (Rummel et al , 2014:888)

Special Regions are regions "within which terrestrial organisms are likely to replicate" as well as "any region which is interpreted to have a high potential for the existence of extant martian life."

At present there are no Special Regions defined by the existence of extant martian life, and this study concentrates only on the first aspect of the definition.

The issue here is that martian life might have capabilities terrestrial life doesn't have through a different biochemistry or even just by having a different salt in the intercellular fluid instead of sodium chloride. We may not need to consider this in much depth for a study on forward contamination but it is essential to consider the possibility of martian life with capabilities different from terrestrial life for backward contamination.

Details here are for a separate paper. It is covered in my preprint under:

2015 review of the 2014 MEPAG report recommends further research into detectability of potential small-scale microbial habitats on Mars as a knowledge gap to be looked at in the future

This is in their Appendix A. (Board, 2015 : 46)

. . .

The need for more research into detectability of potential small-scale microbial habitats

Detectability of Potential Small-Scale Microbial Habitats

Perform in situ investigations in extreme environments on Earth to deepen our knowledge about microbial processes and habitability at micron scales. Adapt and optimize existing technologies and develop new ones to undertake the kind of investigations which may be used in the future exploratory missions to other planets and moons of astrobiological relevance.

Details here are for a separate paper. It is covered in my preprint under:

2015 review of the 2014 MEPAG report recommends further research into viability of terrestrial microbes transported in the dust storms on Mars as a knowledge gap to be looked at in the future

Need for more research into microbial viability of terrestrial life when transported in dust storms

Translocation of Terrestrial Contamination

Undertake investigations of transport mechanisms and microbial viability in Mars simulation chambers—e.g., the Mars Surface Wind Tunnel facility at NASA's Ames

Research Center or the low-pressure recirculating wind tunnels in the Mars Simulation Laboratory at Aarhus University—wherein microbes and spores are exposed to Marsrelevant levels of ultraviolet radiation, desiccation, nutrient deficit, and air movement, to assess the likelihood of survival during transport by, for example, dust storms.

As far as I can tell this research hasn't been done, at least I find no recent studies that cite the older studies on the topic.

In more detail on dust the 2015 report says dust can block UV and make microbes more viable, and microbes often occur in cell clusters and the inner cells would be protected against UV in dust storms

: (<u>Board, 2015</u> : <u>12</u>)

Atmospheric transport can move microbial cells and spores over long distances, as is known from investigations of foreign microbes delivered to North America from Africa via Saharan dust (Chuvochina et al. 2011; Barberàn et al. 2014) and Asia (Smith et al. 2012).

•••

In addition to dilution effects, the flux of ultraviolet radiation within the martian atmosphere would be deleterious to most airborne microbes and spores.

However, dust could attenuate this radiation and enhance microbial viability. In addition, for microbes growing not as single cells but as tetrades or larger cell chains, clusters, or aggregates, the inner cells are protected against ultraviolet radiation. Examples are methanogenic archaea like Methanosarcina, halophilic archaea like Halococcus, or cyanobacteria like Gloeocapsa. This is certainly something that could be studied and confirmed or rejected in terrestrial Mars simulation chambers where such transport processes for microbes (e.g., by dust storms) are investigated. The SR-SAG2 report does not adequately discuss the transport of material in the martian atmosphere.

Also this is all about forwards contamination by terrestrial life. What about Martian life adapted to the dust storms over billions of years? Could it develop adaptations to survive transport in dust storms that terrestrial life doesn't have? I suggest native Martian life could propagate via much larger grains up to half a millimeter in diameter if it can survive the impact shocks of repeated bounces across the Martian landscape.

Details here are for a separate paper. It is covered in my preprint under:

Draft EIS says (MISTAKENLY) Mars life can get to Earth faster and be better protected in meteorites than sample tubes - their cites don't support this - their main cite is about transfer from Mars to its innermost moon Phobos instead of Earth - and didn't look at sterilization during ejection from Mars

This is a central point in their argument (NASA, 2022eis: 3-3):

The natural delivery of Mars materials can provide better protection and faster transit than the current MSR mission concept.

Indeed, if they were able to establish this, there would be no need for containment. For example, the last question in the decision tree for returning samples from small bodies is (<u>NASEM, 1998:17</u>)

Does the preponderance of scientific evidence indicate that there has been a natural influx to Earth, e.g., via meteorites, of material equivalent to a sample returned from the target body?

If the answer is yes, no special precautions are needed. This has been used correctly on other sample return missions, for instance with Hayabusa 1 & 2 the second sample from an artificially induced impact crater was similar to material transferred to Earth through natural processes, and so needed no special treatment (Kminek et al, 1999) (Yano et al, n.d.).

But sadly, NASA don't establish this due incorrect use of their cites. The draft EIS says that potential Mars microbes would be expected to survive ejection forces and pressure (<u>NASA</u>, <u>2022eis</u>: 3-3):

First, potential Mars microbes would be expected to survive ejection forces and pressure (National Academies of Sciences, Engineering, and Medicine and the European Science Foundation 2019), ...

But the paper from 2019 which they cite to support that claim is first of all, a **study on ejection** of materials from Mars to its innermost moon Phobos, not to Earth.

To get from the Martian surface to Phobos requires an escape velocity of 3.8 km/s according to their cite (Board, 2019 : 26). So the shock of ejection would be far less than for materials ejected with enough velocity to reach Earth of 5.03 km / sec (NASA, n.d.mfs).

It's worse than that though. The paper they cite says explicitly that the team did NOT study sterilization during Mars ejecta formation in their analysis ($\underline{Board, 2019}$: $\underline{26}$) :

The SterLim team did not include any sterilization during Mars ejecta formation in its analysis because such investigations were not requested in its study's statement of work.

So, the draft NASA EIS is using this 2019 paper as their only source - on a topic *which the cite itself explicitly says it does NOT cover*. Their cite does briefly look at heating during ejection and it concludes:

Based on heat inactivation tests conducted by the SterLim team, its JAXA counterpart expected that this heating highly sterilizes the ejecta from Mars. In contrast to these impact physics models, the JAXA study did note that some experimental observations of martian meteorites do not show any signatures of shock heating.

In summary, the JAXA team decided to assume a survival rate of 10 percent during Mars ejecta formation, but noted that this assumption may be too conservative.

It does NOT look at the far more important effects of shock. It doesn't even mention the shock of ejection.

For more about this see (below):

 NASA fail to adequately consider the risks from life that can't get to Earth on meteorites - in 2009, the National Research Council examined the possibility of life transferred on meteorites said the risk is significantly greater in a sample return mission and said they can't rule out the possibility of large scale effects in the past due to life from Mars – NASA's EIS instead claims microbes will survive transfer from Mars to Earth more easily in a meteorite than in a sample return mission but their sources don't back this up

Another issue they don't mention is that all the martian meteorites we have in our collections come came from at least 3 meters below the Martian surface (Head et al, 2002:1355),. The subsurface below about 12 cms has a uniform temperature of around 200°K or -73°C (Möhlmann, 2005:figure 2). They were probably thrown up into space after glancing collisions into the Elysium or Tharsis regions, high altitude southern uplands (Tornabene et al, 2006). With such a thin atmosphere, and the low temperatures at 3 meters below the surface, present day life at those altitudes is unlikely (except perhaps for deep subsurface geothermal hot spots).

I cover this in more detail in the supplementary information at the end:

They also don't look at the fireball of re-entry when it reaches Earth, which is the biggest hurdle for photosynthetic life. Although life inside the rock is shielded from the fireball, any photosynthetic life would be on the surface, not inside.

One of the papers they cite (Fajardo-Cavazos et al, 2005) was about re-entry by bacillus subtilis in this passage:

Thus, if potentially harmful microbes were abundant on the Martian surface it is likely they already would have been transferred to Earth by this natural process (Fajardo-Cavazos et al. 2005, Horneck et al. 2008, Howaxrd et al. 2013).

These though are papers on panspermia. What matters for sample return is whether there could be species on Mars that do NOT get to Earth. For panspermia what matters is if ANY species get from Mars to Earth. Charles Cockell showed that photosynthetic life, for instance, has many challenges getting from Mars to Earth and wouldn't survive re-entry in typical position on or near the surface of the rock.

Charles Cockell's paper (which they don't mention) said planetary exchange of photosynthesis might not be impossible but quite specific physical conditions and evolutionary adaptations are needed and the fireball of re-entry is the most important filter to stop photosynthetic life getting to Earth

Charles Cockell, professor of astrobiology at Edinburgh university and author or co-author of numerous papers on astrobiology, is one of many authors who HAVE looked at this question.

Charles Cockell looks at Chroococcidiopsis, a blue-green algae that is astonishingly resistant to UV, dessicationk that can remake its DNA even when chopped to pieces by ionizing radiation, that can live almost anywhere on Earth from the hottest driest deserts to Antarctica, tropical reservoirs, or even over 100 meters below the sea level (it has many alternative metabolic pathways that let it survive without light). It's also one of the top candidates for an Earth microbe that could survive on Mars.

Yet he concluded that Chroococcidiopsis would find it very hard to get from Mars to Earth. This very versatile polyextremophile still can't do it easily.

Charles Cockell concludes that though some shock resistant life can be ejected from Mars and survive, that most photosynthetic life can't get to Earth from Mars in this way on present day

Mars though he leaves open the possibility that it could get here in unusual circumstances. (Cockell, 2008)

QUOTE Few ecological dispersal filters are completely effective. Each of the filters described above could be survived on account of specific physical factors or evolutionary innovations.

He found that it could survive ejection from Mars but only at the lower end of the range. Chroococcidiopsis doesn't form spores and that makes it far harder for it to resist the shock of ejection from Mars than other hardier spore forming microbes.

...In the case of ejection from the planetary surface, the experiments with Chroococcidiopsis sp. show that even these vegetative cells could survive shock pressures at the lower end of that documented in Martian meteorites (~ 5 GPa).

To put this in context just about all the meteorites in our collections have ejection shock pressures larger than 5 GPa. Normally 15 GPa or larger. But from modelling about 1 in 50 should be less than 1 GPa.

Unlike the draft EIS, Cockell refers to planetary ejection as a "potentially strong dispersal filter" - many of the microbes would be killed by ejection. But at lower levels then they can be survivable.

... Thus, although planetary ejection is shown experimentally to be a potentially strong dispersal filter, these same experiments show that shock pressures close to those required to achieve escape velocity, at least for Mars-like planets, can be survived even for vegetative phototrophs without special protection.

But for those that survive the shock of ejection, then there's the fireball of re-entry. It's going to be hard for any photosynthetic life to survive that as they would be living on the surface or else maybe in cracks but still within reach of plasma that would get deep inside the meteorite.

... The dispersal filter of atmospheric transit is the most effective dispersal filter for photosynthesis.

... Thus, the planetary exchange of photosynthesis might not be impossible, but quite specific physical situations and/or evolutionary innovations are required to create conditions where a photosynthetic organism happens to be buried deep within a rock during ejection to survive atmospheric transit.

His argument here looks specifically at Chroococcidiopsis, one of the top candidates for a terrestrial microbe that might be able to survive on present day Mars.

There isn't anything in Cockell's paper to support the thesis of the draft EIS that it is easier for Martian microbes to get to Earth on a meteorite than in a sample tube. (<u>NASA, 2022eis</u>: 3-3):

The natural delivery of Mars materials can provide better protection and faster transit than the current MSR mission concept.

Chroococcidiopsis is an example that shows that a species can be returned via a sample return far more easily than it could get here on a meteoroid ejected from Mars.

NASA's principle is fine. It goes back to Greenberg (Greenberg et. al, 2001)

"As long as the probability of people infecting other planets with terrestrial microbes is substantially smaller than the probability that such contamination happens naturally, exploration activities would, in our view, be doing no harm. We call this concept the natural contamination standard."

But it is applied incorrectly in this draft EIS.

The bottom line here is that we have no examples of life that got to Earth from Mars. It may have happened but we don't know for sure that it ever happened. We are reasoning theoretically about something we can't currently study through observation.

The reasoning we have is based on the capabilities of terrestrial life. We can test various terrestrial microbes extensively. However we know nothing specific about the capabilities of Martian life such as its ability to withstand the shock of ejection, the vacuum of space, and the fireball of re-entry or how likely it is to be able to get onto a meteorite that heads for Earth.

We not only don't know if ALL martian species can get to Earth on meteorites. So far we don't know if ANY martian species can get to Earth on meteorites, if there is life on Mars.

The meteorite argument only works if ALL Martian species can get to Earth on meteorites – European starlings are the invasive species in the Americas, not the barn swallows which can cross the Atlantic – natural processes can't transfer the surface dust, dirt, ice and salts of Mars to Earth unaltered while sample tubes can like a small spaceship for a microbe

This is a point I highlight in my preprint <u>(Walker, 2022b)</u>. If certain species do sometimes get transferred to Earth from Mars it does NOT mean that all species on Mars are safe for Earth.

As an example, barn swallows cross the Atlantic from Europe to the USA, but starlings don't.

Barn swallows are not an invasive species in the USA while starlings are. European starling is an invasive bird in the Americas (<u>US DOA, 2017</u>).

Some microbes may be able to get from Mars to Earth what matters for invasive species are the ones that can't



Some microbes may be able to get from Mars to Earth - what matters for invasive species are the ones that can't.

Barn swallow - can cross Atlantic

Starling - invasive species in the Americas

Starling photo from: (Johnstone, 2017)

Barn swallow photo from (Batbander, 2017)

As an example, in 2012, starlings caused \$189 million in damage to crops of blueberries, wine grapes, apples, sweet cherries and tart cherries in the USA (<u>US DOA, 2017</u>).

Starlings also eat cattle feed and 1000 starlings can represent a loss of \$200 to \$400 in cattle feed. They can also transmit many diseases to cattle via the feeding troughs and their excrement corrodes iron structures including motor vehicles and iron roofs. They are also involved in thousands of bird strikes (US DOA, 2017)..

Example of fresh water diatoms that can't cross oceans on Earth

We have invasive diatoms in the Great Lakes. *Stephanodiscus binderanus* is a nuisance species that clogs water works and introduces foul odours into the water <u>(Spaulding et al, 2010)</u>. The diatom Didymosphenia geminata is an invasive species in New Zealand, possibly brought there on damp sports equipment. <u>(Spaulding et al, 2010)</u>. The long stalked version of Didymosphenia geminata is also an invasive species in the Great Lakes. The short stalked

version doesn't form mats and is presumed to be native to the Great Lakes. There were no records of the long stalked version in the Great Lakes until around 1990. After that this long stalked variant started to spread. It can survive and remain viable for up to 40 days in cool dark damp conditions, so it can be spread place to place on angling equipment, boot tops, neopreme waders and felt-soles.

The mats can be up to 20 cm thick and they trap stream sediment. These can cover the bottom of the stream and smother native plants, insects, mollusks and algae. Streams impacted outside of the Great lakes see the insects decrease and an absence of fish. This may be due to a new genetic variant that started to spread but if so, it hasn't been identified. These two paragraphs summarize / paraphrase some of the information from (Schmidt, n.d.)

This is an example sign in New Zealand warning sailors about the risk of carrying didymo to another lake in New Zealand.



Text on sign: Your boat may now be carrying didymo. Please clean using approved methods. Protect our waters ...

Image from: (Thorney; ?. 2006)

As you can see Didymo can't even move from one lake to another in New Zealand without help from humans carrying it in wet gear. There is no way it could travel between planets. There are salt water diatoms too. But they couldn't travel between planets on meteorites either. If there are diatoms on Mars they have evolved independently and can't be directly related to terrestrial diatoms.

We might even find diatoms on Mars – either preserved in gypsum, or perhaps living in the lakes our orbiters found beneath the polar ice

Perseverance has found gypsum, as did Curiosity <u>(Scheller et al, 2022)</u>., and on Earth, gypsum can preserve viable diatoms for tens of thousands of years and maybe hundreds of millions of years <u>(Benison et al, 2014)</u>. Diatoms evolved late on Earth which could be a reason to suppose they are unlikely to have evolved on Mars (<u>Cabrol et al, 2009</u>). However it isn't impossible as it is hard to generalize given only one example from one planet.

So, even the idea that some day we find a viable diatom on Mars is not as far fetched as it might seem though it would need diatoms to evolve on Mars and for a lake to form with enough water for diatoms to inhabit it in the recent enough past for the diatoms to still be viable today. Such lakes actually do exist in present day Mars but they are deep below the ice at the poles (Orosei et al, 2018). David Wiliams, diatom researcher at the Natural History Museum said that technically diatoms could survive there though he says a more interesting question is whether we'd even be able to identify it as life if it originated on Mars (Davis, 2018):

'Yes, technically tiny life forms such as diatoms and cyanobacteria could survive in these environments. But that is not the question we should be asking.

'A more interesting question is whether we would know what we're looking at, even if we did find something in the lake. Would we even be able to identify it as life, if it originated on Mars itself?'

So, though it's not the most likely example for Jezero crater, it's not impossible we eventually find diatoms on Mars, or maybe some other form of life adapted to a similar life style, and the chance it is able to get to Earth on a meteorite may be very low.

Chroococcidiopsis as an example of a species that wouldn't survive transfer by impacts from modern Mars based on an analysis by Charles Cockell

Some species will be better able than others to withstand the shock of ejection from Mars, the cold and dry and complete vacuum of the transition through space, then the fireball of re-entry to Earth. As an example, most photosynthetic life is killed in this process.

The first challenge is the shock of ejection. Microbes are suddenly accelerated from rest to escape velocity in a fraction of a second. The microbes can be destroyed by cell rupture or by DNA damage. All cells of Chroococcidiopsis are killed at 10 GPa (Nicholson, 2009). To put this in context, ALH84001 experienced a shock of ejection of \sim 35 – 40 GPa. The Nahkalites were least shocked at 15 to 25 GPa. This is still too much for Chroococcidiopsis (Nyquist, 2001)

The microbe also has to survive the fireball of re-entry to Earth.

Cockell inculcated an artificial gneiss rock with Chrooccoccidiopsis at a depth where it occurs naturally, and affixed it to the re-entry shield of a Soyuz rocket. None survived re-entry, nor did any organics.

Cockell concluded that it might not be impossible for photosynthetic life to get to Earth from Mars, but it would need an extraordinary combination of events (Cockell, 2008)

So in this analogy, most photosynthetic life on Mars would be more like the European starling than the Swallow, wouldn't be able to get to Earth on meteorites except possibly in rare very large impacts, and most likely in the early solar system.

Then, the rocks we have in our Martian meteorite collections all come from at least three meters below the surface (Head et al, 2002) . They were probably thrown up into space after glancing collisions into the Elysium or Tharsis regions, high altitude southern uplands (Tornabene et al, 2006). The atmosphere for these high altitude regions on Mars is thin, making ejection to Earth easier. The subsurface below about 12 cms has a uniform temperature of around 200°K or -73°C (Möhlmann, 2005:figure 2). With such a thin atmosphere, present day life at those altitudes is unlikely (except perhaps for deep subsurface geothermal hot spots).

Larger impacts in the recent geological past could send material to Earth from other potentially more habitable parts of Mars. However:

- Many proposed habitats are in surface layers of dirt, ice and salts. These would likely never get into space
- Other proposed habitats are millimeters below the surface of rocks. These layers would ablate away during entry into the Earth's atmosphere

Life on Mars could be extremely localized to only a few square kilometers over the entire planet, for instance, only to the RSL's, or only above geological hot spots, making it less likely that the habitats are hit by an asteroid able to send material all the way to Earth in the large chunks needed for protection from cosmic radiation during the transfer.

Yet life from distant habitats on Mars may be able to get to Jezero crater in dust storms. Of course dust storms can't transport Martian spores or propagules to Earth and the dust can't be

transported to Earth. We have no samples of Martian dust or Martian surface salts or ice in our meteorite collections and these couldn't get to Earth even in the early solar system.

NASA's draft EIS cites previous research incorrectly and as a result fail to properly consider the potential for large scale impacts on the environment

NASA don't cite the European Space Foundation study from 2012 study (Ammann et al, 2012:PG) at all and don't cite the section of the 2009 National Research Council study on large scale impacts (Board et al, 2009: 48).

Not only that, the submitted documents make statements that go against the conclusions of the peer reviewed literature on the topic. Example, let's look at this passage from the MSR safety fact sheet for the Draft Environmental Impact Statement (<u>NASA, 2022msfs</u>):

The question of whether samples from Mars could present a hazard to Earth's biosphere has been studied by several different panels of scientific experts from the United States and elsewhere over the past several decades.

[this much is true]

The reports from these panels have found an extremely low likelihood that samples collected from areas on Mars like those being explored by Perseverance could possibly contain a biological hazard to our biosphere.

[this is not an accurate summary]

The most recent of the thorough Mars sample return studies, from the European Space Foundation in 2012:

"The risks of environmental disruption resulting from the inadvertent contamination of Earth with putative martian microbes are still considered to be low. But since the risk cannot be demonstrated to be zero, due care and caution must be exercised in handling any martian materials returned to Earth"

NASA's MSR Safety fact sheet for the draft EIS again (NASA, 2022msfs):

The evidence includes the absence of any observed harm to Earth's environment from Martian rocks that frequently fall to Earth in the form of meteorites,

National Research Council report in 2009 said (Board et al, 2009: 48).:

Section: Potential for large scale effects [of a Mars Sample Return] "The potential hazards posed for Earth by viable organisms surviving in samples is significantly greater with a Mars sample return than if the same organisms were brought to Earth via impact-mediated ejection from Mars

...Certainly in the modern era, there is no evidence for large-scale or other negative effects that are attributable to the frequent deliveries to Earth of essentially unaltered Martian rocks. However the possibility that such effects occurred in the distant past cannot be discounted."

NASA's MSR Safety fact sheet for the draft EIS again (NASA, 2022msfs):

and the fact that the Mars samples being gathered by NASA's Perseverance Mars rover are from the frst few inches of a planetary surface that is very dry and highly irradiated naturally by the Sun, which would sterilize all known active biology.

The Review from 2015: (Board, 2015)

There are many examples of small-scale and microscale environments on Earth ... that can host microbial communities, including biofilms, which may only be a few cell layers thick. The biofilm mode of growth, as noted previously, can provide affordable conditions for microbial propagation despite adverse and extreme conditions in the surroundings.

NASA fail to adequately consider the risks from life that can't get to Earth on meteorites - in 2009, the National Research Council examined the possibility of life transferred on meteorites said the risk is significantly greater in a sample return mission - and said they can't rule out the possibility of large scale effects in the past due to life from Mars – NASA's EIS instead claims microbes will survive transfer from Mars to Earth more easily in a meteorite than in a sample return mission but their sources don't back this up Let's look at the first of these two statements NASA use to support their conclusion that the activity is very low risk, from the <u>MSR safety fact sheet from this page</u>:

The evidence includes the absence of any observed harm to Earth's environment from Martian rocks that frequently fall to Earth in the form of meteorites,

Then in the draft EIS:

One of the reasons that the scientific community thinks the risk of pathogenic effects from the release of small amounts (less than 1 kilogram [2.2 pounds]) of Mars samples is very low is that pieces of Mars have already traveled to Earth as meteorites.

...

The natural delivery of Mars materials can provide better protection and faster transit than the current MSR mission concept.

They cite the NRC report from 2009 but not on this point. The National Research Council DID look into this question in their "Assessment of Planetary Protection Requirements for a Mars Sample Return". However their conclusion was the opposite of NASA's draft EIS summary.

They were unable to rule out the possibility that life from Mars could have caused past mass extinctions on Earth

The NRC found that most of the meteorites that get to Mars are sterilized during transit. But about 1% get here within 16,000 years and 0.01 percent within 100 years (note none of the meteorites we have from Mars left the planet less than hundreds of thousands of years ago)

This is from Earth (Board et al, 2009: 48).

"Transit to Earth may present the greatest hazard to the survival of any microbial hitchhikers. Cosmic-ray-exposure ages of the meteorites in current collections indicate transit times of 350,000 to 16 million years. However theoretical modeling suggests that about 1 percent of the materials ejected from Mars are captured by Earth within 16,000 years and that 0.01 percent reach Earth within 100 years.

NRC continue that survival of organisms in meteorites is plausible. If they can be shown to survive ejection, entry and impact they can be expected to transfer from Mars to Earth (Board et al, 2009: 48).

"Thus, survival of organisms in meteorites, where they are largely protected from radiation, appears plausible. If microorganisms could be shown to survive conditions of ejection and subsequent entry and impact, there would be little reason to doubt that natural interplanetary transfer of organisms is possible and has, in all likelihood, already occurred.

However that is the big unknown. Can life from present day Mars get onto the meteorites, be ejected from Mars, and then survive the fireball of re-entry to Earth.

The NASA EIS says this (<u>NASA, 2022eis</u>: 3-3):

First, potential Mars microbes would be expected to survive ejection forces and pressure (National Academies of Sciences, Engineering, and Medicine and the European Science Foundation 2019), and, within the interior portions of the rocks, would be protected from elevated radiation levels, and large temperature variations that meteorite surfaces experience during the transit from Mars to Earth (Mileikowsky 2000).

The big hurdles for transfer of life from Mars are the shock of ejection, the fireball of exit from Mars the cold, vacuum and ionizing radiation of the passage to Earth, and the fireball of reentry.

Their cite on ejection pressures is about transport of materials from Mars to the Martian moons for an assessment of sample return missions from those moons. It does NOT look at sterilization during Mars ejecta formation. This is what they say (<u>Board, 2019</u> : <u>26</u>). :

The SterLim team did not include any sterilization during Mars ejecta formation in its analysis because such investigations were not requested in its study's statement of work.

It also looks at only one impact, the ejection from Zunil crater as any ejection from more than a million year ago would not leave surviving microbes close to the surface of the Martian moons due to the ionizing radiation.

It does mention shock heating. It didn't look at the acceleration during ejection from Mars. But the sudden acceleration actually kills most microbes. I cover that below

Second, a significant fraction of natural transits occur on trajectories that require as little as 6 months where the material returned by the MSR mission concept would be in flight for Mars Sample Return Campaign Programmatic EIS over 18 months (Gladman 1997). Thus, if potentially harmful microbes were abundant on the Martian surface it is likely they already would have been transferred to Earth by this natural process (Fajardo-Cavazos et al. 2005, Horneck et al. 2008, Howard et al.2013).

Actually the meteorites we have on Earth all came from at least 3 meters below the surface of Mars. The proposed habitats for present day Mars are on the surface in dust and brine layers. How is life in those layers going to get into a rock at least 3 meters below the surface?

For details see supplementary data below:

Then there's the shock of ejection and the fireball of re-entry to Earth.

• S

NRC 2009 report says the potential for large-scale negative effects on Earth's inhabitants or environments by a returned martian life form appears to be low, but is not demonstrably zero – draft EIS says potential environmental effects would not be significant

What the National Research Council said:

The committee concurred with the basic conclusion of the NRC's 1997 report Mars Sample Return: Issues and Recommendations²⁶ that the potential risks of large-scale effects arising from the intentional return of martian materials to Earth are primarily those associated with replicating biological entities, rather than toxic effects attributed to microbes, their cellular structures, or extracellular products. Therefore, the focus of attention should be placed on the potential for pathogenic-infectious diseases, or harmful ecological effects on Earth's environments.

The committee found that the potential for large-scale negative effects on Earth's inhabitants or environments by a returned martian life form appears to be low, but is not demonstrably zero. Changes in regulations, oversight, and planetary protection controls over the past decade support the need to remain vigilant in applying requirements to protect against potential biohazards, whether as pathogenic or ecological agents. Thus, a conservative approach to both containment and test protocols remains the most appropriate response.

A related issue concerns the natural introduction of martian materials to Earth's environment in the form of martian meteorites. Although exchanges of essentially unaltered crustal materials have occurred routinely throughout the history of Earth and Mars, it is not known whether a putative martian microorganism could survive ejection, transit, and impact delivery to Earth or would be sterilized by shock pressure heating during ejection, or by radiation damage accumulated during transit. Likewise, it is not possible to assess past or future negative impacts caused by the delivery of putative extraterrestrial life, based on present evidence.

What NASA's draft EIS says:

unlikely to pose a risk of significant ecological impact or other significant harmful effects should there be a sample release. The relatively low probability of an inadvertent reentry combined with the assessment that samples are unlikely to pose a risk of significant ecological impact or other significant harmful effects support the judgement that the potential environmental impacts would not be significant.

What the National Research Council said: The committee found that the potential for large-scale negative effects on Earth's inhabitants or environments by a returned martian life form appears to be low, but is not demonstrably zero

Although exchanges of essentially unaltered crustal materials have occurred routinely throughout the history of Earth and Mars, it is not known whether putative martian microorganisms could survive ejection, transit and impact delivery to Earth or would be sterilized by shock pressure heating during ejection or by radiation damage accumulated during transit. Likewise, it is not possible to assess past or future negative impacts caused by the delivery of putative extraterrestrial life, based on current evidence.

(Board et al, 2009: 48).

What NASA's draft EIS says: The relatively low probability of an inadvertent reentry combined with the assessment that samples are unlikely to pose a risk of significant ecological impact or other significant harmful effects support the judgement that the potential environmental impacts would not be significant. (NASA, 2022eis: 3-3):

Going back to the NRC report, they continue that any microbes in martian materials transported to Earth in a sample return mission face very different conditions from those in meteorites (Board et al, 2009: 48).

It should be noted that martian materials transported to Earth via a sample return mission will spend a relatively short time (less than a year) in space - all the while protected in containers. (Note that researchers have yet to discover compelling evidence of life in any meteorite, martian or otherwise.) Thus the potential hazards posed for Earth by viable organisms surviving in samples is significantly greater with a Mars sample return than if the same organisms were brought to Earth via impact-mediated ejection from Mars."

They go on to say that it is simply not possible to determine whether viable Martian life forms have already been delivered to Earth.

They also say that though there is no evidence of large scale or other negative effects (such as extinctions) in the modern era due to the frequent deliveries of Martian rocks, that it is not possible to discount such effects in the distant past. (Board et al, 2009: 48).

"Despite suggestions to the contrary, it is simply not possible, on the basis of current knowledge, to determine whether viable Martian life forms have already been delivered to Earth. Certainly in the modern era, there is no evidence for large-scale or other negative effects that are attributable to the frequent deliveries to Earth of essentially unaltered Martian rocks. However the possibility that such effects occurred in the distant past cannot be discounted."

That's in their section 5, Potential for Large Scale Effects, page 48:

I discuss this passage below in:

• <u>The Great Oxygenation Event which transformed Earth's atmosphere and oceans</u> <u>chemically gives a practical example of a way life from another Mars-like planet could in</u> principle cause large scale changes to an Earth-like planet

•

NASA's draft EIS summarizes this INCORRECTLY as (NASA, 2022eis: 3-3):

The reports from these panels have found an extremely low likelihood that samples collected from areas on Mars like those being explored by Perseverance could possibly contain a biological hazard to our biosphere.

...

The evidence includes the absence of any observed harm to Earth's environment from Martian rocks that frequently fall to Earth in the form of meteorites

Then in the draft EIS they say that the potential environmental impacts from a sample release would not be significant (<u>NASA, 2022eis</u>: 3-16):

The MSR Campaign is the first sample return mission to be classified as Restricted Earth Return, since the term was defined. (The Apollo 11, 12, and 14 missions were subjected to quarantine upon return until lunar samples were assessed and found to pose no hazard.) Prior mission sample return missions at the UTTR (e.g., Stardust, Genesis, and the upcoming return of OSIRIS-Rex) were all classified as Unrestricted Earth Return.

The human health and safety analysis focuses on the precautions taken to provide backward planetary protection. However, the probability of inadvertent or off-39 nominal reentry would be similarly small as those evaluated for these earlier missions (NASA 1998, NASA 2001, NASA 2013), and as stated previously, the samples are unlikely to pose a risk of significant ecological impact or other significant harmful effects should there be a sample release. The relatively low probability of an inadvertent reentry combined with the assessment that samples are unlikely to pose a risk of significant ecological impact or other significant harmful effects support the judgement that the potential environmental impacts would not be significant.

This is all that they say on the topic. There is no further discussion of the potential for large scale effects and this particular sentence isn't cited to any other source.

NASA's draft EIS has no mention of ANY potential for large scale effects on humans or other lifeforms of accidental release on Earth

Another striking omission is that there is no mention of potential effects of accidental release on humans or animals or plants or any other life even locally.

This is extensively studied in the literature on the topic (Pugel et al, 2020):

An extraterrestrial pathogen lacks existing diagnostic testing and medical management protocols. Future health emergency response measures may need to incorporate

knowledge deficits into plans and exercises, and all those responding, including healthcare workers and first responders, will need education and training in advance of the spacecraft's return.

The lack of knowledge surrounding extraterrestrial pathogens, from disinfection to incubation periods, presents a novel situation for which current public health and healthcare emergency preparedness efforts have not been developed. The spectrum of biological threats (natural outbreak, intentional attack, and laboratory accident) does not include a novel pathogen of unknown biological makeup.

There is no discussion of precautions to be taken if there is an accidental release, or if a technician in the facility is accidentally exposed to the samples.

This is all they say on the matter (NASA, 2022eis :3-18)

Overall Health and Safety Impacts

Health and safety impacts are mitigated through the prevention of backward contamination, which is provided by the low probability of failure of the engineered containment systems intended to provide containment of the Mars sample material under all circumstances. Implementation of actions that are in line with accepted procedures used for the isolation of biohazard materials provides additional protection against the release and spread of such material. Given implementation of these precautions and given that Mars materials are not expected to have significant pathological impacts if released into the Earth's biosphere, on-site mission preparation (to include testing, rehearsals, and landing site preparation), EES landing, and EES recovery operations are expected to have minimal direct and/or indirect impacts on human health at the UTTR, the Det-1 location, or in general.

And claims that the risk of accidental release from a BSL-4 can be described as zero. (<u>NASA</u>, <u>2022eis</u>: 3-14):

While not completely analogous, the results of previous NEPA analyses for BSL-4 facilities have concluded that the hazards associated with the operation of BSL-4 facilities are expected to be minimal. Analyses performed in support of recent NEPA documents conclude that the risk from accidental release of material from a BSL-4, even under accident conditions that include the failure of protective boundaries (e.g., reduced effectiveness of ventilation filtration systems) are minute and can be described as zero (NIH/DHHS 2005).

An alternative release path resulting from the contamination of workers leading to direct contact with others (members of the public) was also analyzed. Qualitative risk assessments for this mode of transmission have shown that the risk to the public is negligible. (NIH/DHHS 2005, DHS 2008)

Yet when considering the possibility of studying the samples with humans in orbit they say there is concern about potential health impact (<u>NASA, 2022eis</u>: 2-26):

Additionally, a positive result from the SSAP (Site Safety Assessment Protocol) represents a potential hazard to crew health within a small, enclosed system, plus a contaminated facility that will eventually need to be returned to Earth (or will fall to Earth if there is a system failure).

So they claim a potential hazard to crew health if the samples are studied in orbit, but minimal hazard to human health in case of an accidental release once the samples are returned to Earth.

The only occurrence of the word quarantine is in a reference to the Apollo mission (<u>NASA</u>, <u>2022eis</u>: 3-15):

The MSR Campaign is the first sample return mission to be classified as Restricted Earth Return, since the term was defined. (The Apollo 11, 12, and 14 missions were subjected to quarantine upon return until lunar samples were assessed and found to pose no hazard.)

During the Apollo sample returns, there were several times technicians were accidentally exposed to the samples and had to isolate (Mangus et al, 2004:51). For instance, two technicians had to go into isolation after a leak was found in a sample handling glove for Apollo 11 (Meltzer, 2012:485), and then 11 technicians had to go into isolation in 1969 when a small cut was found in one of the gloves during preliminary examination of one of the samples returned by Apollo 12 (Meltzer, 2012:241).

The draft EIS doesn't discuss what happens if technicians are similarly exposed to the sample materials on Earth, even though they raise it as an issue for astronauts studying the samples in orbit.

A carefully peer reviewed EIS wouldn't have internal inconsistencies like this.

The draft EIS does however describe a need to take precautions at the landing site. They plan to decontaminate the landing site with chlorine dioxide such as is used in drinking water and aldehydes (<u>NASA, 2022eis</u>: 3-35):

After removal of the EES, the entire landing site (consisting of the impact area and extent of ejecta) may be decontaminated as a precautionary measure

The process of retrieving the EES and placing it into the vault would be assumed to generate potentially hazardous biological waste until demonstrated otherwise. As described earlier, the process of placing the EES into containment and then inserting it into the vault would be conducted as in past missions. All the systems used, including personnel protective gear, would be assumed to be contaminated and would either be decontaminated or simply discarded as hazardous waste. Wastes could include plastics and clothing. Any liquids used in the decontamination process would be absorbed onto solids prior to disposal.

Chlorine dioxide is a disinfectant. When added to drinking water, it helps destroy bacteria, viruses and some types of parasites.

Aldehydes are highly effective, broad-spectrum disinfectants, which typically achieve sterilization by damaging proteins. Aldehydes are effective against bacteria, fungi, viruses, mycobacteria and spores.

They explain (<u>NASA, 2022eis</u>: 3-35):

NASA believes these types of decontaminates would be effective given the assumption that any putative Mars life forms would be similar to "life as we know it" with a watermediated carbon-based biochemistry, and that there would not be any "unique" biohazards associated with the Mars samples

This surely needs more thorough study for the special case of extra-terrestrial life from Mars.

These methods rarely achieve 100% reduction. From their cite, this shows the effect of 24 hours of high concentrations of CLO2. It has almost no effect on the top soil or below a depth of one inch below the surface. It is much more effective on clay or sand with a 100 million fold reduction (EPA, 207:36)

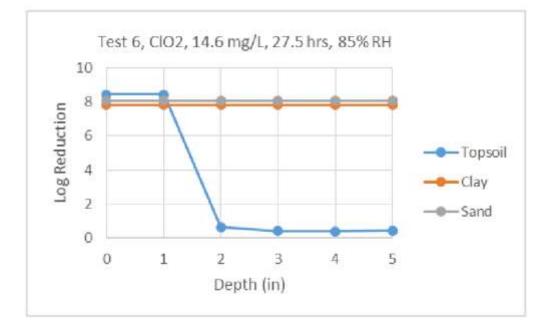


Figure 7. Test 5, CIO2: 9.3 mg/L, 24 hrs, 80% RH, [saturated soil]

Also this is for reduction in "colony forming units" in other words cultivable spores. Many microbes are uncultivable. Also Martian life is adapted to surface conditions with high concentrations of perchlorate. They may well be more resistant to chlorine dioxide than terrestrial life.

Also, what are the contingency plans if Martian life has got into the microbiome of a human, or an insect flies away with it, or it gets blown away from the site in dust in the atmosphere, or into groundwater?

And then – if these precautions are needed for the landing site, why are they not also needed in case of an accidental breach of containment at the BSL-4 facility?

This raises many questions that would likely be asked during a peer review of the draft EIS by independent experts.

I cover issues of effect of release of the sample on humans and of quarantine in in my preprint (Walker, 2022b) under:

- Public health challenges responding to release of an extraterrestrial pathogen of unfamiliar biology
- Failure modes for sample containment
- Complexities of quarantine for technicians accidentally exposed to sample materials

NASA's draft EIS gives no quantitative answer to concerned questions from the general public about how low the risk is for large scale effects from a sample return from Mars handled according to the methods they have outlined – is it 1 in thousand or 1 in a million or 1 in a billion? They just say it is impossible to give a 100% guarantee

This is one of the main questions from the public. Yet NASA don't give anything like a satisfactory answer to it. This answer alone is likely to lead to litigation once the document reaches general public awareness if NASA can't improve on it.

Example, the draft EIS gives this as one of the main questions from the public (<u>NASA, 2022eis</u>: 3-3):

When the consequences of a failure are so great, a 100% guarantee should be required.

The NASA factsheet "The Safety of Mars Sample Return" does address this issue. "Panels have found an extremely low likelihood that samples collected from areas on Mars like those being explored by Perseverance could possibly contain a biological hazard to our biosphere." Just how low is "low likelihood"? Is NASA's goal specification to prevent accidental release of the Mars samples 1 in a thousand? 1 in a million? 1 in a billion?

This is their answer to that question:

No outcome in science and engineering processes can be predicted with 100% certainty. The safety case for MSR safety is based on redundant containment supported by rigorous testing and analysis, the extensive experience of NASA and ESA with very similar activities over the past three decades, as well as independent reviews of program plans by external expert

The draft EIS shows clearly the results of not setting up any advanced planning and oversight agency with experts in legal, ethical and social issues tasked with interfacing NASA decisions and the general public's questions as the top priority – as recommended in numerous papers on Mars sample return missions

Margaret Race made a relevant point here. She says scientists are likely to focus on (Race, 1996)

- 1. technical details
- 2. mission requirements
- 3. engineering details
- 4. costs of the space operations and hardware

General public are likely to focus on

- risks and accidents
- whether NASA and other institutions can be trusted to do the mission
- worst case scenarios
- whether the methods of handing the sample, quarantine and containment of any Martian life are adequate

We see the results of this different focus in the report. It is just not something that greatly occupies the minds of the engineers and scientists who work on space projects, yet it is the main thing on the minds of members of the public.

This shows up clearly the issues with their failure to set up the mechanism to deal with public responses recommended by numerous sample return studies.

• Rummel et al recommend a planning agency set up in advance with experts in legal, ethical and social issues - Uhran et al recommend an advanced planning and oversight agency set up two years before the start of the legal process – and the ESF recommends an international framework should be set up, open to representatives from all countries - NASA don't seem to have done any of this yet

Again it's understandable that engineers whose minds are focused on solving numerous complex technical difficulties with the mission might not understand why there is need to set up a planning and oversight agency two years before the start of the legal process. This wouldn't help solve their engineering problems in any way whatsoever.

But for the general public, it is absolutely essential for the issues that matter most to them.

Answer to the question about how low the risk is - it can't be quantified but is likely very low for the proposed action – since Perseverance is not searching for microhabitats in Jezero crater and will return hardly any dust – level of risk is similar to the risk of building a house without a smoke detector – rather than the risk off outdoor fireworks in your kitchen – but for a house NASA share with nearly 8 billion other people when almost all don't know NASA is considering removing the smoke detectors and they have no say in the decision

I can help here based on my experience working full time (on my own initiative) as a voluntary fact checker for scared people. I am doing this to help anyone who might read this document and panic and expect the worst. E.g. jump instantly to fear of human extinction.

A good analogy, it's more of the order of building a house without a smoke detector - but a house you share with nearly 8 billion people - than setting off outdoor fireworks in the kitchen. This smoke detector analogy is from Margaret Race from her contribution **"No Threat? No Way"** in the Planetary Report **"**(Rummel et al., 2000). In this cite, she is responding to Robert Zubrin, president of the Mars society who thinks we don't need to protect Earth from a Mars sample return. She wrote in 2000:

"He's confident in our impressive technological prowess; he's raring to go and doesn't want anything to slow down or stop our exploration of Mars - especially not burdensome regulations based on very small risks and scientific uncertainty. Yet when he suggests that there's no need for back contamination controls on Mars sample return missions, he's advocating an irresponsible way to cut corners. If he were an architect, would he suggest designing buildings without smoke detectors or fire extinguishers? There are many hurdles for life on Mars to jump to get to Earth.

- The chance of present day life in the geological samples or in the few dust spores attached to the containers is very low.
- •
- The ultramicrobacteria has to be dislodged from the sample
- Then to escape from a BSL-4 facility it has to be a very small microbe such as an ultramicrobacteria or escape due to improper handling. The habitats on Mars may favour ultramicrobacteria because of the low levels of nutrients, ultramicrobacteria have a higher surface to volume ratio so can take up more nutrients per volume with nutrients that diffuse at the same rate through the cell membrane.
- Then there's whether it is pre-adapted to survive on Earth.

An example of a worst case microbe to escape is one that can survive in the rivers and the sea and ends up in water outside the facility, or one that can spread in viable spores in airborne dust.

The easiest case to contain is a microbe with very specialist capabilities that has almost no habitats on Earth it can survive in. It may be possible to stop it spreading even if it escapes.

You can argue both ways.

Mars has conditions sufficiently like Earth on Mars so it's not impossible and the environment would encourage polyextremophiles able to withstand almost anything it encounters. And for a microbe a droplet of brine may be much the same whether it is on Mars or on Earth.

On the other hand Earth has nothing that closely resembles the Martian habitats and it might be that Martian life depends on things Mars has and Earth doesn't such as the perchlorates, say.

As an example, suppose Martian life depends on perchlorates or chlorates in its habitat just as sea life depends on salty water on Earth. In that case it will be easy to stop.

Suppose though that we return a polyextremophile such as an analogue of Chroococcidiopsis which can survive almost anywhere on Earth and can probably survive in almost any Martian habitat suitable for terrestrial life if such exist. That would be impossible to stop once it leaves containment.

Most astrobiologists seem to say things like the chance of returning harmful life is low but not impossible.

I don't see any reasoning for it being a high probability.

But low could be 1 in 10 or 1 in 1000 or anything between or more or less

Large scale effects will be low probability, though nobody can attach a number to it due to us never encountering any other form of life other than terrestrial life.

Cockell has suggested (amongst other possible scenarios) that if early Martian life went extinct, Mars could now have uninhabited habitats, i.e. which life could colonize but with nothing left by way of early Martian life to colonize them <u>(Cockell, 2014)</u>.

For Jezero crater there are several proposed microhabitats but one example would be the possibility of biofilms using the Curiosity brines. For this, see:

• NASA fail to consider at all the potential for microhabitats in Jezero crater not detectable from orbit such as the Curiosity brines which could be habitable to biofilms or martian life able to tolerate conditions too old for terrestrial life

The other main possibility is life transferred in the dust, see:

• NASA fail to consider at all the potential for winds to transfer microbes imbedded in a grain of dust to Jezero crater shielded from the UV by the global dust storms

The main reason this mission is low risk is:

- 1. The mission isn't designed to look for present day life
- 2. If there are microhabitats in Jezero crater for Martian life with greater capability than terrestrial life or even for terrestrial life in biofilms they may be uninhabited
- 3. If these potential microhabitats for martian life are inhabited, this mission is still not likely to return life because it is not going to return the brines Curiosity discovered or any other likely microhabitats
- 4. There might be viable spores in the dust but they are returning hardly any of the dust from the surface. Unless spores are very abundant they are not likely to return a spore in a few grains of dust
- 5. They aren't returning a sample of dirt. So if Viking did find life, they likely won't return it

Then you can go on to consider what kind of life might be on Mars.

- 1. Mars could be potentially habitable to life in some form or uninhabitable.
- 2. Assuming Mars is potentially habitable to life in some form, the habitats could be inhabited or uninhabited
- 3. If there is life it may survive the transfer back to Earth or not survive (as it is significantly different from Martian conditions)
- 4. If there is life, it might spread easily if released on Earth, or it might require a specialist habitat (e.g. chlorates or perchlorates) and be containable.
- 5. It might be early life, at a similar level of evolution to terrestrial life or have evolved further to more complex genomes.
- 6. It might be beneficial, or harmless or harmful.

7. If harmful it might be a minor nuisance (e.g. can make cheese mouldy in a freezer or algal blooms covering lakes), a major nuisance (e.g. harmful to an important agricultural crop), an opportunistic pathogens for humans or animals or plants, or finally, cause major chemical or biological changes to Earth's important ecosystems or biosphere

You can argue that early life in most cases would be made extinct by whatever made it extinct on Earth. But early life on Mars could be

- 8. Related to Earth life
- 9. Unrelated.

If unrelated it could be

- 10. Same chirality
- 11. Mirror chirality.

The combination of unrelated and mirror chirality could give it a competitive advantage even if early life

There is no rigorous way really to assign any probabilities to any of these options though many astrobiologists will have opinions about which ones are most likely. So, just as a way to get started thinking about this, let's make them all equal probability.

First, once more, we have the unknown chance of returning life at all given that Perseverance is not searching for present day life and the site was selected based on past rather than present day life. That is likely low already.

Add to that:

- Habitable ¹/₂
- Inhabited ¹/₂
- Survives ¹/₂
- Can spread on Earth and can't be contained 1/2
- Not early life 2/3 (will do separate list for early life)
- Harmful 1/3
- Causes widespread effects 1/4

So we get 1/16 for the first four points. Then it's an extra 1/72 for it to be harmful. Then another 1/4 for large scale effects.

So we get 1 chance in 16 * 72 * 4 = 1 chance in 4,608 that life returned from Mars has large scale effects. We get 1 chance in 16 * 72 or one chance in 1,152 for some harmful effects all the way down to minor nuisances.

However we haven't accounted for the mirror life so let's do that one.

1/16 for the first four points again – returning life that can spread on Earth and can't be contained once released.

Then mirror life is of concern whether early or recent.

- 1/2 that it is unrelated
- ¹/₂ that it's mirror life

So that then becomes 1 in 64 that we return mirror life that is able to spread on Earth and can't be contained.

This depends very much on how you evaluate the chance that Martian life is unrelated to terrestrial life and how you evaluate the chance that unrelated life is mirror life.

But it does seem a reason for particular care about mirror life even if the chance of it is rather lower than this suggest.

So those are the chances if they tried really hard to return life.

But they aren't, they are returning samples of geological interest with any present day life only there incidentally. The chance of returning life if they do absolutely no changes to the mission - is quite low it depends on whether life is almost everywhere on Mars.,

If the Viking missions did find life on Mars it has a chance. Not a high chance since they aren't planning a scoop of dirt which is what Viking did, but a chance since some of the dirt and dust may get onto the sample tubes.

If the Viking missions didn't find life it's almost no chance since they aren't trying to sample any potential microhabitats in Jezero crater.

Just the very remote chance of a viable spore in the dust. But they don't have a dedicated dust collector so there will be few dust grains, any that get stuck to the outside of the tubes by chance.

And then you have the BSL-4 facility to reduce the risk further.

I don't for a moment want to suggest there is anything rigorous about this calculation. Rather it's like the Drake Equation which tries to work out how many civilizations there are in the galaxy. The aim isn't really to get an answer but for a framework to start to think about the topic.

The Great Oxygenation Event which transformed Earth's atmosphere and oceans chemically gives a practical example of a way life from another Mars-like planet could in principle cause large scale changes to an Earth-like planet

In the quote from the National Research Council, they give no examples when they say "*the possibility that such effects occurred in the distant past cannot be discounted.*" (Board et al, 2009: 48).

:

Certainly in the modern era, there is no evidence for large-scale or other negative effects that are attributable to the frequent deliveries to Earth of essentially unaltered Martian rocks. However the possibility that such effects occurred in the distant past cannot be discounted."

See above:

 <u>NRC 2009 report emphasizes that large scale effects can't be ruled out – it says</u> potential hazards from microbes returned in a sample return mission are significantly greater than hazards from microbes in meteorites and that though there have certainly been no recent large scale effects that could be due to microbes from Mars, the possibility of large scale effects in the distant past can't be disproved – draft EIS says potential environmental effects would not be significant

There are many past extinctions in the geological record that are not well understood. However the Great Oxygenation Event could be relevant. Chroococcidiopsis may be partially responsible for the oxygenation of our atmosphere. One minority view explains the unusual ionizing radiation resistance of Chroococcidiopsis as a natural adaptation of Martian organisms (Pavlov et al, 2006).

This is weak evidence since the ionizing radiation resistance of chroococcidiopsis could be a byproduct of the repair mechanisms that chroococcidiopsis uses for UV resistance and desiccation resistance. Cyanobacteria originated in the Precambrian era. It could have developed these mechanisms back then, when, with no oxygen in the atmosphere, there was no ozone layer to shield out UV radiation (Casero et al, 2020) (Rahman et al, 2014)

However, the early Martian atmosphere was rich in oxygen (Lanza et al, 2016) before Earth and though much of that may well be due to ionizing radiation from solar storms splitting the water it's not impossible that it had photosynthetic life.as well.

Some astrobiologists have hypothesized that terrestrial life originated on Mars. If so, photosynthesis could have developed on Mars first too then transferred to Earth. Whether this happened for Mars and Earth, it does give a practical example of a way that life from another planet such as Mars could in principle cause large scale changes to an Earth-like planet.

So was this an extinction event? The Great Oxygenation Event might have forced rapid evolution rather than extinction. Early anaerobes may have retreated to anaerobic habitats as obligate anaerobes, which we still have today (Lane, 2015).

However, there is some evidence suggesting extinctions. There is evidence of exceptionally large sulfur reducing bacteria from this time, 20 to 265 μ m in size, which also occasionally occur in short chains of cells. This may be part of a diverse ecosystem that predated the GOE (Czaja et al, 2016). If such an ecosystem existed, most traces of it are gone now. However it seems not impossible that the GOE had major impacts on a prior diverse ecosystem.

There are many other confirmed mass extinctions in the fossil record. In many cases the cause is not fully known or debated leaving it not impossible that microbial transfer from Mars could be part of the explanation.

Whether or not this ever happened in the past, this worked example of the Great Oxygenation Event shows how in the worst case scenario, independently evolved life from another planet could lead to large scale transformations of the chemistry of Earth's atmosphere or oceans, climate and ecosystems. Humans with modern technology would surely survive a gradual transformation of our atmosphere and oceans but it could make the planet significantly less habitable in the short term for humans and other species.

If Mars has mirror life, returning it could potentially cause a similar large scale transformation of terrestrial ecosystems by gradually converting organics to mirror organics – an example worst case scenario

An example of a possible large scale transformation could be return of mirror life, if such exists on Mars and has never got to Earth. If it exists on Mars it is likely able to make use of both normal and mirror organics since most of the organics on Mars likely comes from meteorites and comets and interplanetary dust which has organics of both types.

Only a few terrestrial microbes can digest mirror organics so this would be a competitive advantage for the invasive mirror microbe species from Mars. Over time, this single species could diversify and could gradually transform nearly all the organics on Earth to mirror organics and make Earth significantly less habitable for terrestrial life.

Chroococcidiopsis survives on rock + nitrogen + water + sunlight

Mirror chroococcidiopsis could spread on Earth without any support from other life

Chroococcidioopsis survives on rock + nitrogen + water + sunlight

Mirror chroococcidiopsis could spread on Earth without any support from other life.

Photograph shows chroococcidiopsis in a cave at Ares Station, Cantabria in the Iberian peninsula – with a transparent covering of other microbes – it can live on its own or in colonies with other life and it can also live inside rocks. Photo by <u>Proyecto Agua on</u> <u>Flickr</u>

Chroococcidiopsis is a "polyextremonphile" which over hundreds of millions of years hash accumulated numerous metabolic pathways and adaptations adaptations. A mirror life analogue from Mars might be similar. Like Chroococcidiopsis it may be able to survive almost anywhere on Earth from Antarctic cliffs to tropical oceans and reservoirs, and from hot sunny deserts such as the Atacama desert to darkness hundreds of meters below the sea floor. I cover this below in the section:

 <u>A mirror life chroococcidiopsis analogue as a worst case example of a pioneer species</u> that would have adaptations that let it survive almost anywhere on Earth if returned from Mars and that could never be returned safely as it would risk transforming terrestrial organics to mirror organics that most life can't use

This is an example worst case scenario that I consider in my preprint <u>(Walker, 2022b)</u>. The mirror life could also be early life, even mirror life ribocells which may be able to pass through 0.02 micron filters. If it is independently evolved on Mars there is no particular reason to expect it to be normal rather than mirror life. Nanobes such as the ribocells are so small they escape

protozoan grazing and they would also have a much higher surface to volume ratio which is an advantage in habitats with low nutrient availability – so they may have a competitive advantage with more advanced modern life. That was a motivation for searching for a shadow biosphere of nanobes on Earth. None was found but possibly life returned from Mars could establish such a shadow biosphere here.

Scenario based approach – in other scenarios life from another planet is harmless or indeed beneficial

I found many other scenarios, including some where life from another planet could be harmless, or indeed beneficial. The archaea are an example of an entire domain of life that is largely beneficial in it is interactions with other life on Earth. A domain is the highest level of classification, the other domains are the bacteria and Eukarya. All multicellular life belongs to the Eukarya.

On Earth though harmful invasive species get most publicity there are many species that are beneficial or have no effect when they spread to new regions – contributing to the biodiversityh.

It would be possible for Martian life to lead to a more biodiverse and even a more productive biosphere on Earth for instance if they can make better use of low light levels or of nutrient poor regions of the Earth's surface or oceans.

See sections of my preprint (Walker, 2022b)

- Could Martian microbes be harmless to terrestrial organisms?
- Enhanced Gaia could Martian life be beneficial to Earth's biosphere?

But we have no experience of what happens if two biospheres collide in this way. We need to know what is there, on Mars. We need to know if there is life there, and if so, if it is safe to return it or not. This example shows that we can't assume it is safe until we know what it is.

I use a scenario based approach to explore this in my preprint (Walker, 2022b), explained in the introduction in the section:

Scenario based approach to explore the consequences if Earth or Mars develops a
mixed biosphere involving two forms of biochemistry or alien species from the other
planet – such as mirror life, RNA world nanobes, early life cells that cooperate rather
than compete before modern evolution, fungi and molds that our immune systems don't
recognize, or a new domain of life that is largely beneficial to terrestrial ecosystems
similarly to the archaea

A single mission can't resolve this question as it may not return life at all – and life that is safe for Earth may co-exist with other life that can never be returned safely which we could encounter in future missions on a planet with total surface area similar to the land area of Earth – it will take more future missions to resolve this question

We won't be able to resolve this question of whether there is life on Mars or not and whether it is safe for Earth or not with a single mission such as Perseverance returning samples from selected spots from one location on Mars.

Even if we return familiar life, it could have new capabilities acquired on Mars so needs careful study. Even if we prove that the species we returned are safe, they might easily co-exist with other species that can never be returned to Earth such as mirror life, that we will discover with future missions, even the next mission to Mars.

See the sections of my preprint (Walker, 2022b):

- Early discovery of a familiar microbe from Mars such as chroococcidiopsis is not enough to prove the sample is safe as familiar life can have new capabilities
- Discovery of a familiar microbe like chroococcidiopsis does not prove all life in the sample is familiar if terrestrial life originated on Mars, it could have extra domains of life that never got to Earth
- Potential to discover multiple biochemistries such as mirror and non mirror life in the same sample perhaps evolved in disconnected early Martian habitats or unfamiliar life mixed with familiar life transferred from Earth to Mars in the past

Resolving this is a matter for future missions and surely needs to be a priority for space colonization enthusiasts and astrobiologists alike. In my preprint <u>(Walker, 2022b)</u> I look at ways we may be able to do it:

• Resolving these issues with a rapid astrobiological survey, with astronauts teleoperating rovers from orbit around Mars

For space colonization enthusiasts, though discovery of a form of life that can never be returned to Earth such as mirror life would likely mean they can never colonize the Mars surface (at least not if they return to Earth) it would lead to huge interest in the planet which could be safely explored from orbit virtually via telepresence similarly to the way we explore computer game landscapes and from space settlements for instance on the moons Phobos and Deimos, and could be exploited also commercially using telerobotics to export materials to Earth.

A form of life that we can never return safely to Earth such as mirror life can also be one of the most exciting possibilities in terms of expanding knowledge. The mirror biology could easily be

of great commercial value to us. There are many other places in the solar system to explore, settle and perhaps colonize.

I discuss this under:

- Discovery of extant life on Mars could lead to long term interest in the planet, including orbiting colonies using sterile robots as our mobile eyes and hands to explore the planet from orbit via telepresence, and perhaps develop it commercially too, making it more habitable for Martian life
- This could be a stepping stone to human outposts or colonies further afield such as Jupiter's Callisto or Saturn's Titan, and settlements in self contained habitats throughout the solar system, spinning slowly for artificial gravity and built from materials from asteroids and comets

If we want to conclude from the meteorite evidence that microbial species from Mars are safe for Earth we need ALL Martian species to get to Earth on meteorites – example of barn swallows that can cross the Atlantic and are native to North America, while European starlings can't and are non native – natural processes can't transfer the surface dust, dirt, ice and salts of Mars to Earth

This is a point I highlight in my preprint <u>(Walker, 2022b)</u>. If certain species do sometimes get transferred to Earth from Mars it does NOT mean that all species on Mars are safe for Earth.

As an example, barn swallows cross the Atlantic from Europe to the USA, but starlings don't.

Barn swallows are not an invasive species in the USA while starlings are. European starling is an invasive bird in the Americas (<u>US DOA, 2017</u>).

Some microbes may be able to get from Mars to Earth what matters for invasive species are the ones that can't



Some microbes may be able to get from Mars to Earth - what matters for invasive species are the ones that can't.

Barn swallow - can cross Atlantic

Starling - invasive species in the Americas

Starling photo from: (Johnstone, 2017)

Barn swallow photo from (Batbander, 2017)

As an example, in 2012, starlings caused \$189 million in damage to crops of blueberries, wine grapes, apples, sweet cherries and tart cherries in the USA (<u>US DOA, 2017</u>).

Starlings also eat cattle feed and 1000 starlings can represent a loss of \$200 to \$400 in cattle feed. They can also transmit many diseases to cattle via the feeding troughs and their excrement corrodes iron structures including motor vehicles and iron roofs. They are also involved in thousands of bird strikes (US DOA, 2017)..

Example of fresh water diatoms that can't cross oceans on Earth

We have invasive diatoms in the Great Lakes. *Stephanodiscus binderanus* is a nuisance species that clogs water works and introduces foul odours into the water <u>(Spaulding et al, 2010)</u>. The diatom Didymosphenia geminata is an invasive species in New Zealand, possibly brought there on damp sports equipment. <u>(Spaulding et al, 2010)</u>. The long stalked version of Didymosphenia geminata is also an invasive species in the Great Lakes. The short stalked

version doesn't form mats and is presumed to be native to the Great Lakes. There were no records of the long stalked version in the Great Lakes until around 1990. After that this long stalked variant started to spread. It can survive and remain viable for up to 40 days in cool dark damp conditions, so it can be spread place to place on angling equipment, boot tops, neopreme waders and felt-soles.

The mats can be up to 20 cm thick and they trap stream sediment. These can cover the bottom of the stream and smother native plants, insects, mollusks and algae. Streams impacted outside of the Great lakes see the insects decrease and an absence of fish. This may be due to a new genetic variant that started to spread but if so, it hasn't been identified. These two paragraphs summarize / paraphrase some of the information from (Schmidt, n.d.)

This is an example sign in New Zealand warning sailors about the risk of carrying didymo to another lake in New Zealand.



Text on sign: Your boat may now be carrying didymo. Please clean using approved methods. Protect our waters ...

Image from: (Thorney; ?. 2006)

As you can see Didymo can't even move from one lake to another in New Zealand without help from humans carrying it in wet gear. There is no way it could travel between planets. There are salt water diatoms too. But they couldn't travel between planets on meteorites either. If there are diatoms on Mars they have evolved independently and can't be directly related to terrestrial diatoms.

We might even find diatoms on Mars – either preserved in gypsum, or perhaps living in the lakes our orbiters found beneath the polar ice

Perseverance has found gypsum, as did Curiosity <u>(Scheller et al, 2022)</u>., and on Earth, gypsum can preserve viable diatoms for tens of thousands of years and maybe hundreds of millions of years <u>(Benison et al, 2014)</u>. Diatoms evolved late on Earth which could be a reason to suppose they are unlikely to have evolved on Mars (<u>Cabrol et al, 2009</u>). However it isn't impossible as it is hard to generalize given only one example from one planet.

So, even the idea that some day we find a viable diatom on Mars is not as far fetched as it might seem though it would need diatoms to evolve on Mars and for a lake to form with enough water for diatoms to inhabit it in the recent enough past for the diatoms to still be viable today. Such lakes actually do exist in present day Mars but they are deep below the ice at the poles (Orosei et al, 2018). David Wiliams, diatom researcher at the Natural History Museum said that technically diatoms could survive there though he says a more interesting question is whether we'd even be able to identify it as life if it originated on Mars (Davis, 2018):

'Yes, technically tiny life forms such as diatoms and cyanobacteria could survive in these environments. But that is not the question we should be asking.

'A more interesting question is whether we would know what we're looking at, even if we did find something in the lake. Would we even be able to identify it as life, if it originated on Mars itself?'

So, though it's not the most likely example for Jezero crater, it's not impossible we eventually find diatoms on Mars, or maybe some other form of life adapted to a similar life style, and the chance it is able to get to Earth on a meteorite may be very low.

Chroococcidiopsis as an example of a species that wouldn't survive transfer by impacts from modern Mars based on an analysis by Charles Cockell

Some species will be better able than others to withstand the shock of ejection from Mars, the cold and dry and complete vacuum of the transition through space, then the fireball of re-entry to Earth. As an example, most photosynthetic life is killed in this process.

The first challenge is the shock of ejection. Microbes are suddenly accelerated from rest to escape velocity in a fraction of a second. The microbes can be destroyed by cell rupture or by DNA damage. All cells of Chroococcidiopsis are killed at 10 GPa (Nicholson, 2009). To put this in context, ALH84001 experienced a shock of ejection of \sim 35 – 40 GPa. The Nahkalites were least shocked at 15 to 25 GPa. This is still too much for Chroococcidiopsis (Nyquist, 2001)

The microbe also has to survive the fireball of re-entry to Earth.

Cockell inculcated an artificial gneiss rock with Chrooccoccidiopsis at a depth where it occurs naturally, and affixed it to the re-entry shield of a Soyuz rocket. None survived re-entry, nor did any organics.

Cockell concluded that it might not be impossible for photosynthetic life to get to Earth from Mars, but it would need an extraordinary combination of events (Cockell, 2008)

So in this analogy, most photosynthetic life on Mars would be more like the European starling than the Swallow, wouldn't be able to get to Earth on meteorites except possibly in rare very large impacts, and most likely in the early solar system.

Then, the rocks we have in our Martian meteorite collections all come from at least three meters below the surface (Head et al, 2002) . They were probably thrown up into space after glancing collisions into the Elysium or Tharsis regions, high altitude southern uplands (Tornabene et al, 2006). The atmosphere for these high altitude regions on Mars is thin, making ejection to Earth easier. The subsurface below about 12 cms has a uniform temperature of around 200°K or -73°C (Möhlmann, 2005:figure 2). With such a thin atmosphere, present day life at those altitudes is unlikely (except perhaps for deep subsurface geothermal hot spots).

Larger impacts in the recent geological past could send material to Earth from other potentially more habitable parts of Mars. However:

- Many proposed habitats are in surface layers of dirt, ice and salts. These would likely never get into space
- Other proposed habitats are millimeters below the surface of rocks. These layers would ablate away during entry into the Earth's atmosphere

Life on Mars could be extremely localized to only a few square kilometers over the entire planet, for instance, only to the RSL's, or only above geological hot spots, making it less likely that the habitats are hit by an asteroid able to send material all the way to Earth in the large chunks needed for protection from cosmic radiation during the transfer.

Yet life from distant habitats on Mars may be able to get to Jezero crater in dust storms. Of course dust storms can't transport Martian spores or propagules to Earth and the dust can't be

transported to Earth. We have no samples of Martian dust or Martian surface salts or ice in our meteorite collections and these couldn't get to Earth even in the early solar system.

A mirror life chroococcidiopsis analogue as a worst case example of a pioneer species that would have adaptations that let it survive almost anywhere on Earth if returned from Mars and that could never be returned safely as it would risk transforming terrestrial organics to mirror organics that most life can't use

We only need one pioneer species to get to Earth to set up a new ecosystem. Martian life would be likely to be able to survive on Earth. The Martian brines are highly oxidising, with perchlorates and hydrogen peroxides. They are so oxidizing that many terrestrial life forms would find hard to tolerate them. Recent research by Stamenković suggests the cold brines on Mars may be oxygenated too, even with the very low levels of oxygen, in the very cold conditions since oxygen is more soluble in cold water.

Then, though Mars gets very cold at night, in daytime it can sometimes reach above 20°C.

Microbes returned from Mars to Ear may be able to settle in on Earth as a "home from home" even more habitable for them than Earth.

For instance, suppose that Mars has mirror life, which is like the European starling, is not able to get here via panspermia. An example here is Chroococcidiopsis, a blue-green algae found in Antarctic cliffs, also in the Arizona desert near JPL, but also is ubiquitous through Earth, found in the sea, in tropical water supplies, both wet, dry, hot, cold, it's a polyextremophile that has numerous metabolic pathways that let it survive almost everywhere, and it is one of the top candidates for a form of life that could survive on Mars.

A mirror analogue of chroococcidiopsis from Mars could flourish almost anywhere from Antarctic cliffs to the Atacama desert (<u>Bahl et al, 2011</u>) or from Sri Lankan reservoirs (<u>Magana-Arachchi et al, 2013</u>) to the Chinese sea (Xu et al, 201q26:111), and form the foundation of a mirror ecosystem.

It is a pioneer species and a primary producer and doesn't depend on any other life to survive.

Chroococcidiopsis, is an ancient polyextremophile with numerous alternative metabolic pathways it can utilize, including nitrogen fixation, methanotrophy, sulfate reduction, nitrate reduction etc (KEGG, n.d.), even able to grow in complete darkness using a hydrogen-based lithoautotrophic metabolism with viable populations found over 600 meters below the surface (Puente-Sánchez et al, 2018) and in another case 750 meters below the Atlantic sea bed (Li et al, 2020).

In the same way a mirror Martian polyextremophile might retain numerous metabolic pathways from its evolutionary history on Mars that it could use to colonize diverse habitats on Earth. The Martian history would include hydrothermal vents, oxygen rich lakes, and almost any climate condition it could encounter on Earth as well as some conditions not present here naturally such as ultra low temperatures and ultra low atmospheric pressures and far higher levels of UV and ionizing radiation than life encounters on Earth.

So, suppose there is a mirror chroococcidiopsis on Mars.. Or some other pioneer species including ultramicrobacteria, maybe even mirror life ribocells.

Once it was well established, other mirror life could build up a microbial ecosystem based on this and in this way mirror life could start to spread through our ecosystems.

This is a worst case scenario. This does not mean it is inevitable that Martian life would harm Earth. Indeed there are other scenarios where Marian life can be harmless or even beneficial to Earth's biosphere.

Enhanced Gaia - could Martian life be beneficial to Earth's biosphere?

So far we've focused on situations where biosphere collisions are harmful, since the topic is planetary protection, so we need to focus on scenarios where there is indeed a need to protect Earth. However we should also recognize that the introduction of extraterrestrial life to our biosphere could also be beneficial, as Rummel mentioned in his foreword to "When Biospheres Collide" (Meltzer, 2012)

We have examples from multicellular life to show that invasive species aren't always harmful. Schlaepfer et al did a survey of invasive species and in their table 1 they find many non native species that are actually beneficial. Some were deliberately introduced for their value for conservation, but many of the best examples were introduced unintentionally (Schlaepfer et al, 2011).

Schlaepfer doesn't list any microbial examples. What could benign interactions with terrestrial life look like for Martian microbes? Here are a few suggestions:

- More efficient photosynthetic life from Mars could increase the rate of sequestration of CO₂ in the sea and on land, improve soil organic content, and perhaps help with reduction of CO₂ levels in the atmosphere
- More efficient photosynthesis could increase the productivity of oceans
- Most of the surface layers of our oceans are deserts, except near to the coasts, because
 of the limitation of nitrogen, phosphorus, iron and silica (needed for diatom shells)
 (Bristow et al, 2017). If extraterrestrial life has different nutrient requirements, it may be
 able to inhabit these deserts and form the basis of an expanded food web.

- Martian microbes could be better at nitrogen fixation, phosphorus and iron mobilization, and so improve our soils, and help with crop yields as endophytes. Just as Martian microbes could enter the human microbiome, they could also enter plant microbiomes as endophytes and those interactions need not be harmful, many could be beneficial. (Afzal et al, 2019)
- New forms of yeast could be of interest in the food industry (Sarmiento et al, 2015).
- Martian life could increase species richness by gene transfer to Earth microbes, leading to more biodiverse microbial populations.
- Martian extremophiles could colonize microhabitats in deserts and eroded landscapes barely habitable to terrestrial life, helping with reversal of desertification
- More efficient Martian microbes might be useful to generate biofuels from sunlight and water <u>(Schenk et al, 2008)</u>
- Martian life might be accidentally toxic and control harmful microbes or insects
- Martian life might aid digestion or enter into other beneficial forms of symbiosis.
- Martian life could produce beneficial bioactive molecules as part of the human microbiome. These could include molecules that are antiviral, antibacterial, antifungal, insecticides, molecules that kill cancer cells, immunosuppressants, and antioxidants - we get all of those from beneficial microbes that are already in our microbiome. (Borges et al, 2009).
- It could add a new domain of life with almost entirely beneficial interactions similarly to the Archaea
- It could add new forms of multicellular life based on a different biochemistry, or multicellular life in a different domain of life from the eukaryotes, with a more ancient common ancestor.

Even if introducing martian life is largely beneficial, it could still be harmful in some ecosystems or have mixed effects with some harms and some benefits

However even if introducing terrestrial life is largely beneficial we still need caution. There would be not just one encounter in one ecosystem. Martian conditions may well favour polyextremophiles able to survive in a wide range of conditions.

Chroococcidiopsis is perhaps our best analogue for a Martian cyanobacteria and it is a polyextremophile and found in many habitats throughout the world. Also the microbes would evolve eventually, and perhaps quickly, or change gene expression, and eventually find new habitats that they can colonize.

Maybe some of these encounters would be beneficial in some ecosystems, while other ecosystems are degraded, possibly even by the same interactions with the same microbe. Similarly for organisms, some organisms may be benefited and others harmed.

To take an example, even if what we find on Mars is just a new strain of Chroococcidiopsis, it could have toxins, protoxins or accidentally toxic semiochemicals. Chroococcidiopsis is an

The same Martian microbe may also have both harmful and beneficial effects on the same organism, or in the same ecosystem. Generally there might well be a mix of some beneficial and some harmful interactions.

On the other hand the interactions could all be beneficial. To take an example, our planet is not necessarily optimal for global biomass (<u>Kleidon, 2002</u>). Perhaps extraterrestrial life with additional capabilities could enhance the productivity of the terrestrial Gaaia.

Return of Martian life might create a new enhanced Gaia system that has significantly more surface biomass and biodiversity than the one we have today. It might even add new beneficial domains of life like the archaea or a new form of multicellularity which only enhances the diversity of our biosphere.

We have nothing by way of previous experience to guide us here.

Amongst a million extraterrestrial civilizations that return a sample from a nearby biosphere with limited technological capabilities to contain it, we don't know how many would find they have harmed the biosphere of their home world. It might be that

- it is never seriously harmful, it usually leads to an enhanced Gaia, and is almost always a beneficial process.
- Or the worst case may be true that most Extraterrestrial biospheres are seriously degraded after their first unsterilized sample return from a nearby independently evolved biosphere

There is no way to know.

Writings by John Rummel, Joshua Lederberg, Carl Sagan, Claudius Gros and many others emphasize that though putative martin life based on a different biochemistry may not be adapted to us – we also haven't evolved immune defences to them – the reasoning of the sterilizing subcommittee is refuted by these papers which they don't cite

NASA's sterilizing subcommittee on the risk of life from Mars claim that there is near zero probability that a putative martian microorganism could be pathogenic to humans – this is refuted in an extensive literature on ways that Martian life could be harmful to humans and our biosphere which they don't cite.

Their reasoning looks convincing at first if you haven't read the literature. (Craven et al., 2021)

Microorganisms are usually highly adapted to specific biological niches or hosts, and even when novel pathogenicity arises, as in zoonosis or opportunistic infections, it does not represent a major evolutionary gulf. Emerging human pathogens are often the result of zoonosis in which an existing pathogen moves between related species being modified during this transfer such as coronaviruses, Ebola or HIV which all emerged from other mammalian hosts, or influenza which can transmit from avian or mammalian hosts.

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Since any putative Martian microorganism would not have experienced long-term evolutionary contact with humans (or other Earth host), the presence of a direct pathogen on Mars is likely to have a near-zero probability.

There are many simple examples, fungal pathogens of immunocompromised patients aren't adapted to their host. Also, Legionnaire's disease is a pathogen of biofilms that isn't adapted to humans. It uses the same method it uses to infect protozoa in biofilms to infect the macrophages in our lungs

Legionella pneumophila is normally a parasite of freshwater amoebae, which take it up by phagocytosis. When droplets of water containing L. pneumophila or infected amoebae are inhaled into the lung, the bacteria can invade and live inside alveolar macrophages, which, to the bacteria, must seem just like large amoebae. ; .<u>Cell Biology</u> of Infection

Warmflash et al put it like this

In essence, all that a potentially infectious human pathogen needs to emerge and persist is to grow and live naturally under conditions that are similar to those that it might later encounter in a human host. On Mars, these conditions might be met in a particular niche within the extracellular environment of a biofilm, or within the intracellular environment of another single-celled Martian organism.

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To be sure, the genetic similarity between humans and protozoa is much greater than could be expected between humans and the Martian host of a Martian microbe.

However, the L. pneumophila example does bring into question the rationale of the need for host-pathogen coevolution. Even in the context of a planetary bio-sphere that is limited to single-celled life, and even where there is unlikely to have been a co-evolution between agent and host organism, the possibility of infectious agents, even an invasive type, cannot be ruled out.

There are many examples in the literature of experts warning that this argument isn't valid. Here are some quotes from John Rummel, Joshua Ledererg, Carl Sagan, and Claudius Gros, all warning that this reasoning is not correct.

This is how John Rummel put it in the foreword to "When Biospheres Collide":

"Likewise, we don't know what would happen if alien organisms were introduced into Earth's biosphere. Would a close relationship (and a benign one) be obvious to all, or will Martian life be so alien as to be unnoticed by both Earth organisms and human defenses? We really have no data to address these questions, and considerate scientists fear conducting these experiments without proper safeguards. After all, this is the only biosphere we currently know - and we do love it!"

Joshua Lederberg, who got his Nobel prize for his work on microbial genetics was a key figure in the early work on planetary protection <u>(Scharf, 2016)</u>. He first began to give it his attention in 1957 <u>(Lederberg, 1959)</u>. He put it like this:

"Whether a microorganism from Mars exists and could attack us is more conjectural. If so, it might be a zoonosis to beat all others. On the one hand, how could microbes from Mars be pathogenic for hosts on Earth when so many subtle adaptations are needed for any new organisms to come into a host and cause disease? On the other hand, microorganisms make little besides proteins and carbohydrates, and the human or other mammalian immune systems typically respond to peptides or carbohydrates produced by invading pathogens. Thus, although the hypothetical parasite from Mars is not adapted to live in a host from Earth, our immune systems are not equipped to cope with totally alien parasites: a conceptual impasse." (Lederberg, 1999b)

Our immune system and defenses are keyed to various chemicals produced by Earth life. such as peptides and carbohydrates. Mars life might use different chemicals. In the best case (for us), the Martian microbes are unable to make anything of terrestrial biochemistry and give up.

However, in the worst case, it's the other way around. This time, it's our defense systems that are mystified. The microbes don't resemble Earth life and so our defenses don't recognize the attackers as life or attempt to do anything about them.

Carl Sagan put it like this (Sagan, 1973:162):

"Precisely because Mars is an environment of great potential biological interest, it is possible that on Mars there are pathogens, organisms which, if transported to the terrestrial environment, might do enormous biological damage - a Martian plague, the twist in the plot of H. G. Wells' War of the Worlds, but in reverse. This is an extremely grave point. On the one hand, we can argue that Martian organisms cannot cause any serious problems to terrestrial organisms, because there has been no biological contact for 4.5 billion years between Martian and terrestrial organisms. On the other hand, we can argue equally well that terrestrial organisms have evolved no defenses against potential Martian pathogens, precisely because there has been no such contact for 4.5 billion years. The chance of such an infection may be very small, but the hazards, if it occurs, are certainly very high.

The physicist Claudius Gros looks at a clash of interpenetrating biospheres in his paper on a "Genesis project" to develop ecospheres on transiently habitable planets. Gros reasons that the key to functioning of the immune system of multicellular organisms, plants or animals, is recognition of "non-self". He presumes that biological defense mechanisms evolve only when the threat is actually present and they don't evolve to respond to a never encountered theoretical possibility (Gros, 2016).

"How likely is it then, that 'non-self' recognition will work also for alien microbes?"

"Here we presume, that general evolutionary principles hold. Namely, that biological defense mechanisms evolve only when the threat is actually present and not just a theoretical possibility. Under this assumption the outlook for two clashing complex biospheres becomes quite dire."

"In the best case scenario the microbes of one of the biospheres will eat at first through the higher multicellular organisms of the other biosphere. Primitive multicellular organisms may however survive the onslaught through a strategy involving rapid reproduction and adaption. The overall extinction rates could then be kept, together with the respective recovery times, 1–10 Ma, to levels comparable to that of terrestrial mass extinction events."

"In the worst case scenario more or less all multicellular organism of the planet targeted for human settlement would be eradicated. The host planet would then be reduced to a microbial slush in a pre-cambrian state, with considerably prolonged recovery times. The leftovers of the terrestrial and the indigenous biospheres may coexist in the end in terms of 'shadow biospheres' " I don't know of anyone who has gone into this in detail, how it would work. But our body is protected by broad spectrum antimicrobials and then by specific immune responses such as the response to specific fungi that harm humans.

As an example, opportunistic fungi kill an estimated 1.5 million people worldwide every year (Brown et al, 2012). Those are often immuncompromised people as our skin and immune system has natural defences against fungi and especially the three most common genera,

This will be a topic of another paper in this series.

I go into this in a preliminary way in my preprint <u>NASA and ESA are likely to be legally required</u> to sterilize Mars samples to protect the environment until proven safe ... It's in the section

• Microplastics and nanoplastics as an analogue for cells of alien life entering our bodies unrecognized by the immune system

Example of fungi to illustrate how our immune system may not notice an alien fungus with a different biochemistry not recognized by our skin's natural antimicrobials or immune responses – fungi kill 1.5 million people a year, mainly immunocompromised and we may all be immunocompromised to an alien fungus from Mars

Our antibiotics might not work with Martian life. They target specific enzymes and processes within living cells based on Earth's biochemistry (Kapoor et al, 2017). Let's take penicillin as an example. It targets transpeptidase which is essential for cross linking in the final stage of cell wall synthesis to make rigid cell walls (Yocum et al, 1980). It does that by forming a highly stable penicilloyl-enzyme intermediate. One way that microbes develop resistance to this antibiotic is by using different enzymes that perform the same function in the cell (Gordon et al, 2000).

An alien biochemistry likely has different enzymes already, through independent evolution. So antibiotics may not work with it.

It is possible that our skin gives little protection against Martian microbes. Its first line of defence consists of sixteen broad spectrum antimicrobial peptides and the second line of defence consists of T cell responses with inflammatory cascades in the subepithelial tissue (Abdo et al, 2020). The antimicrobials might have no effect on an alien biochemistry, and the immune response might not be triggered by it. If this were to happen, Martian life might penetrate these barriers without being noticed by our skin's defences and enter the underlying flesh and bloodstream.

The endolithic yeast Exophiala jeanselmei can survive simulated Martian conditions, without any source of water except atmospheric humidity (Zakharova et al, 2014).

Exophiala jeanselmei is closely related to opportunistic human pathogens. It can be an opportunistic human pathogen itself, causing superficial and localized infections in humans, in skin, nails, cornea and superficial wounds and is occasionally serious for immunocompromised individuals and is naturally resistant to most antifungals on the market (Urbaniakt al, 2019). Most healthy people have fungi in their sinuses, but these are harmless to them. Sometimes in patients with normal immune systems, these may form "fungal balls" that occupy the empty spaces in our sinuses.

When the immune system is not functioning properly, fungi can penetrate mucosal barriers and the epithelial layer and invade the host tissues and when this happens the results can be serious (Soler et al, 2012). A diverse range of fungal species can cause a lethal infection in immunocompromised hosts and these are often resistant to antibiotics (Pfaller et al, 2004) Opportunistic fungi kill an estimated 1.5 million people worldwide every year (Brown et al, 2012). Our immune system probably stops many fungal infections by recognizing particular patterns, the pathogen-associated molecular patterns (PAMPs). It likely does this using pattern recognition receptors (PRRs) which then trigger the immune response. These are targeted to the molecular patterns from the most common fungi that attack humans, species from three genera: Candida, Aspergillus, and Cryptococcus with different molecular patterns specific to each genera (Kumar et al, 2018).

Our immune system wouldn't have these pattern reception receptors for a martian fungus with an alien biochemistry. It may not have them even for related martian fungal species in a different genera from any terrestrial biology.

We have only a few effective antifungal medicines, making antifungal resistant microbes a problem <u>(Cowen et al, 2015)</u>. Alien life might be naturally antifungal resistant, if they don't have the biochemistry targeted by antifungal medicines.

For alien life we may all be effectively immunocompromised if the broad spectrum antibiotics in our skin and epithelium have no effect on the alien life, and our innate or adaptive immune systems don't recognize it as pathogenic.

Example of a a Shewanella algae to show that alien life might be able to confer antibiotic resistance to synthetic antiobitics even if it is not itself affected by them

When human pathogens develop antibiotic resistance, this often comes from other microbes by horizontal gene transfer, as they arise too quickly for the microbes to evolve it themselves.

These resistance genes are found for every type of antimicrobial (<u>Martínez, 2012</u>). Many of the naturally occurring antibiotic resistance genes probably originate in microbes that make those antibiotics themselves and need the resistance gene to protect themselves from their own antibiotics.

However, the gene that gives antibiotic resistance to quinolones, a new non naturally occurring synthetic antibiotic, seems to have originated in a Shewanella algae which doesn't produce antibiotics itself. So it seems likely to have a different role in it (<u>Martínez, 2012</u>).

In the same way, even related Martian microbes could have antibiotic resistance or confer novel antibiotic resistance to terrestrial microbes through genes evolved for other purposes on Mars that lead to their internal processes changing in ways that make the antibiotics no longer effective.

Examples of exotoxins, protoxins, allergens, secondary metabolites that spoil food, accidentally toxic signalling chemicals (semiochemials) and the possibility that the internal chemistry of alien life, such as perchlorates in place of salt for the intracellular medium could be harmful to terrestrial life

Other issues may arise from secondary metabolites, for instance, *Wallemia, an* airborne extremophile fungus, is found in food, especially highly salted or sweetened food such as salted fish, jams and cake. It is adapted to low water activity, and produces the secondary toxic metabolites wallimidione, walleminol and walleminon. W. sebi is a common cause for spoiled food through its production of secondary metabolites. The most toxic of these is wallimidione (Desroches et al, 2014). Mars conditions are likely to favour life adapted to low water activity levels, and so, as for w. sebi, could be a nuisance particularly for highly salted or sugary foods, where they also might produce secondary metabolites.

Martian life could cause allergic reactions. W. sebi has been found to cause allergic sensitization (<u>Desroches et al, 2014</u>). Another example is the fungus Aspergillus which can trigger asthma, and as an opportunistic infection can also cause the more serious illness of aspergillosis, and death (<u>Latgé, 1999</u>).

The common allergic reaction to poison ivy is due to Urushiol, a Catichol $C_6H_4(OH)_2$ with one or more alkyl chains substituted in the 3 position. It forms antigens by binding to surface proteins of the dermis or epidermis so forming an antigen, which leads to an allergic response on the second exposure (Bryson, 1996, page 680). This again is a simple enough chemical so that it may occur in an alien biology, or something else similar. For another example, sesquiterpines is a toxic signaling chemical (semiochemical) produced by potatoes under stress (<u>Matthews et al, 2006</u>). Could semiochemicals produced by an alien biochemistry be accidentally toxic to Earth life.

Alien biochemistries could also produce, or contain protoxins, which when metabolized break down into toxic products. For instance hypoglycin A, which is not itself toxic, is broken down into the highly toxic MCPA-CoA on digestion and can lead to the fatal Jamaican vomiting sickness after eating the unripe fruit of the Ackee tree, a national foodstuff in Jamaica (Holson, 2015). A more commonplace example is methanol which is converted into toxins when digested (Mégarbane, 2005).

Again, toxicity may be more common if the secondary metabolites or protoxins are based on a different biochemistry.

The chemistry of alien cells may itself be toxic to Earth life. One suggestion is that Martian life might use hydrogen peroxide and perchlorates in its intracellular fluids in place of the chlorides used by Earth life, similarly to the composition of the brines it inhabits <u>(Schulze-Makuch et al, 2010a)</u>. This could adversely affect Earth microbes that interact with Martian cells or scavenge dead Martian life.

Waste products and metabolic intermediaries could also be accidentally toxic or allergenic.

As before all, if humans are unaffected, these effects could still harm other creatures in Earth's biosphere, and harm us indirectly, if other creatures we depend on are affected.

Accidental similarity of amino acids forming neurotoxins such as BMAA which resembles L-serine – a putative cause for the motor neurone disease LouGherig's disease or ALS

Certain algae blooms, including Chroococcidiopsis produce β -N-methylamino-L-alanine or BMAA (table 2 of <u>Cox et al, 2005</u>) which is a neurotoxin which can contaminate drinking water and in worst cases cause death (<u>Cox et al, 2005</u>).

In laboratory experiments BMAA can get misincorporated into proteins in human cells, and is a putative cause for the motor neurone disease ALS, or Lou Gherig's disease (Dunlop et al, 2013). This time BMAA is not produced as an exotoxin. The poisoning is accidental, it gets misincorporated because of its accidental partial resemblance to I-serine.

There are thousands of potential amino acids an alien biology might use. An extraterrestrial biology could use many more amino acids than the 20 encoded in terrestrial life.

There are 140 amino acids that occur naturally in terrestrial biology, but not in proteins (<u>Ambrogelly et al., 2007</u>). 52 amino acids have been identified in the Murchison meteorite

(Cronin, 1983). A computer search turned up nearly 4,000 biologically reasonable amino acids (Meringer, 2013) (Doyle, 2014).

Many of those won't occur in nature, but terrestrial biology also includes non natural amino acids. Meanwhile also many of the natural amino acids don't occur in terrestrial biology and might potentially be used in extraterrestrial biology.

If two biospheres collide that are based on a different vocabulary of amino acids, there may be many such accidental similarities. In the case of BMAA, it's been suggested that proteobacteria in our gut provide some protection by removing it <u>(Baugh et al, 2017)</u>. However there might be no helpful microbes to protect us by removing similarly close analogs of our amino acids from an alien biochemistry.

Example of independently evolved mirror life, evolved from the mirror chemicals to terrestrial life, to expand on the National Research Council and European Space Foundation statements about the potential for large scale environmental impacts of alien biochemistry in the sample return studies

Be

The worst case could transform the Earth's biosphere in a fundamental way. It could perhaps as major a transformation as the Great Oxygenation Event in terms of habitability. It might well be more habitable for some future form of life on Earth that evolves later, but not for us.

Mirror life is a simple example that all astrobiologists agree is biologically plausible, just life that evolved from scratch using chemicals in the opposite, mirror sense to the chemicals used by terrestrial life.

It's not known how terrestrial homochirality evolved, with many proposed mechanisms (Blackmond, 2019). Some experts such as Blackmond and Vlieg have expressed the view that it is just the *"luck of the draw"* and that we could find another planet out there with mirror life (Brazil, 2015). So we have to consider the possibility that technicians could be contaminated by mirror bacteria.

Mirror bacteria are likely to have a survival advantage on Earth. Most terrestrial life would be unable to metabolize most mirror organics such as starches, proteins, and fats (Dinan et al, 2007) (Bohannon, 2010).

Some species of terrestrial microbes might develop the ability to metabolize mirror organics. Our biosphere already has a few species of microbes that can express the isomerases and racemases needed to flip organics into their mirror molecules, to metabolize mirror organics (Pikuta et al, 2006) (Pikuta et al, 2010) (Pikuta et al, 2016).

However, most terrestrial microbes would not be able to do anything with mirror organics. Meanwhile, Martian life could already have the equivalent enzymes to metabolize normal organics. This has to be a possibility, given that some terrestrial microbes can already metabolize mirror organics.

One way this could happen is if Mars already has a biosphere where mirror and non mirror life co-exist. They might for instance have evolved separately in different habitats on early Mars and then two forms of life encounter each other later. Each form of life might then evolve the enzymes to metabolize organics from the other form of life. The result could be that mirror life from Mars is already able to metabolize non mirror starches, proteins and fats, giving it an initial competitive advantage over terrestrial life that has never been exposed to mirror organics.

Mirror Martian life might also need these enzymes to metabolize organics from the infall of meteorites, as these have both normal and mirror forms of carbohydrates, amino acids and other organics.

Most organics on Mars may well come mainly from the infall of meteorites, comets and interplanetary dust (Frantseva et al, 2018) rather than from life processes even if there is life there. If there was no degradation of the organics, Mars should have 60 ppm of organics deposited into the regolith, averaged over its entire surface to a depth of a hundred meters (Goetz et al, 2016:247).

This would lead to a strong selection advantage for life able to make maximal use of both isomers of sugars and amino acids in meteoritic material.

The outcomes for terrestrial ecosystems from release of such a lifeform could be serious, as mirror life gradually converts terrestrial organics to indigestible mirror organics through one ecosystem after another

The worst case is not human extinction but humans having to live essentially in space colonies on Earth growing crops in habitats, preserving tropical jungles, coral reefs etc in vast enclosed biomes with the technology of tomorrow.

Especially if we returned independently evolved mirror life. That might well be adapted to be able to make use of the organics from comets, meteorites and interplanetary dust so would have the isomerases to transform organic food into its mirror image so it can eat it. There are a few terrestrial microbes can do this, can eat mirror organics, but it is a rare capability.

So mirror life from Mars would slowly spread and consume ordinary organics, and transform it into mirror organics. Eventually I think terrestrial microbes would adapt and we'd end with a mix of mirror and ordinary microbes each able to use the opposite sense of organics – but these would be different biochemistries, different capabilities. The proportion of mirror and ordinary microbes would be hard to predict, but it could be mainly mirror organics in a worst case. Higher life couldn't evolve fast enough to make use of the mirror organics and it may well also interfere with its metabolism. Eventually over millions of years Earth's biosphere might well be enhanced as multicellular life evolves again able to use both types of organics and maybe we can accelerate that with genetic manipulation but its not a legacy we'd want to leave to our descendants.

Probably a transformation of our biosphere like this, converting organics to mirror organics, or half in half ordinary and mirror, would unfold slowly. The martian life would be likely slowly replicating anyway, even as polyextremophiles because it's adapted to cold conditions and most psychrophiles have doubling times of months to years.

Then the mirror life has to evolve to exploit niches. But for instance a mirror blue-green algae (or perhaps black on Mars) analogue of chroococcidiopsis as a polyextremophile might already be pre-adapted to live almost anywhere on Earth retaining capabilities from the distant past just as Chroococcidiopsis has somehow achieved and retained the ability to heal itself from large numbers of double strand DNA breaks possibly a capability it develop

These sections are a taster sampler for a future paper to expand on this in more detail as in the preprint

This is a topic for a future paper in this series.

I cover this in my preprint (Walker, 2022b) in the sections:

Many ways present day Martian life could harm terrestrial organisms

Mars could have opportunistic fungi – these kill 1.5 million people on Earth every year

Martian life could be a pathogen of Martian biofilms sufficiently closely adapted to infect protists on Earth – or it might be ignored by the white blood cell phagocytes and live in intercellular spaces of our lungs

Our antibiotics target specific enzymes and processes so might not work with unrelated martian life – meanwhile related life might have naturally evolved accidental antibiotics like the Shewnella algae which seems to be the origin of the gene that confers resistance to quinolones – a new non naturally occurring synthetic antibiotic

Ways that our immune system may not notice an alien biochemistry without the natural antimicrobials or immune responses for alien opportunistic pathogens and other diseases

Could a Martian originated pathogen be airborne or otherwise spread human to human?

Microplastics and nanoplastics as an analogue for cells of alien life entering our bodies unrecognized by the immune system

Exotoxins, protoxins, allergens and opportunistic infection

Accidental similarity of amino acids forming neurotoxins such as BMAA which resembles Lserine – a putative cause for the motor neurone disease LouGherig's disease or ALS

Martian microbes better adapted to terrestrial conditions than terrestrial life, example of more efficient photosynthesis

Example of a mirror life analogue of chroococcidiopsis, a photosynthetic nitrogen fixing polyextremophile

Example of mirror life nanobacteria spreading through terrestrial ecosystems

Possibility of extraterrestrial Martian life setting up a "Diminished Gaia" on Earth

Worst case scenario where terrestrial life has no defences to an alien biology - humans survive by 'paraterraforming' a severely diminished Gaia

Worst case where alien life unrecognized by terrestrial immune systems spreads to pervade all terrestrial ecosystems

The threshold for risk for the terrestrial biosphere should be a decision for the public not NASA when their scientist naturally have a high priority for completing this mission recommendations by many experts to set up an oversight agency in advance with experts in legal, ethical and social issues ideally two years before the start of the legal process – this has not been done

NASA and ESA clearly didn't 'do this or they would have produced a much more thorough EIS and would have engaged in far more outreach to the general public before submitting the EIS.

With so much to be sorted out, Uhran et al recommended that an oversight agency should be set up long before the legal process starts. Uhran et al recommend this is done two years

before filing the environmental impact statement to develop a consensus position on the margin of safety for sample containment (Uhran et al, 2019).

Since the aim is to develop a consensus position, this would need to be based on up to date information. So it would need to include the review of the size limits required in the ESF sample return study (Ammann et al, 2012:PG). The current paper suggests the need to review filter technology and provide a preliminary study of the technological advances needed to achieve the specified size limits, since the technology doesn't seem to exist yet.

Rummel et al say that the oversight committee would need to contain experts in legal, ethical and social issues in addition to the experts in astrobiology, space engineering and mission planning. It should conduct ethical and public reviews. Broad acceptance by the public is essential at an early stage for success of the mission (Rummel et al, 2002).

In more detail, Rummel et al advise that clear communication with the public is essential from an early stage, for success of the mission. (Rummel et al, 2002).

Pages 94-5: As part of sample return planning, it will be important to develop an organized communication plan which will lay a strong foundation in public understanding and acceptance prior to the mission, and allow for an open dialogue with all sectors of the public. Such a plan should include consideration of the diverse questions, concerns, and issues likely to be raised, including those related to the mission and spacecraft operations, the sample return and Biohazard testing, the administrative and legal matters associated with the effort, and to the potential implications of discovering extraterrestrial life.

Plans should be developed well in advance in order to avoid a frenzied, reactive mode of communications between government officials, the scientific community, the mass media, and the public.

They recommend that this should avoid a NASA centric focus and include links with other government agencies and international partners and external organizations

Any plan that is developed should avoid a NASA-centric focus by including linkages with other government agencies, international partners, and external organizations, as appropriate. It will also be advisable to anticipate the kinds of questions the public might ask, and to disclose information early and often to address their concerns, whether scientific or non-scientific.

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Evaluations of the proposal should be conducted both internal and external to NASA and Centre National d'Etudes Spatiale (CNES) and the space research communities in the nations participating in the mission.

They talk about the need for an ethical review which needs to be made publici early in the process.

An ethical review should be conducted at least at the level of the Agencies participating and these reviews made public early in the process (in France, the national bioethics committee, Comité Consultatif National d'Ethique pour les Sciences de la Vie et de la Santé, CCNE, is the appropriate organization).

They talk about the need to announce the final protocol broadly to the scientific community and they say broad acceptance at both lay public and scientific levels is essential for success.

The final protocol should be announced broadly to the scientific community with a request for comments and input from scientific societies and other interested organizations.

Broad acceptance at both lay public and scientific levels is essential to the overall success of this research effort.

They highlight the issues that could arise later on if extraterrestrial life is discovered. Including legal ownership questions

In the long term, the discovery of extraterrestrial life, whether extant or extinct, in situ or within returned sample materials, will also have implications beyond science and the SRF per se. Such a discovery would likely trigger a review of sample return missions, and plans for both robotic and human missions. Legal questions could arise about ownership of the data, or of the entity itself, potentially compounded by differences in laws between the United States and the countries of international partners.

Ethical, legal and social issues should be considered seriously. I think here where it says "in any event" it means whether or not they find life?

In any event, ethical, legal and social issues should be considered seriously. Expertise in these areas should be reflected in the membership on appropriate oversight committee(s).

They say that a central question is whether any protocol can be guaranteed to be risk free [I argue in my paper that study above GEO in a telerobotic facility fulfils this condition as does sterilizing all samples] and ask what counts as an acceptable level of risk

Page 96: Central to an understanding of the arguments is the question of risk, i.e., Can any protocol be guaranteed to be absolutely risk-free? If not, what is an acceptable level of risk (for example, one that approximates the risk from the natural influx of martian materials into Earth's biosphere)?

And, is there any treatment method that can eliminate all risks from the returned samples, while preserving them for the detailed scientific study envisaged by the scientific community?

They also talk about the need to have a communication plan to address concerns and perceptions about the associated risks:

Page 101: **Communications** Unusual or unprecedented scientific activities are often subject to extreme scrutiny at both the scientific and political levels. Therefore, a communication plan must be developed as early as possible to ensure timely, and accurate dissemination of information to the public about the sample return mission, and to address concerns and perceptions about associated risks.

They talk about how the public and stakeholders need to be able to participate in an open, honest dialogue.

The communication plan should be pro-active and designed in a manner that allows the public and stakeholders to participate in an open, honest dialogue about all phases of the mission with NASA, policy makers, and international partners. Risk management and planetary protection information should be balanced with education/outreach from the scientific perspective about the anticipated benefits and uncertainties associated with Mars exploration and sample return.

They go on to talk about the process of informing the public of any discoveries. This must be decided well in advance

The communication plan should also address how the public and scientific community will be informed of results and findings during Life Detection and Biohazard testing, including the potential discovery of extraterrestrial life. Because of the intense interest likely during initial sample receipt, containment, and testing, procedures and criteria should be developed in advance for determining when and how observations or data may be designated as "results suitable for formal announcement."

Details about the release of SRF information, the management of the communication plan, and its relationship to the overall communications effort of the international Mars exploration program should be decided well in advance of the implementation of this protocol

They warn that potentially the sample return mission, and the facility, could also attract intentionally disruptive events, by bioterrorists, or by "radical" groups opposed to sample return (Rummel et al, 2002).

Page 93: Concerns about security should also be reconsidered, especially in view of the potential disruptive activities of any terrorists or 'radical' groups that may be opposed to sample return.

[NOTE] I can't find it now, I thought Rummel at al warned about the sharing of viral misinformation. Maybe it was someone else. Does anyone reading this know the cite? That clearly is a concern after what happened in the COVID pandemic whoever it was that said it. Perhaps this may need to be managed based on the emerging discipline of infodemiology (WHO, 2020wic).

Similarly the ESF recommends that since negative consequences from an unintended release could be borne by countries not involved in the program, a framework should be set up at the international level open to representatives of all countries, with mechanisms and fora dedicated to ethical and social issues of the risks and benefits from a sample return (Ammann et al, 2012:59).

RECOMMENDATION 3

Potential risks from an MSR are characterised by their complexity, uncertainty and ambiguity, as defined by the International Risk Governance Committee's risk governance framework. As a consequence, civil society, the key stakeholders, the scientific community and relevant agencies' staff should be involved in the process of risk governance as soon as possible.

In this context, transparent communication covering the accountability, the benefits, the risks and the uncertainties related to an MSR is crucial throughout the whole process. Tools to effectively interact with individual groups should be developed (e.g. a risk map).

RECOMMENDATION 4

Potential negative consequences resulting from an unintended release could be borne by a larger set of countries than those involved in the programme. It is recommended that mechanisms and fora dedicated to ethical and social issues of the risks and benefits raised by an MSR are set up at the international level and are open to representatives of all countries.

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This again would be best done before the start of the legal process to make sure everyone is on the same page before it starts.

. As Randolph put it (Randolph, 2009:292).

The risk of back contamination is not zero. There is always some risk. In this case, the problem of risk - even extremely low risk - is exacerbated because the consequences of back contamination could be quite severe. Without being overly dramatic, the consequences might well include the extinction of species and the destruction of whole ecosystems. Humans could also be threatened with death or a significant decrease in life prospects

In this situation, what is an ethically acceptable level of risk, even if it is quite low? This is not a technical question for scientists and engineers. Rather it is a moral question concerning accepting risk. Currently, the vast majority of the people exposed to this risk do not have a voice or vote in the decision to accept it. Most of the literature on back contamination is framed as a discourse amongst experts in planetary protection. Yet, as I've already argued, space exploration is inescapably a social endeavor done on behalf of the human race. Astronauts and all the supporting engineers and scientists work as representatives of all human persons.

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In this situation to treat persons with dignity and justice means that everyone should have the opportunity to voice their opinion concerning whether humans should accept the risk.

- 1. The best practices of planetary protection must be followed. ... Yet pursuing best practices by itself does not necessarily guarantee an ethically acceptable level of risk.
- 2. There should be opportunities for open comment by those individuals or groups that have concerns about the risks of back contamination. These comments should be taken seriously and NASA should publicly respond to these concerns.
- 3. A committee of neutral or disinterested persons should review the planetary protection measures for return of spacecraft and samples. This committee should include persons with a diversity of expertise, including ecology, biology, chemistry, specialists in risk analysis, and ethicists. The ethicists should represent a diversity of philosophical and religious perspectives.
- 4. The entire process of soliciting comment, analysing the risk factors and deciding on whether the risk levels are ethically acceptable should be transparent to the interested public.

NASA did set up a review board for sample return missions on August 14th 2020 (<u>NASA</u>, <u>2020nebmsr</u>). However, from the draft EIS and the responses to the public within it, it is clear that it can't have been set up to consider these wide ranging issues, or include experts in legal, ethical and social issues, as recommended by (<u>Rummel et al</u>, 2002) and (<u>Randolph</u>, 2009:292).

Indeed, from the content of the draft EIS and the reactions in comment replies, it seems unlikely that these issues have been considered at all in the process of developing the EIS.

Once the potential for large scale effects is recognised this leads to a legal process that is likely to extend by many more years with involvement of CDC, DOA, NOAA, OSHA etc., legislation of EU and members of ESA, international treaties, and international organizations like the World Health Organization – NASA don't seem to be prepared for this or even mention potential international ramifications

The EIS as it stands now essentially says that they are certain there is no life on Mars and that they are doing these precautions out of an "abundance of caution". If this is the final decision, other agencies in the USA as well as other countries and international organizations will likely

. . .

conclude that there is nothing here for the DOA, CDC. NOAA, OSHA, WHO, FOA, UNEP etc. to look at.

There is still the presidential directive NSC-25 requires a review of large scale effects which is done after the NEPA process is completed. (Race, 1996)

This directive says (Whitehouse, 1977):

"It should be understood that experiments which by their nature could be reasonably expected to result in domestic or foreign allegations that they might have major or protracted effects on the physical or biological environment or other areas of public or private interest, are to be included under this policy even though the sponsoring agency feels confident that such allegations would in fact prove to be unfounded.

So these other agencies may develop an interest as a result of that directive if the EIS isn't challenged and goes through.

There is potential for many delays in the legal process after the filling of the EIS (EPA, n.d.). First, since there is a potential for damage to Earth's environment, various executive orders mandate NASA itself, as a federal agency, to consider such matters as (NASA, 2012fdg):

- impact on the environment,
- impact on the oceans,
- impact on the great lakes,
- escape of invasive species,
- lab biosecurity against theft

After the environmental impact statement is filed, Uhran et al mention many other agencies likely to declare an interest such as the <u>(Uhran et al, 2019)</u> (Meltzer, 2012:454)

- CDC (for potential impact on human health),
- Department of Agriculture (for potential impact on livestock and crops),
- NOAA (for potential impact on oceans and fisheries after a splashdown in the sea)
- Occupational Safety and Health Administration, to consider questions of quarantine if a scientist or technician gets contaminated by a sample
- Department of Homeland Security,
- Federal Aviation Administration because the sample returns through the atmosphere
- Department of Transportation for bringing the sample to the receiving laboratory from where it touches down and to distribute to other laboratories
- Occupational Safety and Health Administration for any rules about quarantine for technicians working at the facility

- U.S. Customs and Border Protection and the Coast Guard to bring back sample in case of an water landing or the Department of Defense if it lands on land, likely the Utah Test & Training Ranges
- Department of the Interior which is the steward for public land and wild animals which could be affected by release of Martian microbes
- Fish and Wildlife Service for the DoI who maintain an invasive species containment program and may see back contamination as a possible source of invasive species
- National Oceanic and Atmospheric Administration (NOAA)'s fishery program for sea landing in case it could affect marine life and NOAA fisheries
- Integrated Consortium of Laboratory Networks (ICLN) for laboratories that respond to disasters a partnership of the Department of Agriculture, Department of Defense, Department of Energy, Department of Health and Human Services, Department of Homeland Security, Department of the Interior, Department of Justice, Department of State, and Environmental Protection Agency
- The state where the receiving laboratory is stationed may have regulations on invasive species, environmental impacts, disposal of waste, and possession of pathogens, similarly also for any states the sample may have to transit to from the landing site to the facility

As the process continues it is possible to stop the activity. It's the same process that is used for instance. to stop oil pipelines across tribal lands in the USA or almost any US environmental legal action.

The Congressional Research Service explains (<u>Congressional Research Service, 2021</u>) that NEPA doesn't provide for judicial review directly. But it's often a ground for litigation on the basis that the process hasn't been carried out properly.

For instance judicial review can be requested because

- the agency failed to consider some of the impacts
- the agency failed to properly consider the weight of the impacts under review

During the litigation the court can issue injunctions that

• bar all or part of a proposed action

The result of the court case is usually

- referred back to the agency (such as NASA) for further proceedings and the court can say what those are
- It can order equitable relief which vacates the action i.e. stops the project going ahead
- Or issue some other action.

The "ordinary" remedy is to just vacate the Federal action so it can't go ahead, but the courts consider the "seriousness" of the deficiencies in the EIS and the "disruptive consequences" of vacating the action (<u>Congressional Research Service, 2021)</u>.

So the courts can just stop the whole thing - or they could require some injunction on NASA. In this case, one example injunction might be that NASA have to sterilize all samples returned to Earth until proven to be safe, if they assess that NASA haven't taken account of all possible impacts or they haven't sufficiently considered the weight of the impacts.

Meanwhile, since this is a joint NASA / ESA mission, it involves ESA. Most of the ESA member states are in the EU (ESA, n.d.MS) so the EU will get involved.

This leads to a separate legal process in Europe, starting with the Directive 2001/42/EC <u>(EU, 2001)</u>. I haven't located any academic reviews for the European process, but as for the case in the USA, this would spin off other investigations which would involve the European Commission (Race, 1996).

The UK, as a member of ESA but not in the EU, might also be involved in a separate process with its domestic laws. Canada also sits on the governing council of ESA, so perhaps may get involved. These countries are all members of ESA and also all potentially impacted by an adverse outcome.

However it wouldn't stop at the USA and ESA. All other countries are potentially impacted in the worst case. These potential impacts on the environment of Earth, and on human health world-wide bring many international treaties into play (Uhran et al, 2019),

In an address given to the Space Studies Board Task Group on Issues in Sample Return in 1996, attorney George Robinson presented a list of 19 treaties or international conventions and 10 domestic categories of law, including the rights of individual states and municipalities to quarantine, that may affect return missions.

These lists include treaties governing the use of the air and sea, environmental protection treaties, the constitution of the World Health Organization (WHO), and treaties related to outer space as well as the Administrative Procedure Act (Robinson, 1996).

[Need to find out more details here]

Also several international organizations are likely to be involved such as the WHO (Uhran et al. 2019).

We will see below that the very worst case scenarios involve degradation of Earth's environment (such as by mirror life).

It seems unlikely that these worst case scenarios would be ignored as the legal proceedings continue. If the legal discussions expand to focus on these scenarios, this could involve many other organizations.

The Food and Agriculture Organization (UN, 1945) could become involved, especially if the potential for alien exobiology such as mirror life is considered, because of potential impact on agriculture and fisheries and global food supplies, and the World Health Organization because of effects on human health globally if a new organism is returned that can be spread to other countries.

In the USA, the Environmental Protection Agency partners with the United Nations Environment Program (UNEP), and Arctic Council, so they'd likely get involved (<u>EPA, n.d.pwio</u>).

Indeed, there would be few aspects of human life that would not be relevant in some way in discussions of the very worst case scenarios. As the legal process continues, surely there would be open public debate about these scenarios, and if the discussion expands in this way, potentially it might lead to much wider involvement in the international community. It would be necessary to convince the public, and interested experts in all these agencies that this is a safe mission and that all their concerns have been answered.

Race <u>(Race, 1996)</u> says that experts will have challenges deciding in advance whether the sample should be classified as potentially:

- an infectious agent
- an exotic species outside its normal range
- a truly novel organism (as for genetic engineering)
- a hazardous material

The choices here would change which laws and agencies would be involved.

Presidential directive NSC-25 requires a review of large scale effects which is done after the NEPA process is completed. (Race, 1996)

There are numerous treaties conventions and international agreements relating to environmental protection or health that could apply.

Including those to do with (Race, 1996)

- protection of living resources of the sea
- air pollution (long range pollution that crosses country boundaries)
- world health, etc

Individual groups in other countries could invoke domestic laws such as laws on accidents at sea or on land if they argue back contamination of Earth can cause measurable damage. (Race, 1996)

Race says scientists are likely to focus on (Race, 1996)

- 12. technical details
- 13. mission requirements

- 14. engineering details
- 15. costs of the space operations and hardware

General public are likely to focus on

- risks and accidents
- whether NASA and other institutions can be trusted to do the mission
- worst case scenarios
- whether the methods of handing the sample, quarantine and containment of any Martian life are adequate

The legal process and public debate for NASA's mission as precedent for China's mission to return a sample too – perhaps as soon as 2030 – with sterilization a likely solution for a country that wants to be first to return a sample

China currently plans to launch a mission possibly as soon as 2028, to return a sample by 2030. It would consist of two rockets, one with a lander and ascent vehicle, and the other with an orbiter and reentry capsule to return the sample to Earth, using two Long March rockets (Jones, 2021)

China had one of the most rigorous of all responses to the COVID pandemic. Professor Bruce Aylward, leader of the joint team that studied their response (<u>McNeil, 2020</u>) put it like this in the press briefing about their findings (<u>United Nations, 2020</u>)

They [the Chinese] approached a brand new virus [that] has never been seen before that was escalating and quite frightening in January ... and they have taken very basic public health tools ... and applied these with a rigor and an innovation of approach on a scale that we've never seen in history

If China considers the Mars sample return to be potentially hazardous it is likely to be especially careful just as it has been especially careful with COVID.

The debate that is sure to happen with the NASA mission will help bring widespread awareness of the issues of a sample return and the need to be careful.

Suplementary data

Could Martian life have got to Earth on meteorites? Our Martian meteorites come from at least 3 m below the surface in high altitude regions of Mars

If Earth frequently encounters Martian life, then we have no need to protect Earth with special precautions, by Greenberg's "Natural Contamination Standard" (Greenberg et al, 2001).

However, our Martian meteorites all come from at least 3 meters below the surface (Head et al, <u>2002:1355</u>), and left Mars over a period spanning 20 million years. They were probably thrown up into space after glancing collisions into the Elysium or Tharsis regions, high altitude southern uplands (Tornabene et al, 2006). The atmosphere for these high altitude regions on Mars is thin, making ejection to Earth easier. The subsurface below about 12 cms has a uniform temperature of around 200°K or -73°C (Möhlmann, 2005:figure 2). With such a thin atmosphere, present day life at those altitudes is unlikely (except perhaps for deep subsurface geothermal hot spots).

So it seems unlikely that any life has got to Earth in the last few million years. The Martian meteorites we have are from one of the least likely to be habitable regions on Mars, the subsurface of the high altitude Martian uplands.

It is not totally impossible life could get into the Martian meteorites, but would require a high measure of luck. Some Martian volcanoes have been active in the geologically recent past, as recent as 2 million years ago. Olympus Mons also shows signs of glacial activity as recent as four million years ago which suggests it likely has ice protected beneath the dust on its slopes. . (Neukam et al., 2004)

A lucky asteroid impact on Mars could throw up material from a subsurface cave, or a geothermal hot spot, or fumarole. But such events would surely be rare.

So, it's possible that some exceptionally hardy life has got here, even in geologically recent times. Perhaps life from geothermal vents after a lucky strike of a meteorite into a geologically active geothermal system on the flanks of Olympus Mons.

It's not impossible that a lucky asteroid impact could send back life from Mars from a cave or a geothermal vent just below the surface, but most wouldn't send any life this way.

Just as there are many species on Earth that could never get to Mars on a meteorite, if Mars has a diversity of microbial species, there are likely to be many species on Mars that could never get to Earth that way.

Larger impacts could send material to Earth - but unlikely to transfer fragile surface dirt, ice and salts

Larger impacts in the recent geological past could send material to Earth from other potentially more habitable parts of Mars. However:

- Many proposed habitats are in surface layers of dirt, ice and salts. These would likely never get into space
- Other proposed habitats are millimeters below the surface of rocks. These layers would ablate away during entry into the Earth's atmosphere
- Life on Mars could be extremely localized to only a few square kilometers over the entire planet, for instance, only to the RSL's, or only above geological hot spots, making it less likely that the habitats are hit by an asteroid able to send material all the way to Earth in the large chunks needed for protection from cosmic radiation during the transfer.

It was easier for Mars to exchange life with Earth in the early solar system. However even the ejecta from an impact into a Martian ocean need not necessarily transmit life to Earth.

The first challenge is the shock of ejection. Microbes are suddenly accelerated from rest to escape velocity in a fraction of a second. The microbes can be destroyed by cell rupture or by DNA damage. All cells of Chroococcidiopsis are killed at 10 GPa (Nicholson, 2009)

ALH84001 experienced a shock of ejection of \sim 35 – 40 GPa. The Nahkalites were least shocked at 15 to 25 GPa. This is still too much for Chroococcidiopsis (Nyquist, 2001)

Some deep subsurface layers are sent to orbit with much less shock especially for the larger impacts. These low levels of shock arises from interaction between the shock wave moving away from the forming crater and a reflected shock wave moving backwards. The shock moving back is 180 degrees out of phase so the two shock waves cancel, creating a lightly shocked "spall" zone where the two interact. The spall zone depth is proportional to the radius of the impactor, so a large impactor would have a thicker spall zone. Some of the ejecta would survive shock of less than 1 GPa (Mileikowsky, 2000: 393)

For the Mars meteorites, from modelling, about 2% of the ejecta is lightly shocked in this way. (Nyquist, 2001:147).

More shock resistant microbes can survive better. Of the order of 1 in 10,000 of microbes of b. subtilis and the photobiont and microbiont partners in the lichen X Elegans could survive 40 to 50 GPa (Nicholson, 2009). In one paper, samples of a marine photosynthetic algae

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nannochloropsis oculata frozen in ice were able to survive 6.93 km / sec impacts into water with approximate shock pressure of 40 GPa (Pasini, 2014).

The Martian life then has to survive the fireball of exit from the Martian atmosphere. The lower gravity reduces the Martian escape velocity from 11.19 to 5.03 km / sec (NASA, n.d.mfs), but the Martian atmosphere has to have nearly three times the mass of the Earth's atmosphere for the same surface pressure, and the Martian atmosphere was likely several bars for early Mars (Mileikowsky et al, 2000: 423).

It then has to survive the cold and vacuum conditions of space and cosmic radiation. Cosmic radiation sterilizes the surface of a meteorite to a depth of 2 cm within 100,000 years by breaking up the nucleic acids . That's below the maximum depth you'd expect to find photosynthetic life in normal circumstances, even in fine cracks.

It is theoretically possible for some rocks to get to Earth as soon as ten years after ejection from Mars. But most take between a hundred thousand and ten million years to get there. Assuming a maximum ejection velocity of 6 km / sec, in a simulation with 2100 particles, incorporating the gravitational effects of all the planets from Venus through to Neptune, most took over 100,000 years in transit. The fastest transfer in the simulation was 16,000 years (Gladman et al, 1996).

It also has to survive the fireball of re-entry to Earth, Cockell inculcated an artificial gneiss rock with Chrooccoccidiopsis at a depth where it occurs naturally, and affixed it to the re-entry shield of a Soyuz rocket. None survived re-entry, nor did any organics. He concluded that it might not be impossible for photosynthetic life to get to Earth from Mars, but it would need an extraordinary combination of events (Cockell, 2008)

Some terrestrial extremophiles might survive these processes but the fireball of re-entry would sterilize most of them.

The interior of a rock can be better protected. The interior of ALH84001 never got hotter than 40°C during entry into our atmosphere (Weiss et al, 2000). But how does the photosynthetic life get deep into a Martian rock? It can flourish in cracks, if light filters in through them - but that also would give cracks that channel hot gases into the interior of the rock during re-entry. Cracks like that would also be places where the rocks are quite likely to break apart during ejection from Mars or re-entry to Earth.

Charles Cockell's concludes that it might not be impossible for photosynthetic life to get to Earth from Mars, but it would need a rather extraordinary combination of events (Cockell, 2008):

"Thus, the planetary exchange of photosynthesis might not be impossible, but quite specific physical situations and/or evolutionary innovations are required to create conditions where a photosynthetic organism happens to be buried deep within a rock during ejection to survive atmospheric transit."

His final conclusion is that photosynthetic life has the potential to make dramatic changes to a planet, but that this transfer of photosynthetic life is less likely than for heterotrophs (which use

organic carbon) or chemotrophs (which use chemical reactions as a source of energy and synthesize all their organics from carbon dioxide, living in places such as hydrothermal vents).

In addition, panspermia experiments are based on capabilities of terrestrial life. Capabilities of any native Martian life are unknown. Many Earth microbes could not survive this journey.

It's not impossible that Martian life made the transition. However, even if there has been some transfer of life from Mars to Earth, there are likely to be many species of Martian life that don't have the capability to get to Earth in this way, as for their Earth counterparts, either because they live in fragile habitats like dust and salts that can't be transferred via meteorites, or because they don't have the extremophile adaptations needed to be able to survive the transfer.

So we can't apply the Greenberg "Natural Contamination Standard" (Greenberg et. al, 2001) for microbial life from Mars. It's possible that a sample return could return microbes that wouldn't be able to get to Earth on meteorite impacts.

References

7th Circuit, 1997, Simmons v. U.S. Army Corps of Engineers, 120 F.3d 664

Abdo, J.M., Sopko, N.A. and Milner, S.M., 2020. <u>The applied anatomy of human skin: a model</u> for regeneration. Wound Medicine, 28, p.100179.

Approximately every 28 days, fully differentiated cuboidal basal keratinocytes with large nuclei, abundant organelles, and a phospholipid membrane migrate apically from the basal layer through the spinous and granular layers [4]. During this turnover process, an accumulation of keratin and lipids ensues which then undergoes terminal differentiation to form the stratum corneum

Skin is an active immunological organ, and dysfunctional innate defenses have serious clinical implications. Products of the stratum corneum, including free fatty acids, polar lipids, and glycosphingolipids accumulate in the intercellular spaces and horny layer, exhibiting antimicrobial properties, and functioning as a first line of defense. Antimicrobial peptides (AMPs) exhibit potent and targeted resistance against a wide spectrum of common pathogens. When this barrier is breached, second lines of protection are provided by inflammatory cascades in the subepithelial tissue. Approximately sixteen AMPs have been shown to be expressed in the skin (Table 1) Afzal, I., Shinwari, Z.K., Sikandar, S. and Shahzad, S., 2019. <u>Plant beneficial endophytic</u> <u>bacteria: Mechanisms, diversity, host range and genetic determinants</u>. Microbiological research, 221, pp.36-49

Ammann, W., Barros, J., Bennett, A., Bridges, J., Fragola, J., Kerrest, A., Marshall-Bowman, K., Raoul, H., Rettberg, P., Rummel, J. and Salminen, M., 2012. <u>Mars Sample Return backward</u> <u>contamination–Strategic advice and requirements</u> - Report from the ESF-ESSC Study Group on MSR Planetary Protection Requirements.

ATSB (Australian Transport Safety Beaureau), n.d., Black box flight recorders fact sheet

Avila-Herrera, A., Thissen, J., Urbaniak, C., Be, N.A., Smith, D.J., Karouia, F., Mehta, S., Venkateswaran, K. and Jaing, C., 2020. <u>Crewmember microbiome may influence microbial</u> <u>composition of ISS habitable surfaces</u>. PloS one, 15(4), p.e0231838.

Bahl, J., Lau, M.C., Smith, G.J., Vijaykrishna, D., Cary, S.C., Lacap, D.C., Lee, C.K., Papke,
R.T., Warren-Rhodes, K.A., Wong, F.K. and McKay, C.P., 2011. <u>Ancient origins determine</u> <u>global biogeography of hot and cold desert cyanobacteria</u>. Nature communications, 2(1), pp.1-6.
Batbander, K., 2020, <u>A Barn Swallow in Flight</u>, Wikimedia Commons

Benison, K.C. and Karmanocky III, F.J., 2014. <u>Could microorganisms be preserved in Mars</u> <u>gypsum?</u> Insights from terrestrial examples. Geology, 42(7), pp.615-618.

Some clusters of dozens of diatoms appear pristine, suggesting that they had been living in the salar pool immediately before being trapped as the gypsum crystal grew.

Could microfossils and/or viable microorganisms be trapped in gypsum on Mars as they are in gypsum on Earth? It is likely that abundant sulfate sand grains on Mars contain fluid inclusions similar to those in the acid-precipitated bottom-growth and reworked gypsum we discuss here.

We suggest that gypsum on Mars would have entrapped, as solid inclusions and within fluid inclusions, any microorganisms and/or organic compounds that were present in its parent waters. Therefore, fluid inclusions and solid inclusions hosted by salt minerals may be the best place to continue the search for life on Mars.

Some of these entrapped microorganisms remain viable for at least tens of thousands of years ... and possibly for hundreds of millions of years

Benner, S. and Davies, P., 2010, <u>'Towards a Theory of Life'</u>, in Impey, C., Lunine, J. and Funes, J. eds., *Frontiers of astrobiology*. Cambridge University Press.

Bilen, M., Dufour, J.C., Lagier, J.C., Cadoret, F., Daoud, Z., Dubourg, G. and Raoult, D., 2018. <u>The contribution of culturomics to the repertoire of isolated human bacterial and archaeal</u> <u>species</u>. *Microbiome*, *6*(1), pp.1-11.

Blackmond, D.G., 2019. <u>The origin of biological homochirality</u>. *Cold Spring Harbor perspectives in biology*, *11*(3), p.a032540.

Bianciardi, G., Miller, J.D., Straat, P.A. and Levin, G.V., 2012. <u>Complexity analysis of the Viking</u> <u>labelled release experiments</u>. International Journal of Aeronautical and Space Sciences, 13(1), pp.14-26.

Board, S.S. and National Research Council, 1999. *Size limits of very small microorganisms: proceedings of a workshop*. National Academies Press.

Board, S.S. and National Research Council, 2009. *Assessment of planetary protection requirements for Mars sample return missions*. National Academies Press. <u>page 48</u>

5, Potential for Large Scale Effects

"Despite suggestions to the contrary, it is simply not possible, on the basis of current knowledge, to determine whether viable Martian life forms have already been delivered to Earth. Certainly in the modern era, there is no evidence for large-scale or other negative effects that are attributable to the frequent deliveries to Earth of essentially unaltered Martian rocks. However the possibility that such effects occurred in the distant past cannot be discounted."

Board, S.S., European Space Sciences Committee and National Academies of Sciences, Engineering, and Medicine, 2015. <u>Review of the MEPAG report on Mars special regions</u>. National Academies Press. <u>10</u>: "*SR-SAG2 Finding 3-1:* Cell division by Earth microbes has not been reported below – 18°C (255K). **"Revised Finding 3-1:** Cell division by Earth microbes has not been reported below – 18°C (255K). The very low rate of metabolic reactions at low temperature result in doubling times ranging from several months to year(s). Current experiments have not been conducted on sufficiently long timescales to study extremely slow-growing microorganisms."

Board, S.S., 2019. <u>Planetary protection classification of sample return missions from the Martian</u> <u>moons</u>, European Space Sciences Committee and National Academies of Sciences, Engineering, and Medicine

Boeder, P.A. and Soares, C.E., 2020, August. <u>Mars 2020: mission, science objectives and build.</u> <u>In Systems Contamination: Prediction, Control, and Performance 2020</u> (Vol. 11489, p. 1148903). International Society for Optics and Photonics

Bohannon, J., 2010. <u>Mirror-image cells could transform science-or kill us all</u>. Wired, Accessed at: https://www.wired.com/2010/11/ff_mirrorlife/

Kasting: "After doing some rough calculations on the effects of a mirror cyanobacteria invasion, Jim Kasting isn't sure which would kill us first—the global famine or the ice age. "It would quickly consume all the available nutrients," he says. "This would leave fewer or perhaps no nutrients for normal organisms." That would wipe out the global ocean ecology and starve a significant portion of the human population. As the CO₂ in the ocean was incorporated into inedible mirror cells, they would "draw down" CO₂ from the atmosphere, Kasting says. For a decade or two, you would have a cure for global warming. But Kasting predicts that in about 300 years the bugs would suck down half of Earth's atmospheric CO₂. Photosynthesis of most land plants would fail. "All agricultural crops other than corn and sugar cane would die," he says. (They do photosynthesis a little differently.) "People might be able to subsist for a few hundred years, but things would be getting pretty grim much more quickly than that." After 600 years, we'd be in the midst of a global ice age. It would be a total evolutionary reboot—both Kasting and Church think mirror predators would evolve, but whatever life existed on Earth by that point wouldn't include us..

Borges, W.D.S., Borges, K.B., Bonato, P.S., Said, S. and Pupo, M.T., 2009. <u>Endophytic fungi:</u> <u>natural products, enzymes and biotransformation reactions</u>. Current Organic Chemistry, 13(12), pp.1137-1163.

Borojeni, I.A., Gajewski, G. and Riahi, R.A., 2022. <u>Application of Electrospun Nonwoven Fibers</u> <u>in Air Filters</u>. Fibers, 10(2), p.15. Boshuizen, H.C., Neppelenbroek, S.E., van Vliet, H., Schellekens, J.F., Boer, J.W.D., Peeters, M.F. and Conyn-van Spaendonck, M.A., 2001. <u>Subclinical Legionella infection in workers near</u> the source of a large outbreak of Legionnaires disease. *The Journal of infectious diseases*, *184*(4), pp.515-518.

Brazil, R., 2015, The origin of homochirality, Chemistry World.

Bristow, L.A., Mohr, W., Ahmerkamp, S. and Kuypers, M.M., 2017. <u>Nutrients that limit growth in</u> the ocean. *Current Biology*, *27*(11), pp.R474-R478.

BS, 2009, BS EN 1822-1:2009 <u>High efficiency air filters (EPA, HEPA and ULPA), Part 1:</u> <u>Classification, performance testing, marking</u>

Byrd, A.L., Belkaid, Y. and Segre, J.A., 2018. <u>The human skin microbiome</u>. *Nature Reviews Microbiology*, *16*(3), p.143. 5).

Cabrol, N.A., McKay, C.P., Grin, E.A., Kiss, K.T., Ács, É., Tóth, B., Grigorszky, I., Szabó-Taylor, K., Fike, D.A., Hock, A.N. and Demergasso, C., 2009. <u>Signatures of habitats and life in</u> <u>Earth's high-altitude lakes: clues to Noachian aqueous environments on Mars</u>.

Diatoms-like organisms are unlikely to have ever evolved on Mars as they appeared late in Earth's biological history. However, by their rate of mutation, adaptation, and or extinction, they, and other microorganisms, can provide important clues for the search for life on Mars. Both habitat and life are can leave characteristic geo- and biosignatures that we might learn to recognize on distant Martian shores

Cabrol, N.A., 2021. Tracing a modern biosphere on Mars. Nature Astronomy, 5(3), pp.210-212.

Carrier, B.L., Bass, D., Gaubert, F., Grady, M.M., Haltigin, T., Harrington, A.D., Liu, Y., Martin, D., Marty, B., Mattingly, R. and Siljeström, S., 2019. <u>Science-Driven Contamination Control</u> <u>Issues Associated with the Receiving and Initial Processing of the MSR Samples</u> Carrier, B.L., Beaty, D.W., Meyer, M.A., Blank, J.G., Chou, L., DasSarma, S., Des Marais, D.J., Eigenbrode, J.L., Grefenstette, N., Lanza, N.L. and Schuerger, A.C., 2020. <u>Mars Extant Life:</u> <u>What's Next? Conference Report.</u> (<u>html</u>)

802: Future missions would therefore benefit from the development of instruments capable of direct and unambiguous detection of extant life in situ, and improvements are needed in capabilities for sample preparation to optimize biosignature detection. Spacecraft resources should support a sufficient number of sample analyses to support replicate analyses, positive and negative controls. Contamination control should be coupled with contamination knowledge so that Earth-sourced material can be eliminated as a possible source of any biological material discovered in Martian samples.

Cheruy, F., Dufresne, J.L., Aït Mesbah, S., Grandpeix, J.Y. and Wang, F., 2017. <u>Role of soil</u> <u>thermal inertia in surface temperature and soil moisture-temperature feedback</u>. *Journal of Advances in Modeling Earth Systems*, 9(8), pp.2906-2919.

Church, F.S., n.d. <u>Opened up a Pandora's box</u>

Clark, B., 2009, Cultybraggan nuclear bunker

Cleland, C.E., 2019. <u>The Quest for a Universal Theory of Life: Searching for Life as we don't</u> <u>know it (</u>Vol. 11). Cambridge University Press.

Cockell, C.S., 2008. <u>The Interplanetary Exchange of Photosynthesis</u>. Origins of Life and Evolution of Biospheres, 38(1), pp.87-104.

Cockell, C.S., 2014. Trajectories of Martian habitability. Astrobiology, 14(2), pp.182-203.

Congressional Research Service, 2021, <u>National Environmental Policy Act: Judicial Review and</u> <u>Remedies</u> Conley, C (2016), interviewed by Straus, M., for National Geographic, <u>Going to Mars Could</u> <u>Mess Up the Hunt for Alien Life</u>. Available at: <u>https://www.nationalgeographic.com/news/2016/09/mars-journey-nasa-alien-life-protection-humans-planets-space/</u> (accessed 1 July 2020)

From the perspective of planetary protection, Conley is also concerned about terrestrial organisms that can absorb water from the air. She recalls fieldwork she did in the Atacama Desert in Chile, which is one of the driest places on Earth, with less than 0.04 inch of rain a year.

Even in this dessicated place, she found life: photosynthetic bacteria that had made a home in tiny chambers within halite salt crystals. There's a small amount of water retained inside the halite and, at night, it cools down and condenses both on the walls of the chambers and on the surface of the organisms that are sitting there.

Council on Environmental Quality, n.d. NEPA Modernization

Crisler, J.D.; Newville, T.M.; Chen, F.; Clark, B.C.; Schneegurt, M.A., 2012. <u>"Bacterial Growth at the High Concentrations of Magnesium Sulfate Found in Martian Soils"</u>. Astrobiology. **12** (2): 98–106

Daderot, 2017, <u>Oregon Space Ball, probably from the equipment module of Gemini 3, 4, or 5</u> <u>mission, titanium</u> - Oregon Air and Space Museum - Eugene, Oregon

Davies, P.C., Benner, S.A., Cleland, C.E., Lineweaver, C.H., McKay, C.P. and Wolfe-Simon, F., 2009. <u>Signatures of a shadow biosphere</u>. Astrobiology, 9(2), pp.241-249.

Davies, P., 2014, The key to life on Mars may well be found in Chile, The Guardian

Davila, A.F., Skidmore, M., Fairén, A.G., Cockell, C. and Schulze-Makuch, D., 2010. <u>New</u> priorities in the robotic exploration of Mars: the case for in situ search for extant life. *Astrobiology*, *10*(7), pp.705-710.

Davis, J., 2018, Liquid water found beneath the surface of Mars, Natural History Museum,

The conditions in the lake are expected to be extreme. To stay liquid beneath more than a kilometre of ice, it is likely that the water is hypersaline (very salty). Usually this would make it difficult for life to exist - but it's not beyond the realms of possibility.

<u>Dr David Williams</u>, a Researcher of Diatoms at the Museum, says 'Yes, technically tiny life forms such as diatoms and cyanobacteria could survive in these environments. But that is not the question we should be asking.

'A more interesting question is whether we would know what we're looking at, even if we did find something in the lake. Would we even be able to identify it as life, if it originated on Mars itself?'

Deighton B., 2016, Life could exist on Mars today, bacteria tests show, Horizon, EU research and Innovation Magazine

Deo, P.N. and Deshmukh, R., 2019. <u>Oral microbiome</u>: Unveiling the fundamentals. *Journal of oral and maxillofacial pathology: JOMFP*, 23(1), p.122.

de Vera, J.P., Schulze-Makuch, D., Khan, A., Lorek, A., Koncz, A., Möhlmann, D. and Spohn, T., 2014. <u>Adaptation of an Antarctic lichen to Martian niche conditions can occur within 34 days</u>. *Planetary and Space Science*, *98*, pp.182-190. See also summary Koh Xuan Yang, 2014, <u>Adaptation of Antarctic Lichens to Conditions on Mars</u>, Beyond Earthly Skies

Dinan, F.J. and Yee, G.T., 2007. <u>An adventure in stereochemistry: Alice in mirror image land</u>. New York: National Center for Case Study Teaching in Science, University at Buffalo, State University of New York.

EMW, ISO 29463 - <u>New test standard for HEPA Filters</u> At: <u>https://www.emw.de/en/filter-</u> <u>campus/iso29463.html</u> Accessed on 7 July 2020

In 1998 <u>EN 1822</u> came into effect. This was the first standard, which established a filter classification system for <u>HEPA filters</u> based on filtration process theory. EN 1822 also introduced the evaluation criterion MPPS (Most Penetrating Particle Size). MPPS is the particle size at which the air filter has its lowest arrestance. Not just a whim of nature, MPPS relates directly to physical mechanisms in the <u>filtration process</u>.

The U.S. takes a different approach for filter classification of HEPA filters. The mother of all test procedures for these filters in the U.S. is MIL-STD-282, which was introduced in 1956. Other test procedures include e.g. IEST-RP-CC001 and IEST-RP-CC007. Each test procedure specifies certain particle sizes at which efficiency is evaluated. Depending on the filter class evaluated, this is done at 0.3 μ m, 0.1 - 0.2 μ m or 0.2 - 0.3 μ m.

Eninger, R.M., Honda, T., Reponen, T., McKay, R. and Grinshpun, S.A., 2008. What does respirator certification tell us about filtration of ultrafine particles?. Journal of occupational and environmental hygiene, 5(5), pp.286-295.

EPA. 2017, <u>Assessment of the Decontamination of Soil Contaminated with Bacillus anthracis</u> <u>Spores Using Chlorine Dioxide Gas, Methyl Bromide, or Activated Sodium Persulfate</u>. EPA/600/R-17/343. U.S. Environmental Protection Agency.

EPA, n.d., What is the National Environmental Policy Act?

ESA, n.d.MS, ESA member states

EPA, n.d.pwio., Partnering with International Organizations.

EU, 2001, Directive 2001/42/EC of the European Parliament and of the Council of 27 June 2001 on the assessment of the effects of certain plans and programmes on the environment

Fajardo-Cavazos, Patricia, Lindsey Link, H. Jay Melosh, and Wayne L. Nicholson, 2005,. "Bacillus subtilis spores on artificial meteorites survive hypervelocity atmospheric entry: implications for lithopanspermia." *Astrobiology* 5, no. ; 726-736.

Fajardo-Cavazos, P., Morrison, M.D., Miller, K.M., Schuerger, A.C. and Nicholson, W.L., 2018. <u>Transcriptomic responses of Serratia liquefaciens cells grown under simulated Martian</u> <u>conditions of low temperature, low pressure, and CO 2-enriched anoxic atmosphere</u>. Scientific reports, 8(1), pp.1-10.

Federal Transit Administration, n.d., <u>Record of Decision</u>.

Fischer, E., Martinez, G., Elliott, H.M., Borlina, C. and Renno, N.O., 2013, December. <u>The</u> <u>Michigan Mars Environmental Chamber: Preliminary Results and Capabilities</u>. In AGU Fall Meeting Abstracts (Vol. 2013, pp. P41C-1928).

.Fischer, E., Martínez, G.M., Elliott, H.M. and Rennó, N.O., 2014. <u>Experimental evidence for the</u> <u>formation of liquid saline water on Mars</u>. *Geophysical research letters*, *41*(13), pp.4456-4462.

Flemming, H.C., Neu, T.R. and Wozniak, D.J., 2007. <u>The EPS matrix: the "house of biofilm cells</u>". Journal of bacteriology, 189(22), pp.7945-7947.

Frantseva, K., Mueller, M., ten Kate, I.L., van der Tak, F.F. and Greenstreet, S., 2018. <u>Delivery</u> of organics to Mars through asteroid and comet impacts. Icarus, 309, pp.125-133

Goetz, W., Brinckerhoff, W.B., Arevalo, R., Freissinet, C., Getty, S., Glavin, D.P., Siljeström, S., Buch, A., Stalport, F., Grubisic, A. and Li, X., 2016. <u>MOMA: the challenge to search for organics</u> and biosignatures on Mars. International Journal of Astrobiology, 15(3), pp.239-250

Goodsell, D., 2000 PDB101: Molecule of the Month: Ribosomal Subunits

Greenberg, R. and Tufts, B.R., 2001. <u>Macroscope: Infecting Other Worlds</u>. *American Scientist*, *89*(4), pp.296-299

Grant, J.A., Golombek, M.P., Wilson, S.A., Farley, K.A., Williford, K.H. and Chen, A., 2018. <u>The</u> <u>science process for selecting the landing site for the 2020 Mars rover</u>. *Planetary and Space Science*, *164*, pp.106-126.

Gronstall, A., 2014, Liquid Water from Ice and Salt on Mars, NASA astrobiology magazine.

Grove, G.L. and Kligman, A.M., 1983. <u>Age-associated changes in human epidermal cell</u> <u>renewal</u>. Journal of Gerontology, 38(2), pp.137-142.

Ghuneim, L.A.J., Jones, D.L., Golyshin, P.N. and Golyshina, O.V., 2018. <u>Nano-sized and</u> <u>filterable bacteria and archaea: biodiversity and function</u>. Frontiers in microbiology, 9, p.1971. See section: **Selective Pressures for Small Size**

Head, J.N., Melosh, H.J. and Ivanov, B.A., 2002. <u>Martian meteorite launch: High-speed ejecta</u> <u>from small craters</u>. *Science*, *298*(5599), pp.1752-1756.

Page 1355: Nishiizumi et al. (1986) found that all cosmogenic nuclide data indicate that the shergottites were ejected from>3 m depth. This conclusion was supported by Reedy (1989) stating that the Shergottite-Nakhdite-Chassignite group meteorites (SNCs), especially the shergottites, must have been buried >5 m in any previous parent object (corresponding to a shielding depth of >1500 glcm²)

Hendrickson, R., Urbaniak, C., Minich, J.J., Aronson, H.S., Martino, C., Stepanauskas, R., Knight, R. and Venkateswaran, K., 2021. <u>Clean room microbiome complexity impacts planetary</u> protection bioburden. *Microbiome*, *9*(1), pp.1-17.

Hoarfrost, A., Aptekmann, A., Farfañuk, G. and Bromberg, Y., 2022. <u>Deep learning of a bacterial and archaeal universal language of life enables transfer learning and illuminates microbial dark matter</u>. *Nature communications*, *13*(1), pp.1-12.

Hoff, B., Thomson, G. and Graham, K., 2007. <u>Ontario: Neurotoxic cyanobacterium (blue-green alga) toxicosis in Ontario</u>. *The Canadian Veterinary Journal*, *48*(2), p.147.

Hogle, J.M., 2002. <u>Poliovirus cell entry: common structural themes in viral cell entry pathways</u>. Annual Reviews in Microbiology, 56(1), pp.677-702.

Horne, W.H., Volpe, R.P., Korza, G., DePratti, S., Conze, I.H., Shuryak, I., Grebenc, T., Matrosova, V.Y., Gaidamakova, E.K., Tkavc, R. and Sharma, A., 2022. <u>Effects of Desiccation</u> <u>and Freezing on Microbial Ionizing Radiation Survivability: Considerations for Mars Sample</u> <u>Return.</u> *Astrobiology*.

Hospodsky D, Yamamoto N, Nazaroff W, Miller D, Gorthala S, Peccia J. <u>Characterizing airborne</u> <u>fungal and bacterial concentrations and emission rates in six occupied children's classrooms</u>. Indoor air. 2015;25(6):641–52.

Hsu, J., 2009, Keeping Mars Contained, NASA Astrobiology Magazine.

Jakosky, B., Amato, M., Atreya, S., Des Marais, D., Mahaffy, P., Mumma, M., Tolbert, M., Toon, B., Webster, C. and Zurek, R., 2021. <u>Scientific value of returning an atmospheric sample from</u> <u>Mars</u>. Bulletin of the AAS, 53(4).

In the implementation involving gas compression, existing technology could be utilized. For example, MOXIE on Mars 2020 uses an Air Squared compressor (2.3 kg, 100 W) designed for large gas amount, flow rates; a miniature scroll pump by Creare (350 g, 5W) developed for Mars under SBIR. The compressor could be mounted on the lander and not be a part of sample-canister mass that is returned to Earth; for example, it could utilize a solenoid release/separation mechanism, with Schrader-like input valve in series with microvalve seal. Airborne dust also could be collected with addition of 3 valves and a dust filter (Figure 6). After gas reservoir is filled and reservoir valves closed, large volumes of Mars air would be pumped through filter to collect and trap dust and its valves closed.

With consideration of upcoming Mars-targeted missions, we conclude that gas collected in a newly designed and purpose-built valved sample-tube sized vessel, which could be flown on either SFR or SRL, would be considered of higher priority than either the head space gas or a sealed M2020 sample tube. Conceptually, this vessel would require no more physical space to return than a sealed empty sample tube and alleviate concerns about the manufacturing and history of a non-purpose-built vessel, and the valving would provide a more robust mechanism for sealing the vessel and testing the seal upon return.

Johnstone, J., 2017, Starling, Wikimedia Commons

Jones, A., 2021, China is planning a complex Mars sample return mission, SpaceNews

Joyce, G.F., 2007. <u>A glimpse of biology's first enzyme</u>. Science, 315(5818), pp.1507-1508.

Joyce, G.F. and Szostak, J.W., 2018. <u>Protocells and RNA self-replication</u>. *Cold Spring Harbor Perspectives in Biology*, *10*(9), p.a034801.

JPL, 2016, <u>NASA Weighs Use of Rover to Image Potential Mars Water Sites</u>, available at: <u>https://www.jpl.nasa.gov/news/news.php?feature=6542</u>, accessed on: July 18, 2020

KEGG, n.d., <u>Metabolic pathways - Chroococcidiopsis thermalis</u>, Kyoto Encyclopedia of Genes and Genomes

Kim, H.J., Kim, H.N., Raza, H.S., Park, H.B. and Cho, S.O., 2016. <u>An intraoral miniature X-ray</u> tube based on carbon nanotubes for dental radiography. *Nuclear Engineering and Technology*, *48*(3), pp.799-804.,

The tube voltage is 50 kV and the electron beam current is 200 μ A in the calculation

Kleidon, A., 2002. <u>Testing the effect of life on Earth's functioning: how Gaian is the Earth system?</u>. *Climatic Change*, *52*(4), pp.383-389.

Klusman, R.W., Luo, Y., Chen, P., Yung, Y.L. and Tallapragada, S., 2022. <u>Seasonality in Mars</u> <u>atmospheric methane driven by microseepage, barometric pumping, and adsorption</u>. Icarus, p.115079.

Kminek, G. and Bada, J.L., 2006. <u>The effect of ionizing radiation on the preservation of amino</u> acids on Mars. *Earth and Planetary Science Letters*, *245*(1-2), pp.1-5.

- Kminek et al's paper uses more than double the radiation levels now known from Curiosity, 200 mGy instead of 76 mGy for surface radiation but the reasoning is the same

Kminek, G., Fellous, J.L., Rettburg, P., Moissl-Eichinger, C., Sephton, M.A., Royle, S.H., Spry, A., Yano, H., Chujo, T., Margheritis, D.B. and Brucato, J.R., 2019. <u>The international planetary protection handbook</u>. See: section "Case Study Planetary Protection Category V Unrestricted Earth Return: Hayabusa-1&2"

Kminek, G., Benardini, J.N., Brenker, F.E., Brooks, T., Burton, A.S., Dhaniyala, S., Dworkin, J.P., Fortman, J.L., Glamoclija, M., Grady, M.M. and Graham, H.V., 2022. <u>COSPAR Sample Safety Assessment Framework (SSAF)</u>.

Korr, M., 2020. <u>Mary Mallon: First Asymptomatic Carrier of Typhoid Fever</u>. Rhode Island Medical Journal, 103(4), pp.73-73.

Kumpitsch, C., Koskinen, K., Schöpf, V. and Moissl-Eichinger, C., 2019. The microbiome of the upper respiratory tract in health and disease. *BMC biology*, *17*(1), p.87.

Kun, Á., 2021. <u>Maintenance of Genetic Information in the First Ribocell</u>. Ribozymes, 1, pp.387-417.

Lachance, J.C., Rodrigue, S. and Palsson, B.O., 2019. <u>Synthetic biology: minimal cells,</u> <u>maximal knowledge</u>. *Elife*, *8*, p.e45379.

Lerner, L, 2019, <u>Salt deposits on Mars hold clues to sources of ancient water</u>, University of Chicago news.

Leung, W.W.F. and Sun, Q., 2020. <u>Charged PVDF multilayer nanofiber filter in filtering</u> <u>simulated airborne novel coronavirus (COVID-19) using ambient nano-aerosols</u>. *Separation and Purification Technology*, 245, p.116887.

Levin, G.V. and Straat, P.A., 2016. <u>The case for extant life on Mars and its possible detection by</u> the Viking labelled release experiment. *Astrobiology*, *16*(10), pp.798-810.

Li, J., Mara, P., Schubotz, F., Sylvan, J.B., Burgaud, G., Klein, F., Beaudoin, D., Wee, S.Y., Dick, H.J., Lott, S. and Cox, R., 2020. <u>Recycling and metabolic flexibility dictate life in the lower oceanic crust</u>. *Nature*, *579*(7798), pp.250-255.

Lingam, M., 2021. <u>Theoretical constraints imposed by gradient detection and dispersal on</u> <u>microbial size in astrobiological environments</u>. *Astrobiology*, *21*(7), pp.813-830.

Liu, J., Li, B., Wang, Y., Zhang, G., Jiang, X. and Li, X., 2019. <u>Passage and community changes</u> of filterable bacteria during microfiltration of a surface water supply. *Environment international*, *131*, p.104998

McDaniel, L.D., Young, E., Delaney, J., Ruhnau, F., Ritchie, K.B. and Paul, J.H., 2010. <u>High</u> <u>frequency of horizontal gene transfer in the oceans</u>. *Science*, *330*(6000), pp.50-50.

McKay, C et al, 2014, How to Search for Life on Mars, Scientific American

McKay, C., (2015) interviewed by David, L. for Space News <u>Q&A with Chris McKay, Senior</u> <u>Scientist at NASA Ames Research Center</u>. Available at: <u>https://spacenews.com/qa-with-chris-mckay-senior-scientist-at-nasa-ames-research-center/</u>

[Downloaded highest resolution graphic here: https://hdqwalls.com/mars-planet-view-4k-wallpaper, need to locate original source]

McNeil, D.G., 2020, Inside China's All-Out War on the Coronavirus, New York Times.

Magana-Arachchi, D.N. and Wanigatunge, R.P., 2013. <u>First report of genus Chroococcidiopsis</u> (cyanobacteria) from Sri Lanka: a potential threat to human health. Journal of the national science foundation of Sri Lanka, 41(1).

Mahlen, S.D., 2011. <u>Serratia infections: from military experiments to current practice</u>. *Clinical microbiology reviews*, *24*(4), pp.755-791.

Mangus, S. and Larsen, W., 2004. Lunar Receiving Laboratory Project History

Marraffa, L., Kassing, D., Baglioni, P., Wilde, D., Walther, S., Pitchkhadze, K. and Finchenko, V., 2000. <u>Inflatable re-entry technologies: flight demonstration and future prospects</u>. *ESA bulletin*, pp.78-85.

Martínez, G.M. and Renno, N.O., 2013. <u>Water and brines on Mars: current evidence and</u> <u>implications for MSL</u>. *Space Science Reviews*, *175*(1-4), pp.29-51. Section numbers refer to the pdf rather than the online html version of the article.

Martín-Torres, F.J., Zorzano, M.P., Valentín-Serrano, P., Harri, A.M., Genzer, M., Kemppinen, O., Rivera-Valentin, E.G., Jun, I., Wray, J., Madsen, M.B. and Goetz, W., 2015. <u>Transient liquid</u> water and water activity at Gale crater on Mars. *Nature Geoscience*, *8*(5), p.357. Summary: "Evidence of liquid water found on Mars (BBC). NASA press release: <u>NASA Mars Rover's</u> Weather Data Bolster Case for Brine and University of Copenhagen press release, <u>Mars might</u> have liquid water, quotes Morten Bo Madsen, associate professor and head of the Mars Group at the Niels Bohr Institute at the University of Copenhagen. :

"We have discovered the substance calcium perchlorate in the soil and, under the right conditions, it absorbs water vapour from the atmosphere. Our measurements from the Curiosity rover's weather monitoring station show that these conditions exist at night and just after sunrise in the winter. Based on measurements of humidity and the temperature at a height of 1.6 meters and at the surface of the planet, we can estimate the amount of water that is absorbed. When night falls, some of the water vapour in the atmosphere condenses on the planet surface as frost, but calcium perchlorate is very absorbent and it forms a brine with the water, so the freezing point is lowered and the frost can turn into a liquid. The soil is porous, so what we are seeing is that the water seeps down through the soil. Over time, other salts may also dissolve in the soil and now that they are liquid, they can move and precipitate elsewhere under the surface," explains Morten Bo Madsen, associate professor and head of the Mars Group at the Niels Bohr Institute at the University of Copenhagen.

Mattingly, R, 2010, <u>Mission Concept Study</u>, <u>Planetary Science Decadal Survey</u>, <u>MSR Orbiter</u> <u>Mission (Including Mars Returned Sample Handling)</u>

Maxmen, A., 2010. <u>Virus-like particles speed bacterial evolution</u>. *Nature doi: 10.1038/news.2010.507*

Meltzer, M., 2012. <u>When Biospheres Collide: A History of NASA's Planetary Protection</u> <u>Programs</u>. Government Printing Office, After Splashdown: Plans To Safely Transport the Apollo Astronauts, Command Module, and Samples to the Recovery Ship, Page 217 and following

Meyer, M.A., Kminek, G., Beaty, D.W., Carrier, B.L., Haltigin, T., Hays, L.E., Agree, C.B., Busemann, H., Cavalazzi, B., Cockell, C.S. and Debaille, V., 2022. <u>Final Report of the Mars</u> <u>Sample Return Science Planning Group 2</u> (MSPG2).

Miller, J.D., Straat, P.A. and Levin, G.V., 2002, February. <u>Periodic analysis of the Viking lander</u> <u>Labelled Release experiment</u>. In *Instruments, Methods, and Missions for Astrobiology IV* (Vol. 4495, pp. 96-108). International Society for Optics and Photonics.

A temperature-regulated change in CO₂ solubility could at least partially account for the amplitude of the LR oscillation. However, the HT oscillation phase leads the LR oscillation by as much as two hours, an unusual circumstance if this were simply a chemical oscillation driven by thermal fluctuation.

(Admittedly there is uncertainty concerning the delay between change in temperature at the head end assembly, perhaps one inch over the 0.5 cc soil sample, and soil sample temperature per se. However, a two-hour lag seems quite long for what is presumably a convective and radiative process. Similarly, thermal-induced movement of gas between the soil sample and the beta detector requires only about 20 minutes.)

Furthermore, the LR oscillation does not slavishly follow the thermal variation; rather, it seems that the LR rhythm is extracted from the HT oscillation, while high frequency noise is not. This is very common in terrestrial organisms in which a low frequency periodic stimulus (i.e., a zeitgeber) such as a 12:12 light/dark cycle can entrain a circadian rhythm, while high frequency transients in the same stimulus are ignored (e.g., turning on the light in the bathroom at night for a minute or two does not alter normal entrainment to the light/dark cycle).

Furthermore, there is abundant evidence that as little as a 2° C temperature cycle can entrain circadian rhythms in terrestrial organisms such as lizards, fruit flies, and bread molds and entrainment can be preferential to the diminution phase of the temperature cycle, in analogy to the temperature fall that occurs at sunset on Mars).

Miteva, V.I. and Brenchley, J.E., 2005. <u>Detection and isolation of ultrasmall microorganisms</u> <u>from a 120,000-year-old Greenland glacier ice core</u>. *Applied and Environmental Microbiology*, 71(12), pp.7806-7818.

Möhlmann, D., 2005. <u>Adsorption water-related potential chemical and biological processes in</u> <u>the upper Martian surface</u>. *Astrobiology*, *5*(6), pp.770-777.

Morozova, Daria; Möhlmann, Diedrich; Wagner, Dirk (2006). <u>"Survival of Methanogenic Archaea</u> <u>from Siberian Permafrost under Simulated Martian Thermal Conditions"</u> (PDF). Origins of Life and Evolution of Biospheres. **37** (2): 189–200

National Research Council. 2009. <u>Assessment of Planetary Protection Requirements for Mars</u> <u>Sample Return Missions (Report)</u>. p. 59.

"It has been estimated that the planning, design, site selection, environmental reviews, approvals, construction, commissioning, and pre-testing of a proposed SRF will occur 7 to 10 years before actual operations begin. In addition, 5 to 6 years will likely be required for refinement and maturation of SRF-associated technologies for safely containing and handling samples to avoid contamination and to further develop and refine biohazard-test protocols. Many of the capabilities and technologies will either be entirely new or will be required to meet the unusual challenges of integration into an overall (end-to-end) Mars sample return program."

NASA, 2005npr<u>, NPR 8020.12D</u>, Planetary Protection Provisions for Robotic Extraterrestrial Missions. Washington , DC: Office of Safety and Mission Assurance

NASA, 2005odt, Opportunity Discovers Tiny Craters on Mars,

NASA, 2008grcg, Genesis Return Capsule on the Ground

NASA, 2012fdg, NASA Facilities Design Guide

NASA, 2015, Mars - Viking 1 Lander

NASA, 2016hmossf, How Mold on Space Station Flowers is Helping Get Us to Mars

NASA, 2022eis, <u>MSR Campaign Programmatic EIS, DRAFT Mars Sample Return (MSR)</u> <u>Campaign Programmatic Environmental Impact Statement</u> NASA, 2022msfs, MSR safety fact sheet ,MSR PEIS Fact Sheets

NASA, 2022msr, public comments, MSR, PEIS

NASA, 2020nebmsr, NASA Establishes Board to Initially Review Mars Sample Return Plans

NASA, n.d.hsp, Health Stabilization Program

NASA, n.d.mfs, Mars Fact Sheet

NASA, n.d.pmm, Phoenix Mars Mission, Astrobiology Magazine

NASA, n.d.pst, Perseverance Sample Tube 266

NASA, n.d., SEH,, System Engineering Handbook, see particularly

2.5 Cost Effectiveness considerations

3.3 Project Pre-Phase A: Concept Studies

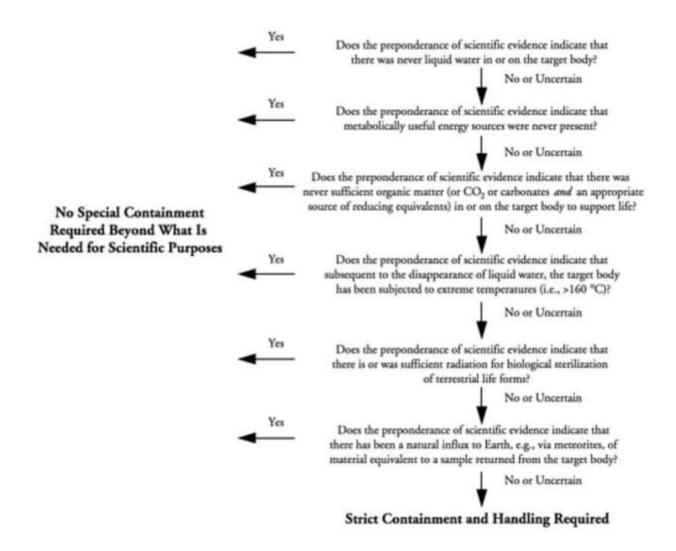
3.4 Project Phase A: Concept and Technology Development

3.5 Project Phase B: Preliminary Design and Technology Completion

National Academies of Sciences, Engineering, and Medicine. 1998. <u>Evaluating the Biological</u> <u>Potential in Samples Returned from Planetary Satellites and Small Solar System Bodies:</u> <u>Framework for Decision Making</u>. Washington, DC: The National Academies Press..

This is the decision tree:

https://nap.nationalacademies.org/read/6281/chapter/3#18



NEPA, 2022, <u>Comments submitted in May in response to NASA's first request for comments on</u> their plans

Neukum, G., Jaumann, R., Hoffmann, H., Hauber, E., Head, J.W., Basilevsky, A.T., Ivanov, B.A., Werner, S.C., Van Gasselt, S., Murray, J.B. and McCord, T., 2004.. <u>Recent and episodic volcanic and glacial activity on Mars revealed by the High Resolution Stereo Camera</u>. *Nature*, *432*(7020), pp.971-979.

Orosei, R., Lauro, S.E., Pettinelli, E., Cicchetti, A.N.D.R.E.A., Coradini, M., Cosciotti, B., Di Paolo, F., Flamini, E., Mattei, E., Pajola, M.A.U.R.I.Z.I.O. and Soldovieri, F., 2018. Radar evidence of subglacial liquid water on Mars. *Science*, *361*(6401), pp.490-493.

Pikuta, E.V., Hoover, R.B., Klyce, B., Davies, P.C. and Davies, P., 2006, September. <u>Bacterial</u> <u>utilization of L-sugars and D-amino acids</u>. In *Instruments, Methods, and Missions for Astrobiology IX* (Vol. 6309, p. 63090A). International Society for Optics and Photonics.

Pikuta, E.V. and Hoover, R.B., 2010, September. <u>Utilization of alternate chirality enantiomers in</u> <u>microbial communities</u>. In *Instruments, Methods, and Missions for Astrobiology XIII* (Vol. 7819, p. 78190P). International Society for Optics and Photonics.

Pikuta, E.V., Menes, R.J., Bruce, A.M., Lyu, Z., Patel, N.B., Liu, Y., Hoover, R.B., Busse, H.J., Lawson, P.A. and Whitman, W.B., 2016. <u>Raineyella antarctica gen. nov., sp. nov., a</u> <u>psychrotolerant, d-amino-acid-utilizing anaerobe isolated from two geographic locations of the</u> <u>Southern Hemisphere</u>. *International journal of systematic and evolutionary microbiology*, *66*(12), pp.5529-5536.

Pires, F. 2015, <u>"Mars liquid water: Curiosity confirms favorable conditions"</u>, Michigan news. "Life as we know it needs liquid water to survive. While the new study interprets Curiosity's results to show that microorganisms from Earth would not be able to survive and replicate in the subsurface of Mars, Rennó sees the findings as inconclusive. He points to biofilms—colonies of tiny organisms that can make their own microenvironment."

Puente-Sánchez, F., Arce-Rodríguez, A., Oggerin, M., García-Villadangos, M., Moreno-Paz, M., Blanco, Y., Rodríguez, N., Bird, L., Lincoln, S.A., Tornos, F. and Prieto-Ballesteros, O., 2018. <u>Viable cyanobacteria in the deep continental subsurface</u>. *Proceedings of the National Academy of Sciences*, *115*(42), pp.10702-10707

Pugel, B., Popescu, S. and Madad, S., 2020. <u>Restricted and Uncontained: Health</u> <u>Considerations in the Event of Loss of Containment During the Restricted Earth Return of</u> <u>Extraterrestrial Samples</u>. Health security, 18(2), pp.132-138.

Race, M. S., 1996, <u>Planetary Protection, Legal Ambiguity, and the Decision Making Process for</u> <u>Mars Sample Return</u> Adv. Space Res. vol 18 no 1/2 pp (1/2)345-(1/2)350

Randolph, R. 2009, <u>Chapter 10, A Christian Perspective</u>, in Bertka, C.M. ed., 2009. Exploring the Origin, Extent, and Future of Life: Philosophical, Ethical and Theological Perspectives (Vol. 4),. Cambridge University Press.

Renno, N., 2014, <u>How liquid water forms on Mars</u>, YouTube video, <u>University of Michigan</u> <u>Engineering</u> (transcript from <u>1:48 onwards</u>)

Renno, N. n.d.bio, faculty bio at the University of Michigan.

Richmond, J.Y. and McKinney, R.W., 2000. <u>Primary containment for biohazards: selection,</u> installation and use of biological safety cabinets.

Roberts, D. and Marks, R., 1980. <u>The determination of regional and age variations in the rate of desquamation: a comparison of four techniques</u>. Journal of Investigative Dermatology, 74(1), pp.13-16. See figures 3-4.

Rummel, J., Race, M., Nealson, K., "No Threat? No Way", The Planetary Report Nov/Dec. 2000

Contains:

- **A Case for Caution** by John Rummel, NASA'S planetary protection officer at the time, and previously, NASA senior scientist for Astrobiology
- *Hazardous Until Proven Otherwise*, by Margaret Race, a biologist working on planetary protection and Mars sample return for the SETI Institute and specialist in environment impact analysis
- **Practical Safe Science** by Kenneth Nealson, Director of the Center of Life Detection at NASA's JPL at the time.

Rummel, J.D., Race, M.S., DeVinenzi, D.L., Schad, P.J., Stabekis, P.D., Viso, M. and Acevedo, S.E., 2002. <u>A draft test protocol for detecting possible biohazards in Martian samples returned to Earth</u>.

Rummel, J.D., Beaty, D.W., Jones, M.A., Bakermans, C., Barlow, N.G., Boston, P.J., Chevrier, V.F., Clark, B.C., de Vera, J.P.P., Gough, R.V. and Hallsworth, J.E., 2014. <u>A new analysis of Mars "special regions": findings of the second MEPAG Special Regions Science Analysis Group (SR-SAG2)</u>

Rummel, J. D., Conley C. A, 2017, <u>Four fallacies and an oversight: searching for Martian life</u> *Astrobiology*, *17*(10), pp. 971-974.

Sarmiento, F., Peralta, R. and Blamey, J.M., 2015. <u>Cold and hot extremozymes: industrial</u> <u>relevance and current trends</u>. Frontiers in bioengineering and biotechnology, 3, p.148. While isolating psychrophilic strains would likely provide a better analog for the Martian surface, the generation times are prohibitively slow for research purposes in such exploratory experiments

Scheller, E.L., Hollis, J.R., Cardarelli, E.L., Steele, A., Beegle, L.W., Bhartia, R., Conrad, P., Uckert, K., Sharma, S., Ehlmann, B. and Asher, S., 2022, March. <u>First-results from the</u> <u>Perseverance SHERLOC Investigation: Aqueous Alteration Processes and Implications for</u> <u>Organic Geochemistry in Jezero Crater</u>, Mars. In LPSC 2022. The observed carbonates co-occur with hydrated materials, gypsum, and potentially aqueously-formed phases, amorphous silicates and phosphate

Schenk, P.M., Thomas-Hall, S.R., Stephens, E., Marx, U.C., Mussgnug, J.H., Posten, C., Kruse, O. and Hankamer, B., 2008. <u>Second generation biofuels: high-efficiency microalgae for</u> biodiesel production. *Bioenergy research*, *1*(1), pp.20-43.

page 37: Normal wild-type algae have large chlorophyll-bindingLHCII antenna systems and consequently the culture is dark green. Cell lines with small LHCII antenna systems yield cultures which are a much lighter green at the same cell density (Fig.7a). In the wild-type case, algal cells at the illuminated surface of the bioreactor that are exposed to high light levels capture the bulk of the light, but waste upto~90% of the energy as fluorescence and heat [122,134].

As a result the wild-type cells located deeper in the culture are exposed to ever decreasing levels of light the further they are from the illuminated surface (see"Open PondSystems"section). These shaded cells are prevented from capturing enough solar energy to drive photosynthesis efficiently. This in turn drastically reduces the efficiency of the overall culture. In contrast, small antenna cell lines with reduced LHCIIIevels have the advantage that they improve the light penetration into the bioreactor (Fig.7a) and better match itto the energy requirements of each photosynthesizing cell. Thus small antenna cells at the bioreactor surface absorb only the light that they need, largely eliminating fluores-cence of excess energy. This in turn allows more light (i.e.the light wasted in wild-type as fluorescence and heat) to penetrate into the bioreactor so that even cells deeper in the culture have a near optimal exposure to light

Schlaepfer, M.A., Sax, D.F. and Olden, J.D., 2011. <u>The potential conservation value of non-native species</u>. *Conservation Biology*, *25*(3), pp.428-437.

Shekhtman, L., 2019, <u>With Mars methane mystery unsolved</u>, <u>Curiosity serves scientists a new</u> one: Oxygen

Schuerger, A.C., Ulrich, R., Berry, B.J. and Nicholson, W.L., 2013. <u>Growth of Serratia</u> <u>liquefaciens under 7 mbar, 0 C, and CO₂-enriched anoxic atmospheres</u>. Astrobiology, 13(2), pp.115-131 Sieber, J.R., McInerney, M.J., Plugge, C.M., Schink, B. and Gunsalus, R.P., 2010. <u>Methanogenesis: syntrophic metabolism</u>. In *Handbook of Hydrocarbon and Lipid Microbiology*.

Schmidt, M., n.d. <u>Species Profile - Didymosphenia geminata</u>, aquatic non indigenous species, Great Lakes Information system.

Historically the species was restricted to low-nutrient waters but has recently seen large range expansions reportedly occurring in eutrophic rivers, showing much greater tolerance for nutrient and flow conditions than previously expected. This may be attributed to a genetic variant with broader tolerances than the original species.

Means of Introduction: Didymosphenia geminata has been shown to survive outside of the stream environment. Cells are able to survive and remain viable for 40 days in cool, dark, damp conditions. Angling equipment, boot tops, neoprene waders, and felt-soles provide a particularly suitable environment for cells to remain viable. Cells can hitchhike on this equipment and other recreational equipment into new bodies of water (Spaulding and Elwell 2007). Freshwater diatoms are dispersed through the flow of water and transport by other organisms, primarily waterfowl (Kristiansen 1996).

Blooms of Didymosphenia geminata form mats which can be over 20 cm thick. Extracellular stalks trap fine sediment, changing the nature of substrate and have potential long lasting effects due to the apparent resistance of stalks to degradation by bacteria and fungi

...

These mats are capable of engulfing the stream bottom, smothering native species of plants, insects, mollusks, and algae, and reducing habitat for insects for aquatic insects and fis

Streams outside the Great Lakes region harshly impacted by these mats have seen invertebrate populations decrease, macrophyte elimination, and absence of fish

It has been hypothesized that a new strain of Didymosphenia geminata is now dominant (Bothwell et al., 2006), and is responsible for the invasive behaviour. However, the presence of a new genetic strain has not been established....

Some clusters of dozens of diatoms appear pristine, suggesting that they had been living in the salar pool immediately

before being trapped as the gypsum crystal grew.

Could microfossils and/or viable microorganisms be trapped in gypsum on Mars as they are in gypsum on Earth? It is likely that abundant sulfate sand grains on Mars contain fluid inclusions similar to those in the acid-precipitated bottom-growth and reworked gypsum we discuss here.

We suggest that gypsum on Mars would have entrapped, as solid inclusions and within fluid inclusions, any microorganisms and/or organic compounds that were present in its parent waters. Therefore, fluid inclusions and solid inclusions hosted by salt minerals may be the best place to continue the search for life on Mars.

Some of these entrapped microorganisms remain viable for at least tens of thousands of years ... and possibly for hundreds of millions of years

Singh, R., Bhadouria, R., Singh, P., Kumar, A., Pandey, S. and Singh, V.K., 2020. <u>Nanofiltration</u> <u>technology for removal of pathogens present in drinking water</u>. In Waterborne Pathogens (pp. 463-489). Butterworth-Heinemann.

Sivasubramaniam, R. and Douglas, R., 2018. <u>The microbiome and chronic rhinosinusitis</u>. *World journal of otorhinolaryngology-head and neck surgery*, *4*(3), pp.216-221.

Solden, L., Lloyd, K. and Wrighton, K., 2016. <u>The bright side of microbial dark matter: lessons</u> learned from the uncultivated majority. *Current opinion in microbiology*, *31*, pp.217-226.

Stamenković, V., Ward, L. M., Mischna. M., Fischer. W. W.. "O₂ solubility in Martian nearsurface environments and implications for aerobic life" — <u>Nature</u>, October 22, 2018 - see also Vlada Stamenkovic. "<u>Origins of Life & Habitability - authors website with bibliography - and</u> <u>author shared link to the article</u>", sharing is via <u>Nature Sharedit</u> — <u>Habilabs</u>

Stillman, E, 2018, Chapter 2 - <u>Unraveling the Mysteries of Recurring Slope Lineae</u> in Soare, R.J., Conway, S.J. and Clifford, S.M. eds., 2018. *Dynamic Mars: Recent and Current Landscape Evolution of the Red Planet*. Elsevier. <u>Page 81</u>: "No proposed RSL mechanism can adequately describe all the observations ... We suggest RSLs that are scored excellent and very good and sites that do not typographically preclude aquifer fed springs are likely caused by a wet-dominated mechanism while numerous other sites are caused by dry granular flow"

Swindle, T.D., Atreya, S., Busemann, H., Cartwright, J.A., Mahaffy, P.R., Marty, B., Pack, A. and Schwenzer, S.P., 2021. <u>Scientific Value of Including an Atmospheric Sample as part of Mars Sample Return</u>. Astrobiology, (ja).

(2) Collecting gas in a newly-designed, valved, sample-tube-sized vessel that is flown on either the Sample Fetch Rover (SFR) or the Sample Retrieval Lander (SRL)

•••

The triple oxygen isotope composition of atmospheric CO2, O2, H2O, and CO would provide a unique picture of Martian atmospheric photochemistry and allow an understanding of the anomalous signatures in Martian minerals and water.

Thorney; ?, 2006, Didymo signage on Waiau river, Wikipedia

Tornabene, L.L., Moersch, J.E., McSween Jr, H.Y., McEwen, A.S., Piatek, J.L., Milam, K.A. and Christensen, P.R., 2006. Identification of large (2–10 km) rayed craters on Mars in THEMIS thermal infrared images: Implications for possible Martian meteorite source regions. *Journal of Geophysical Research: Planets*, *111*(E10).

Trainer, M.G., Wong, M.H., Mcconnochie, T.H., Franz, H.B., Atreya, S.K., Conrad, P.G., Lefèvre, F., Mahaffy, P.R., Malespin, C.A., Manning, H.L. and Martín-Torres, J., 2019. <u>Seasonal variations in atmospheric composition as measured in Gale Crater</u>, Mars. *Journal of Geophysical Research: Planets*, *124*(11), pp.3000-3024. See also <u>Supporting information</u>

Surprisingly, however, we have found that O_2 does not demonstrate the predictable seasonal behavior of the other major components. Surface O_2 measurements by SAM yield abundances that vary between 1300 and 2200 ppmv; when corrected for the annual global mean pressure, O_2 varies from 1300 to 1900 ppmv. Despite large instrument backgrounds, these are the first precise in situ measurements of O2, revealing a surprising seasonal and interannual variation that cannot be accounted for in current chemical models. Though Mars has the potential to generate significant O_2 release due to abundances of oxidants in/at its surface, the mechanisms by which O_2 could be quickly generated and then quickly destroyed are completely unknown. As with all surprising results, we hope that continued in situ, experimental, and theoretical results may shed light on this intriguing observation.

United Nations, 2020, <u>Press Briefing: Coronavirus Outbreak (COVID - 19)</u>: WHO Update (25 February 2020) at <u>10:80</u>

Urbaniak, C., Massa, G., Hummerick, M., Khodadad, C., Schuerger, A. and Venkateswaran, K., 2018. <u>Draft genome sequences of two Fusarium oxysporum isolates cultured from infected</u> <u>Zinnia hybrida plants grown on the international space station</u>. Genome announcements, 6(20).

Venier, C.G., Jones Jr, W.R., Jansen, M.J. and Marchetti, M., 2003, September. <u>Comparative</u> <u>physical and tribological properties of three Pennzane® fluids, SHF X-1000, SHF X-2000, and</u> <u>SHF X-3000</u>. In 10th European Space Mechanisms and Tribology Symposium (Vol. 524, pp. 337-340).

Wall, M., 2018, "Salty Martian Water Could Have Enough Oxygen to Support Life" — Space.com,

WhiteHouse, 1977, <u>NSC-25: Scientific or Technological Experiments with Possible Large-Scale</u> Adverse Environmental Effects and Launch of Nuclear Systems into Space

Wickett, R.R. and Visscher, M.O., 2006. <u>Structure and function of the epidermal barrier</u>. American journal of infection control, 34(10), pp.S98-S110.

In SC [stratum corneum] that is desquamating at its normal rate, corneocytes persist in the SC for approximately 2 weeks, depending on body site, before being shed into the environment. On average, about one layer of corneocytes is shed each day from the surface and replaced by keratinocytes at the SG. The corneocytes that are shed each day can have a significant bacterial load and may be a source of contamination of the environment.

WHO, 2020tosi, <u>Transmission of SARS-CoV-2: implications for infection prevention precautions</u>, Science Brief

Smith, D.H., Canup, R.M. and Christensen, P.R., 2022, May. Origins, Worlds, and Life: A Decadal Strategy for Planetary Science and Astrobiology. In *2022 Astrobiology Science Conference*. AGU.

Spaulding, S.A., Kilroy, C.A.T.H.Y. and Edlund, M.B., 2010. <u>Diatoms as non-native species</u>. *The diatoms: applications for the environmental and earth sciences*, pp.560-569.

Uhran, B., Conley, C. and Spry, J.A., 2019. <u>Updating Planetary Protection Considerations and</u> <u>Policies for Mars Sample Return</u>. Space Policy, 49, p.101322.

UN, 1945, Constitution of the United Nations Food and Agriculture Organization (FAO)

US DOA, 2017, European Starling

van Schaik, W. (2020) interviewed by Science Media Centre, expert reaction to questions about COVID-19 and viral load, accessed at: <u>https://www.sciencemediacentre.org/expert-reacti</u>

Vítek, P., Edwards, H.G.M., Jehlička, J., Ascaso, C., De los Ríos, A., Valea, S., Jorge-Villar, S.E., Davila, A.F. and Wierzchos, J., 2010. <u>Microbial colonization of halite from the hyper-arid</u> <u>Atacama Desert studied by Raman spectroscopy</u>. *Philosophical Transactions of the Royal Society of London A: Mathematical, Physical and Engineering Sciences*, *368*(1922), pp.3205-3221.

Walker, R., 2022a, <u>Comment submitted on May 15th by Robert Walker to NASA's first request</u> for comments on their plans.

Later updated with:

- <u>Comment submitted on November 28^h by Robert Walker to NASA's second request for</u> <u>comments on their draft EIS.</u>
- Comment submitted December 5th
- <u>Comment submitted December 13th</u>

Walker, R., 2022b, NASA and ESA are likely to be legally required to sterilize Mars samples to protect the environment until proven safe – technology doesn't yet exist to comply with ESF study's requirement to contain viable starved ultramicrobacteria that are proven to pass through 0.1 micron nanopores - proposal to study samples remotely in a safe high orbit above GEO with

<u>miniature life detection instruments – and immediately return sterilized subsamples to Earth.</u> (preprint, not peer reviewed)

Westall, F., Loizeau, D., Foucher, F., Bost, N., Betrand, M., Vago, J. and Kminek, G., 2013. <u>Habitability on Mars from a microbial point of view</u>. Astrobiology, 13(9), pp.887-897.

WHO, 2003, Laboratory Biosafety Manual Second Edition (Revised)

WHO, 2020wic, 1st WHO Infodemiology Conference

Winn, W.C., 1988. <u>Legionnaires disease: historical perspective</u>. *Clinical Microbiology Reviews*, *1*(1), pp.60-81

Xu, Z., Chen, Y., Meng, X., Wang, F. and Zheng, Z., 2016. <u>Phytoplankton community diversity is</u> <u>influenced by environmental factors in the coastal East China Sea</u>. *European Journal of Phycology*, *51*(1), pp.107-118.

Yano, H., Chujo, T. JAXA/ISAS, <u>Case Study Planetary Protection Category V Unrestricted Earth</u> <u>Return: Hayabusa-1&2</u> Japan and the Hayabusa-1 and Hayabusa-2 Teams

Yin, W., Wang, Y., Liu, L. and He, J., 2019. <u>Biofilms: The Microbial "Protective Clothing" in</u> <u>Extreme Environments</u>. International journal of molecular sciences, 20(14), p.3423.

Yung, Y.L., Chen, P., Nealson, K., Atreya, S., Beckett, P., Blank, J.G., Ehlmann, B., Eiler, J., Etiope, G., Ferry, J.G. and Forget, F., 2018. <u>Methane on Mars and habitability: challenges and responses</u>. Astrobiology, 18(10), pp.1221-1242.

Zakharova, K., Marzban, G., de Vera, J.P., Lorek, A. and Sterflinger, K., 2014. <u>Protein patterns</u> of black fungi under simulated Mars-like conditions. *Scientific reports*, *4*, p.5114.

Zhou, B. and Shen, J., 2007. Comparison Of HEPA/ULPA Filter Test Standards Between America And Europe. In *Proceedings of Clima*.