NASA's draft EIS does NOT establish that life from Mars can get to Earth faster and better protected in a meteorite - or that ANY life on Mars can get here in this way – it does NOT overturn previous warnings of the potential for large scale effects – and these mistakes have MAJOR public interest and 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/uy4rw</u>, doi 10.31219/osf.io/ uy4rw

This version dated 30th December 2022.

Section titles are written like mini-abstracts with the basic argument and conclusion. So you can get a good overview of the paper simply by reading the contents list. You can then drill down into any sections of interest for more details.

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Abstract

This paper focuses on the arguments in NASA's draft EIS which claims that life can be transferred more easily from Mars to Earth in meteorites than in the sample tubes. It also claims that there is no significant risk of effects on the environment These statements are at odds with the cites they themselves cite on these topics. This paper shows that the literature doesn't support their conclusions and that there are many papers that raise significant concerns for the potential for large scale effects on the environment which they don't refer to.

This paper focuses on the literature on whether life can be transferred from Mars to Earth on meteorites, the literature on the potential for large scale effects, on public involvement and on the legal process.

This paper also includes for motivation a brief summary of why the published literature says that there is potential for extant life in Jezero crater either in microhabitats in a seemingly uninhabitable desert in biofilms similarly to the seemingly uninhabitable Mars analogues of the McMurdo dry valleys and the hyperarid core of the Atacama desert. This will be expanded on in a follow up paper.

As Rummel at al wrote (Rummel et al, 2002:96).,

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

This is part of a series of papers in which we find that the cites for NASA's draft EIS are so flawed that a clean restart is needed with a properly peer reviewed study to assess the environmental impact correctly. If NASA uses this as their final EIS, and it gets taken to the courts, NASA won't have a case, It will fail basic review, just through checking the EIS's own cites.

However, I suggest with some changes the proposed action can go ahead in a way that is safe for the environment and maximizes return for astrobiology and geology.

What this EIS proposes is similar to building a house without smoke detectors. But a house for nearly 8 billion people, most of whom are not aware that this decision is being made for them by NASA. This smoke detector analogy is from Margaret Race from her contribution **"No** *Threat? No Way*" (Rummel et al., 2000)

We need to examine this properly and if it is needed we need to install those smoke detectors. To do this requires an adequate EIS that uses the sources correctly and it needs to consider alternatives designed to protect Earth with 100% safety, and not just "no action"

The issues that need to be addressed in this draft EIS are so many and so serious that they can't be covered adequately in a single paper. The reason for writing these papers is to have

credibility during the NEPA review process, for any potential legal appeals, and if necessary, for the presidential review for large scale effects.

Other issues will be covered in subsequent papers.

Notes for readers and reviewers – I wish to submit these papers to peer review to support my final comment on the NASA draft Environmental Impact Statement for a Mars sample return – their aim is to finalize it by spring / summer 2023 which gives little time for academic responses – although it is based on claims counter to the existing literature on planetary protection including their own cites – and has numerous very serious errors in it as we'll see in these papers

I hope to get this paper accepted for publication before completion of the NEPA process in spring / summer 2023. I don't expect it to complete peer review by then (though it will be great if it can be).

If it is accepted for publication this will help persuade NASA and if necessary any justices, that this is indeed something that needs to be looked at.

I was able to write this analysis quickly because I've been working for two years (with early draft in 2015) on another paper about planetary protection issues for NASA's Mars sample return mission (Walker, 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 miniature life detection instruments – and immediately return sterilized subsamples to Earth.

Preprint DOI 10.31219/osf.io/rk2gd

For latest version please visit: (url https://osf.io/rk2gd)

Because of the work I did on that paper beforehand - I was able to produce a rapid study of NASA's draft EIS preprint (Walker, 2022c). which I submitted as an attachment to my last public comment on the draft EIS, the comment posted on December 20th (Walker, 2022a):.

Now that the public comments period is over, I am in the process of converting my longer analysis of NASA's draft EIS into a series of shorter papers, which I plan to submit for publication, unless NASA reverse course on this EIS.

The aim with these papers is to help support any legal challenge by getting my academic analysis of their EIS peer reviewed – and also hopefully help direct the justice to find an equitable solution such as requiring pre-sterilization of samples that NASA returns to Earth rather than just a request to abort the mission altogether.

I very much hope NASA will reverse course, in which case I have no need to submit these papers for publication and I'll return to work on my original paper <u>(Walker, 2022b)</u> for submission for publication in good time, with a great sense of relief!

N.B. the citation list here is longer than needed as it merges the citations of those two preprints, (Walker, 2022c) and (Walker, 2022b). If accepted for publication the citation list can be filtered down to the cites actually used here with a few hours of work.

Colour coding – pale blue for titles of sections in the original main paper – my public comments – and proposed titles of preprints in this series – and orange for quotes from the NASA draft EIS and associated documents

I often refer to sections of my original main paper for additional details like this:

For more details see the section in my main paper preprint (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, also 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 colour-blindness except monochromats who will see both types of text as a pale gray according to <u>this simulator</u> – but for them also it distinguishes them from the other quotes and the context makes it clear which is which, they aren't easily confused.

Note on use of language – this paper is designed to be maximally accessible – by careful use of vocabulary and grammatical structures, but never with loss of precision in the meaning of the text

I have written this paper to be maximally accessible to everyone - theologians, philosophers, lawyers, politicians, decision makers, the general public and autistic people.

I wish this choice to survive through to the final version of this paper if possible. Examples:

- use the most widely accessible vocabulary available to convey the desired meaning
- replace technical by non technical terms when it can be done with no loss of precision
- use non scientific terms and non mathematical language whenever if it is available with the same precision.

Examples of using non scientific terms when there is no loss of precision:

- Million instead of 10⁶
- "Didymo" instead of Didymosphenia geminate
- Signaling chemicals instead of semiochemicals

Where there are no ordinary language equivalents I explain the term in ordinary language as far as is possible when it is first introduced. I may later use a shorter definition of the same term as a reminder. Example:

"[GPa stands for Gigapascals, a unit of pressure equivalent to a billion pascals, and 1 GPa is a little under 10,000 times atmospheric pressure, or the pressure at a depth of 100 km of water. In this context it refers to a sudden increase of shock pressure which is harder to withstand than constant pressure]"

Reminder:

[shock pressure of 1 GPa]

That's first used in the section:

 Charles Cockell's paper (which they don't mention) said that though planetary exchange of photosynthesis might not be impossible - 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

As for the choice to make this paper maximally accessible to autistic people - I am used to working with scared people, many autistic and have learnt how to use simple and self contained sentence structures that even quite severely autistic people can understand quickly when in the middle of a panic attack.

This is a "win win" situation as I find this approach usually makes the sentences shorter, with fewer words and makes the text easier for everyone to parse quickly.

I did a blog post on the difference between how autistic and non autistic people preferentially parse sentences which may help the reader understand the choices I make in sentence structure, see (Walker, n.d.)

Introduction – natural division into four shorter papers

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.

It also has an introductory section on the potential for sampling martian life in Jezero crater, since this is needed to motivate the rest of it.

If this paper is considered too long, it can be divided into sub-papers. I have suggested four papers:

1. Potential for sampling martian life in Jezero crater, Provisional title: NASA's draft EIS incorrectly excludes the

Provisional title: NASA's draft EIS incorrectly excludes the possibility of present day martian life in Jezero crater - it omits the MEPAG review which overturned their MEPAG cite and highlighted the unexplored potential for terrestrial life to inhabit biofilms in microhabitats or to get transferred in dust – this is even more important for Martian life with biology adapted to Mars for billions of years.

- 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.
 Provisional title: NASA's draft EIS does NOT establish that life from Mars can get to Earth faster and better protected in a meteorite or that ANY life on Mars can get here in this way
- 3. Potential of likely low risk for large scale harm for public health or the environment (partly summarized in this paper) NASA's draft EIS for samples from Mars does NOT overturn previous warnings of the potential for likely low risk of large scale harm for public health or the environment – in some scenarios life can NEVER be returned safely from Mars to Earth – in other scenarios Martian life doesn't exist, or is harmless or beneficial –

we need to know which scenario we have in our solar system

4. Legal implications once NASA recognizes the potential for a likely low risk of large scale harm for public health or the environment (partly summarized in this paper)

Provisional title: NASA's many mistakes in its EIS for samples from Mars have MAJOR public interest and legal implications – because of the need to recognize the potential for a likely low risk of large scale harm to public health or the environment from an unsterilized sample return done incorrectly • Legal implications

Provisional title: NASA's many mistakes in its EIS for samples from Mars have MAJOR public interest and legal implications – because of the need to recognize the potential for a low risk of large scale harm to public health or the environment from an unsterilized sample return done incorrectly

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.

The MEPAG review study which they don't cite finds some potential even for terrestrial life to be able to survive in Jezero crater in a seemingly uninhabitable desert similar to other seemingly uninhabitable but inhabited Mars analogue deserts on Earth or transferred there in the dust

This will be a topic for a separate paper but a brief summary is included here.

This paper expands on points 9, 10 and 11 in my final comment to NASA's draft EIS

This is to follow on from the issues raised in the comment I submitted to the EIS posted on 20th - December. This was the summary which I also supported with multiple attachments to my final comment (<u>Walker, 2022a</u>):

I recommend this draft Environmental Impact Statement is stopped, and a new one prepared after doing the necessary size limits review, and fixing whatever led to its many errors.

- 1. The BSL-4 recommendation in this EIS is out of date, based on science of 1999.
- 2. This EIS does not mention the most recent Mars Sample Return study from 2012 by the European Space Foundation which reduced the 1999 size limit from 0.2 microns to 0.05 microns to contain ultramicrobacteria and required 100% containment at that size.
- 3. A BSL-4 is not designed to this standard. In recent reviews of filter technology, I find NO AIR FILTERS with that capability and no evidence anybody is working on them. Air filters for larger particles remove some of these very small particles kicked out of the airstream by jostling of air molecules by Brownian motion but can't remove all. It is an unusual requirement.
- 4. NASA haven't responded to my comment in May which alerted them to this omission. They still don't cite the ESF study. Also, the ESF said their limit needs to

be updated periodically. An update is certainly due a decade later.

- 5. The EIS has an overnarrow scope in the Purpose and Need section it requires samples to be returned unsterilized to terrestrial labs for "safety testing". This won't work. NASA believe they reduced the most abundant biosignatures to 0.7 nanograms per gram of returned rock sample this guarantees a positive test. There will be no way to know if tubes contain safe terrestrial life or potentially unsafe martian life.
- 6. This narrow scope improperly excludes the reasonable alternative of presterilizing samples before they reach Earth's biosphere which achieves virtually the same science return and keeps Earth 100% safe. By a 1997 case in the 7th circuit this alone probably invalidates the EIS.
- 7. The high levels of forward contamination make astrobiology almost impossible. I recommend bonus samples of dirt, dust and atmosphere collected in a STERILE container with no terrestrial organics, brought to Mars especially on the ESA fetch rover.
- **8.** I recommend returning these bonus astrobiology samples to a safe orbit above GEO where they can be tested for life
- 9. The EIS's reasoning for no significant environmental effects contradicts the conclusion of the NRC study from 2009 which they do cite, which says the risk of even large-scale impacts on human health or environment is likely low but not demonstrably non zero. It also warns against the meteorite argument that they use. I found multiple errors in my analysis.
- 10. Returned life COULD be harmful. Example, fungi kill crops, other life and sometimes immunocompromised humans. Botulism, ergot disease, tetanus, all are the results of exotoxins not adapted to the lifeforms they kill, similarly some algal blooms kill dogs and cows that eat them. BMAA misincorporated for L-serine causes protein misfolding and is a neurotoxin implicated in some cases of the disease that affected Steven Hawking an alternative biochemistry may have many different amino acids similar enough to terrestrial amino acids to be misincorporated. Or perhaps martian life evolved from scratch from mirror chemicals as mirror life the effect on our biosphere can't be predicted. I give many such examples in my preprint. Or it could be harmless like microbes from a terrestrial desert, or indeed beneficial. But we DON'T KNOW. So we need to find out first
- 11. What matters for invasive species are the ones that can't 'get here, like starlings that can't cross the Atlantic rather than barn swallows. The freshwater diatom "Didymo" is invasive in New Zealand and can't get from one

freshwater lake to another without humans. A microbe adapted to briny seeps on Mars and to spreading in dust storms shielded from UV, may well not get to Earth in a meteorite, while a sealed sample tube including Martian atmosphere, at Mars atmospheric pressure, is like a mini spaceship.

- 12. Quarantine of humans can't keep out a fungal disease of crops, mirror life etc.
- 13. So any unsterilized samples will need to be studied remotely via telerobotics which also greatly reduces forwards contamination (issues with filtering ultramicrobacteria will go both ways).
- 14. astrobiologists now have tiny instruments that can go from sample preparation to life detection, even to a gene sequence, operated remotely on Mars. They could send hundreds of these in each 7 ton payload of the Ariane 5 to above GEO.

Let's make this an even better mission and SAFE for Earth. Thanks!

The current paper expands on points 9, 10 and 11 along the lines outlines in the attachments to that submission.

NASA haven't responded to my public comments on the draft EIS, or my follow up email to the Planetary Protection Office on any of these issues so they can't be resolved by dialog

NASA's draft EIS didn't mention my previous public comment on May 28th or answer the issues I found already back then. (<u>Walker, 2022a</u>):

My earlier public comment on 28th particularly focused on issues 1, 2 and 3

NASA's proposed action seems likely to fail legal review, since a BSL-4 facility can't comply with the 2012 European Space Foundation study's limit (Ammann et al, 2012:14ff):

"The release of a single unsterilized particle larger than 0.05 μ m is not acceptable under any circumstances".

Their rationale: viable starvation limited ultramicrobacteria can pass through a 0.1 micron filter (Miteva et al, 2005).

This limit is easier to achieve in water under high pressure. One study achieved 100%

removal of 0.03 micron polioviruses using carbon nanotubes loaded with silver. (Kim et al, 2016) (Singh et al, 2020:6.3).

However aerosol filters are less effective. Even ULPA level 17 filters remove only 99.999995%. Also those filters are only tested to 0.12 microns (BS, 2009:4). At the ESF's 0.05 microns, an experimental 6-layer charged nanofiber filter for coronaviruses filtered out 88% of ambient aerosol particles (Leung et al, 2020), far from 100% containment.

The ESF also said the chance of release of even a single unsterilized particle at 0.01 microns must be less than 1 in a million, to stop gene transfer agents which readily transfer novel capabilities to unrelated species of archaea overnight in sea water (Maxmen, 2010).

In that submission in May I also uploaded the current version at the time of my large paper on NASA's Mars sample return mission (Walker, 2022b) which identified most of the other points I cover here as issues for NASA's mission but I didn't specifically tie them to their proposed EIS which wasn't yet published at the time.

Then the other issues were identified in my comments after the draft EIS was published, focusing on the text of the EIS . (Walker, 2022a).

The agency concerned, NASA, haven't contacted me via email on either occasion, although I provided my email address when I submitted the comments.

NASA also haven't responded to the email I sent to the planetary protection office about these issues. So it is not possible to enter into a dialog with NASA on this topic at the current time.

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

I plan other papers on:

1. Impossibility of containing 0.05 / 0.01 microns in a BSL-4, need for review of the size limit and survey of literature on air filters showing none currently meet the size limit requirements set by the ESF in 2012.

Provisional title: NASA improperly assume samples from Mars can be contained in a Biosafety Level 4 laboratory - this would NOT comply with the size limit set by the European Space Foundation in 2012, - this is the only Mars sample return size limit update since 1999 – yet it isn't cited in the draft EIS 2. Unexplored potential for sampling martian life in Jezero crater (partly summarized in this paper)

Provisional title: NASA's draft EIS incorrectly excludes the possibility of present day martian life in Jezero crater - it omits the MEPAG review which overturned their MEPAG cite and highlighted the unexplored potential for terrestrial life to inhabit biofilms in microhabitats or to get transferred in dust – this is even more important for Martian life with biology adapted to Mars for billions of years.

- 3. Not established that all or even any putative martian life on or near the surface in Jezero crater can get back to Earth faster and more easily in a meteorite (this paper).. Provisional title: NASA's draft EIS does NOT establish that life from Mars can get to Earth faster and better protected in a meteorite or that ANY life on Mars can get here in this way
- Unknown potential for microbes from Mars to cause large scale harm for public health or the environment - likely low risk but not demonstrably zero (partly summarized in this paper)

NASA's draft EIS for samples from Mars does NOT overturn previous warnings of the potential for likely low risk of large scale harm for public health or the environment – in some scenarios life can NEVER be returned safely from Mars to Earth – in other scenarios Martian life doesn't exist, or is harmless or beneficial – we need to know which scenario we have in our solar system

 Legal and public interest implications once NASA recognizes unknown potential for microbes from Mars to cause large scale harm for public health or the environment (partly summarized in this paper)

Provisional title: NASA's many mistakes in its EIS for samples from Mars have MAJOR public interest and legal implications – because of the need to recognize the potential for a likely low risk of large scale harm to public health or the environment from an unsterilized sample return done incorrectly

6. 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

Provisional title for 4+5: Terrestrial biosignatures in NASA's samples from Mars guarantee false positive life detection - the required "Safety testing" achieves nothing – and improperly rules out the reasonable alternative of samples sterilized before they reach Earth which keeps Earth 100% safe 7. We can't protect Earth from martian life using human quarantine in a human operated space station until we know what is there

Provisional title: Human quarantine can't protect Earth from harm from martian microbes unless we know what we are protecting against and what its capabilities are – in some scenarios humans exposed to martian life can never return to Earth in a way that's safe for our biosphere

- 8. Building on previous papers a request for NASA to include a sterilized return as a reasonable alternative in the EIS
- Even without forward contamination, difficulty of testing a sample for presence of life non destructively – need for Sagan's "vigorous program of unmanned Martian exobiology" before we can know if it is safe or not to return any unsterilized materials to Earth
- 10. A request to NASA to include as a reasonable alternative 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 with a proposal to also add a pebble from the Martian surface collected with a presterilized marscopter as a first step towards future return of rock samples from Mars totally free from terrestrial contamination and proposal to target recently excavated craters as there is a near certainty to find a crater that excavated to a depth of at least 2 meters within reach of the rovers..

Provisional title for 8 - 10: How NASA's MSR mission can be transformed into a 100% safe mission for Earth that retains virtually all science interest, while bonus astrobiology samples returned in a STERILE container sent to Mars can boot up the first stage of Carl Sagan's "vigorous program of unmanned Martian exobiology and terrestrial epidemiology".

Annotated copy of NASA's draft Environment Impact Statement

This is in the form of a word document <u>NASA_EIS_annotated.docx</u> - you don't need Word to read it - though sadly it leaves out formatting and graphics from the comments. If it gets stuck on the Word icon try a refresh of the page and it will likely show up.

Also, sadly I couldn't get the page numbers to match in Word so you need to search for the text to find the same passage in my annotated version.

If you have Word you can download the pdf complete with annotations either from that page or via this direct link. <u>NASA_EIS_annotated.docx (for download)</u> or as a <u>zip (only slightly smaller)</u> which then has all the formatting and graphics for the annotations.

[For 1st separate paper] Yes there are significant reasons to protect Earth from life returned from Jezero crater on Mars – every step in the chain of arguments in NASA's EIS are invalid – to be expanded on in other papers in this series

This is to motivate the rest of this paper. If NASA's draft EIS was correct that there is no life in Jezero crater, there is nothing to protect against. But we do NOT know that.

Provisional title:

"There IS potential for NASA to return martian life from Jezero crater – the MEPAG review overturned the MEPAG cite NASA's draft EIS relies on and highlighted the unexplored potential for terrestrial life to inhabit biofilms in microhabitats or to get transferred in dust – this is even more important for Martian life with biology adapted to Mars for billions of years".

EVERY statement in this chain of reasoning is improperly cited:

- that Mars has been uninhabitable for millions of years

 their most recent cite is about a search for CURRENT small scale and micro habitats on Mars so the sentence in the EIS flatly contradicts its most recent cite.
- that if there is life on the surface of Mars elsewhere, it can't get to Jezero crater

 the cite in the EIS does say this but it was overturned by the MEPAG review commissioned by ESA and NASA which said it is necessary to investigate the potential for terrestrial life to establish itself in apparently uninhabitable regions using local microhabitats and biofilms or to be transported in the dust to distant parts of Mars (which means transfer back is also possible)

Also both MEPAG and the MEPAG review are only about the capabilities of terrestrial life not life evolved on Mars possibly independently with differences in biology, and adapted to Martian conditions for billions of years

• that if there is life in Jezero crater it can get to Earth faster and better protected in a meteorite

- their cite does establish that it MIGHT be possible for some exceptionally hardy forms of life to get to Earth from Mars rarely. It does NOT establish that less hardy life can get here at all never mind faster or better protected.

One of their cites indeed gives reasons why conclusions about meteorites shouldn't be applied to a Mars sample return mission - in a section they don't cite (subject of this paper)

• that if life is returned in a sample that didn't get here on a meteorite, the potential for environmental harm or large scale harm on human health is not significant and can be ignored.

- their cite is internal to the NASA sterilizing subcommittee and it represents a minority view in astrobiology

The sterilizing subcommittee doesn't mention any of the many astrobiologists who said this reasoning is false, dating back to Carl Sagan (Sagan, 1973:162), and Joshua Lederberg (Lederberg, 1999b). They said we can't know what Mars microbes would do to humans or our biosphere, and that there IS potential for significant long term and large scale harm.

Nor does it cite the National Research Council report in 2009 which said (Board et al, 2009: 48).

"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

... 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"

Also as we'll see, there are many specific examples in the literature of types of martian life could potentially cause large scale harm, such as fungi, diseases of biofilms or more generally new forms of life based on an independently evolved biology introduced to our biosphere. These examples aren't discussed.

Also, each statement in this chain in the EIS is one you can only consider if the previous statements were all invalid.

NASA's intention is clearly to build confidence by showing that each stage is an additional proof that no harm is possible. However this very long chain of steps where each one can only be considered if none of the previous steps are valid is likely to have the opposite effect of reducing confidence even on a reader who doesn't detect the issues with the cites. And in reality as we'll see ALL the steps in this chain of reasoning are invalid.

The way they do improper citing include:

- missummarize the papers they cite
- cite papers that say explicitly they didn't study the topic they are cited for
- omit later papers that overturn the results they cite.
- present the views of a minority without citing the views of a majority and without mentioning reasons the majority give for their views

Meanwhile, here is a brief summary for the first two issues and the last issue is covered at the end of this paper.

[For 1st separate paper] 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 (<u>NASA</u>, <u>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 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, 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.

Here is a screenshot.

Chapter 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 here 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:

[For 1st separate paper] Published views of astrobiologists – some think there is a high chance the surface of Mars is inhospitable to terrestrial life but none go as far as certainty – and others say it may have small niches suitable for microbial life over much of the surface – and a minority think Viking sampled life already in the 1970s – it could have uninhabited habitats but it isn't easy to find a source from this century stating EIS's claim of "existing credible evidence" that it is uninhabitable

Many astrobiologists have expressed a view that present day Mars may well be habitable to terrestrial life in part. This need not mean that there is life there, it could 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>. Some astrobiologists do say that Mars has a high chance to be inhospitable but not certainty and many think Mars may have small niches suitable for life, similar to niches found in the soil or rocks of our driest coldest deserts which often have small communities of microbes, even if they are only habitable at microbial scales.

Many astrobiologists also think it could have extant Martian life. A few think there is a possibility that Viking discovered life in the 1970s.

There is no consensus for any of these positions. But so far I haven't found NASA's supposed "consensus" as a published point of view of any astrobiologist in any of the papers I've looked at. I am interested if anyone knows of such a source from a reputable peer reviewed journal so I can add it to the range of points of view of astrobiologists.

This quote is from a paper about planetary protection in the forwards direction by Rummel and Conley, both former planetary protection officers for NASA (Rummel et al, 2017)

"Claims that reducing planetary protection requirements wouldn't be harmful, because Earth life can't grow on Mars, may be reassuring as opinion, but the facts are that we keep discovering life growing in extreme conditions on Earth that resemble conditions on Mars. We also keep discovering conditions on Mars that are more similar—though perhaps only at microbial scales—to inhabited environments on Earth, which is where the concept of Special Regions initially came from."

Here are a few example statements:

Davila et al. (Davila et al, 2010).

"We argue that the strategy for Mars exploration should center on the search for extant life. By extant life, we mean life that is active today or was active during the recent geological past and is now dormant. As we discuss below, the immediate strategy for Mars exploration cannot focus only on past life based on the result of the Viking missions, particularly given that recent analyses call for a re-evaluation of some of these results. It also cannot be based on the astsumption that the surface of Mars is uniformly prohibitive for extant life, since research contributed in the past 30 years in extreme environments on Earth has shown that life is possible under extremes of cold and dryness."

Westall (Westall, 2013:192)

"This presupposes that the ephemeral surface habitats could be colonized by viable life forms, that is, that a subsurface reservoir exists in which microbes could continue to metabolize and that, as noted above, the viable microbes could be transported into the short-lived habitat

.... Although there are a large number of constraints on the continued survival of life in the subsurface of Mars, the astonishing biomass in the subsurface of Earth suggests that this scenario as a real possibility."

Morozova (Morozova et al, 2006)

"The observation of high survival rates of methanogens under simulated Martian conditions supports the possibility that microorganisms similar to the isolates from Siberian permafrost could also exist in the Martian permafrost"

Crisler et al (Crisler et al, 2012)

Our results indicate that terrestrial microbes might survive under the high-salt, low-temperature, anaerobic conditions on Mars and present significant potential for forward contamination. Stringent planetary protection requirements are needed for future life-detection missions to Mars

Renno (Renno, 2014):

"This is a small amount of liquid water. But for a bacteria, that would be a huge swimming pool - a little droplet of water is a huge amount of water for a bacteria. So, a small amount of water is enough for you to be able to create conditions for Mars to be habitable today'. And we believe this is possible in the shallow subsurface, and even the surface of the Mars polar region for a few hours per day during the spring."

Stamenković (Wall, 2018)

There is still so much about the Martian habitability that we do not understand, and it's long overdue to send another mission that tackles the question of subsurface water and potential extant life on Mars, and looks for these signals

De Vera et al (de Vera et al, 2014)

"This work strongly supports the interconnected notions (i) that terrestrial life most likely can adapt physiologically to live on Mars (hence justifying stringent measures to prevent human activities from contaminating / infecting Mars with terrestrial organisms); (ii) that in searching for extant life on Mars we should focus on "protected putative

(ii) that in searching for extant life on Mars we should focus on "protected putative habitats"; and

(iii) that early-originating (Noachian period) indigenous Martian life might still survive in such micro-niches despite Mars' cooling and drying during the last 4 billion years"

Cockell (Deighton, 2016)

Most microbes can grow in different types of extremes and the extremes that we are looking at, things like radiation, perchlorate salts and also sulphate salts (found on Mars), they will grow in that. It's just a question of trying to determine what the limits are and that's the work we're doing at the moment. Anywhere where we've gone to the deep subsurface (on earth) today, where there is liquid water, there is a high chance that environments are habitable,

Simply because Mars is a planet of volcanic rock, and when volcanic rock weathers that provides an environment for microbes to grow and reproduce, I think we can already say there is a high chance there are habitable environments. 'At the moment we just don't know what the origin of life requires, going from simple chemicals to self-replicating microbe,' Edinburgh's Prof. Cockell said. 'If we looked at many planets, many environments and didn't find life, then that would tell us that life is extremely rare and that early spark was an unusual event. 'And then we'd have to try and find out exactly why it was, and what happened in those early stages of life that was unusual on the earth.'.

Cabrol (Cabrol, 2021)

Arguably, dispersal does not imply seeding, but it provides the potential for it and, if life started on Mars, odds are that not only is it still there, but it is everywhere it can be where conditions allow dormancy or metabolic activity. Here, terrestrial analogues in extreme environments show that 'everywhere it can be' does not, however, mean easy to see. Hidden oases are often measured in centimetres to micrometres, their presence intimately linked to the subtle interplay and feedback mechanisms between living things and their environment.

Bianciardi et al (Bianciardi et al, 2012)

"These analyses support the interpretation that the Viking LR experiment did detect extant microbial life on Mars"

Miller et al (Miller et al, 2002).

"Did Viking Lander biology experiments detect life on Mars? ... Recent observations of circadian rhythmicity in microorganisms and entrainment of terrestrial circadian rhythms by low amplitude temperature cycles argue that a Martian circadian rhythm in the LR experiment may constitute a biosignature."

Levin et al (Levin et al, 2016)

"It is concluded that extant life is a strong possibility, that abiotic interpretations of the LR data are not conclusive, and that, even setting our conclusion aside, biology should still be considered as an explanation for the LR experiment. Because of possible contamination of Mars by terrestrial microbes after Viking, we note that the LR data are the only data we will ever have on biologically pristine martian samples"

In the 2020 conference Mars extant life: what's next? (<u>Carrier et al, 2020</u>) a significant fraction of the participants thought that there is a possibility Mars has extant life.

Primary conclusions are as follows: A significant subset of conference attendees concluded that there is a realistic possibility that Mars hosts indigenous microbial life. A powerful theme that permeated the conference is that the key to the search for martian extant life lies in identifying and exploring refugia ("oases"), where conditions are either permanently or episodically significantly more hospitable than average. Based on our existing knowledge of Mars, conference participants [For 1st separate paper] 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 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 source (<u>Rummel et al</u>, 2014) is a study of "special regions", regions on Mars that terrestrial life might be able to colonize.

This is not a consensus position. Even as that 2014 report by Rummel et al was in publication, NASA and ESA commissioned a review (<u>Board, 2015</u>) which overturned many of its findings including ALL the ones relevant to Jezero crater. For instance it said that Rummel et al doesn't adequately discuss transport in the atmosphere, that would include dust storms (<u>Board, 2015</u>: $\underline{12}$):

The SR-SAG2 report does not adequately discuss the transport of material in the martian atmosphere. The issue is especially worthy of consideration because if survival is possible during atmospheric transport, the designation of Special Regions becomes more difficult, or even irrelevant.

It also says that MEPAG only briefly considered the implications of our lack of knowledge about the potential for microhabitats within apparently uninhabitable regions (<u>Board, 2015</u> :<u>12</u>):

Physical and chemical conditions in microenvironments can be substantially different from those of larger scales. Although the SR-SAG2 report considered the microenvironment (Finding 3-10), the implications of the lack of knowledge about microscale conditions was only briefly considered.

The MEPAG review also has a long section on biofilms and the ability of microbes to modify microhabitats by surrounding themselves with "extrapolymeric substances" - proteins, polysaccharides, lipids, DNA and other molecules.

These EPS can modify the microhabitat and make it much more habitable for microbes and help them cope with stressors in the environment. (Board, 2015:11)

Also, MEPAG 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. MEPAG did NOT an attempt to explore possible locations for extant native martian life returned FROM Mars.

MEPAG 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.

Dirk Schulze Makuch et al suggested that native life on Mars might have evolved to use the cold brines on Mars with a novel cold adapted biochemistry, using perchlorates or hydrogen peroxide internally, in place of the chloride salts in our cells (Schulze-Makuch et al, 2010a).

The Mars surface also has many chaotropic agents which could reduce the minimum temperatures for cell division, including MgCl₂, CaCl₂, FeCl₃, FeCl₂, FeCl, LiCl, chlorate, and perchlorate salts (<u>Rummel et al., 2014</u>).

I cover this in more detail in my original paper (Walker, 2022b) under:

 How Martian life could make perchlorate brines habitable when they only have enough water activity for life at -70 °C – biofilms retaining water at higher temperatures - chaotropic agents permitting normal life processes at lower temperatures – and novel biochemistry for ultra low temperatures

[For 1st separate paper] 2015 MPEG2 review of the 2014 MEPAG report revises ALL its conclusions relevant to Jezero crater – and says maps made from orbit only provide information at the scale of the map - and

"can only represent the current (and incomplete) state of knowledge for a specific time"

The biggest change between on <u>(Rummel et al , 2014)</u> and <u>(Board, 2015)</u> was on the utility of maps to map out the special regions. The review board said that maps made from orbit can only provide information at the scale of the map and so are a generalization. They say that maps can only represent the current (and incomplete) state of knowledge for a specific time.

They say that this knowledge is subject to change as new information is obtained: (Board, 2015 : 28) (page number here links directly to the relevant page on the site).

Another potential source of misinterpretation related to the use of maps in Special Region studies is the issue of scale. Identification of a Special Region needs a multiscale approach (see also the discussion in Chapter 2, "Detectability of Potential Small Scale Microbial Habitats," and thus, as far as missions to Mars are concerned, conservatism demands that each landing ellipse be scrutinized on a case-by-case basis.

Maps, which come necessarily at a fixed scale, can only provide information at that scale and are, therefore, generalizations

•••

In general, the review committee contends that the use of maps to delineate regions with a lower or higher probability to host Special Regions is most useful if the maps are accompanied by cautionary remarks on their limitations. **Maps that illustrate the distribution of specific relevant landforms or other surface features can only represent the current (and incomplete) state of knowledge for a specific time knowledge that will certainly be subject to change or be updated as new information is obtained.**

In more detail, (<u>Board, 2015</u>:<u>12</u>) the temperature and humidity is only measured on large scales, and microhabitas can be substantially different physically and chemically.

The definition of Mars Special Regions is based on temperature and humidity conditions that are measured on spatial scales that do not reflect these conditions within microscale niches that can be potential habitats for microbial communities. Physical and chemical conditions in microenvironments can be substantially different from those of larger scales. Although the SR-SAG2 report considered the microenvironment (Finding 3-10),

the implications of the lack of knowledge about microscale conditions was only briefly considered.

There are many examples of these small-scale and microscale environments with microbial communities on Earth. The biofilm, a mix of many species of microbes, can make the conditions suitable for microbial propagation despite adverse and extreme surrounding conditions.

There are many examples of small-scale and microscale environments on Earth (see e.g., Lindsay and Brasier 2006) 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.

This is something that has become more obvious on Earth in recent years. We need a better understanding of this for Mars.

On Earth, the heterogeneity of microbial colonization in extreme environments has become more obvious in recent years (e.g., Azúa-Bustos et al. 2015). To identify Special Regions across the full range of spatial scales relevant to microorganisms, a better understanding of the temperature and water activity of potential microenvironments on Mars is necessary.

They give examples of microenvironments - craters, and even microenvironments underneath rocks could become special regions when the temperature and humidity on the larger landscape-scale doesn't permit terrestrial life to flourish on Mars.

For instance, the interior of the crater Lyot in the northern mid-latitude has been described as an optimal microenvironment with pressure and temperature conditions that could lead to the formation of liquid water solutions during periods of high obliquity (Dickson and Head 2009). Craters, and even microenvironments underneath and on the underside of rocks, could potentially provide favorable

[For 1st separate paper] 2015 review of the 2014 MEPAG report recommends further research into detectability of potential smallscale 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.

[For 1st separate paper] 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 (Board, 2015 : 46)

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 Mars-relevant 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.

[This paper] 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 was about transfer from Mars to its innermost moon Phobos instead of Earth - didn't look at sterilization during ejection from Mars - and specifically said that its conclusions should NOT be used for Mars Sample Return missions

Provisional title: NASA's draft EIS does NOT establish that life from Mars can get to Earth faster and better protected in a meteorite - or that ANY life on Mars can get here in this way

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.

But this directly contradicts their own cites. For instance the NRC in 2009 (Board et al, 2009: 48).

"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

And then later on the same page in their discussion of large scale effects

... Thus it is not appropriate to argue that the existence of martian meteorites on Earth negate the need to treat as potentially hazardous any samples returned from Mars by robotic spacecraft.

Their Sterlim cite makes a similar point on page 2, right near the start of the study (<u>Board, 2019</u>:2), it discusses how it would be much harder for life in Jezero crater to get to Earth on a meteorite. It's own conclusions are for life that has already been ejected from Mars. So it concludes:

Therefore, the committee finds that the content of this report and, specifically, the recommendations presented in it do not apply to future sample return missions from Mars itself

Yet NASA applies that very report to its own mission without mentioning that caveat.

This is used elsewhere in the draft EIS, for instance in the same section:

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.

They make the same argument in the MSR safety fact sheet for the Draft Environmental Impact Statement (<u>NASA, 2022msfs</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,

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 (NASEM, 1998:17)

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. First a minor point, but it illustrates the incorrect use of cites that we find throughout this document. 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 a **study on ejection of materials from Mars to its innermost moon Phobos, not to Earth.** The paper they cite **says explicitly that the team did NOT study sterilization during Mars ejecta formation in their analysis** (<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.

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

There are many cites that do cover this topic so it is somewhat bizarre that NASA would select a cite that specifically says it doesn't cover this topic. This is a minor issue however.

More importantly, their cite in its summary also says specifically that its recommendations should NOT be used for a Mars sample return missions, because the MSR sampling sites are specifically selected to maximise sampling of evidence of extinct or extant life, and might come from sites that cannot mechanically survive ejection (such as the dirt and dust): (<u>Board, 2019</u>:<u>2</u>)

Implications for Mars sample return—What implications for a Mars sample return (MSR) mission can be drawn from this study and the work of the JAXA and SterLim teams? The main differences between MSR and Phobos/Deimos sample return missions are as follows:

- MSR sampling sites will be specifically selected to maximize sampling of evidence of extinct or extant life, whereas materials deposited on the martian moons originates from randomly distributed crater impact sites.
- Martian material present in a Phobos/Deimos sample would have undergone several physical sterilization processes (e.g., excavation by impact, collision with Phobos, and exposure to radiation), before it is actually sampled. Material collected on the surface of Mars will not have undergone such processes.
- MSR material might come from sites that mechanically cannot survive ejection from Mars and thus any putative life-forms would de facto not be able to survive impact ejection and transport to space. Such mechanical limitations do not apply for material collected on Mars.

Therefore, the committee finds that the content of this report and, specifically, the recommendations presented in it do not apply to future sample return missions from Mars itself

This is a far more major issue. To expand on that issue raised by JAXA and Sterlim, the NASA EIS doesn't mention 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 section (below):

 <u>Could Martian life have got to Earth on meteorites (in more detail)? Our Martian</u> meteorites come from at least 3 meters below the surface in high altitude regions of <u>Mars</u>

One of the papers NASA's EIS cites, <u>(Fajardo-Cavazos et al, 2005)</u> 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.

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

NASA's draft EIS 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.

This is important for the large question of whether life from Mars has ever got to Earth and if so, when and how often.

Charles Cockell's paper (which they don't mention) said that though planetary exchange of photosynthesis might not be impossible - quite specific physical conditions and evolutionary adaptations are needed - and the fireball of reentry 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).

[GPa stands for Gigapascals, a unit of pressure equivalent to a billion pascals, and 1 GPa is a little under 10,000 times atmospheric pressure, or the pressure at a depth of 100 km of water. In this context it refers to a sudden increase of shock pressure which is harder to withstand than nstant pressure]

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

Here our attention should be on the species that are NOT able to get from Mars to Earth or not get here easily. As an example, 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>).



Text on graphic: 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

Didymosphenia geminatum invasive diatom in Great Lakes and New Zealand, can't even cross oceans.

Starling photo from: (Johnstone, 2017)

Barn swallow photo from (Batbander, 2017)

Didymosphenia geminata (Lyngb.) from (Schmidt, n.d.)

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).

It's not only birds. You might think microbes can surely all cross the Atlantic. But no. It's far harder for a microbe that can only survive in fresh water to cross an ocean. There are diatoms in the sea too, but diatoms can't survive long when dried out and fresh water diatoms can't survive in the sea.

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 general public refer to Didymosphenia geminate as "Didymo" so we will use the same word to refer to it here.

The long stalked version of Didymo 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.)

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 a shock pressure of 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.

JAXA team's analysis of their sample return from Phobos in 2029 used the natural contamination standard correctly and is a much simpler analysis than for NASA's mission since any viable microbes that reach Phobos already left Mars – though they explain that this does not apply to the Mars sample return study as we already mentioned

Japan also plan a Mars sample return from the Moon of Phobos in 2029. Since JAXA are not returning samples from the Martian surface their calculation is much simpler. Any microbes that get to Phobos have already got into rocks below the surface in the high dry southern uplands at the location of Zunil crater, and survived ejection from Mars.

As they themselves explain this approach does NOT apply to a sample return from the Martian surface see above

 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 was about transfer from Mars to its innermost moon Phobos instead of Earth - didn't look at sterilization during ejection from Mars - and specifically said that its conclusions should NOT be used for Mars Sample Return missions

There may be a minor error in JAXA's analysis but one that doesn't seem likely to change their conclusion. This is to do with how their analysis deals with the difference in heating between the surface and exterior of the rocks during re-entry.

They make a minor adjustment to survival %s due to this effect (Board, 2019 : 40)..

Next, Phobos organisms needed to survive impact on a solid surface at cosmic velocities. About one in 10⁴ microbes are expected to survive. Somewhere between 10 and 100 percent of the microbes in a sample survive passage through Earth's atmosphere. This survival rate comes from the numerical analysis undertaken by the JAXA team.

With an atmospheric entry velocity of ~5 km/s, the JAXA team used the same atmospheric sterilization model that it used for Mars and the same sterilization criterion

(i.e., heating to 773 K for 0.5 second) to show that meteorites >10 cm across suffer a survival rate of between 20 and 80 percent. This fraction is somewhat misleading, as parts of the Mars rock are strongly heated and sterilized, while other parts of the rock remain unheated and unscathed

So, they converted a survival rate of 20% to 80% into a survival rate of 10% to 100% because of the uneven heating of the rock.

However, this adjustment may not be enough to take account of the growing habits of photosynthetic life which leads it to colonize the surfaces of rocks or cracks that sunlight can penetrate which the plasma of the fireball of re-entry can also penetrate which can lead to a survival rate for photosynthetic life of 0%, as we saw above in:

<u>Charles Cockell's paper (which they don't mention) said that though planetary exchange of photosynthesis might not be impossible - 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</u>

However, it may not be a serious error in this case. As we'll see in the next section, it seems likely from our meteorite collections and modelling that rocks ejected from Mars for the last 20 million years come from at least 3 meters below the surface of Mars.

So, this doesn't seem to be a significant issue since photosynthetic life is highly unlikely at such depths, and if it does occur there, its growing patterns would be similar to other life at such depth.

The polyextremophile blue-green algae Chroococcidiopsis is able to grow in complete darkness using a hydrogen-based lithoautotrophic metabolism with viable populations found over 600 meters below the surface (<u>Puente-Sánchez et al, 2018</u>) and in another case 750 meters below the Atlantic sea bed (<u>Li et al, 2020</u>).

However even if a Mars analogue polyextremophile got into rocks by this method, for instance in a geothermal hot spot sampled by Zumil crater, its colonization patterns would resemble other microbes living in the same habitat. As it wouldn't be using light to grow, Cockell's argument that it would preferentially colonize the surfaces or cracks in ejected fragments of rock no longer applies.

So, the reasoning seems sound in this case, and this seems to be a minor omission which wouldn't change the assessment of a Phobos sample return as unrestricted Category V, i.e. an unrestricted sample return.

Could Martian life have got to Earth on meteorites (in more detail)? Our Martian meteorites come from at least 3 meters 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 sub-surface 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.

[2nd separate paper could start here] 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

Provisional title: NASA's draft EIS for samples from Mars does NOT overturn previous warnings of the potential for likely low risk of large scale harm for public health or the environment – in some scenarios life can NEVER be returned safely from Mars to Earth – in other scenarios Martian life doesn't exist, or is harmless or beneficial – we need to know which scenario we have in our solar system

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 (NASA, 2022eis: 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?

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

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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 any potential environmental effects would not be significant – i.e. that there is no significant risk of ANY environmental effects

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> <u>principle cause large scale changes to an Earth-like planet</u>
- ٠

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 (<u>NASA, 2022eis</u> :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 which doesn't answer the question

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

This and other answers in 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 engineering type answer which deflects from the question without answering it shows up issues with their failure to set up the mechanism to deal with public responses, as recommended by numerous sample return studies.

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

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.

Illustration of a more informative answer to this question from the general public 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 – the 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 provide this response, to help anyone who might read this document and panic and expect the worst. E.g. jump instantly to fear of human extinction. This section has been tested with scared people and they say that it helps. For instance for members of the public who are not very strongly grounded in science, it is important to supplement your answers with meaningful analogies that they can relate to.

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.

- Mars could be potentially habitable to life in some form or uninhabitable.
- Assuming Mars is potentially habitable to life in some form, the habitats could be inhabited or uninhabited
- If there is life it may survive the transfer back to Earth or not survive (as it is significantly different from Martian conditions)
- 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.

- It might be early life, at a similar level of evolution to terrestrial life or have evolved further to more complex genomes.
- It might be beneficial, or harmless or harmful.
- 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

- Related to Earth life
- Unrelated.

If unrelated it could be

- Same chirality
- 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 ½
- Inhabited 1/2
- Survives ¹/₂
- Can spread on Earth and can't be contained ½
- 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:

NRC 2009 report emphasizes that large scale effects can't be ruled out – it says
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 to the Great Oxygenation Event 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 (Walker, 2022b) 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

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 (Bahl et al, 2011) or from Sri Lankan reservoirs (Magana-Arachchi
et al, 2013) 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.

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 NASA's 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.

...

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.

However, this seems to be a minority view and this working group doesn't cite the many papers by experts warning of the potential for major issues.

There are many examples in the literature of experts warning that the argument used by the NASA's sterilizing subcommittee is not 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" (Meltzer, 2012):

"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.

In the same book he wrote (Sagan, 1973)

Because of the danger of back-contamination of Earth, I firmly believe that manned landings on Mars should be postponed until the beginning of the next century, after a vigorous program of unmanned Martian exobiology and terrestrial epidemiology. I reach this conclusion reluctantly. I, myself, would love to be involved in the first manned expedition to Mars. But an exhaustive program of unmanned biological exploration of Mars is necessary first.

I reach this conclusion reluctantly. I, myself, would love to be involved in the first manned expedition to Mars. But an exhaustive program of unmanned biological exploration of Mars is necessary first. **The likelihood that such pathogens exist is probably small, but we cannot take even a small risk with a billion lives.** Nevertheless, I believe that people will be treading the Martian surface near the beginning of the twentyfirst century.

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' "

The perception that microbial life from Mars would be safe for Earth seems to be a minority view amongst microbiologists. Here is a survey of 201 microbiologists attending a five-session colloquium "Prospecting for Extraterrestrial Microorganisms and the Origin of Life: An Exercise in Astrobiology" in 1998 (MacGregor et al, 2001)

89.5% said all sample returns should be considered hazardous until proven safe, 9% said they shouldn't be considered hazardous, statement, based on whether they agreed or strongly agreed versus those who disagreed or strongly disagreed with this statement:

"All materials brought to Earth from Mars should be considered hazardous until proven otherwise"

79.1% said our current understanding of microbiology is not sufficient to predict how life elsewhere in our solar system could impact on the environment of Earth. 18.4% said it is sufficient, based on this statement:

"Our current scientific understanding of microbiology is sufficient to predict with reasonable certainty how life elsewhere in our solar system, should it exist, could impact our environment if returned in samples to Earth"

22.9% said if there is life on Mars it poses no threat to life on our planet, while 34.4% disagreed or strongly disagreed with this statement?

"If there is life on Mars, it poses no threat to life on our planet."

I couldn't find detailed research into how an extraterrestrial biology would evade our immune responses so I've looked at it in a preliminary way myself.

First, let's look at fungi. Our body is protected by broad spectrum antimicrobials and then by specific immune responses such as the response to specific fungi that harm humans.

Let's look at some of these issues:

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

There are many simple examples that contradict the findings of the sterilizing subcommittee that martian life would be harmless because it hasn't adapted to human hosts, such as fungal pathogens of immunocompromised patients. 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, Candida, Aspergillus, and Cryptococcus (Kumar et al, 2018). We may

not have natural defences against unrelated genera of fungi from Mars. More on this in the next section.

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

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 (Baugh et al, 2017). 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 legionnaire's disease which is adapted to biofilms and amoebae - but uses the same methods to invade human lungs

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 <u>(Alberts et al 2002)</u>:

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.

Warmflash et al put it like this (Warmflash, 2007)

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.

Warmflash et al look at many potential pathogens of humans in the context of human astronauts on Mars. However their conclusion was that though it isn't risk free, we should accept the risks because in their view they are outweighed by the benefits of human exploration of Mars. They say we should try to limit the risks by biological containment on Mars and quarantine on return to Earth. (Warmflash, 2007).

Since the discovery and study of Martian life can have long-term benefits for humanity, the risk that Martian life might include pathogens should not be an obstacle to human exploration.

Since this potentially affects all humans on Earth in the worst case, this is surely a matter for wider discussion than colonization enthusiasts, whether we all wish to take these risks and are of the view that the benefits of humans on Mars outweigh the risks of pathogens returned from Mars.

As we saw, Carl Sagan was of the view we shouldn't take such risks, even if they are likely low, but should use unmanned missions to Mars to find out what is there first.

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 <u>(Frantseva et al, 2018)</u> 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 (<u>Goetz et al, 2016:247</u>).

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

There are numerous other examples of ways Martian life could harm Earth – plans for a future paper to expand on this in more detail using material from the larger preprint

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

I cover this in my preprint <u>(Walker, 2022b)</u> in these sections, some of which have been included in this paper:

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

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.

- Martian life might aid digestion or enter into other beneficial forms of symbiosis with humans
- 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).
- 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 (Schenk et al, 2008)
- Martian life might be accidentally toxic and control harmful microbes or insects
- 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. As an example of this, microalgae produce accidental hepatotoxins that can damage livers of cattle and dogs that eat algal mats, a common occurrence in the Great Lakes (Hoff et al, 2007). Such toxins wouldn't harm humans, since we don't eat the algal mats, but are harmful to other creatures. Similarly even a new strain of chroococcidiopsis that developed in the very different conditions of Mars with the cold conditions, UV and ionizing radiation might produce accidental toxins that the terrestrial strains don't produce and this would not be something one could decide from a gene sequence.

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 (Kleidon, 2002). 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 civilizations that return unsterilized uncontained samples from biospheres of neighbouring planet, it could be almost all benefit their home biosphere, all the way through to almost all harm their home biosphere – currently we have insufficient knowledge to decide

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 uncontained unsterilized sample return from a nearby independently evolved biosphere

There is no way to know.

Given the many examples we have seen of ways that independently evolved life could be harmful, the first possibility may seem implausible at first, that it's almost always a beneficial process - but we simply don't have the information needed to assign a probability.

There are many potential explanations that could make the first case more plausible. Moving from the ones that rely most to the ones rely least on terrestrial evolution being optimal:

• Perhaps terrestrial biology is optimal in ways we can't yet understand. Perhaps life throughout the universe always or almost always has the same mirror sense, the same genetic bases, the same amino acids, even the same codon table and almost identical genetic language, error correction for the DNA, same ribosomes, same semiochemicals, same metabolites, etc. Perhaps all life evolves chemically almost identical rhodopsin, carotene, chlorophyll etc. What seems to us to be haphazard may be optimal in some way we can't understand yet over all the other possibilities. Where terrestrial life has evolved multiple solutions such as the reason plants on Earth are green and early archaea are purple, perhaps this also happens identically in other star systems.

This is hardest to motivate. The hardest to explain step is the selection of ordinary over mirror life as it is hard to see why either should be preferred biologically – but there have been attempts to explain this by abiotic physical processes. Many other steps seem implausible such as the codon table, amino acids, etc. However, based on current knowledge, it is also likely impossible to assign a probability to this.

- Perhaps all life in our part of the universe, including neighbouring galaxies, is evolved from life that developed in the first few hundred million years at the time that the universe for a few tens of millions of years was universally at temperatures suitable for terrestrial life and after that it evolved the same way because terrestrial life is optimal
- Perhaps all the stars in our birth star cluster were seeded by an earlier star that passed through it and then evolved in the same way because terrestrial life is optimal from then on.
- Perhaps life develops the capability to transfer between planets early enough and this panspermia s frequent enough so that typically all the life in the same star system, or at least in the terrestrial planets of that star system is sufficiently synchronized to be compatible without major issues, and perhaps microbes that can't be transferred in this way evolve in compatible ways because terrestrial life is optimal from then on
- Perhaps all life in most of the universe or in our galaxy has been seeded by an early advanced civilizations that filled the galaxy with self replicating automated seeder robots that work to ensure all life in all planets evolves in the same way and is compatible with

each other, and this technology continues to operate, with the robots continuing to seed new forms of life every few million years in our solar system, possibly long after the originating civilization lost interest in the project and possibly maintained by the civilizations that are benefited by it.

- Perhaps planets evolve multiple different biochemistries, but there are underlying principles of biology that we don't yet understand that make those exobiologies almost always compatible and mutually beneficial when the biospheres collide, to the extent that harmful interactions almost never occur.
- It could be that almost always the other planets in star systems have only prebiotic chemistry, or life that is so early in development not even mirror life could compete on the home planet. This last solution requires no optimization of terrestrial life.

One way this could happen is if the early steps of evolution to the use of proteins, ribosomes and efficient fast replication rarely happen, and nearly all terrestrial planets have only abiotic organic chemistry, prebiotic synthesis, protocells, Woese's transformable cells with competition only at the level of the RNA and not at the cellular level,Ostwald's chrystals, naked RNA on clay, and other forms of early life or prebiotic chemistry that can't compete with more advanced life. If early life ribocells are possible, they may have doubling times of years even in warm conditions, and replication may be imperfect with many errors, with most daughter cells not surviving replication, making it hard for them to compete with modern life at all even with mirror biology

That still leaves the potential of life that spread from Earth to Mars and then evoled their independently since then. However we may have overestimated the potential for panspermia. Also, panspermia is far harder from a high gravity planet such as Earth to Mars, so it could also be that no life has got to Mars since the early solar system and early life on Earth wasn't capable of panspermia. That would be specific to civilizations in star systems where the other terrestrial planet became much harder to seed early in evolutionary history.

This is also the scenario with greatest risk for forward contamination. Mars is our only nearby terrestrial planet and our only planet where we may be able to study prebiotic synthesis, abiotic organic chemistry. It might be that forward contamination could erase modern active traces of all these things, leaving only the possibility of studying them in sealed caves or subglacial lakes cut off from connection with the surface, or entombed in gypsum or halite, or in subsurface organics maintained at -73 C, in which case some of it may be revivable but it would be difficult to near impossible to study the complexity of modern dynamic processes at a planetary scale after forward contamination as this would be erased.

In this scenario there is also some risk of accidentally reducing habitability of Mars to

humans in the forwards direction, for instance by inadvertently introducing methanogens that transform subsurface water reservoirs to calcite.

What we discover on Mars and in the oceans of Europa, Enceladus and so on may help us to investigate the various possibilities, but so far we simply lack the data to do this.

Likely need for some planetary protection if Mars does have a biosphere and has many species of life that could spread on Earth

By analogy with Didymo, if there is life on Mars, even closely related, though many species may be harmless or beneficial, we may need to protect Earth from some of them. For KNOWN risks, protection could vary from

- As for Didymo, astronauts need to be careful not to bring damp equipment back from Mars
- Astronauts need to wash thoroughly with soap and sterilize surfaces with disinfectant and UF or fumigate the spacecraft
- There may be human pathogens but if the quarantine period is less than 6 months they could be detected during the journey back and the crew put into isolation if found The six months is long enough even for diseases with quarantine periods of months such as rabies. However as Carl Sagan said, the latency period of leprosy is decades and then there's the issue of symptomless carriers like typhoid Mary

However, when we don't know what is on Mars we don't know how to protect Earth. The examples of mirror life, fungi of crops that are symptomless in humans, and initially slowly doubling cold loving psychrophiles show that in some cases there may simply be no way to protect Earth from the potential for life returned by astronauts.

So it is vital to find out what is on Mars first before we can intelligently devise procedures to protect Earth and our astronauts. It will then probably not be an easy task to evaluate the impact of species from Mars on Earth and its environment.

It will be far easier to prove an independently evolved lifeform unsafe than safe, and that's likely to be true even for a species that's closely related with some minor genetic differences until we know their implications, such as the example of a novel strain of chroococcidiopsis that we looked at above in <u>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</u>

[3rd separate paper could start here] 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

Provisional title: NASA's many mistakes in its EIS for samples from Mars have MAJOR public interest and legal implications – because of the need to recognize the potential for a likely low risk of large scale harm to public health or the environment from an unsterilized sample return done incorrectly

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 (NASA, 2020nebmsr). 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 (Rummel et al, 2002) and (Randolph, 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. (<u>Race, 1996</u>)

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 (<u>Uhran et al, 2019</u>),

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 (Race, 1996) 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)

- technical details
- mission requirements
- engineering details
- 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 – and any other countries that might do so - 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.

References (some quotations included to assist verification)

[Uses Harvard reference style, but in this draft, instead of a, b, c etc., I use unique ids like (NASA, 2020tesgs) - the idea is to search / replace these ids with a, b, c etc once the list is complete, after peer review]

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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)

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Andrew, R.G., 2019, <u>NASA's Curiosity Rover Finds Unexplained Oxygen on Mars</u>, Scientific American

On Earth, photosynthesis and respiration by living things cause tiny fluctuations in our planet's otherwise steady oxygen concentration. We shouldn't expect this on Mars, though. "That's far out," Telling says: Mars appears too inhospitable for a critical mass of life capable of sustaining either process. "It's almost certainly going to be a nonbiological chemical reaction."

Trainer herself does not rule out a biological explanation, but nevertheless underscores its unlikeliness. "People in the community like to say that it will be the explanation of last resort, because that would be so monumental," she says. There are abiotic mechanisms aplenty, both known and unknown, to rule out first before leaping to any more sensational claims.

Andrews, R.G., 2020, Rocks, Rockets and Robots: <u>The Plan to Bring Mars Down to Earth</u>, Scientific American

A single U.S. facility ticking all of these boxes could cost around \$500 million, Dreier says. And it is not yet clear if others will be built in Europe

...

MSR's masters are foregoing parachutes because the devices cannot be guaranteed to work, Vijendran says—something immortalized in 2004 by the solar-wind-particlegathering Genesis mission, whose sample capsule broke open after an unintentional hard landing. In this case, it is simpler to build a rigid capsule that can withstand such a landing. "It just comes in, and, wham, it hits the ground," Vago says. "That's going to be an interesting one."

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"In this White Paper we argue that it is not yet time to start down the MSR path. We have by no means exhausted our quiver of tools, and we do not yet know enough to intelligently select samples for possible return. In the best possible scenario, advanced instrumentation would identify biomarkers and define for us the nature of potential sample to be returned. In the worst scenario, we would mortgage the exploration program to return an arbitrary sample that proves to be as ambiguous with respect to the search for life as ALH84001."

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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 2016. <u>The cosmic zoo: the (near)</u> inevitability of the evolution of complex, macroscopic life. Life, 6(3), p.25.

Photosynthesis is primarily useful for providing energy for the reduction of environmental carbon ...

There are six known pathways for fixing carbon dioxide, of which the Calvin Cycle used in oxygenic phototrophs is the least efficient in terms of the energy and the reducing equivalents (electrons) required per mole of fixed CO_2 ...

However, the great advantage provided by oxygenesis was its capacity to liberate life from the need to find rare electron donors such as sulphide, hydrogen or Fe(II) to support the reduction of carbon dioxide, giving oxygenic photosynthesisers an advantage over all other forms of life ...

There are six known pathways for fixing atmospheric carbon, of which the Calvin Cycle used in oxygenic phototrophs is the least efficient in terms of the energy and the reducing equivalents (electrons)required per mole of fixed CO_2 . Rubisco has a very low turnover for fixing carbon, and its carboxylase activity is compromised by opposing oxygenase activity that uses molecular oxygen to break down Ribulose-1,5-bisphosphate rather than fix CO_2 into it. Despite this, the first inventor of water-splitting was successful, and filled the niche ...

Oxygenesis evolved only once. There are two possible explanations for this. One is that it is a Random Walk process, requiring a sequence of unlikely evolutionary steps, which would not have evolved elsewhere. The hypotheses on the origins of oxygenesis above hint this may not be the case, but do not prove it. The other explanation is that the evolution of oxygenesis is a Many Paths process, one which has a high probability of occurring, but is also a Pulling Up the Ladder event, such that once oxygenesis evolved once that evolution removed the preconditions for its evolution again, in this case filling the niche of a photosynthesiser freed from limitation of an electron donor supply. The biochemistry of oxygenic photosynthesis points toward this second explanation. Bandfield, J.L., Glotch, T.Dleast tens of thousands of years ... and possibly for hundreds of millions of years
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These small, spherical, lipid membrane-bound structures typically range in size from ~20 to 200 nm diameter and provide a means for cells to interact with their environment over both spatial and temporal scales

Perhaps one of the most striking features of extracellular vesicles is that they can contain nucleic acids (Dorward et al., 1989; Valadi et al., 2007; Rumbo et al., 2011; Biller et al., 2014). DNA fragments of diverse sizes, ranging from hundreds of bp to >20 kb have been reported in vesicles from Gram-negative bacteria, Gram-positive bacteria, archaea and eukaryotes, and include genomic, plasmid and viral DNA (Dorward and Garon, 1990; Klieve et al., 2005; Soler et al., 2008; Biller et al., 2014; Gaudin et al., 2014; Jiang et al., 2014; Grande et al., 2015; Yáñez-Mó et al., 2015). As such, vesicles can function as vehicles of horizontal gene exchange (Yaron et al., 2000; Renelli et al., 2004; Klieve et al., 2005). Shotgun sequencing of vesicle-associated DNA from ocean samples has revealed sequences from diverse bacteria, archaea and eukaryotes (Biller et al., 2014), suggesting that vesicles could be an important mechanism mediating gene transfer among marine microbes.

Billi, D., Staibano, C., Verseux, C., Fagliarone, C., Mosca, C., Baqué, M., Rabbow, E. and Rettberg, P., 2019a. <u>Dried biofilms of desert strains of Chroococcidiopsis survived prolonged</u> exposure to space and Mars-like conditions in low Earth orbit. Astrobiology, 19(8), pp.1008-1017.

Our results suggest that bacteria might indeed survive on Mars if shielded from UV, for instance by martian dust, since it is known that a few millimeters of soil is enough for UV protection (Mancinelli and Klovstad, 2000; Cockell and Raven, 2004). In view of the resistance of desert strain of Chroococcidiopsis to ionizing radiation (Billi et al., 2000; Verseux et al., 2017), the exposure in LEO to a total dose of 0.5 Gy of ionizing radiation did not affect biofilm survival. Hence, based on the dose of 76 mGy/year measured by the Curiosity rover at Gale Crater's surface (Hassler et al., 2013), dried biofilms would survive on Mars more than half a decade. In addition, since the UV dose received in LEO corresponds to approximately 8 h under a Mars UV flux at the equator (Cockell et al., 2000), the speculated biofilm survival supports the possible dissemination of viable

organisms. If carried, for instance, by winds at 5 m/sec (Gomez-Elvira et al., 2014) with the average flux mentioned above, they could travel more than 100km without dying. However, other factors found on Mars need to be taken into account so as to reduce the planetary protection risk, such as the presence of perchlorates that have been shown to be highly damaging to life (Wadsworth and Cockell, 2017)

Billi, D., Verseux, C., Fagliarone, C., Napoli, A., Baqué, M. and de Vera, J.P., 2019b. <u>A desert</u> cyanobacterium under simulated Mars-like conditions in low Earth orbit: implications for the habitability of Mars. *Astrobiology*, *19*(2), pp.158-169.

In this experiment, survival of the Chroococcidiopsis strain occurred only with those cells that were mixed with martian regolith simulant and plated as thin layers (about 15– $30 \mu m$, corresponding to 4–5 cell layers).

... Our finding suggests that a putative microbial life-form at least as resistant to desiccation and radiation as the investigated desert cyanobacterium could withstand some exposure to UV on the martian surface.

... Our findings support the hypothesis that opportunistic colonization of protected niches on Mars, such as in fissures, cracks, and microcaves in rocks or soil, could have enabled life to remain viable while being transported to a new habitat

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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."

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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..

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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.

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Section 3.3: In the Moon's formation temperatures of proto-Earth and progenitor impactor material in simulations grow to thousands of Kelvins, sufficient to drive off the great majority of all volatiles, but these are not necessarily the only masses in the system. Either body might have been orbited by satellites containing appreciable

volatiles, which would likely not be heated to a great degree and which would have had a significant probability of being incorporated into the final moon. Furthermore, there is recent discussion of significant water being delivered to Earth/Moon distances from the Sun in the minerals themselves (Lunine et al. 2007, Drake & Stimpfl 2007), and these remaining mineral-bound even at high temperatures up to 1000K (Stimpfl et al. 2007). The volume of surface water on Earth is at least 1.4×109 km3, so even if the specific abundance of lunar water is depleted to 10-6 terrestrial, one should still expect over 1010 tonnes endogenous to the Moon, and it is unclear that later differentiation would eliminate this. This residual quantity of water would be more than sufficient to concern us with the regolith seepage processes outlined above.

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<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.

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As Woese has written, "The ancestor cannot have been a particular organism, a single organismal lineage. It was communal, a loosely knit, diverse conglomeration of primitive cells that evolved as a unit, and it eventually developed to a stage where it broke into several distinct communities, which in their turn become the three primary lines of descent [bacteria, archaea and eukaryotes]." In other words, early cells, each having relatively few genes, differed in many ways. By swapping genes freely, they shared various of their talents with their contemporaries. Eventually this collection of eclectic and changeable cells coalesced into the three basic domains known today. These domains remain recognizable because much (though by no means all) of the gene transfer that occurs these days goes on within domains.

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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.

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Improved instrumentation on rovers that might detect and identify a diversity of potential in situ biosignatures, including ancient organic molecular biosignatures, designed with the ability to differentiate biotic and abiotic signals in micro- or macrostructures. Instrumentation could also be better attuned to the unique complications of biosignature preservation on Mars (e.g., deeper drilling to access potentially better preserved organics)

The fluorescence spectrometers on SHERLOC can detect condensed carbon and aromatic organics by deep UV-induced fluorescence, and SHERLOC's Raman spectrometer will allow classification of aromatic and aliphatic organics. Raman spectrometry can also be used to detect minerals relevant to aqueous chemistry. While these measurements would allow us to identify reduced carbon compounds, there may not be sufficient structural information to distinguish between a biological signal and extraterrestrial organic input.

A major knowledge gap that will directly impact our ability to choose an appropriate landing site is what terrestrial analog environments might look like—what the biosignature signals might be—if photosynthetic microorganisms had not evolved and instead the environments were only inhabited by chemosynthetic microorganisms

4.4. Strategies and priorities

In many of the environments discussed, there is a dichotomy between habitability and preservation—many of the conditions that make an environment more habitable are destructive to one or more of the biosignatures of interest. For example, fluid flow in the subsurface of hydrothermal environments helps create the redox gradients that support communities that inhabit the outflow channel. Fluids are also essential for lithification and the associated decrease in permeability essential for long-term preservation. Preservation is enhanced by rapid burial and mineral precipitation that encases and lithifies biological materials in less permeable matrices—in these cases, silica from hydrothermal environments, or silica-enriched aqueous environments, is an important material for preservation. However, these same fluids can degrade biosignatures such as mineralogy, chemistry, and micro- and macrostructures. One strategy for astrobiological exploration has to be to seek out a "sweet spot" where these two balance each other so that long-term preservation is possible. This sweet spot may occur as conditions change through time.

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As shown in Table 2, when a sample containing 100,000 spores was analyzed, either Bleach Rite® or 10% bleach was able to dramatically reduce (<0.0001% remaining) the number of viable spores at the earliest time point, and no viable spores were detected after 20 minutes of treatment. Complete sterilization was not attained until 20 minutes

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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

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On Spirit there is a yearly pattern with steady dust deposition throughout roughly the colder half year from late southern summer to late southern winter, which encompasses the Martian aphelion, and overall dust removal during the warmer and windier perihelion season from late southern winter to late southern summer.

On Opportunity ... the overall variation between highs and lows is smaller, and there are two periods of overall dust deposition and two periods of overall dust removal every year. The deposited dust thickness peaks once in the middle of the northern hemisphere spring. This peak recurs very regularly 6 times. ... There is also a peak roughly in the middle of the southern spring. This peak is clear in the first year, but the pattern becomes more irregular later in the mission and is entirely absent in the last year.

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LM: Pamela and I disagree on this, but there's a provision in the Outer Space Treaty, Article VI, which says that each country must supervise and authorize the activities of its nongovernmental entities. This is not a self-executing provision, and the U.S. Supreme Court has held that a non-self-executing treaty is not domestically enforceable. ...

PM: I disagree with Laura on this. Article VI of the Outer Space Treaty provides that all state parties to the treaty are responsible for their activities in outer space, whether they're carried out by government agencies or private companies. Countries are required to subject private companies within their jurisdiction that engage in space activities to an authorization requirement and continuing supervision. So, the United States is responsible for compliance with the Outer Space Treaty by our private companies or entities that go into space. Kleidon, A., 2002. Testing the effect of life on Earth's functioning: how Gaian is the Earth system?. *Climatic Change*, *52*(4), pp.383-389.

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The resistance of D. radiodurans is not exclusive to radiation and desiccation but extends also to many toxic chemicals and conditions. Therefore, Dra is called a polyextremophile, a robust "generalist," to be distinguished from specialized extremophiles with an evolutionary redesign of their proteome (e.g., proteins purified from thermophiles are thermostable in vitro). Unlike specialized extremophiles, Dra does not thrive on extreme conditions—indeed, it does not grow while desiccated or when heavily irradiated—but it can reproduce under standard growth conditions after recovering from damage inflicted by chronic moderate, or acute intense, exposures to cytotoxic conditions.

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(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).

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After 7 years of air-drying, Chroococcidiopsis not only avoided genome degradation but preserved at least a sub-set of mRNAs and 16S ribosomal RNA.

... In the present work, the occurrence of survivors in dried biofilms and dried-UVirradiated biofilms was proved by growth after transfer into liquid BG-11 medium (not shown) and by INT reduction after 72 h of rewetting.

Reshaping the boundaries of Chroococcidiopsis desiccation and UV tolerance has implications in the search for extra-terrestrial life since it contributes to defining the habitability of Mars and planets orbiting other stars. In fact, the UV dose used here corresponds to that of a few hours at Mars's equator (Cockell et al., 2000). Hence, considering that survivors occurred in the bottom layers of the biofilms (Baqué et al., 2013), it might be hypothesized that if a biofilm life form ever appeared during Mars's
climatic history, it might have been transported in a dried state under UV radiation, from niches that had become unfavorable to niches that were inhabitable (Westall et al., 2013). The reported survival also suggests that intense UV radiation fluxes would not prevent the presence of phototrophic biofilms or their colonizing of the landmass of other planets.

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3.4 Project Phase A: Concept and Technology Development

3.5 Project Phase B: Preliminary Design and Technology Completion

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Strict Containment and Handling Required

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Having a large light-harvesting antenna would allow photosynthesis and growth at light intensities that could not support the growth of algae with smaller antenna sizes optimized for growth at higher light intensities. In fact, algae that grow at extreme depths in the oceans have among the largest light-harvesting antenna sizes known in photosynthetic organisms (Yamazakiet al., 2005).

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A critical nutrient to the expansion of both subsurface and surface life on any planet is the availability of nitrogen as an aqueous species. On Earth, microorganisms evolved the ability to fix N2 into ammonia with the development of nitrogenase to overcome this constraint. Nitrogenases, Nif proteins, are complex enzymes, utilizing iron, molybdenum, and/or vanadium, that exist in both bacterial and archaeal domains. Phylogenetic comparison of genes that comprise nitrogenases and a complement of proteins required for their regulation indicate that nitrogenases emerged in anoxic sulfidic environments on Earth within obligate anaerobic thermophilic methanogens and were transferred to obligate anaerobic clostridia (Boyd et al., 2015), both common subsurface microorganisms. As Nif proteins were adopted first by the aerobic diazotrophic lineage Actinobacteria and then by the more recently evolved aerobic Proteobacterial and Cyanobacterial lineages, the Nif protein suite became more complex to protect the core MoFe-bearing proteins from O2 (Boyd et al., 2015). Although it is not clear whether the emergence of the more complex protein occurred prior to or after the Great Oxidation Event, it is certain that the ancestral protein emerged in an anoxic environment when the demands for aqueous nitrogen species exceeded the abiotic supply. The implications for martian ecosystems are that nitrogenase would have also likely emerged within an anaerobic subsurface environment, not in the oxic surface environment.

Experiments on the effects of low pN2 on diazotrophic nitrogen-fixing soil bacteria have shown that they could grow in N2 partial pressures of 5 mbar but not 1 mbar (Klingler et al., 1989). This result suggests that further experiments on wild-type species are required to determine whether the evolution of pN2 in the martian atmosphere was a significant deterrent to the expansion of early life, especially after Mars lost most of its atmosphere. Analyses of the nitrogen budget and of nitrogen cycling from deep subsurface environments in South Africa indicate that the pN2 is higher at depth than on the surface, that most of this N2 originates from the rock formations through nitrogen cycling, and that N2 is being actively fixed in the subsurface by microbial communities (Silver et al., 2012; Lau et al., 2016b). Given the presence of a cryosphere barrier to diffusion on Mars, the nitrogen availability and perhaps even the pN2 of subsurface brines are likely to be higher there than on the martian surface.

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Meter-sized Fe(II)-rich carbonate/iron oxide concretions (Fig. 4) are found in Jurassic sandstone deposits of southwest Colorado that were formed at hundreds of meters' depth between 2 and 0.5 Ma as the Colorado River Basin was uplifted (McBride et al., 2003; Loope et al., 2010). Similar-sized ferroan calcite and siderite concretions occur in Late Paleocene/Early Eocene Wasatch Group sandstones, and siderite nodule-bearing cores from the formation (Lorenz et al., 1996) yielded thermophilic Fe(III)-reducing bacteria that were capable of producing prodigious quantities of siderite (Roh et al., 2002). In subaqueous systems unconstrained by rock matrix, authigenic carbonate mounds at CH4 and hydrocarbon seeps, formed from carbon mobilized by methane- and alkane-oxidizing microorganisms (Greinert et al., 2001; Formolo et al., 2004; Ussler and Paull, 2008), can be hundreds of meters tall and more than a kilometer wide (Klaucke et al., 2008).

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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>.

Pages 94-5: Questions about the adequacy of the SRF to maintain the new life form must also be addressed, including the possible need to add equipment, change operations, review emergency plans, or upgrade the facilities because of what has been found.

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. The advisability of allowing distribution of untested sample material outside the *SRF2684* may need to be reconsidered, as well.

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. 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.

...

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. 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). 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.

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. 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).

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. 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.

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.

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

Rummel, J.D. and Conley, C.A., 2018. <u>Inadvertently Finding Earth Contamination on Mars</u> <u>Should Not Be a Priority for Anyone</u>. *Astrobiology*, *18*(2), pp.108-115.

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S

Sagan, C, 1961. Organic matter and the Moon., National Academy of Sciences.

<u>Page 23</u>: It is remarkable that the depth at which surviving lunar organic matter is expected to be localized (section II) is just the depth at which temperatures appear to be optimum for familiar organisms (section IV). At such temperatures and depths, some moisture should be expected, arising from meteoritic and organic bound water. Watson, Murray and Brown (1961) have recently pointed out that ice could have been retained on permanently shaded areas of the Moon. These circumstances provide all the survival requirements of many terrestrial organisms - water and their metabolites, appropriate temperature, and negligible radiation. That autochthons evolving with the changing environment could also survive under these conditions is far from inconceivable.

Sagan, C., Levinthal, E.C. and Lederberg, J., 1968. <u>Contamination of Mars</u>. *Science*, *159*(3820), pp.1191-1196.

"The prominent dust storms and high wind velocities previously referred to imply that aerial transport of contaminants will occur on Mars. While it is probably true that a single unshielded terrestrial microorganism on the Martian surface ... would rapidly be enervated and killed by the ultraviolet flux, ... The Martian surface material certainly contains a substantial fraction of ferric oxides, which are extremely strongly absorbing in the near ultraviolet. ... A terrestrial microorganism imbedded in such a particle can be shielded from ultraviolet light and still be transported about the planet."

..

"A single terrestrial microorganism reproducing as slowly as once a month on Mars would, in the absence of other ecological limitations, result in less than a decade in a microbial population of the Martian soil comparable to that of the Earth's. This is an example of heuristic interest only, but it does indicate that the errors in problems of planetary contamination may be extremely serious."

Sagan, C., 1973, The Cosmic Connection - an Extraterrestrial Perspective

I reach this conclusion reluctantly. I, myself, would love to be involved in the first manned expedition to Mars. But an exhaustive program of unmanned biological exploration of Mars is necessary first. **The likelihood that such pathogens exist is probably small, but we cannot take even a small risk with a billion lives.** Nevertheless, I believe that people will be treading the Martian surface near the beginning of the twentyfirst century.

Sagan, C., 1977. <u>Reducing greenhouses and the temperature history of Earth and Mars</u>. *Nature*, 269(5625), pp.224-226.

Sagan, C., 1980., *Cosmos: The Story of Cosmic Evolution, Science and Civilisation* full quote:

The surface area of Mars is exactly as large as the land area of the Earth. A thorough reconnaissance will clearly occupy us for centuries. But there will be a time when Mars is all explored; a time after robot aircraft have mapped it from aloft, a time after rovers have combed the surface, a time after samples have been returned safely to Earth, a time after human beings have walked the sands of Mars. What then? What shall we do with Mars?

There are so many examples of human misuse of the Earth that even phrasing this question chills me. If there is life on Mars, I believe we should do nothing with Mars. Mars then belongs to the Martians, even if the Martians are only microbes. The existence of an independent biology on a nearby planet is a treasure beyond assessing, and the preservation of that life must, I think, supersede any other possible use of Mars.

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Salisbury, F.B., Gitelson, J.I. and Lisovsky, G.M., 1997. <u>Bios-3: Siberian experiments in</u> <u>bioregenerative life support</u>. *BioScience*, *47*(9), pp.575-585.

Salvatore, J.O. and Ocampo, C.A., DirecTV Group Inc, 2000. <u>Free return lunar flyby transfer</u> method for geosynchronous satellites having multiple perilune stages. U.S. Patent 6,149,103.

See Table 1, final row, delta v 1230.6 m/s. This patent is based on the rescue mission for the HGS-1 geostationary satellite using a lunar flyby described in (Ocampo, 2005)

Sapkota, A, 2020, Citrate Utilization Test- Principle, Procedure, Results, Uses, Microbe Notes

Sarmiento, F., Peralta, R. and Blamey, J.M., 2015. <u>Cold and hot extremozymes: industrial</u> relevance and current trends. 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

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The observed carbonates co-occur with hydrated materials, gypsum, and potentially aqueously-formed phases, amorphous silicates and phosphate

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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

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HEPA Filtration is the "Best Available Control Technology" at 99.99 percent at 0.3micron efficiency level and is "Generally Accepted Control Technology" at 99.97 percent at 0.1-micron efficiency level. The added feature of the new 0.1-micron advanced filters is the "gel" seal and micro fiberglass construction that allows combining these filters with UV light disinfection. HEPA filters combined with charcoal and prefilters are the highest approved filters available for NIOSH-certified respirators.

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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

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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>

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The most prominent and conclusive organic biosignature observed is the presence of chlorophyll and carotene detected in the UV-VIS-NIR and Raman spectra in samples S3 and S4 ... However, apart from the carotene and chlorophyll absorption features below ~800 nm, there are no other indications of organic compounds observed in the reflectance spectra of any of the samples. While this most likely evidence of present endolithic life, the detection of such molecules may have implications for Mars as they have been shown to be somewhat stable under Martian surface conditions. However, this stability and preservation potential is dependent on their endolithic habitat, and so detection requires a fresh surface exposed by abrasion (e.g., RAT (rock abrasion tool)) or sample crushing.

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(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.

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