Space agencies must keep Earth safe from microbes in Martian dirt – a review of the planetary protection literature with new worst case mirror life scenarios

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Titles of sections are like mini-abstracts and summarize the details of the section. For a first overview of this paper read the section titles.

This preprint is not yet peer reviewed and is currently in the process of development. This preprint is not intended for widespread sharing at present. I plan to write a shorter self contained 7,000 word version of it next, as this version will likely take a while to complete peer review.

This preprint is uploaded here for convenience so that anyone commenting on it has access to the latest version at any time. I will upload any changes here in response to comments as I check it with the original authors of the research.

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Abstract

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In the late 2020s to 2030s, China, NASA / ESA and Japan plan to return samples from Mars. We need to keep Earth's biosphere safe from any Martian microbes. Japan's agency JAXA has the simplest mission, to return samples from the top few centimeters of Mars's innermost moon Phobos.

JAXA can safely return unsterilized samples without any precautions, because any microbes already withstood ejection from Mars, most recently, 700,000 years ago. They then experienced conditions on the surface of Phobos similar to conditions inside martian meteorites arriving at Earth today from that ancient impact.

JAXA warned their meteorite argument is not valid for surface samples never ejected from Mars. NASA's draft EIS incorrectly says any life from Jezero crater can get here faster and better protected in a meteorite than in a sample tube. Surface dirt and dust can't get here at all.

NASA's EIS also proposes to return its samples to a Biosafety Level 4 facility. However, the European Space Foundation study in 2012 set size limits well beyond capabilities of a BSL-4 and indeed beyond any current air filter capabilities.

We can avoid all these issues and keep Earth 100% safe by sterilizing samples before they get here. with the equivalent of a few hundred million years of Mars surface ionizing radiation. This has virtually no effect on geology, while Perseverance's forward contamination makes most astrobiology impossible.

We can greatly increase science value with bonus samples in a sterile container returned to a martian gravity centrifuge in an unmanned satellite above GEO, to start Sagan's "vigorous program of unmanned exobiology".

This is a short review of central results in planetary protection literature, with new worst case scenarios of mirror life, to encourage space agencies to ensure Earth's biosphere is adequately protected when they return samples from Mars.

Review of central results in the recent planetary protection literature for Mars Sample Return missions for attention of space agencies

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This review focuses on NASA's draft EIS only because it is the first environmental impact statement for a Mars sample return ever published. If NASA and ESA can make mistakes of this order, when they paid so much attention to planetary protection in the past, other space agencies could easily do the same.

For a list of the main issues found in the draft EIS see:

- Questions for NASA
- Reasons for these questions: controversial or mistaken statements in NASA's draft EIS and the report of the sterilizing subcommittee

It would be a major omission if this review didn't mention at least some of the many new findings since the last comprehensive Mars sample return study in 2009 (SSB, 2009).

- This paper frequently covers recent research findings – because if it didn't it would be 13 years out of date – however it is not itself a comprehensive review and shouldn't be used as such

It would be impossible to do a comprehensive review here, and instead the review focuses on material likely to be of especial interest to space agencies based on the mistakes in NASA's draft EIS.

- Scope of this review – material likely to be of especial interest to space agencies, based on mistakes in NASA's draft EIS – rather than any attempt at a comprehensive review

Sections or paragraphs are prefixed with the year of the research if they are:

- relevant to a Mars sample return from after the last major review in 2009.
- Relevant to forward contamination from after the last major study used by the EIS in 2014.

Some other material may be new to this paper, such as the new mirror life scenario, labelled NEW.

This paper is written to be maximally accessible. See:

- 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

The meteorite argument can't be used for potential life in surface dust, salts and dirt as these materials can't mechanically survive ejection from Mars

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Let's start with the meteorite argument. NASA's Environmental Impact Statement (EIS) argues that (NASA, 2022: 3-3):

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

However, the NRC Mars Sample Return study in 2009 said (SSB, 2009: 48)

> *The potential hazards posed for Earth by viable organisms surviving in samples [are] significantly greater with a Mars sample return than if the same organisms were brought to Earth via impact-mediated ejection from Mars*

The NRC goes on to say, in its 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.*

So, how did NASA's EIS come to such a different conclusion? It reasons that potential Mars microbes would be expected to survive ejection from Mars:

> First, potential Mars microbes would be expected to survive ejection forces and pressure (National Academies of Sciences, …, 2019), …

The background here is that the Japanese space agency JAXA plan to return samples from Mars' innermost moon Phobos. In their cite, JAXA conclude that any microbes ON PHOBOS would have already survived ejection from Mars because they had to, to get to Phobos.

However JAXA specifically say that this reasoning does ***not*** apply to Mars sample return missions because, amongst other reasons, any putative lifeforms in Mars samples might come from sites that mechanically can't survive ejection from Mars (SSB, 2019 : 4) saying:

> *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 [Mars Sample Return] 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~~ Phobos.*

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.

[The report has a typo as shown, it says “Mars” where “Phobos” was clearly intended.]

Martian surface dust, salts, and dirt couldn’t mechanically survive ejection, as they would burn up in the atmosphere before reaching escape velocity.

NASA’s EIS get their conclusion through mistaken citing, as they don’t mention this caveat, or the NRC statement.

For planetary protection, what matters are any species that can’t get to Earth – invasive starlings can’t fly across the Atlantic and the invasive freshwater diatom Didymo can’t cross the seas to New Zealand – similarly there could be species in Martian surface dust or dirt, perhaps living in a biofilm, that can’t get here in a meteorite

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NASA’s cites do say some very hardy terrestrial microbes such as b. subtilis might rarely survive transfer from Mars to Earth in a meteorite. However, this doesn’t prove all or even any martian life ever got to Earth from Mars, as we don’t know its capabilities, if it exists. European Barn swallows were in the Americas already, but European starlings are an invasive species in the USA which can’t fly across the Atlantic.

Starling photo from: (Johnstone, 2017)

Barn swallow photo from (Batbander, 2020)

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

For a microbial example, the invasive freshwater diatom "Didymo" (Didymosphenia geminatum) in New Zealand can't get from one freshwater lake to another on the same island without human help (Spaulding et al, 2010). It could never get from Mars to Earth.

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

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

Image from: (Thorney¿?. 2006)

Any diatoms on Mars, perhaps in the lakes beneath the polar ice (Davis, 2018), may well be unable to tolerate six months of vacuum or the extreme shock of ejection from Mars, and if so, likely evolved independently from terrestrial diatoms.

[2015, 2020] For an example of how we could encounter a similar situation on Mars, Curiosity found very cold brines which form in the late evening / early morning on the surface and 5 cm below the surface in Gale crater (Martin-Torres et al, 2015:fig 3b). Perseverance doesn't have Curiosity's Dynamic Albedo of Neutrons (DAN) instrument, so can't detect these brines in situ. But according to models these brines should be stable for even longer in Jezero crater than in Gale crater (Chevrier et al, 2020: figure 7).

[2015] The surface warms as the day approaches, and may get above 0°C, and as it warms these brines get warm enough for life but get too salty and then dry out completely. At other times they have enough water but are far too cold at -73°C for even the most cold tolerant Earth microbes to flourish, (Martin-Torres et al, 2015).

[2015] However, Nilton Renno suggested life could use biofilms to make these brines habitable (Pires, 2015), a strategy often used by terrestrial life to live in microhabitats in deserts (SSB, 2015 :11). In this way they could prevent the water from evaporating. They could also use various chemicals to help them function at lower temperatures and also grow very slowly with doubling times of millennia (Rummel et al , 2014:897).

[2015] The Curiosity brines were still found on the surface through to spring, see Martin-Torres et al, 2015:fig 3b). The last day in the year shown with surface brines (0 cm) also shows them through to 6 am, in the northern autumn, at solar longitude of Ls ~ 219° [the solar longitude specifies the position in the Martian year (NASA, n.d.)], By then, the surface at Gale crater in the middle of the day reached 288 °K = 15 °C (Martin-Torres et al, 2015:fig 2a). These are very warm conditions for Mars, for any microbes in the biofilms able to retain the water through to mid-day.

[2019] If Jezero crater does have biofilms like this, they might transfer in larger propagules, or perhaps as windblown fragments of a biofilm (Billi et al, 2019a), (Billi et al, 2019b), which could help start up a new biofilm faster. Also solitary microbial spores in these biofilms might transfer to other seeps, perhaps attached to a dust grain and shielded from UV in dust storms (Bak et al., 2019). They could spread like this even with only one spore or biofilm fragment succeeding every few millennia. This possibility is discussed below in:

- 2019: A thin (0.03 microns thick) fragment of desiccated biofilm of chroococcidiopsis would be still viable after blowing 100 km in moderate winds (5 meters per sec) in full Martian sunlight (and following sections)

These spores, propagules or biofilm fragments don’t need to be able to survive the journey to Earth in a meteorite to survive and propagate on Mars. They don’t need the capabilities to resist extreme shock, live below the surface of a rock, survive extended periods of vacuum, and so on. Also, living on the surface, they may never get into the rocks that are ejected to Earth. Similarly to Didymo, some or all putative martian species might have no way to get to Earth on a meteorite. For them, a sealed sample tube is like a miniature spaceship complete with dust, shielding from UV, and a small amount of martian atmosphere, much less desiccating than a vacuum.

It is safe for Japan to return unsterilized samples from Phobos without any special precautions because any life in samples from the most recent impact already survived ejection from Mars

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JAXA did establish it is safe to return samples from Phobos because they will have a similar history to the martian meteorites arriving today. (SSB, 2019 : 38 ff).

They compared two chains of events:

> Ejection from Mars → Impact on Phobos → Remains in top 10 cm of the Phobos surface for 800,000 years → returned to Earth in the Phobos sample return
>
> Ejection from Mars → spends 800,000 years in space traveling from Mars to Earth → reenters Earths atmosphere and delivered to Earth.

For all except the last in the chain of events the amount of sterilization is similar for the meteorites ejected from Mars and the returned samples. he ejection velocity is higher from Mars to Earth than from Mars to Phobos but a small percentage of the ejecta is only weakly shocked so this difference has a modest effect.

Then for the last step they found that any microbes from Mars would be far more sterilized as a result of the impact on Phobos than the reentry fireball of Earth because only the surface of the rock is heated

They estimate that about 100 kilograms of Martian meteorites arrives every year and that about 100,000 tons of material have been delivered to Earth from the Zumil impact in the last million years.

This is the backward contamination version of Greenberg's "Natural contamination standard". If Earth frequently encounters Martian life, we have no need to protect Earth with special precautions, more precisely (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."*

The main points in this argument are that:

1. Any material from Mars on the surface of Phobos already survived ejection from Mars.
   - Doesn't apply to a Mars sample return

2. Our martian meteorites last left Mars at least 700,000 years ago (ejection ages between 0.7 and 18.5 million years ago (Udry et al, 2020:table S4))

3. Any material they return from Phobos from that collision spent those 700,000 years on the surface of Phobos had a similar amount of ionizing radiation to our martian meteorites from the same collision
   - Doesn't apply to a Mars sample return (any viable life in the sample may have been growing somewhere on the surface of Mars not long before collection)

4. That leaves the fireball of re-entry to Earth's atmosphere but the heat of re-entry doesn't penetrate far into the meteorites arriving from Mars (and Phobos has the extra impact shock). The committee assumed a 10% survival of microbes (underestimating a likely 80 to 100% survival) (SSB, 2019 : 40).

There may be a slight omission here for photosynthetic life, since their 10% survival figure wouldn't apply for photosynthetic life that lives on or near the surface of rocks. In an experiment with a re-entry aeroshell Cockell found that not only Chroococcidiopsis but all associated organics were destroyed.

But if so, this is a relatively minor issue – because the Martian microbes come from at least 3 meters below the surface in the high Southern Uplands where photosynthetic life is unlikely, and if it is found there, would be using alternative metabolic pathways in darkness, so would still have no reason to live on the surface of rocks. The JAXA analysis seems correct with this minor tweak to account for photosynthetic life. The more eyes that look at these studies the better given how important it is to protect Earth's biosphere. For details see supplementary information:

- New: extending the JAXA analysis to photosynthetic life on or near the surface of any Martian meteorites

2015: Jezero crater seems uninhabited from orbit – but so do terrestrial Mars analogue deserts – the EIS doesn't cite the 2015 MEPAG review which overturned all the main conclusions from 2014 relevant to Jezero crater and drew attention to transport in dust storms, microhabitats, and biofilms that can make deserts locally more habitable

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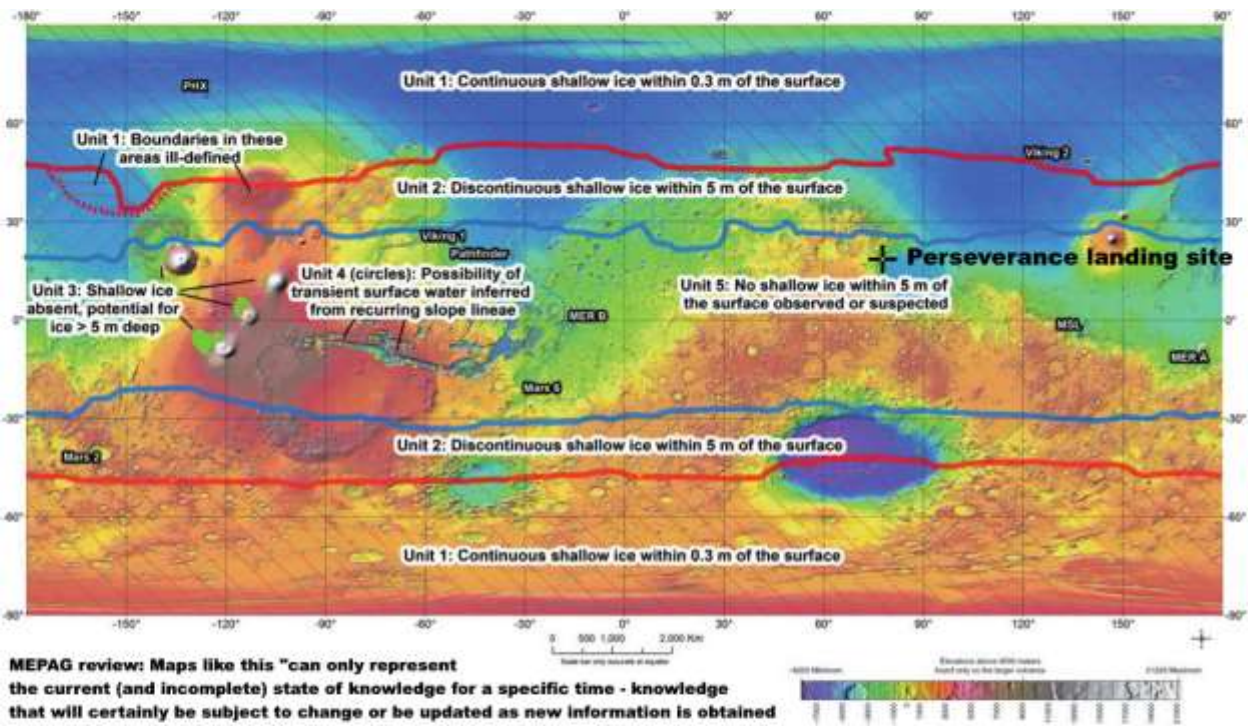
NASA say the Martian surface is especially inhospitable for life in Jezero crater, where Perseverance is collecting samples, arguing that the choice of landing site helps keep Earth safe, saying (NASA, 2022: S-4)

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).

NASA's draft EIS refers to SR-SAG2 (Rummel et al , 2014), but NASA and ESA commissioned a review in 2015, the MEPAG review (SSB, 2015 which modified all the main conclusions that NASA relies on for this statement about Jezero crater. NASA's draft EIS doesn't cite this review.

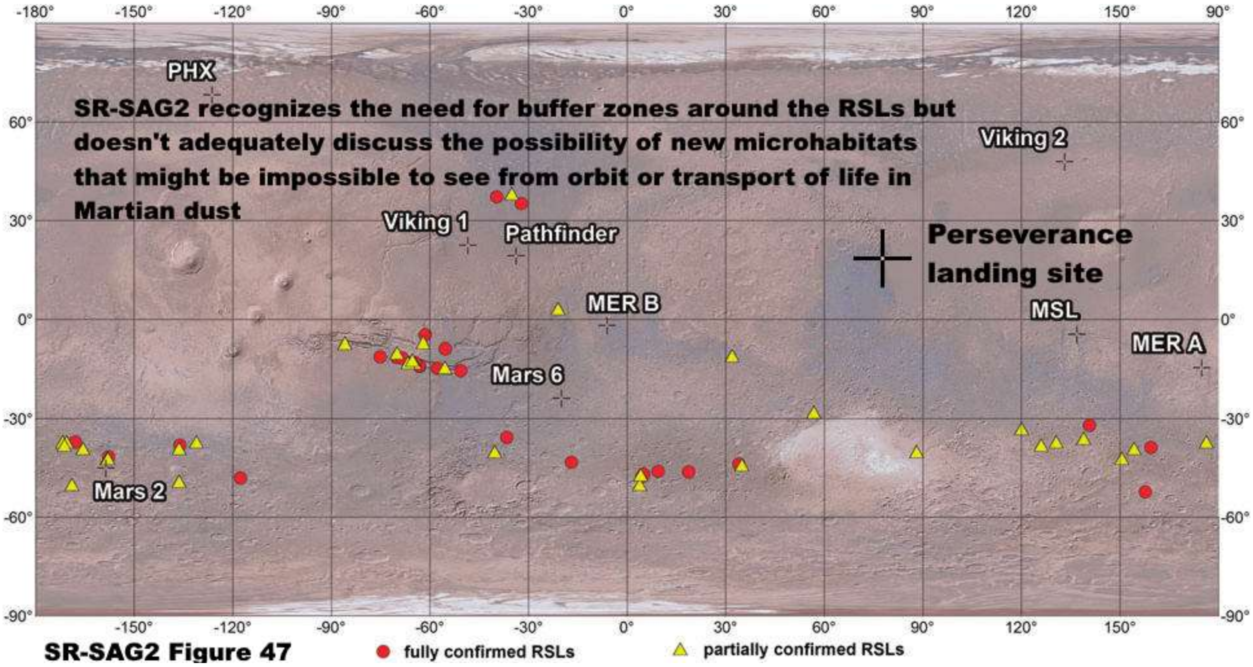
SR-SAG2 relies on maps like this. This map shows that the equatorial region of Mars including Perseverance's landing site in Jezero crater has no shallow ice observed or suspected



Source: (Rummel et al , 2014 : Fig. 45) Colour coding shows elevation. Perseverance landing site shown at 18.44°N 77.45°E (NASA, 2022)

It then maps out the RSLs. These are potentially habitable features that grow in spring, expand through the summer and fade away in the autumn on sun facing slopes on Mars (McEwen, 2011). Stillman suggests some may be explained by a wet dominated mechanism while numerous other sites are caused by dry granular flow (Stillman, E., 2018:81). The ones with a wet dominated mechanism might be habitable.

These can be detected from orbit and SR-SAG2 recognizes the need for buffer zones around them.



Source: (Rummel et al , 2014 : Fig. 47)

The 2014 report uses these maps to map out “Special regions” which are defined as regions (SSB, 2015 :6).

> “within which terrestrial organisms are likely to propagate, or a region which is interpreted to have a high potential for the existence of extant martian life forms.”

The 2015 MEPAG Review of this 2014 report says that such maps can only represent the current (and incomplete) state of knowledge for a specific time (SSB, 2015 :28).

> *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.*
>
> *…*
>
> *Identification of a Special Region needs a multiscale approach ... and thus, as far as missions to Mars are concerned, conservatism demands that each landing ellipse be scrutinized on a case-by-case basis.*

Jezero crater does seem uninhabited from orbit, but there are many ways it could have habitats not easy to detect from so far away.

Many potential microhabitats would be undetectable from orbit, for instance it wouldn't be possible to detect the micropores in the gypsum or salt pillars in the Atacama desert from orbit. It is hard even to detect fossil evidence of past microbial habitats from orbit.

[2018] To reliably detect potential evidence of past microbial habitats from orbit an orbital visible imager would need to have a resolution of 1 cm per pixel [This is feasible for Mars unlike Earth because of the thin atmosphere]. A resolution of 10 to 15 cms per pixel could identify bulk features consistent with a habitat but wouldn't be diagnostic (Cabrol, 2018).

The MEPAG review single out two ways terrestrial life could potentially contaminate Mars from regions that seem safe from these maps from orbit. First through dust transport and secondly, the desert might not be as uninhabitable as it seems from orbit because of microhabitats and because of the way microbes can use biofilms to inhabit regions that are otherwise uninhabitable.

Research since then backs that up their conclusions. Since there hasn't been a comprehensive forward contamination review since then, this paper looks at some of the highlights of the most recent research, as otherwise it would be 8 years out of date.

Then there are several other possibilities available to Martian life which they can safely ignore in these forward contamination studies, as they don't apply to the few microbes, short timescale and known biology of the terrestrial life on our rovers. Those haven't been looked at in a comprehensive review since 2009.

- Life on Mars may be adapted to conditions beyond the range of terrestrial life.
- Martian life has had time to colonize habitats that may take thousands of years to colonize. The SR-SAG2 had a limited time frame and said it didn't need to consider forward contamination that takes more than 500 years to get established (Rummel et al , 2014:894).
- Martian life could use fragments of biofilms blown in the winds to create its own microhabitat in a region that is otherwise inhospitable to life (Billi et al, 2019b). It could do that even if local conditions don't permit it to establish a biofilm by slowly growing from a few microbes today, so long as some time in the past biofilms were able to form, propagating ever since then using these broken off fragments (Mosca et al, 2019). The small numbers of microbes on spacecraft sent to Mars so far can't build up biofilms and the MEPAG Review says whether there are enough terrestrial microbes on a spacecraft to build up a biofilm is a central question in forward contamination (SSB, 2015 :11).

Then there is another possibility for both terrestrial and martian life that's been researched since the MEPAG review

- Microbes can be protected inside larger dust grains up to half a millimeter in size which can travel hundreds to thousands of kilometers by bouncing across the sand dunes a few meters at a time in the dust storms (Kok, 2010:4). (Bak et al., 2019).

All these factors mean that we can't use SR-SAG2 to exclude the possibility of extant martian life in Jezero crater. We will look at this in detail in the following sections:

- 2015: MEPAG2 review draws attention to potential for viable life transported through the atmosphere (for instance in dust storms)

    - 2019: A thin (0.03 microns thick) fragment of desiccated biofilm of chroococcidiopsis would be still viable after blowing 100 km in moderate winds (5 meters per sec) in full Martian sunlight
    - 2019: Curiosity found UV radiation fell by 97% at the start of the 2018 dust storm, which could increase Billi et al's 100 km to 1000s of kilometers in Martian dust storms
    - 2017: individual microbes can travel in dust storms imbedded in a dust grain for extra protection from UV
    - 2019: Microbes can be protected by bouncing sand grains up to half a millimeter in diameter traveling meters in each bounce, and some (less than 1 in 1000) b. subtilis spores remain viable after hundreds to thousands of kilometers of travel in simulation experiments
    - New: Martian life could evolve new strategies for dust storm transport such as spores with extra layers to protect against UV, and fruiting bodies for higher life that are detached by strong winds and may be better protected against UV than terrestrial life

- 2015: MEPAG2 review draws attention to potential for local microenvironments to provide habitats for life that can't be detected in large scale surveys

    - 2021: Potential for melting frost to form a "dew" of microns thick layers of fresh liquid water even in Jezero crater – as an example to show the potential for future surprise microhabitats

- 2015: MEPAG review also draws attention to biofilms that microbes may need to use to create conditions favourable to them in otherwise uninhabitable microniches – this reduces the risk for forward contamination for spacecraft with low bioloads – however this argument doesn't work for backwards contamination – such niches could be inhabited by Martian life that already lives in biofilms

    - Martian life could be more capable of coping with Martian conditions than terrestrial life
    - NEW: Life adjusted to Mars has had millions of years to set up biofilms – and slowly colonize microhabitats we may not yet know exist

- A Martian biofilm might consist of many species that evolved together to the local conditions over millions of years, similarly to the Atacama Desert grit crust [2020]
- 2016: NASA discovered potential for current habitats for terrestrial life in Gale crater AFTER Curiosity's landing

The general picture here is that research since 2015 would seem to support the idea that there is some potential for life in Jezero crater, either local life using biofilms or microhabitats or life brought there from distant regions of Mars in the dust storms.

If you wish to skip to the next main section it is:

- NASA's draft EIS argues that existing credible evidence suggests Mars has not been habitable to Earth life for millions of years — yet their cite for this sentence is about a search for current localized habitable regions on Mars – another conclusion reached through a citing error

In more detail on the potential for returning Martian life from Jezero crater:

2015: MEPAG2 review draws attention to potential for viable life transported through the atmosphere (for instance in dust storms)

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The MEPAG review says the SR-SAG2 report doesn't adequately discuss transport of material in the atmosphere (e.g. dust storms). (SSB, 2015 : 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."*

That last point is that if life can be transported from almost anywhere on Mars to almost anywhere it would be impossible to distinguish any particular regions as safe for forward contamination. They would all be regions (SSB, 2015 :6).:

> "within which terrestrial organisms are likely to propagate, or a region which is interpreted to have a high potential for the existence of extant martian life forms."

It highlights the potential for dust to attenuate the UV and for microbes to be protected growing as aggregates (SSB, 2015 : 12)

> *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 … and Asia …*
>
> …

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.

Both reports are about terrestrial life, for forward contamination. They don't examine the possibility of indigenous martian microbes better adapted to the Martian conditions than terrestrial life.

2019: A thin (0.03 microns thick) fragment of desiccated biofilm of chroococcidiopsis would be still viable after blowing 100 km in moderate winds (5 meters per sec) in full Martian sunlight

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In 2019, Billi et al found that a fragment of dried biofilm of the blue-green algae chroococcidiopsis mixed with regolith only 0.015 to 0.03 millimeters thick could survive 469 days of Mars surface UV in conditions of partial shade on Mars simulated using a 0.1% filter (Billi et al, 2019b). They calculate that this dose is equivalent to 8 hours of full sunlight on Mars, and that eight hours is enough time to transport the biofilm more than 100 km at typical wind speeds of 5 m/s, though they add that they only tested for the effects of UV and not the effects of perchlorates (Billi et al, 2019a).

Perchlorates in the dust may make this more challenging for terrestrial life because UV has been shown to reduce the survival times, at least for b. subtilis, perhaps by converting them to the more reactive chlorates and chlorites. However, this is not tested for polyextremophiles that may be more resilient (Wadsworth et al, 2017). Also, dust storms would reduce UV reducing this effect and increase the survival times for individual microbes. The biofilm mixed with the regolith would also shelter microbes within it from UV. Also, Martian microbes would have had the opportunity to evolve to become more resilient to the martian conditions.

Billi et al conclude Mars (Billi et al, 2019b).

… 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

In this way martian biofilms could hop from one microhabitat to another a few tens of kilometers at a time, similarly to the way desert nomads use oases to cross deserts.

2019: Curiosity found UV radiation fell by 97% at the start of the 2018 dust storm, which could increase Billi et al’s 100 km to 1000s of kilometers in Martian dust storms

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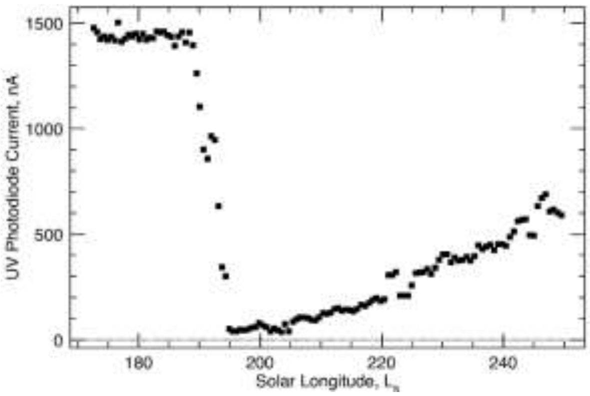
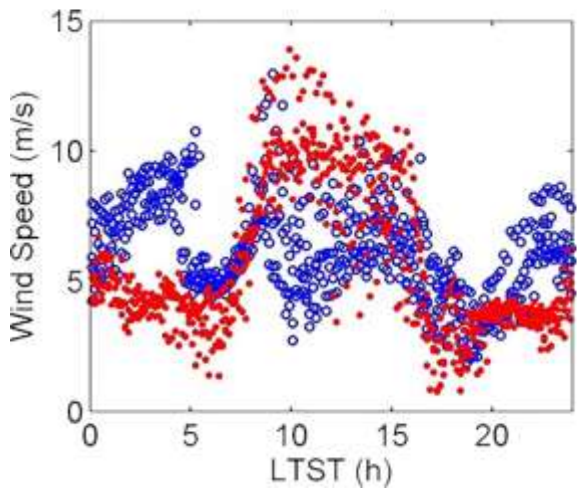
Occasional global dust storms cover much of Mars (Shirley, 2015). They typically start in the south, in the southern spring or summer, encircle the planet in southern latitudes then extend north across the equator. Curiosity was able to give direct observations of surface UV during the 2018 global dust storm, and found that it fell by 97% at the start of this storm, and remained at similar low levels for about three weeks (solar longitude 195 to 205) (Smith, 2019).



UV measurements by upward pointing photodiodes on the REMS instrument suite on Curiosity. The UV fell by 97% at the onset of the dust storm (Figure 5 of Smith, 2019)

Scaling up from Billi et al’s 100 km in 8 hours of full sunlight (Billi et al, 2019a), this suggests a single dust storm could transport biofilm fragments protected from UV for thousands of kilometers, similarly to the dust transport from the Gobi desert to Japan (Maki et al, 2019).

[New] Small biofilm fragments like these can continue moving at night too, when there is no UV radiation. Even during the dust storms, the wind speeds continue at night at 3-4 meters per second increasing to 10 meters per second or more in the day time – at least as measured from the Insight lander for the dust storm in 2019. Before the dust storms, the night wind speeds were above 5 meters per second for most of the night, increasing to a maximum of around 10 meters per second just before dawn at around 4 am. So there seems significant potential for transport of biofilms for large distances at night.



Blue dots show the wind speeds 5 days before the 2019 dust storm as measured from the Insight lander site, about 5 meters per second at night, increasing to 10 meters per second in the middle of the day.

Red dots show the wind speeds 5 days after onset which range from around 3 meters per second most of the night to above 10 meters per second in the middle of the day. (Viúdez-Moreiras et al., 2020: figure 5).

Mars doesn't need to have conditions to form biofilms from single cells today. Mosca et al suggest that if such a biofilm ever formed in Martian history, it could continue to be transported from niches that become unfavourable to more favourable niches on Mars through to the present. (Mosca et al, 2019). So, Mars could continue to have life propagated using biofilm fragments from the same ancestral biofilm, potentially even millions of years after the ancestral biofilm first formed.

This gives a way native evolved Martian life might be able to survive on Mars in conditions a single microbe or spore, Martian or terrestrial, could never colonize. This gives another way for Martian life to colonize apparently uninhabitable regions in Jezero crater.

2017: individual microbes can travel in dust storms imbedded in a dust grain for extra protection from UV

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Individual microbes might also be able to get transported in the dust including in storms. Microbes can get attached to dust grains in tests (van Heereveld et al, 2017) (Osman et al 2008). Sagan suggested a viable microorganism could be imbedded in a dust grain and be protected from the UV by the iron oxides in the dust (Sagan et al, 1968).

The protection from UV by the dust grain could also help with protection from the harmful effects of UV activated perchlorates along with the other factors discussed above in:

- 2019: A thin (0.03 microns thick) fragment of desiccated biofilm of chroococcidiopsis would be still viable after blowing 100 km in moderate winds (5 meters per sec) in full Martian sunlight

2019: Microbes can be protected by bouncing sand grains up to half a millimeter in diameter traveling meters in each bounce, and some (less than 1 in 1000) b. subtilis spores remain viable after hundreds to thousands of kilometers of travel in simulation experiments

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The Martian sand dunes have typical grain sizes of half a millimeter, or 500 microns. The Martian winds, far too weak in the thin atmosphere to suspend these grains, can still pick up these half millimeter diameter grains in a bouncing motion, called saltation.

Once the grains start bouncing, Mars's low gravity and lower vertical drag lets them travel higher and further with each bounce than on Earth. A strong wind can lift the grains a few meters horizontally with each bounce and lift them to a height of 10s of cms, (Kok, 2010:fig.3b).

There are two important wind speeds for this process. The wind needs to go above the fluid threshold to start grains bouncing, and after that it has to stay above the lower impact threshold to keep the bounces going after each impact. The impact threshold on Mars is approximately a tenth or less of the fluid threshold (Kok, 2010:fig.1). That's a much bigger difference than for Earth where the wind speeds can get fast enough to set the grains bouncing at only a little over the impact threshold, with a ratio of 0.82 for loose sand.

This means that on Mars, if a gust of wind just over the fluid threshold detaches a particle from the surface, it will continue to bounce across the dunes until the wind speed drops to below the much lower impact threshold (Kok, 2010). The upshot is that even though the winds are much weaker on Mars, with occasional gusts to get them started, dust grains can then move continuously in the winds on Mars much as they can on Earth (Kok, 2010).

> *The small ratio of the impact and fluid thresholds allows Martian saltation to occur for much lower wind speeds than previously thought possible. Indeed, once saltation is initiated by a localized wind gust, it will continue downwind until the wind speed falls by approximately an order of magnitude to a value below the impact threshold*

As an example, Proctor crater has typical wind speeds at only a third of the fluid threshold, however the instantaneous wind speed will occasionally exceed the fluid threshold and then the dust grains keep going until wind speeds falls to below the impact threshold which is far less than typical wind speeds in this crater (Kok, 2010:4).

So, once a dust grain on Mars starts bouncing it typically continues for a long time.

The bounces themselves can destroy spores, through mechanical stress. In one Mars simulation experiment, with spores of b. subtilis, in grains of Icelandic granite to simulate Martian granite, half of the spores were killed in a minute. These spores were completely destroyed (Bak et al., 2019).

However, Bak et al found some spores viable after days of bouncing. Perhaps these spores were protected inside cavities in the dust grains (Bak et al., 2019). Bak et al found the number of viable spores is reduced more than 1000 fold after 5 days of bounces (Bak et al., 2019:4). Based on the saltation particle speed of 3 to 10 meters per second for a particle of 400 microns in diameter (Kok, 2010:fig.3a), a propagule could travel 250 to 850 kilometers a day. So a similar propagule on Mars could travel 1,300 – 2,150 km in 5 days (rounded to nearest 50 km) before the numbers of viable spores are reduced a thousand-fold.

This suggests that though most b. subtilis spores would be killed in the bouncing martian dust, a small proportion of spores could be transported thousands of kilometers, and remain viable. Also, they only tested one species in the experiment. Their choice, b. subtilis was a reasonable Mars analogue organism for this experiment as it is highly resistant to radiation and oxidizing chemicals. However, spores of native martian life would have evolved to resist these bounces and might be able to survive for more than 5 days in these conditions.

New: Martian life could evolve new strategies for dust storm transport such as spores with extra layers to protect against UV, and fruiting bodies for higher life that are detached by strong winds and may be better protected against UV than terrestrial life

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This is a speculative section about possible adaptations of native Martian life to transport in dust storms. From the previous sections, especially Billi et al’s experiments (Billi et al, 2019a), (Billi et al, 2019b), it seems likely Martian life could propagate anyway as fragments of biofilms with similar capabilities to terrestrial life. However, after billions of years of evolution, martian life might well be better adapted than terrestrial life.

Terrestrial spores are already adapted for protection from UV. They encounter stronger UV high in Earth’s atmosphere and in mountainous and polar regions. Spores are protected from reactive chemicals by multiple coat and crust layers (Cortesão et al, 2019). This makes them far more resistant to oxidizing agents, bactericidal agents, chlorites, hypochlorites etc than vegetative cells (Sella et al, 2014). Spores are also more UV resistant than vegetative cells. Some terrestrial spores can withstand many hours of UV radiation on Mars, including one strain still viable after 28 hours of simulated direct UV radiation in Mars simulation surface conditions (Galletta et al, 2010). Adapted to such extreme conditions, Martian spores could have more layers of protection than terrestrial spores and resist UV for even longer than those 28 hours.

Martian life might develop colonial ways of surviving in dust storms in the larger clusters or aggregates of microbes like the terrestrial analogues described by the MEPAG review (SSB, 2015 :12). Martian microbial life could have evolved larger bacterial fruiting bodies similarly to those of the myxobacteria. These have some bacteria that altruistically develop into non reproductive cells to protect the spores inside (Muñoz-Dorado et al, 2016).

Multicellular martian life could reproduce by fragmentation in dust storms, similarly to fungi reproducing through hyphal fragments or red macroalgae (rhodophyta) which often propagate using multicellular propagules. These propagules would be fragments of the parent plant, a vegetative multicellular structure, that breaks off from the parent thallus and gives rise to a new individual (Cecere et al, 2011). The evolution could begin with fragments broken off in winds due to the impact splashes of the half millmeter sand grains bouncing on the dunes of the previous section and the natural movements of the dunes. Bacteria might evolve strategies to create propagules extended above the surface that detach in response to stronger winds in dust storms or dust devils.

Combining this with the results of the previous section, a propagule half a mm in diameter could potentially be transported great distances in dust storms by the saltation bounces. Of course there is no way to know if Martian life has evolved such a method of propagation but it suggests a possibility for increased survival over terrestrial life for native life adapted to martian conditions.

A half millimeter diameter propagule, or 500 microns in diameter, typical in size for saltation on Mars (Kok, 2010:fig.3b) can contain many spores. At a maximal packing density of $\frac{\pi}{3\sqrt{2}} \approx 0.74048$, which is the densest packing density possible for congruent spheres (Hales, 1998) (Hales et al, 2017), it can contain $\frac{\pi}{3\sqrt{2}} \times \frac{4}{3}\pi \times 250^3 = \frac{\pi^2 \times \sqrt{2} \times 250^3}{9}$= 24. 2 million spherical spores or microbes in dormant state at 1 micron diameter.

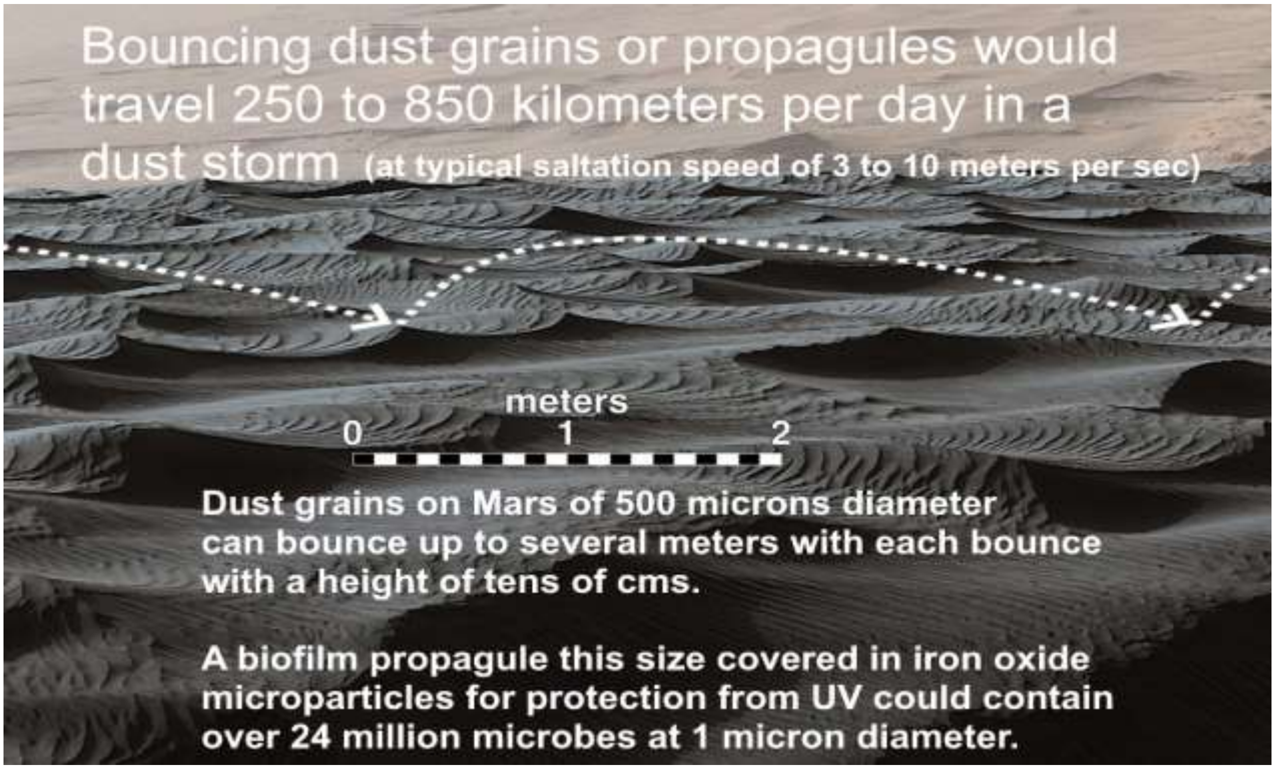

Figure 32: Bouncing dust grains or propagules would travel 250 to 850 kilometers per day in a dust storm (at typical saltation speed of 3 to 10 meters per sec).

Dust grains on Mars of 500 microns diameter can bounce up to several meters with each bounce with a height of tens of cms. A biofilm propagule this size covered in iron oxide microparticles for protection from UV could contain over 24 million microbes at 1 micron diameter.

Artist's impression of a typical bounce based on figure 2b from (Almeida et al, 2020) superimposed on photograph of the top of a large sand dune taken by Curiosity on December 23, 2015 (NASA, 2016)

As with the biofilm fragments, such comparatively large propagules could help martian life to get off to a head start in an environment where a single microbe might not be able to survive.

Any fruiting bodies would have an evolutionary advantage if they evolved extra protection against UV, perhaps an agglutinated external cyst of iron oxides to protect themselves from UV held together by secreted organics similarly to the external cysts of foraminifera (Heinz et al, 2005).

Speculating further, Martian life might also perhaps evolve specialized biomaterials with much stronger protective layers than iron oxide to protect themselves from both UV and damage from impacts during the saltation bounces. To take one very speculative example [new]: chitin is an essential component of the cell walls of fungi and the fungal component of lichens (Lenardon et al, 2010). The same material is also used in insect exoskeletons and jaws. Chitin has a Mohs hardness of 7 – 7.5 (Zhang et al, 2020) similar to quartz (King, n.d.). Chitin nanofibers have a Young's modulus of elasticity of more than 150 GPa (Vincent et al, 2004), higher than copper or titanium alloy and not far below wrought iron or steel (Engineering ToolBox, 2003). A protective chitin coating could do a lot to protect a bouncing propagule from damage during saltation bounces.

2015: MEPAG2 review draws attention to potential for local microenvironments to provide habitats for life that can't be detected in large scale surveys

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The 2014 report considers microenvironments in (Rummel et al , 2014:904).

> ***Finding 3-10:*** *Determining the continuity/heterogeneity of microscale conditions over time and space is a major challenge to interpreting when and where Special Regions occur on Mars.*

It then gives a list of seven naturally occurring microenvironments on Mars:

- **Vapor-phase water available** Vapor or aerosols in planet's atmosphere; within soil cavities, porous rocks, etc.; within or beneath spacecraft or spacecraft debris
- **Ice-related** Liquid or vapor-phase water coming off frost, solid ice, regolith or subsurface ice crystals, glaciers
- **Brine-related** Liquid water in deliquescing salts, in channels within ice, on the surface of ice, within salt crystals within halite or other types of ''rock salt''
- **Aqueous films on rock or soil grains** Liquid water on regolith particles of their components such as clay minerals, on surface of ice, on and within rocks, on surfaces of spacecraft
- **Groundwater and thermal springs** (macroenvironments) Liquid water
- **Places receiving periodic condensation or dew** Liquid water on regolith particles of their components such as clay minerals, on surface of ice, on and within rocks, on surfaces of spacecraft
- **Water in minerals** Liquid water bound to minerals

The 2015 MEPAG review says that though SR-SAG2 considered these microenvironments it only briefly considered the implications of our lack of knowledge of them (SSB, 2015 :12).

> *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.*
>
> *…*
>
> *Craters, and even microenvironments underneath and on the underside of rocks, could potentially provide favorable conditions for the establishment of life on Mars, potentially leading to the recognition of Special Regions where landscape-scale temperature and humidity conditions would not enable it.*
>
> *…*
>
> *The review committee agrees with Finding 3-10 of the SR-SAG2 report but stresses the significance of the microenvironment and the role it might play on the definition of a Special Region in areas that (macroscopically speaking) would not be considered as such.*

Also (SSB, 2015 :28).

> *Identification of a Special Region needs a multiscale approach ... 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*

To give an example here, relevant to Jezero crater, microbes can use spontaneous capillary condensation of water vapour in micropores in salt deposits in deserts when the air is otherwise too dry (Vítek et al, 2010) (Wierzchos, 2012). Cassie Conley (Conley, 2016) and, separately, Paul Davies (Davies, 2014) have suggested these micropores as potential habitats on present day Mars.

Microbes can use micropores in gypsum too. In one study of the hyperarid core of the Atacama desert, microbes imbibed water when the external humidity was above 60% and gradually became more desiccated when it was below that (Wierzchos et al, 2011: figure 1).
Jezero crater doesn't have the large bright salt deposits of Mount Sharp (Lerner, 2019), but micropores in a small deposit could still be a potential microhabitat for a martian microbe.

Any martian life would have millions of years to find these habitats and colonize them. Then let's look at one other suggestion to illustrate the potential for future surprises as we find out more about Mars.

2021: Potential for melting frost to form a "dew" of microns thick layers of fresh liquid water even in Jezero crater – as an example to show the potential for future surprise microhabitats

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This idea fits one of the examples from S-SRAG2, liquid water from melting frost (Rummel et al , 2014:904):

- Ice-related **Liquid** or vapor-phase **water coming off frost,** solid ice, regolith or subsurface ice crystals, glaciers

Fresh water is stable against freezing and boiling over 29% of the surface of Mars, but it is not stable against evaporation because the partial pressure of water vapour in the Martian atmosphere is two orders of magnitude too low (Martinez et al., 2013:2). However fresh water could form temporarily in special conditions, if there is some buffering of the water vapour. This could happen after rapid melting of ice, if it melts faster than the evaporation rate. This may be possible, on: (Martinez et al., 2013:2.1).

> ***"slopes facing the sun, under clear sky and calm wind conditions, at locations with low surface albedo and low soil conductivity"***

However there is another way that liquid water could form as the frost melts. Mars gets a temperature inversion of warm air over cold air, which prevents convec tion and can trap the water vapor close to the surface as the frost melts.

The Viking 2 lander (NASA, 1997) and Phoenix lander (NASA, 2008) both imaged daytime frosts on the surface. The other rovers haven't photographed them but there are estimates that a few tens of microns of frost could have formed in Gale crater at night (Martínez et al., 2013). That's enough to be useful water for a microbe as it melts.

There is possible direct detection of frosts in Gale crater a few microns thick (Gough et al., 2020). Perseverance is currently attempting to detect frost in Jezero crater using Laser Induced Breakdown Spectroscopy (LIBS) and Raman spectroscopy of nearby rocks which they have named Snowy Mountain and Red Mountain, as well as by using the microphone to detect the sound of the LIBS laser "zap" (NASA, n.d.).

> *If frost exists on the Snowy Mountain target, we should detect hydrogen in the LIBS spectra and O-H bonds in the Raman spectra in greater quantities than in the Red Mountain spectra. We're also listening for a soft acoustic signal in the first LIBS shot of Snowy Mountain which could indicate a frost layer as thin as ~10 microns.*

This idea goes back to Gilbert Levin and his son Ron Levin, who suggested a cool humid layer could be trapped near the surface as dawn approaches, in a temperature inversion, overlain by a layer of warm air. This might lead to thin films of water that form briefly in the early morning then evaporate. Chris McKay, agrees that this process could form a layer of liquid though it may not last long (Abe, 2001).

The frost would form at night, and melt in daytime, but the temperature inversion would trap the moisture long enough for it to survive evaporation for up to several hours.

Experimental work shows that this is a plausible scenario. This experiment simulated conditions in Gale crater (Ramachandran et al, 2021)

Our experiments show how a pool of water is formed and remains stable for about 3.5 to 4.5 h while evaporating and releasing water to the dry atmosphere.

These experiments simulate evaporation under wind-free conditions. This scenario is not unrealistic as, according to REMS observations, the night-time to sunrise winds may be very mild with speeds under 2 m/s

There is another way that Mars could surprise us with a discovery of present day fresh liquid water at 0 °C, which could be relevant for microbes transported in the dust storms from much further away. Most of the meltwater in Antarctica actually forms due to the solid state greenhouse effect. Ice and snow is optically transparent and traps heat, so it tends to melt a layer of liquid water about half a meter below the surface where the balance of the amount of light received and the thermal insulation is optimal.

The same process on Mars would melt liquid water at a depth of about 5 cms even with surface temperatures on Mars as low as 180 °K (-93 °C). This habitat should exist on sun facing slopes in polar regions in summer, if Mars has snow or ice with similar optical and thermal properties to the Antarctic snow and ice, and there is no particular reason to suppose it wouldn't be. See below:

- 2009, 2014: Possible future surprise discovery of very terrestrial conditions on Mars: most Antarctic meltwater is melted inside the ice through the solid state greenhouse effect when the surface is far below 0 °C – so long as Mars has similarly optically transparent snow and ice, its ice caps should also have large amounts of internal fresh meltwater at 0 °C, forming even when the surface is colder than -90 °C – and it could also have miniature melt ponds around sun warmed dust grains

2015: MEPAG review also draws attention to biofilms that microbes may need to use to create conditions favourable to them in otherwise uninhabitable microniches – this reduces the risk for forward contamination for spacecraft with low bioloads – however this argument doesn't work for backwards contamination – such niches could be inhabited by Martian life that already lives in biofilms

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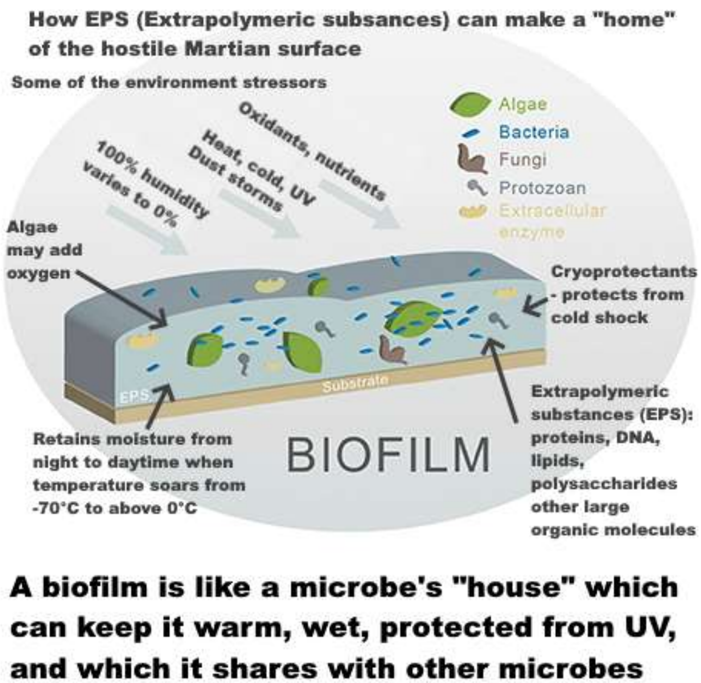
The 2015 MEPAG review also discusses how microbes in biofilms modify microhabitats by surrounding themselves with "extrapolymeric substances" – proteins, polysaccharides, lipids, DNA and other molecules. These can make microenvironments far more habitable for microbes and help them cope with environmental stressors (SSB, 2015 :11)



Graphic adapted from figure 2 of (Sabater et al., 2016)

Microbes in biofilms can use those extrapolymetric substances (EPS) to inhabit ecological niches that would otherwise be uninhabitable (SSB, 2015 :11)

> *The majority of known microbial communities on Earth are able to produce EPS, and the protection provided by this matrix enlarges their physical and chemical limits for metabolic processes and replication. EPS also enhances their tolerance to simultaneously occurring multiple stressors and enables the occupation of otherwise uninhabitable ecological niches in the microscale and macroscale.*

This helps with planetary protection in the forward direction, as a spacecraft from Earth may need to carry enough terrestrial life with them to Mars to establish a biofilm (SSB, 2015 :11)

> *Given the wide distribution and advantages that communities of organisms have when they live as biofilms enmeshed in copious amounts of EPS, it is likely that any microbial stowaways that could survive the trip to Mars would need to develop biofilms to be able to establish themselves in clement microenvironments in Special Regions so that they could grow and replicate.*

> *So when asking if spacecraft could be sources of forward contamination on Mars a central question is whether the spacecraft has enough terrestrial life on it to be able to establish a biofilm on Mars. It's not about the species only but about how many microbes*

there are to establish a "beachhead" on the martian surface for terrestrial life to start growing there.

However this is a study of forwards contamination and any native Martian life may have already established this beachhead millions of years ago.

Martian life could be more capable of coping with Martian conditions than terrestrial life

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The MEPAG and MEPAG review studied forwards contamination, so didn't look at potentially more capable martian life. Any life on Mars has had billions of years to evolve to survive transfer better in dust storms or to adapt to colder temperatures.

Martian life might also use novel biochemistry (Schulze-Makuch et al, 2010a) (Houtkooper et al, 2006), or use the abundant martian "chaotropic agents" such as the perchlorates, which speed up a cell's chemical processes at low temperatures and can reduce the lowest temperatures for cell division for many microbial species (Rummel et al , 2014:897).

NEW: Life adjusted to Mars has had millions of years to set up biofilms – and slowly colonize microhabitats we may not yet know exist

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Also in such very cold conditions, the Martian surface life may colonize very slowly. There is evidence species of lichens and mosses are still in the process of recolonizing Antarctica since the last ice age, with species diversity dependent on distance from the nearest geothermally active sites that provided refuges during the ice ages (Fraser et al, 2014).

We need to consider microhabitats that might take many millennia to slowly colonize in the very cold martian conditions as well as that idea of present day biofilms spread via fragments that originated from biofilms that first formed in slightly more habitable conditions a few million years ago (Mosca et al, 2019).

SR-SAG2 had a 500 year time-frame for forward contamination. They didn't consider processes that could lead to colonization over millennia. (Rummel et al , 2014:894) *(Sun et al, 1999)*..

> *The actual low temperature limits of terrestrial organisms are currently unknown, primarily due to technological constraints of detecting extremely low rates of metabolism and cell division. But even if the actual low temperature limits of terrestrial organisms are lower than the currently known empirically deetermined limits, the actual limits may not be relevant to defining Special Regions for the given 500-year time frame because cell division and metabolism would be so slow.*

For example, cryptoendolithic microbial communities of the Antarctic Dry Valleys (where temperatures rarely exceed 0°C) successfully invade and colonize sandstones over 1000 to 10,000 years (Sun and Friedmann, 1999). Therefore, we examined the currently known empirically determined limits of cell division and metabolism at low temperatures and did not consider theoretical limits or extrapolations based on current knowledge [edited 10^3 to 10^4 to 1000 to 10,000]

Martian life has had millions of years to colonize any potential microhabitats and billions of years of evolution to develop species and biofilms adapted to the Martian conditions.

We can't know that Jezero crater is uninhabitable everywhere for martian life without detailed study looking for:

- Biofilms that might modify conditions in Jezero crater to make it habitable to martian life, such as Nilton Renno's biofilms (Pires, 2015)
- Fragments of biofilms or native propagules in the dust
- Life in the micropores in salt deposits suggested by (Conley, 2016) and, separately, Paul Davies (Davies, 2014)
- Life in other potential local habitats and microhabitats,
- Searching with an open mind with versatile instruments so that we can find microhabitats in situ that could be new to science, and potentially inhabited by a novel exobiology.

Mars may have many surprises for us still, as we saw with the melting frost example.

- 2021: Potential for melting frost to form a "dew" of microns thick layers of fresh liquid water even in Jezero crater – as an example to show the potential for future surprise microhabitats

Whether or not we find that particular microhabitat, we could get many future surprises once we send instruments to Mars better able to detect microhabitats like this, and search them for life.

A Martian biofilm might consist of many species that evolved together to the local conditions over millions of years, similarly to the Atacama Desert grit crust [2020]

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[2020] The Atacama grit crust got its name because it grows around and within small grit sized pebbles about 6 mm in diameter, turning them black over hundreds of square kilometers of the desert (Jung et al, 2022). Biocrusts are common in deserts but this particular biocrust has interesting special adaptations relevant to Mars. It is tolerant to high UV, and adapted to rapid changes of temperature and humidity. It can photosynthesize with the lowest amount of water known for any such community worldwide (Jung et al, 2022). Also, though frequent wetting and drying is normally lethal for similar communities, it causes no problem for the grit crust which can respond rapidly to fogs that blow in over the desert (Jung et al, 2020).

It is made up of a mix of blue-green algae (similar to chroococcidiopsis), green algae such as chlorella, black rock inhabiting fungi, lichens such as Pleopsidium chlorophanum and other microbes. The climate in the Atacama desert has been stable for at least 150 million years giving time for the Atacama gritcrust to evolve to adapt to it. (Jung et al, 2022).

The Atacama grit crust has been considered as a possible pioneer biofilm for preparing extraterrestrial soils for human colonization (Jung et al, 2022). Perhaps the likely evolution of the gritcrust over 150 million years may also be a analogue for a native Martian biofilm if such exists.

An analogous biofilm on Mars would have had billions of years to adapt to the current Martian conditions as the planet slowly became less habitable, with the species that are able to work together for greater resilience forming the most successful biofilm colonizers.

That would be especially so for Mosca's idea of a biofilm that is no longer able to propagate as individual microbes colonizing one by one, but can only establish a foothold as biofilm fragments (Mosca et al, 2019). The most successful biofilm fragments would include organisms that cooperate well with each other for mutual support.

The lichen Pleopsidium chlorophanum, one of the lichens found in the grit crust, is one of the top candidates for a terrestrial lichen that might be able to survive on Mars. See:

- 2014: Example of an alpine lichen Pleopsidium chlorophanum found in places like California and the Alps that also grows in Mars analogue conditions in Antarctica and can survive and even grow in Mars simulation conditions – this shows even higher life from Mars could be adapted to live on Earth

In addition to the lichen Pleopsidium Chloraphpanum, there are many black rock inhabiting fungi that may be able to survive on Mars, and some blue-green algae, especially chroococcidiopsis, so a Martian biofilm potentially might also have close analogues to the components of the Atacama gritcrust.

- Several candidate terrestrial microbes and even higher organisms such as lichens may be able to survive on Mars, with promising results in Mars simulation chambers, suggesting a possibility that their Mars analogues may be able to live on Earth

2010: Martian life could inhabit caves that vent to the surface – and there are many types of cave that can only be detected by in situ observation unlike the easier to detect lava tube skylights already known about

SR-SAG2 and the MEPAG review both discuss caves and agree that there is potential for forward contamination of them. They are treated as (SSB, 2015 : 24)

> SR-SAG2 Finding 4-11: On Earth, special geomorphic regions such as caves can provide radically different environments from the immediately overlying surface environments providing enhanced levels of environmental protection for potential contaminating organisms. The extent of such geomorphic regions on Mars and their enhancement (if any) of habitability are currently unknown.

MEPAG Review looks at the potential for life to survive in such caves writing:

> Although their number and sizes are largely unknown, caves and other subsurface cavities on Mars would represent environments with ambient conditions (e.g., temperature, humidity, exposure to radiation) that are very different from those at the surface, and most probably, those conditions are likely to be favorable for microbial colonization. Consideration of caves and subsurface cavities is paramount for two reasons. First, they provide a protected environment (e.g., from extremely low temperatures and radiation). Second, they can provide a means by which terrestrial contamination can access martian subsurface environments.
>
> …
>
> In conclusion, there could be a number of possible primary sources of the necessary ingredients for life inside caves and subsurface cavities on Mars, and therefore, they are best classified as Uncertain Regions and treated as Special Regions until proven otherwise.

For backwards contamination we need to consider the possibility that such caves are already inhabited by Martian life and that life from the caves could get into the samples via dust transport.

The 2009 NRC review didn't look at caves – there is no occurrence of the search term in a search of the report. (SSB, 2009: cave)

Penelope Boston lists these as some of the types of cave possible on Mars, many of these could be connected to the surface (Boston, 2010):

1. **Solutional caves** (e.g. on Earth, caves in limestone and other materials that can be dissolved, either through acid, or water)
2. **Melt caves** (e.g. lava tubes and glacier caves)
3. **Fracture caves** (e.g. due to faulting)
4. **Erosional cave**s (e.g. wind scoured caves, and coastal caves eroded by the sea)
5. **Suffosional caves** - a rare type of cave on the Earth, where fine particles are moved by water, leaving the larger particles behind - so the rock does not dissolve, just the fine particles are removed.

She points out a few processes that may be unique to Mars. Amongst many other ideas she suggests:

1. **For the solutional caves**, the abundance of sulfur on Mars may make sulfuric acid caves more common than they are on Mars. There's also the possibility of liquid CO2 (which forms under pressure, at depth, e.g. in a cliff wall) forming caves.
2. **For the melt caves**, then the lava tubes on Mars are far larger than the ones on the Earth.
3. **Mars could have sublimational caves** caused by dry ice and ordinary ice subliming directly into the atmosphere.

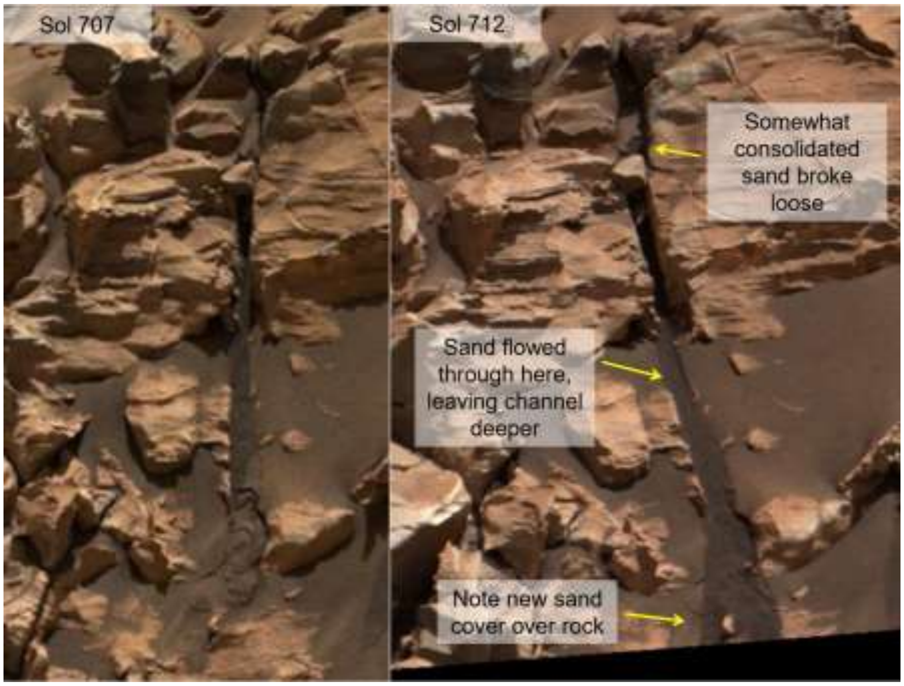
She talks in more depth about the possibility of sulfuric acid caves on Mars and suggests the methane plumes found by Curiosity could come from subsurface caves in (Boston et al, 2006)

The solutional cave and rock fracture caves are particularly challenging to detect from orbit. (Boston et al, 2006). For backwards contamination what matters most is the potential for nearby undetected caves that might be able to spread Martian microbes to the samples in dust storms or dust devils.

On Earth many caves are not easy to find as with the example of the Lascaux cave paintings. Ravidat's dog Robot got entangled in a toppled Juniper tree and he discovered the hole that lead to the cave as a result of going to his dog's rescue. The toppled Juniper revealed the hole (Eshleman et al, 2008).

A concealed cave on Mars might be revealed as a result of a slump of sand. The cave entrances also would be hard to detect remotely. Many caves on Earth can only be seen if you walk right up to them.

This shows a sand slump detected by Curiosity



Slide 15 from (Vasaveda, 2015)

A slump like that could reveal an entrance to a previously unknown cave. In addition, a large subsurface cave with only a small entrance to the surface could exist in complex terrain such as shown in that photo and often found in Jezero crater or Gale crater. It could be undetected not only from orbit but also undetected by the rover or even marscopters.

A new review on back contamination risks for a Mars sample return updating (SSB, 2009) would surely have an extensive section discussing the potential for back contamination from caves similarly to the sections for forward contamination in SR-SAG2 which has over 900 words on it (Rummel et al , 2014:920-21) and the MEPAG review which has over 700 words on it (SSB, 2015 : 24).

2016: NASA discovered potential for current habitats for terrestrial life in Gale crater AFTER Curiosity's landing

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We have an example already of how our knowledge of a landing site can change after a rover lands. Like Perseverance, Curiosity is not sufficiently sterilized to visit regions where terrestrial life could spread. NASA thought Gale Crater had no risk for forward contamination. But then they discovered potential habitats for terrestrial life in Gale Crater after Curiosity's landing (JPL, 2016).

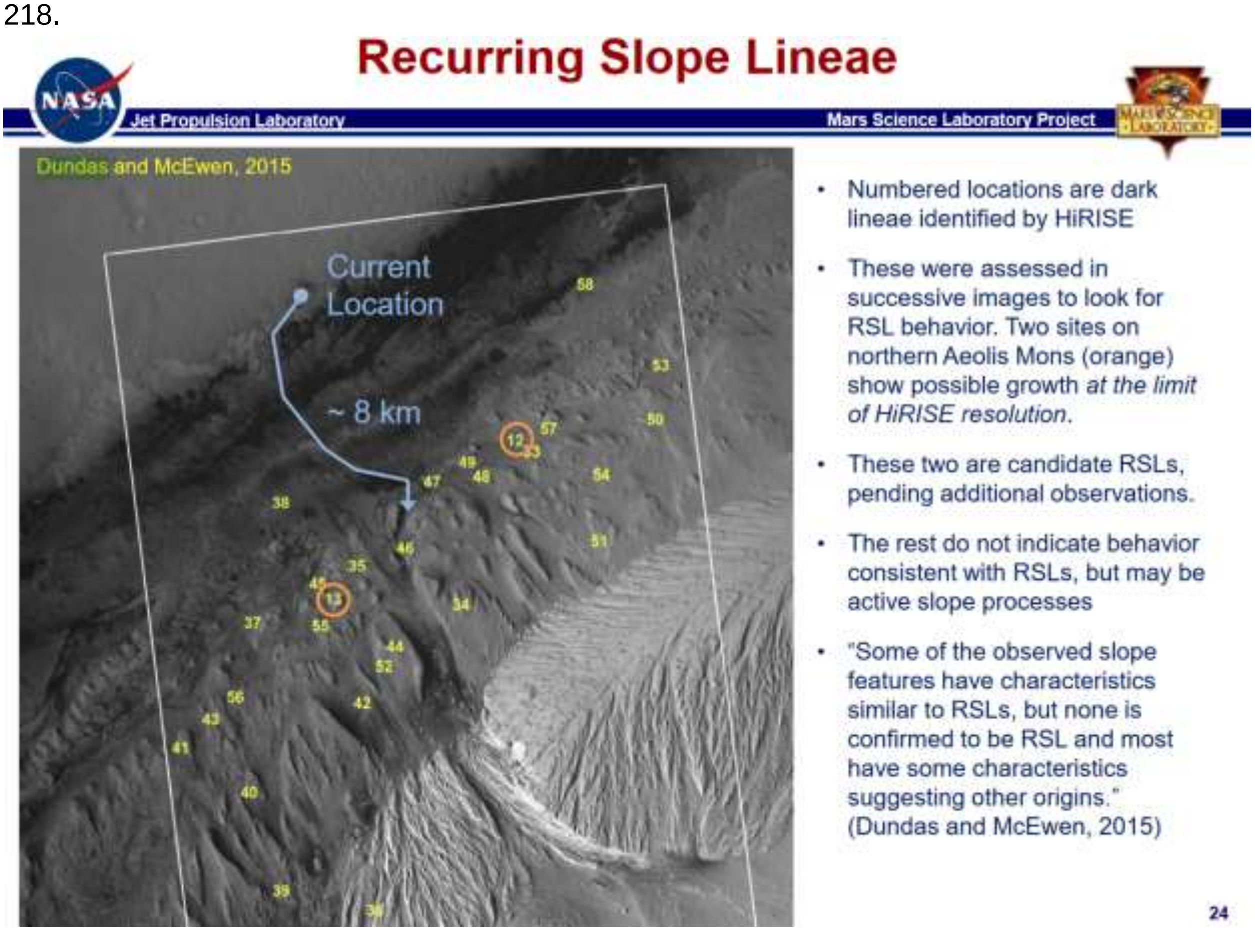
These may be the RSLs, features that grow in spring, expand through the summer and fade away in the autumn on sun facing slopes on Mars (McEwen, 2011). There are two models for them, dry formation by dust flows and a wet formation by brine seeps. Neither model explains them fully. Stillman suggested in 2018 that the ones that score highest are explained by a wet dominated mechanism while numerous other sites are caused by dry granular flow (Stillman, E., 2018:81). In 2021 Stillman et al continued to find problems with all the models they looked at but said that the model where sediment is brought to the top of the RSL externally, perhaps by saltation (bouncing dust grains) is the best model (Stillman et al., 2021):

> *Nonetheless, we suggest that the mixed success of the external sediment transport model is still quantitatively better than any competitor (including water), and that we simply lack the model and data resolution to treat RSL at the required meter scales.*

Although the features close to the rover were ambiguous and not definitely RSLs, mission planners were concerned that Curiosity was not sufficiently sterilized to approach them because of the risk of forward contamination by terrestrial life, in case terrestrial life might be able to inhabit them. After discussion they made a tentative decision that it could approach within a couple of kilometers to image them but not study close up (Witze, 2016).

Of numerous candidates, only two were considered to resemble RSLs sufficiently for concern (Dundas et al., 2015) (Vasaveda, 2015).

Dundas, C.M. and McEwen, A.S., 2015. Slope activity in Gale crater, Mars. *Icarus*, *254*, pp.213-218.



Slide 24 from (Vasaveda, 2015)

Curiosity is currently exploring the region of the possible RSLs but hasn't approached either of those candidates (NASA, n.d.).

Recent research favours the dry explanations more than the wet explanations, for the RSLs. For instance Mars analogue wet streaks in the McMurdo dry valleys fade over multiple years (Toner et al, 2022). The RSLs on Mars became more active after the 2018 global dust storm and though this is also consistent with a wet mechanism supplemented by dust, the observations seem more consistent with a dust mechanism (McEwen et al, 2021). The apparent connection with hydrated salts now seems an artefact of the data processing. The RSLs are found in locations not consistent with groundwater discharge such as the peaks of mountains. Frosts and deliquescing salts can't supply enough water to be the main mechanism. They may be caused by dust and bouncing sand grains and triggered by the dust devils, most of which don't produce tracks detectable from orbit (McEwen et al, 2021).

Even so, none of the models fully explain the RSLs, and the questions can't be regarded as resolved yet. We don't have the surface resolution yet to be able to investigate them in detail and we can't directly test for humidity as the effects on the atmosphere would be too small to detect with current capabilities (Kurokawa et al., 2022).

NASA's draft EIS argues existing credible evidence suggests Mars has not been habitable to Earth life for millions of years – yet their most recent cite for this sentence is about searches for currently habitable environments on Mars – another conclusion reached through a citing error

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Another central argument in NASA's draft EIS is that Mars is lifeless anyway. The draft EIS says (NASA, 2022: 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 (… National Research Council 2022).

Yet their most recent source for this sentence is about searches for currently habitable environments on Mars! (Smith et al, 2022: 393)

> Section title: "Are There Chemical, Morphological and / or Physiologic / Metabolic or Other Biosignatures in **Currently Habitable Environments** in the Solar System
>
> The exploration of … Mars (Curiosity, Perseverance) will help establish whether localised habitable regions **currently exist** within these seemingly uninhabitable worlds.

[Emphasis on “currently” mine]

(cited by NASA’s EIS as National Research Council 2022)

Once more, as for the meteorite argument, NASA got to this conclusion through a citing error.

It’s a surprising error given NASA itself was involved in extensive discussions about whether to divert Curiosity away from potential current habitats for terrestrial life in Gale crater (JPL, 2016) as we saw in the previous section . Although, as we saw, the RSLs are looking increasingly unlikely to be candidates for habitats themselves, this of course doesn’t mean that the entirety of Mars is uninhabitable for terrestrial life, never mind uninhabitable for potentially better adapted indigenous martian life. We don’t yet know even that the RSLs are uninhabitable. It just means that RSLs are less favoured as candidates for habitats on Mars than previously. It also won’t exclude any of the many other candidate habitats if the RSLs turn out not to be habitable.

However, Curiosity continues to take precautions in case the RSLs are habitable for terrestrial life, which makes this statement in NASA’s draft EIS puzzling especially as it doesn’t even mention the RSLs or any of the other candidate Mars surface habitats and microhabitats.

Astrobiologists have a range of views on whether current habitats exist on Mars – sometimes revising their assessments after discoveries suggesting new microhabitats on Mars or new ways that life can grow in extreme conditions

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Astrobiologists have a wide range of views, but most of the published statements are on the lines that current habitats suitable for terrestrial life likely do exist on Mars. Many say habitats are more likely underground, in the deep subsurface or in caves, but others say there could be microhabitats for life over much of the surface of Mars.

It’s a separate question whether any habitats if they exist could be inhabited already by native martian life. There again we have a range of views, all the way to a few who think we may have detected the effects of life already with the Viking labelled release experiment in the 1970s. A significant number of astrobiologists think there is a realistic possibility that Mars has native microbial life today (Carrier et al, 2020:804).

Astrobiologists often revise their assessments about the possibility of habitats on Mars after they make discoveries for new potential habitats or ways that life could survive on Mars.

Rummel and Conley, both former planetary protection officers for NASA, put it like this: (Rummel et al , 2014)

> *"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."

[2014] Renno and his team used the Michigan Mars Environmental Chamber (Fischer et al., 2013) to simulate droplets similar to the ones on the Phoenix lander' legs and found they formed within a few tens of minutes at -50 °C when salt overlaid ice (Fischer et al., 2014). After they achieved this, Renno said (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.

These are the droplets they simulated

Possible droplets on the legs of the Phoenix lander – they appeared to merge and sometimes fall off. In this sequence of frames, the rightmost of the two droplets – highlighted in green on this black and white image – grows and seems to do so by taking up the water from its companion to the left, which shrinks (Gronstall, 2014)

Renno doesn't go into details but though the temperature of the droplets in their experiment is very cold at -50 °C (Fischer et al., 2014), it is not as cold as the Curiosity brines, and as with the Curiosity brines it's possible life could exploit them with biofilms, and also grow at lower temperatures using the chaotropic agents which speed up some biochemical reactions, etc (Rummel et al , 2014:897).

[2014] De Vera et al. showed that an Antarctic lichen not only survived but grew in Mars simulation conditions similar to Antarctica with no liquid water just water vapor, and after that they wrote: (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 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

For the background see below

- 2014: Example of an alpine lichen Pleopsidium chlorophanum found in places like California and the Alps that also grows in Mars analogue conditions in Antarctica and can survive and even grow in Mars simulation conditions – this shows even higher life from Mars could be adapted to live on Earth

[2018] Stamenković with his modelling found new possibilities for brines on Mars to take up substantial amounts of oxygen in cold conditions (Stamenković et al, 2018) and after that he said (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*

[2020] In the 2020 conference "Mars extant life: what's next?" a significant fraction of the participants thought that there is a possibility Mars has extant life (Carrier et al, 2020:Abstract):.

> *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 highlighted four potential martian refugium (not listed in priority order): Caves, Deep Subsurface, Ices, and Salts.*

Also later in the report (Carrier et al, 2020:804):

> *A significant subset of the actively publishing Mars science community who are experts in various disciplines of relevance to interpreting habitability and astrobiology concluded that there exists a realistic possibility that Mars hosts indigenous microbial life and that there are testable hypotheses for seeking it.*

The report from the conference looks into those four categories. It singles out surface and near surface salts as one of the priority targets for future rover missions because of the potential of finding extant life, including life that uses the sunlight for energy, phototrophs (Carrier et al, 2020:797)

> *One major advantage of salts as a potentially habitable microenvironment is that they may provide a protected environment for extant life on Mars very close to the surface and may harbor phototrophs …. The salts themselves may serve as a UV shield, while allowing the limited sunlight to be accessible to the microbes.*

They single out salts as of great interest for an astrobiological sample return so long as it can be free of forward contamination (Carrier et al, 2020: *797*)

Cockell: There is a high chance of habitable environments on Mars – if we look at many planets and don't find life we will have to try to find out what happened that was unusual on Earth

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Cockell, who has written extensively about the possibility of uninhabited habitats on Mars (Cockell, 2014) says that there is a high chance that there are habitable environments. But we don't know what the origin of life requires, so it's not possible to say if there is life there, if we look for at many planets and many environments, and don't find life, it will mean life is very rare (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,'
>
> '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.'

The Viking landers in the 1970s remain our only attempt to search for life on Mars – a few astrobiologists think its labelled release may have already detected life in the 1970s – while others say the data can be explained by complex chemistry – we haven't sent the follow up experiments needed to finally resolve this debate and we can't deduce anything about whether Perseverance might return life even if the Viking experiment did find complex chemistry

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We have only sent two spacecraft to Mars to search for life in situ, the two Viking landers in the 1970s. The results were ambiguous, confused by the reactive chemistry on Mars.

The most sensitive experiment, the Viking labelled release, seemed to detect life. This experiment added dirt to organics labelled with carbon 14 and tested for radioactive evolved gases (such as carbon dioxide or methane). It was so sensitive it could find life in a half gram sample from the Victoria valley in Antarctica which had only 50 cultivable cells in it (colony forming units) (Levin et al, 1976).

A second life detection experiment didn't detect life, but was less sensitive than the labelled release.

However, the Viking chemical analysis experiment (TV-GC-MS) didn't detect any likely looking organics. It heated the samples to 200°, 350°, and 500°C to evaporate small organic molecules and break up large ones which it then analysed by separating them chemically and then by mass. The only organics it found were:

- Dichloromethane CH2Cl2 (Viking Lander 1).
- Chloromethane CH3Cl (Viking Lander 2)

The experimenters dismissed these as likely due to terrestrial contamination, even though they weren't detected in blank runs on Mars, because the chlorine 37 / 35 isotope ratios were similar to Earth isotope ratios.

However we now know that Mars has perchlorates (first discovered by Phoenix in 2009). These chlorohydrocarbons are exactly what you'd get from 0.1% organics reacting with the perchlorates when heated in the Viking ovens for analysis (Navarro-González et al, 2010).

Perchlorates also figure in the most developed non biological theory for the apparent detection of life. Quinn et al in 2013 suggested that the perchlorates in the soil were decomposed through gamma radiation to hypochlorite (ClO^-), trapped oxygen, and chlorine dioxide. Then the hypochlorite reacted with the ^{14}C-labelled alanine to produce chloroalanine which then decomposed to produce the ^{14}C-labelled CO_2. (Quinn et al, 2013). This didn't explain everything and a follow up paper by Georgiou et al filled out the picture some more but is still not a complete explanation (Georgiou et al, 2017).

However there are points in favour of the hypothesis of life too. Levin and Straat in a paper published in 2016 review some of the issues they have found with this and other abiotic proposals (Levin et al, 2016).

1. Two of the labelled release experiments got inactivated after storage in darkness for several months
2. Activity of the soil is significantly reduced if heated first to 50 °C.

Miller's reanalysis of the old Viking data in 2002 found an offset of the evolved gases from the diurnal maximum temperature by two hours. This is especially hard to explain by abiotic processes, as the evolved gases would take only 20 minutes to reach the detector. As an expert on circadian rhythms, Miller said they look like circadian rhythms (Levin et al, 2016) (Miller et al, 2002). He suggested this may be a biosignature in the data. A later complexity analysis seemed to support this interpretation (Bianciardi et al, 2012)

So, it can't be regarded as settled yet either way. Probably most astrobiologists would say it's very complex chemistry.

However these new insights lead some astrobiologists to say they think there is a strong chance the Viking landers detected life on Mars already.

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."*

Bianciardi et al (Bianciardi et al, 2012)

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

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."*

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

> *"... 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 assumption 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."*

What the Viking landers found was either life or very complex chemistry. Some day we'll know the answer. We need follow up experiments to help resolve this question definitively, but we haven't sent them yet.

If the Viking landers did find life it must be widespread on Mars, either in microhabitats or dispersed in the dust, since they got similar results in two sites thousands of miles apart.

If they didn't find life, this doesn't mean there is no life on Mars, as they were stationary landers with no capability to explore and go looking for life. They were limited to whatever they might reach using a scoop extended from the lander.

We can't deduce anything from this either way about whether or not Perseverance could return life bearing samples. Even if somehow we resolve this debate, and show that the Viking landers didn't find life in the 1970s, this wouldn't rule out the possibility of a viable spore or propagule in the Perseverance sample return, blown in the dust or even local indigenous life in a micrometers thick biofilm microhabitat in the dirt which Perseverance sampled by chance.

2012: The European Space Foundation study reduced the size of particle to contain at 1 in a million from 0.2 microns to 0.01 microns, and also said a particle of 0.05 microns or larger shouldn't be released under any circumstances – a BSL-4 can't achieve this and NASA's recommendation is based on the science of 1999

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The draft EIS says they would use many of the basic principles of a Biosafety level 4 facility (BSL-4): (NASA, 2022: S-4):

> ***The material would remain contained until examined and confirmed safe or sterilized for distribution to terrestrial science laboratories. NASA and its partners would use many of the basic principles that Biosafety Level 4 (BSL-4) laboratories use today to contain, handle, and study materials that are known or suspected to be hazardous.***

Here NASA make no mention of the European Space Foundation study in 2012 which reduced the size of particle we need to contain at the 1 in a million level from 0.2 microns to 0.01 microns.

This is how the 2012 ESF report explained its decision at the time study (Ammann et al, 2012:3):

> ***The value for the maximum particle size was derived from the NRC-SSB 1999 report 'Size Limits of Very Small Microorganisms: Proceedings of a Workshop', which declared that 0.25 ± 0.05 μm was the lower size limit for life as we know it (NRC, 1999). However, the past decade has shown enormous advances in microbiology, and microbes in the 0.10–0.15 μm range have been discovered in various environments. Therefore, the value for the maximum particle size that could be released into the Earth's biosphere is revisited and re-evaluated in this***

> ***report. Also, the current level of assurance of preventing the release of a Mars particle is reconsidered.***

They made this change after a discovery of fast horizontal gene transfer to distantly related archaea in sea water via Gene Transfer Agents (GTA) (Ammann et al, 2012:19):

> **Surprisingly, it is now estimated that GTA transduction rates are more than a million times higher than previously reported for viral transduction rates in marine environments. Clearly, GTAs are a major source of genetic diversity in marine bacteria.**

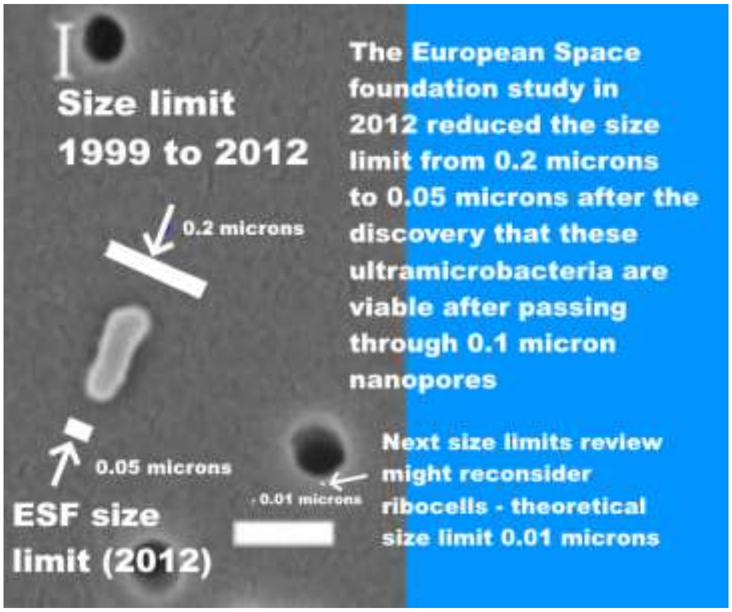
The ESF base this on research that showed that archaea can readily transfer novel capabilities to other distantly related species of archaea overnight in sea water (Maxmen, 2010) (McDaniel, 2010).

Though the EIS doesn't cite the ESF study, the sterilizing subcommittee does cite it (Craven et al., 2021:4) but doesn't mention the update on the limit of size and doesn't mention the gene transfer agents. Instead they have an extensive discussion of prions, which is not cited to any of the previous planetary protection literature. Prions are not listed as a risk by the ESF study (Ammann et al, 2012) or the NRC study (SSB, 2009).

The ESF also said a particle of 0.05 microns or larger shouldn't be released under any circumstances because of the discovery that ultramicrobacteria remain viable after passing through 0.1 micron nanopores (Ammann et al, 2012:21):

> ***"the release of a particle larger than 0.05 µm in diameter is not acceptable in any circumstances"***

This is a visual comparison of the change in the size limit. The 0.01 microns bar is shown as the potential theoretical size limit a future review might decide on to contain early life ribocells. This is also the size limit for the GTAs for the one in a million threshold.



SEM of a bacterium that passed through a 100 nm filter (0.1 microns), larger white bar is 200 nm in length (Liu et al, 2019).

The ESF (Ammann et al, 2012:15): cited two studies that found ultramicrobacteria were still viable after passing through 0.1 micron nanopores in freshwater from Greenland (Miteva et al, 2005), and eight different sites in Switzerland (Wang et al, 2007).

The ESF study also approached this theoretically and found that a minimal size free living cell based on terrestrial biology has a diameter of 0.15 to 0.2 microns if it's spherical but can have a width of less than 0.1 microns and a variable length greater than 0.2 microns. They also say that it's possible smaller cells exist which have an obligatory requirement to co-exist with other organisms as the source of the required genes or gene products (Ammann et al, 2012:15). The ultramicrobacteria that pass through 0.1 micron nanopores for instance in the images by Liu et al are indeed elongated (Liu et al, 2019). Less than 0.1 microns in diameter, but 0.2 microns in length.

NASA's sterilizing subcommittee doesn't consider ultramicrobacteria or the size limit for small organisms (Craven et al., 2021:4).

The ESF requirement is beyond the range for testing HEPA filters – only tested down to 0.1 to 0.2 microns

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The requirements for a BSL-4 facility depend on standards for HEPA filters. Typically it will use biosafety class III cabinets (which can be used for all biosafety levels). A biosafety class III cabinet has to be exhausted to the outside air through two HEPA filters or a HEPA filter and an air incinerator (Richmond et al, 2000:37). We will look at the alternative of an air incinerator in the next section.

These HEPA filters are required to trap 99.97% of particles of 0.3 microns in diameter and 99.99% of particles of greater or smaller size (WHO, 2003:35). These standards don't set any size limit for 100% containment.

In the US, HEPA filters are tested down to 0.1- 0.2 microns (depending on the class of filter, some are tested only at 0.3 microns). In Europe they are tested at the most penetrating particle size which may vary depending on the filter. In both cases, the filters are tested according to probabilities (Zhou et al, 2007) (EMW n.d.).

There is a higher standard than HEPA available. ULPA level 17 filters are rated to filter out 99.999995 percent of particles (BS, 2009:8) in the range 0.12 microns to 0.25 microns (BS, 2009:4), according to BS EN 1822-1:2009, the British implementation of the European standard (BS, 2009).

However even ULPA filters don't comply with the ESF standard of no release of a 0.05 micron particle in any circumstances. They aren't even tested in this size range.

Alternative of an air incinerator for the second HEPA filter – would need to be evaluated for containment of putative Martian life likely more resilient than standard test terrestrial spores – and for 100% containment

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The alternative acceptable approach for a biosafety class III cabinet is to pass the exhaust through a HEPA filter and an air incinerator (Richmond et al, 2000:37). From the NIH guidelines for research involving recombinant or synthetic nucleic acid molecules, the guidelines are to test the air incinerator to make sure it inactivates any viable spores, with a challenge aerosol of at least 100 million bacterial spores per cubic foot. Test spores used are *Bacillus subtilis* var. *niger* or *Bacillus stearothermophilus* (Meyer et al., 2019)

If this approach was used for Martian life, research would be needed into whether the same criteria are suitable for putative Martian life. The issue is that any martian life has had billions of years of evolution in conditions that may well select for higher desiccation resistance and extra protective coatings against UV than these terrestrial challenge spores, see above:

- New: Martian life could evolve new strategies for dust storm transport such as spores with extra layers to protect against UV, and fruiting bodies for higher life that are detached by strong winds and may be better protected against UV than terrestrial life

These extra adaptations could lead to additional protection against air incineration.

There may well be other issues to look at. For instance, any martian life in the samples may be already imbedded in Martian dust grains, see

- 2017: individual microbes can travel in dust storms imbedded in a dust grain for extra protection from UV

Even small dust grains could give extra protection from air incineration. Also even a 100 billion fold reduction per particle might not be the same as 100% assurance of non release of a single particle of 0.05 microns in the lifespan of the facility or a 1 in a million chance of release of a single particle at 0.01 microns in the facility lifespan.

It would also be important to look into maintenance of the incinerator and replacement, that this can be done in a way that doesn't permit release of a single unsterilized particle of 0.05 microns.

NEW: If the ESF requirement is met using air filters it seems to need new breakthrough technology rather than incremental improvements

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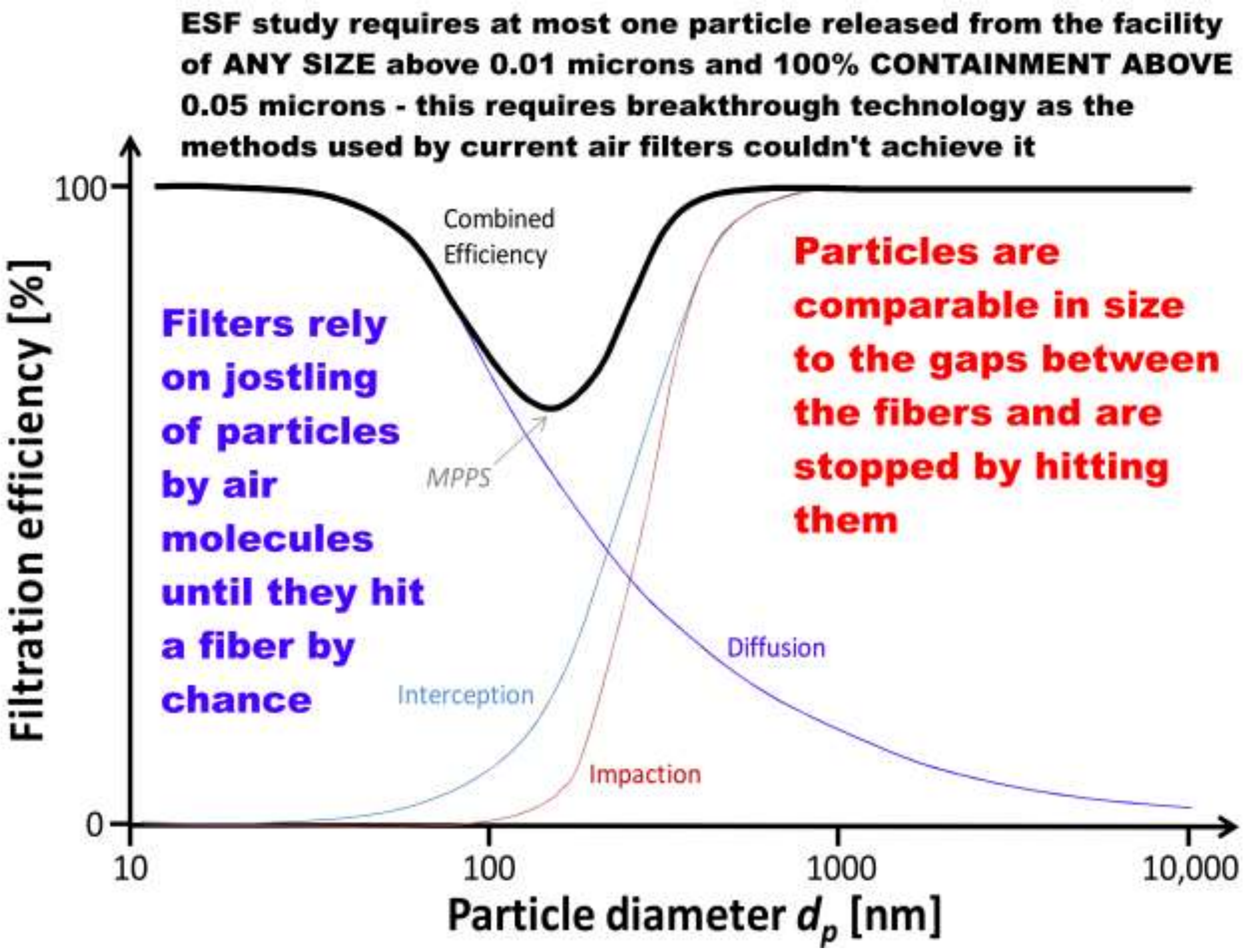
Current air filters can't achieve 100% containment at any small particle size.



From (Todea et al, 2020: fig 1)

Filters generally have a maximum penetrating particle size at around 0.1 microns. Below this size the nanoparticles are far smaller than the gaps between the fibers, so they rely on Brownian motion – the random jostling of the particles by air molecules until some by chance hit the fibers. Above this size they rely more on the fibers directly stopping the particles. See section 4.3 and figure 5 from (Borojeni et al, 2022:7) and (Todea et al, 2020: fig 1)

Recent air filter technology reviews don't mention any attempts to achieve 100% containment above any size. Also they don't mention anything approaching 1 in a million chance of releasing a single particle in the lifetime of a facility at all sizes above 0.01 microns (Borojeni et al, 2022:7). The 100% requirement would seem to need some new breakthrough technique rather than incremental changes such as more layers of filters or varying the spacing as those couldn't get it all the way to 100% containment of such small particles.

It may be possible to achieve 100% containment of 0.05 micron particles in water under high pressure. A 2020 review of the literature found several studies that achieve a million fold reduction or more of small viruses in water. (Singh et al, 2020:6.3). That doesn't quite meet the target but Singh et al found one study using carbon nanotubes loaded with silver that achieved

100% removal of very small viruses such as the polio, noro and Coxsackie viruses (Kim et al, 2016), (Singh et al, 2020:6.3).. The poliovirus is only 0.03 microns in diameter (Hogle, 2002).

However these filters for smaller nanoparticles for water treatment are easily damaged, through chemical and biological deterioration by aging, scratches by particle like substances, or fouling of the membrane (Singh et al, 2020:8). If we achieve filtration at this standard in the air, the air filters may also have similar maintenance issues.

Also there is an issue with testing filters over this very small size range. The filters are tested with challenge aerosols such as dioctylphthal (DOP) generated on the intake side of the filter, and measured with a photometer on the discharge side (Richmond et al, 2000:33). These photometers have limited sensitivity to nanoaerosols below the 100 nm limit.

In a study of a DOP aerosol using TSI model 8130 Automated Filter Tester in 2008 (table III of Eninger et al, 2008), particles below 100 nm (0.1 microns) constituted 10% of the count of particles in the test aerosol, and 0.3% of the mass. However they provided almost none of the light scatter in the testing photometer (less than 0.01%).

Any new filter technology would need to specify how they will be checked and replaced. Biosafety level III cabinets need to be checked annually (Richmond et al, 2000:33) and equipment will sometimes need to be repaired. HEPA filters often fail these annual tests and need replacement. When these filters are changed, the Biological Safety Cabinets (BSCs) must be decontaminated (WHO, 2003:35). The method for decontamination would also need to be devised as well as the method for keeping the Mars samples undamaged during decontamination.

This technology may not be impossible. But by analogy with the situation for HEPA filters, it would seem to require a significant research program that hasn't yet been started to:

- develop filters to achieve the ESF requirement,
- design ways to test the filters,
- design methods to maintain them and replace them while preserving containment of the samples,
- set the necessary standards that the filters must meet, and
- confirm that the required standards have been achieved

ESF study said values for required level of assurance and the size limit need to be revisited periodically based on changes in scientific knowledge and risk perception

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The ESF study said that future reductions in the size limit are indeed possible. They expected later reductions to happen at a slower pace, but say the size limit will need to be reviewed in the future, adding (Ammann et al, 2012:21):

> *Based on our current knowledge and techniques (especially genomics), one can assume that if the expected minimum size for viruses, GTAs or free-living microorganisms decreases in the future, and this is indeed possible, it will be at a slower pace than over the past 15 years*
>
> *However, no one can disregard the possibility that future discoveries of new agents, entities and mechanisms may shatter our current understanding on minimum size for biological entities. As a consequence,* ***it is recommended that the size requirement as presented above is reviewed and reconsidered on a regular basis.***
> *[bolding as in original cited text]*
> *…*
> *RECOMMENDATION 8: Considering that (i) scientific knowledge as well as risk perception can evolve at a rapid pace over the time, and (ii) from design to curation, an MSR mission will last more than a decade, the ESF-ESSC Study Group recommends that values on level of assurance and maximum size of released particle are re-evaluated on a regular basis*

By 2022, a decade later, another review is certainly required.

The next review may examine new research into extremely small early life cells such as ribocells with enzymes made from fragments of RNA instead of proteins (Kun, 2021). Steven Benner and Paul Davies say the small 0.01 micron diameter structures in the martian meteorite ALH84001 are consistent with RNA world cells (Benner et al, 2010: 37). Panel 4 for the 1999 workshop estimated a minimum size of 12 nm in diameter and 120 nanometers in length for early life RNA world cells, if there is an efficient mechanism for packing its RNA (SSB, 1999: 117).

As we learn more about the mystery of the first cell, these researches may lead to a review of the size limit to accommodate new ideas (Kun, 2021).

> *We are a long way from solving the mystery of the first cell, but more and more of the puzzle- pieces are known. The problems, both dynamical and structural, have been identified, and for some, solutions proposed. Here we have reviewed some of the dynamical problems the first cell needed to overcome via having the right set of ribozymes cooperating with each other.*

This review would need to be done first before developing the filter and / or air incinerator technology and relevant testing requirements, as the requirements could change as a result.

Draft EIS does mention a 0.05 micron limit – but not for the BSL-4, only for the return capsule – and without mentioning the ESF study

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My comment of May 15th alerted NASA to this issue. I said in its first two paragraphs (Walker, 2022a)

> ***Are you aware of the ESF Mars Sample Return study (Ammann et al, 2012:14ff)? It said "The release of a single unsterilized particle larger than 0.05 µm is not acceptable under any circumstances". This is to contain starvation limited ultramicrobacteria which pass through 0.1 micron filters (Miteva et al, 2005). Any Martian microbes may be starvation limited.***
>
> ***This 100% containment at 0.05 microns is well beyond capabilities of BSL4 facilities. Even ULPA level 17 filters only contain 99.999995 percent of particles tested only to 0.12 microns (BS, 2009:4).***

NASA didn't respond to this comment in the section of the draft EIS where they respond to public comments. They did mention a 0.05 micron limit but not in the context of the ESF Study or a BSL-4 so it wasn't a response to my comment.

They mention a 0.05 micron limit in their response to this question from members of the general public: (NASA, 2022: Section 4-7):

> What is the smallest Mars particle that is forbidden to be on the capsule carried to Earth? Dust level, bacteria level, virus level, prion level?

They respond that the minimum size is 50 nm – for the capsule on the journey back to Earth (NASA, 2022: Section 4-7):.

> A number of studies (National Research Council 1999, Heim et al. 2017) have estimated the minimum sizes for life forms from fundamental inputs such as the genetic material required to permit a cell to perform basic functions [e.g., (Glass et al. 2006)], observations in extreme environments [e.g., (Comoli et al. 2009)] or theoretical constraints that would apply to astrobiology investigations (Lingam 2021).
>
> Values from such studies have been used to inform findings on best practices for sample return missions and MSR has considered those findings in selecting 50 nm for engineering requirements.

Their first cite to the National Research Council gives a minimum size of 250 nm ± 50 nm for panel 1 for spherical cells(SSB, 1999: 2), which remained the minimum size through to the 2012 ESF study (Ammann et al, 2012:3):.

However, panel 3 which looked into hypothetical early life RNA world cells finds spherical cells of 50 nm diameter and elongated cells down to 12 nm in diameter (SSB, 1999: 117). This is the only occurrence of a 50 nm figure that I found in the cites.

Heim et al gives 250 nm as the minimum diameter for a prokaryote just based on the NRC cite (Heim et al, 2017).

Lingam gives a 0.2 micron limit in diameter for spherical microbes able to sense chemical gradients and move in response to them (Lingam, 2021:17). It assumes the cells are spherical, and elongated cells could be smaller in minimum diameter (Lingam, 2021:3). It can't be an input for the other studies as it is their most recent cite. Lingam may not be relevant to the smallest cell size for Mars, as many microbes are not able to move by themselves, including most fungi, and many blue-green algae. For instance chroococcidiopsis, a top candidate for a terrestrial microbe able to survive on Mars can't move by itself though the daughter cells (baeocytes) of a similar cyanobacteria Myxosarcina are able to move by gliding across surfaces for a short while (Sanders et al, 2021).

NASA's other two cites don't try to estimate minimal sizes for cells. Glass et al is about a search for a minimal genome but doesn't estimate the size of the cells except that they mention that they passed through 0.22 micron nanofilters to break up clumps into single cells (Glass et al, 2006), and Comoli et al is a paper about a successful attempt to image a particular sub-micron microbe with inner membrane fitted to an ellipsoid of 402 nm by 442 nm by 312 nm in diameter associated with much smaller particles that they concluded are probably viruses (Comolli et al, 2009).

It's not clear from these cites why they selected 0.05 microns for engineering requirements for particle release from the return capsule. But apparently nothing to do with the ESF study and they make no connection with HEPA filters or the recommendation to use a BSL-4.

NASA's draft EIS says there is no significant risk of environmental effects – yet the NRC study in 2009 warned the potential for even large scale harm to human health and the environment isn't demonstrably zero and the potential for negative impacts can't be assessed – NASA don't cite the NRC study on this, only the report of their sterilizing subcommittee

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Another major change made by NASA's EIS compared to previous published work on planetary protection is a finding of no significant risk of environmental effects for life returned from Mars. This is what the draft EIS says (NASA, 2022: 3-3):

> 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.**

The National Research Council's 2009 study said it is not possible to assess the potential for negative impacts.

The 2009 NRC study also said the potential for [even] large-scale negative effects appears to be low but is not demonstrably zero (SSB, 2009 : 48)

> 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**
>
> … **it is not possible to assess past or future negative impacts caused by the delivery of putative extraterrestrial life**, based on current evidence.

In this case the discrepancy is based on an assessment by NASA's sterilizing subcommittee (Craven et al., 2021).

The EIS doesn't alert the reader to the discrepancy between the sterilizing subcommittee's conclusions and the conclusions of the National Research Council study in 2009. As we'll see, it also doesn't alert the reader to many other cites that came to the same conclusion as the NRC that we need to consider the potential for even large scale harm to the environment or to human health.

The sterilizing subcommittee do mention the NRC study but single out a different passage, from the section of the NRC study discussing the potential for large scale negative pathogenic effects on humans (Craven et al., 2021:4).

> '...the potential for large-scale pathogenic effects arising from the release of small quantities of pristine Mars samples is still regarded as being very low.' ...'extreme environments on Earth have not yet yielded any examples of life forms that are pathogenic to humans'

The passage they quote continues with a "However" saying that though extreme environments on Earth haven't yielded any human pathogens so far, they ***have*** yielded microbes with interesting evolutionary connections with human pathogens. The passage concludes that since the potential risk for life returned from Mars can't be reduced to zero a conservative approach to planetary protection is essential (SSB, 2009: 46)

> "… It follows that, since the potential risks of pathogenesis cannot be reduced to zero, a conservative approach to planetary protection will be essential, with rigorous

requirements for sample containment and testing protocols of life forms that are pathogenic to humans'

As we'll see, papers since then on planetary protection have agreed with this conclusion and found several specific examples of ways Martian life can be pathogenic to humans not mentioned in the NRC report, with analogies with Legionnaires' disease, tetanus, and others.

An updated version of the NRC Mars Sample Return report would have a far more extensive section on the potential for large scale negative pathogenic effects on humans, based on the research done since then.

The sterilizing subcommittee report argues martian life didn't co-evolve with us so can't harm us and that martian life would be extremophile not able to survive on Earth – these arguments were previously presented in an op. ed. by Zubrin in 2000 – and planetary protection experts at the time found many errors in them and said it was like a recommendation to build a house without smoke detectors

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The basic arguments for the sterilizing sub committee are

- that Martian life didn't co-evolve with humans so it can't harm us
- that Martian life would be extremophile, only able to survive in the extreme conditions on Mars

We will see both these arguments miss out important exceptions, such as diseases such as tetanus and legionnaires' disease from microbes that didn't co-evolve with humans or other higher life, and polyextremophiles such as chroococcidiopsis that can survive in multiple extreme conditions and many can also survive in more normal conditions.

The only previous source I have been able to find for these arguments is a non peer reviewed op. ed. by the space engineer and Mars colonization proponent Robert Zubrin, who presented identical arguments in 2000 (Zubrin, 2000).

Zubrin got an immediate response in the next edition of the planetary report, from planetary protection experts. John Rummel, NASA's planetary protection officer at the time, gave radiodurans as an example of a microbe that is able to colonize nuclear power plant environments, an environment that didn't exist before the 1940s and quite unlike its natural habitats. He also gave examples of botulism and ergot disease as Earth organisms that while not coevolved pathogens are dangerous to humans. He used Robert Zubrin's own example of dutch elm disease to show that pathogens of other organisms also have potential to cause environmental and economic damage, and wrote: (Rummel et al., 2000).

NASA ' s current policy, as recommended by the US National Research Council, is not extreme. Rather, it is based on the sound principle that a sample from Mars should be contained until scientists find it does not contain a biohazard …

Still, he insists that Mars life unrelated to Earth organisms couldn't possibly cause harm. How does he know, when we have precisely zero experience with life unrelated to Earth life? … How ought others judge the cost-benefit ratio of Mars exploration if we don't take simple precautions to avoid potentially harmful consequences? Harshly, I suspect.

Margaret Race said we do need to take care and that his proposal to drop planetary protection is like building a house without smoke detectors (Rummel et al., 2000).

Background graphics:

Smoke detector (Rockmelder, 2007) House on fire (LAFD, 2018)

I work (on my own initiative) as a voluntary fact checker for scared people. For anyone who might read this paper and panic and expect the worst, and perhaps instantly jump to fear of human extinction, Margaret Race's smoke detector analogy may help. Most people will never get their house burnt down, but it is still wise to install smoke detectors. We need to look at worst case scenarios just as the designer of a smoke alarm has to look at house fires.

This particular mission is not searching for present day life, has no way to identify it on Mars, is exploring one of the regions of Mars considered by NASA to be unlikely to have life, is not looking for micohabitats, and is not sterilized sufficiently to approach any location where terrestrial life might be able to establish a foothold on Mars.

Unless life is very common on Mars, perhaps as spores or propagules or native biofilm fragments in the dust or in the dirt, this mission is unlikely to return present day life at all. Also if there is life on Mars, it may be a harmless "drop in" replacement for its terrestrial analogue, or even beneficial, or there may be no life there, or early life that is so primitive it is unable to compete with modern life, or only life precursors such as protocells and prebiotic synthesis.

But as we'll see, it's not a foregone conclusion that putative life on Mars is safe. So we have to take precautions. We also need to take precautions as a precedent for future potentially more risky samples returned from other areas of Mars. Just as it is wise to install smoke detectors in all houses rather than just the most at risk houses, it is also wise to set a precedent for future safe sample returns from Mars by NASA and other space agencies.

NASA's draft EIS and the report of the sterilizing subcommittee don't cite Zubrin, so there is nothing to suggest a direct influence. However, there is a striking parallel. It is the same for the meteorite argument that we began this article with. It occurs in Zubrin's op ed but not in the planetary protection literature for Mars (only for the JAXA mission which is a different situation).

There may be a common background leading to this striking similarity. We explore this more in:

- Factors for space agencies to look out for that may lead to them assigning planetary protection of Earth much less significance and attention than the general public

Many in the space exploration / colonization community have been convinced by Zubrin's arguments, and so would likely find the conclusions of the sterilizing subcommittee convincing too. It may help to go into some detail on this point, why the arguments don't work

Argument by sterilizing subcommittee that martian pathogens wouldn't be adapted to humans or other Earth hosts has a major omission – legionnaires' disease, a disease of biofilms that opportunistically infects human lungs

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First, arguing from many examples of pathogens adapted to humans, the sterilizing subcommittee's assessment says the risk of a direct pathogen of humans is near-zero (Craven et al., 2021:6)

> *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.***

The human pathogens they mention include two that humans catch as a result of handling diseased animals, Ebola (from handling a bat or non human primate) (CDC, n.d.) and HIV (from hunting chimpanzees) (CDC, n.d.). They also mention two diseases that infect humans via mosquitoes, malaria (most variants are an obligate parasite of humans) (CDC, n.d.) and yellow fever (a viral disease of monkeys that also infects humans) (CDC, n.d.). They also mention Kaposi sarcoma, a cancer caused by an infection with a virus called human herpesvirus 8 and

Schistosomiasis also known as bilharzia, a parasitic worm transmitted from freshwater snails in tropical conditions (CDC, n.d.). We can agree on this, none of these are credible analogues for a pathogen from Mars.

NEWCITES

CDC, n.d. Ebola
CDC, n.d. HIV
CDC, n.d. Malaria
CDC, n.d., Schistosomiasis
CDC, n.d. Yellow Fever virus

Mayo clinic, n.d., Kaposi Sarcoma

Stout, J.D., n.d., Protozoa and the Soil

They give two other examples which are less convincing which we'll look at in the following sections. For Escherichia coli strain 0157:H see:

- The sterilizing subcommittee's report gives an example of an e. coli strain that they say became toxic by coexisting with humans – however the NRC report gave an example of human pathogens with close evolutionary connections with microbes in hydrothermal vents – meanwhile Łoś et al suggested their example, e. coli, strain 0157:H7, might have actually developed Shiga's toxin to deter protozoan grazing in biofilms and only uses it opportunistically in humans
  [and following sections]

For Candidiasis yeast infections, see below:

- NEW: Subcommittee report's gives an example of an opportunistic fungal pathogen, Candidiasis, adapted to humans – the omission here is Aspergillus which is not adapted to humans and is invasive due to adaptations to survive rapid dehydration and rehydration, rapid changes of temperature etc. many of which may be shared by life adapted to Mars – with an estimated 200,000 life threatening Aspergillus infections a year – mortality 30% to 95%
  [and following sections]

Their discussion of potential for pathogens of humans has no cites to the planetary protection literature, not even to the NRC study which in the section on the potential for large scale negative pathogenic effects on humans concluded (SSB, 2009: 46) :

> "… It follows that, since the potential risks of pathogenesis [disease causing infection of humans] cannot be reduced to zero, a conservative approach to planetary protection will be essential, with rigorous requirements for sample containment and testing protocols of life forms that are pathogenic to humans.

They quote from that cite earlier but don't mention its conclusion (Craven et al., 2021:4)

Indeed the only cite for the entire section of the report about human pathogens is to a paper about the last common bilaterian ancestor which they use to support their statement that mosquitoes (which transmit malaria and yellow fever) and snails (which transmit schistosomiasis) had a last common ancestor 600 to 1,200 million years ago (Erwin et al., 2002) which presumably is to back up their argument that neither malaria nor schistosomiases are plausible analogues for a martian pathogen.

However, their list of human pathogens has a major omission, Legionnaire's disease, an example from (Warmflash, 2007) of a disease of biofilms and protozoa that is also infects human lungs and sometimes can kill us, yet it's evolved to live in biofilms, not to attack humans. Researchers into Legionella say that to the microbe Legionnella pneumophila, human lungs must seem like biofilms, and the macrophages in our lungs must seem like large protozoa *(Alberts et al 2002)*.

Legionnella pneumophila isn't an exact analogue for a microbe we could return from Mars, as it needs an oxygen rich aquatic environment to survive, can't survive drying and can't form spores. But it does show that a martian microbe could be preadapted to live in human lungs without ever encountering anything except biofilms. It can do that because of a close resemblance in relevant details between the conditions it encounters in lungs and biofilms.

Warmflash used Legionnaire's disease to challenge whether there is a need for human pathogens to co-evolve with us (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.*
>
> *It is important to note the numerous biofilms observed aboard the Mir space station, which were found on surfaces and within water plumbing. These films were often multi-species and included bacteria, fungi, and protozoa.*
>
> *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.*
> *Even in the context of a planetary biosphere 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.*

The sterilizing subcommittee's report gives an example of an e. coli strain that they say became toxic by coexisting with humans – however the NRC report gave an example of human pathogens with close evolutionary connections with microbes in hydrothermal vents – meanwhile Łoś et al suggested their example, e. coli, strain 0157:H7, might have actually developed Shiga's toxin to deter protozoan grazing in biofilms and only uses it opportunistically in humans – the origin of its virulence remains an open question

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The sterilizing subcommittee's report also mentions that microbes that co-exist with humans can take on a new mutation that leads to them expressing a toxin that harms humans (Craven et al., 2021:6).

> *Existing microorganisms that coexist with humans over long periods of time can also cause new diseases when the organism takes on new pathogenicity, such as the Escherichia coli strain 0157:H7 that acquired a gene for Shiga toxin, …*

If this hypothesis is true, that the e. coli strain 0157:H7 acquired its toxicity by coexisting with humans, it would still highlight a potential concern, since martian microbes can acquire new genes too by coexisting with us. A new microbe from Mars is a change in Earth's biosphere, not just for us but for future generations.

The 2009 NRC review which NASA's EIS refers to elsewhere adds an example (SSB, 2009: 46) of hydrothermal vent organisms (SSB, 2009: 46)

> "**However**, it is worth noting in this context that interesting evolutionary connections between alpha proteobacteria and human pathogens have recently been demonstrated for natural hydrothermal environments on Earth … it follows that, since the potential risks of pathogenesis cannot be reduced to zero, a conservative approach to planetary protection will be essential, with rigorous requirements for sample containment and testing protocols of life forms that are pathogenic to humans'

Their cite is to two deep sea vent strains of the class epsilon-Proteobacteria, (Nakagawa et al, 2007) now reclassified as Epsilonbacteraeota (Waite et al, 2017), that though not pathogenic themselves, share many virulence genes with pathogenic relatives

> *Although they are nonpathogenic, both deep-sea vent epsilon-Proteobacteria share many virulence genes with pathogenic epsilon-Proteobacteria, [they give a list of virulence genes, and other capabilities that enhance virulence]*
> *… these provide ecological advantages for hydrothermal vent epsilon-Proteobacteria who thrive in their deep-sea habitat and are essential for both the efficient colonization and persistent infections of their pathogenic relatives.*

Their pathogenic relatives are Helicobacter, which can cause stomach ulcers and Campylobacter which can cause acute gastrointestinal disease in humans (Cornelius et al, 2012). So the suggestion here is that they may have gained many of their virulence genes for humans through adaptations to hydrothermal vents.

[2013] Also, the strain of e-coli the sterilizing subcommittee's report mentions might not have evolved its toxicity for humans by coexisting with us. Łoś et al argue that it is rare for strains that express Shiga's toxin to be transmitted human to human except during outbreaks. Łoś et al hypothesize that Shiga's toxin evolved to defend against protozoan grazing and then turned out to be opportunistically useful in humans. With protozoa it may kill the protozoa that attempt to graze on it. But in humans Shiga's toxin kills the e. coli bacteria that produces the toxin – and only benefits other e. coli altruistically by destroying the white blood cells (phagocytes) that attack it (Łoś et al, 2013).

[2018] Experiments since then have led to conflicting results. Some studies find Shiga's toxin has a survival advantage for e. coli to resist the protozoa *T. pyriformis* where it helps it survive in food vacuoles, and also helps it to kill the protozoa *A. castellanii and T. thermophila. B*ut other studies found it has no benefit in *A. castellanii or can even decrease its ability to resist predation by A. castellanii (Sun et al, 2018)*

In short, it's not clear but it's possible that E. coli strain 0157:H7 actually evolved its virulence in biofilms to resist protozoan grazing *(Sun et al, 2018):*

> *In conclusion, evolution of mechanisms that allow for survival within protozoa may have selected for traits that also allow bacteria to escape that harmful effects of phagocytes [in humans].*

If E. coli strain 0157:H7 really did evolve this virulence in biofilms, it would be a similar example to Legionnaire's disease, a pathogen that evolved its capabilities in a biofilm rather than through coexisting with humans. Also, whether or not this is finally shown to be why E. coli developed Shiga's toxin, the proposed mechanism provides a plausible analogue of a process that may occur in martian life.

Sterilizing subcommittee's report doesn't mention clear examples of microbes which express accidental toxins without coevolution with humans or higher life, such as neonatal tetanus which kills thousands of unvaccinated children every year

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The sterilizing subcommittee's report has another major omission in its discussion of Shiga's toxin (Craven et al., 2021:6):

Existing microorganisms that coexist with humans over long periods of time can also cause new diseases when the organism takes on new pathogenicity, such as the Escherichia coli strain 0157:H7 that acquired a gene for Shiga toxin, …

The origin of shiga's toxin is unclear as we saw in the previous section. But there are many clear examples of microbe that harm us indirectly through secreting accidental toxins which they most certainly didn't acquire through co-evolving with humans or other higher lifeforms. Examples include tetanus, and botulism, (Warmflash, 2007).

Even with tetanus vaccines widely available, neonatal tetanus still kills thousands of children every year in weaker economies (WHO, n.d.) . Tetanus is a common anaerobic (non oxygen using) soil bacteria, and tetanus toxin is made by a plasmid (pE88), a small self-contained circular DNA molecule it got from somewhere else, but its origins are unclear as it seems to be unique to Cloristidium tetani (Brüggemann et al., 2003).

Warmflash et al. also mention ergot disease, a disease of some crops (Warmflash, 2007). The ergot alkaloids are perhaps the best known example of many toxins produced by molds (mycotoxins). Ergot alkaloids are produced by fungi in the Claviceps genus (Miedaner et al, 2015). These are often accidentally harmful to humans, and damage crops, causing millions of dollars of economic loss per year (Hussein et al., 2001). Microbes in the claviceps genus are specialized to infect ovaries of grasses (Miedaner et al, 2015).

However one of the most widespread mycotoxins in food from molds is Aspergillus which is an opportunistic pathogen in both plants and animals. *Aspergillus fumigatus* (the most common pathogen for humans) and *Aspergillus nidulans*, have no adaptations to a pathogenic lifestyle. *A. Flavus* has generalist adaptations to infect wounds in plant, animal and insect hosts though not adapted to any specific species (St. Leger et al., 2000). Aspergillus species also often kill immunocompromised humans which we'll cover in the next section (Navale et al., 2021). Incidentally Aspergillus Niger happens to have high resistance to UV for a terrestrial microbe, higher than two microbes selected from Mars analogue environments. It was selected for a Mars analogue as a human pathogen previously detected in the ISS as well as a microbe with resilient spores that might travel to Mars on the outside of a spacecraft. It was tested in a Mars analogue experiment in a high altitude stratospheric balloon and after 5 hours of UV at levels similar to the Mars surface (Cortesão et al, 2021:table 3) and ionizing radiation between a third and a quarter of Mars surface conditions,.

There are many other forms of accidental toxins and allergens expressed by terrestrial life.

NEW: An unrelated exobiology may produce many novel bioactive compounds which could be of great benefit, but the difference in biochemistry could also lead to more accidental toxins than terrestrial life, and in some scenarios, the internal chemistry of an unfamiliar exobiology could be accidentally toxic

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Additionally, an exobiology could itself be based on chemistry toxic to Earth life, or the difference in biochemistry could lead to more accidental toxins or allergens than terrestrial life

- Allergens, e.g. Aspergillus can trigger asthma (Latgé, 1999) and is not adapted to humans (McCormick et al, 2010).
- Secondary metabolites, e.g. which inhibit the growth of other microbes, Wallemia which is adapted to low water activity in salt or sugary solutions spoils food with secondary metabolites, the most toxic is wallimidione (Desroches et al, 2014).
- protoxins, which when metabolized break down into toxic products., such as methanol which is converted into toxins when digested (Mégarbane, 2005), or hypoglycin A, which 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).
- The chemistry of alien cells may itself be toxic to Earth life. 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 (Schulze-Makuch et al, 2010a).
- As well as the internal chemistry, the waste products and metabolic intermediaries could also be accidentally toxic or allergenic.

So, if we do find Martian life, even unrelated, it would be no great surprise to find they produce bioactive compounds which affect humans in various ways, sometimes beneficial, sometimes harmful.

Biochemicals from unrelated or distantly related martian life may be of great value to us. Many modern medicines are based on bioactive compounds.

Indeed botulism toxin itself, properly used, has many medical benefits (Jankovic, 2004). Extremophile fungi may be a source of bioactive compounds for medically useful drugs (Chávez et al., 2015). However bioactive compounds for medicine have to be screened for toxicity (Madariaga et al., 2019).

If we do find martian life, it may bring new medicines, indeed it may benefit us in many ways. See:

- NEW: Enhanced Gaia – ways that introduced Martian life could be beneficial to humans, ecosystems and Earth's biosphere

However for the topic of back contamination and what we need to do to protect Earth, what matters is whether it can also harm us. Whether we want to introduce martian life itself to Earth would depend on what the effects would be on our biosphere and on humans and the animals within it.

If martian life has potential for mixed effects on Earth's biosphere, with some positive effects, and some negative, again we might prefer to leave it on Mars and exploit it on Mars. We will have a difficult decision about whether or not to return an alien exobiology to Earth if this means some terrestrial human communities and ecosystems will be positively benefited but others negatively impacted.

NEW: Sterilizing subcommittee's report gives an example of an opportunistic fungal pathogen, Candidiasis, adapted to humans – the omission here is Aspergillus which is not adapted to humans and is invasive due to adaptations to survive rapid dehydration and rehydration, rapid changes of temperature etc. many of which may be shared by life adapted to Mars – with an estimated 200,000 life threatening Aspergillus infections a year – mortality 30% to 95%

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In the next section we'll look at warnings by Sagan, Lederberg and others that there is a possibility that our immune system can't detect alien pathogens generally.

First, let's look at a particular example of this, opportunistic fungal pathogens. Could our immune system detect an alien fungus?

The sterilizing subcommittee report said that fungal infections such as candidiasis yeast opportunistically infected people with compromised immune systems as a result of coexisting with humans for a long time (Craven et al., 2021:6):

> *Existing microorganisms that coexist with humans over long periods of time can also …*
>
> *opportunistically infect a host with a weakened or compromised immune system such as candidiasis yeast infections*

As they say there,

- candidiasis yeast adapted to humans (Alves et al., 2010) .

The second main genus of opportunistic fungal infections that kill humans is adapted to mammals:

- cryptococcus adapted to mammalian hosts (Kronstad et al, 2012).

However that's not true of the third main genus of opportunistic fungal infections that kill humans

- aspergillus fumigatus [the main aspergillus pathogen of humans] is not adapted to a pathogenic lifestyle [in any other organism] (McCormick et al, 2010).

  According to our current knowledge A. fumigatus lacks sophisticated virulence factors that are solely dedicated to permit a pathogenic lifestyle.

So once more they choose an example that supports their conclusion but there is a major omission in the form of an example that disproves it.

Aspergillus is pathogenic in humans because of factors that make the fungi very resilient in extreme conditions, stress resistant, able to respond rapidly to dehydration and rehydration, able to form biofilms and penetrate tissues mechanically with filaments, which also break off and can spread through the body, able to withstand low oxygen in damaged lung tissue and so on. These are not adaptations to humans and many of them are also likely to be shared by Martian fungi.

Paulussen et al. put it like this (Paulussen et al, 2017):

> *Collectively, the aspergilli are remarkable fung. … there are numerous aspects of* Aspergillus *cell biology and ecology (including their metabolic dexterity when adapting to nutritional and biophysical challenges) which contribute to their status as, arguably, the most potent opportunistic fungal pathogens of mammalian hosts.*
>
> *Aspergillus species are able to utilize a wide range of substrates, highly efficient at acquiring such resources, and can store considerable quantities of nutrients within the cell; all traits which contribute to their energy‐generating capacity and competitive ability*
>
> *…*
>
> *Species of Aspergillus are also among the most stress‐tolerant microbes thus far characterized in relation to, for example, low water activity, osmotic stress, resistance to extreme temperatures, longevity, chaotropicity, hydrophobicity and oxidative stress*

It may help to give a summary of some of the key points in their section: "Biophysical capabilities and ecophysiology of pathogenic *Aspergillus* species" (Paulussen et al, 2017) along with (Paulussen et al, 2017: table 1) and (Paulussen et al, 2017: table 2),

Aspergillus species are able to be pathogenic in humans because of many adaptations in extreme environments, as a result of which they are:

- Capable of rapid recovery from dessication –inhaled dessicated spores can rapidly recover to colonize the respiratory tract
- Have large amounts of melanin which can protect the cell membranes from breakdown (lysis) by the immune system
- Produce enzymes that break down proteins (proteases)
- Produce branching filaments (hyphae) which mechanically penetrate tissues and can also break up into fragments that can spread rapidly through immunocomromised patients
- All these activities require a lot of energy and Aspergillus species are able to colonize a wide range of media, highly efficient at taking up nutrients and converting it to energy
- Amongst the most stress tolerant of species, able to resist extreme high and extreme low temperatures oxidative stress (including hydrogen peroxide), UV, and ionizing radiation (Paulussen et al, 2017: table 2),
- Able to protect themselves from chaotropic agents like urea and ethanol, by stabilizing their proteins, producing more proteins, more energy, and modifying their membrane composition and increase production of enzymes that remove reactive oxygen species. [there are many chaotropic agents on Mars such as the perchlorates]
- Able to tolerate low oxygen levels in the lungs (as low as 1% partial pressure in inflamed tissue) and some strains can function without oxygen (Paulussen et al, 2017: table 2)
- Many specific adaptations to stress including ability to produce EPS for biofilms, accumulate large amounts of melanin in the cell walls, and protein stabilization mechanisms, and they can synthesize solutes like glycerol for protection from the environment around them. The glycerol can protect against freeze thawing (Paulussen et al, 2017: table 2)
- Naturally resistant to antifungals generally - some species of aspergillus can pump antifungals out of the cell (using efflux pump transport proteins) and also secrete plastic like substances (polymeric substances) to reduce contact with the antifungals, the melanin in the cell walls can bind to antifungals and they can use heat shock responses to reduce the entry of antifungals (Paulussen et al, 2017: table 1)

These are all capabilities that could be shared by a new genus of fungi from Mars.

Aspergillus is capable of micro-evolution as it spreads through the body after it infects a host (Ballard et al, 2018). The same could be true of any putative Martian fungus after it infects a human host.

There are many people who have a weakened or compromised immune system. There are an estimated 200,000 life threatening Aspergillus infections a year with mortality rates varying from 30 to 95% (Brown et al, 2012) .

There seems to be some potential that a martian fungus might already be well adapted for spreading through a human host because of adaptations to extreme stress conditions on Mars including low oxygen, UV, and ionizing radiation and rapid changes in temperature and humidity and likely evolutionary adaptations for rapid recovery and rehydration after dessication.

More generally, looking at the larger picture over a timescale of our future generations, the other two main genera of fungi that infect us may be relevant too, candidiasis and cryptococcus. Shouldn't we look at the possibility for a new genus of fungi from Mars to gradually adapt to human hosts if they are not immediately able to be more than a minor nuisance? We don't know how quickly this adaptation might occur.

NEW: Many terrestrial fungi do well in Mars simulation chambers – a fungal disease from Mars would be likely to be hard to distinguish from tuburculosis through testing or medical imaging - and with likely no effective antifungals available initially or for some time

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[2015] Fungi capable of living on Earth seem to be a realistic possibility for Mars as we have many terrestrial fungi which do well in Mars simulation chambers. Many are rock inhabiting black fungi that are able to adapt to extreme environments, hot and cold and other extremes such as high salinity, acidity, and dessication, and many have been able to colonize rocks in Antarctica (Selbmann et al, 2015). One of these black fungi, Cryomyces antarcti was tested in the BIOMEX experiment simulating a Martian atmosphere, exterior to the ISS. At the end of the experiment it was not only still viable but showed only slight damage too fine to see with optical microscopy (Pacelli et al, 2017). They are given many names in the literature including “black yeasts” and “micro-colonial fungi”.

[2014] One of these is closely related to human pathogens. That's *Exophiala jeanselmei MA 2853,* a rock inhabiting black fungus in moderate climates, which turned out to have the potential to survive and grow in the Mars simulation chamber of the German aerospace center with daily temperature changes from below -40°C to above 15°C and also simulating the day to night humidity cycle (Zakharova et al, 2014). Its close relatives are sometimes serious pathogens for immunocompromised individuals, and naturally resistant to most antifungals on the market (Urbaniakt al, 2019).

Fungal diseases are hard to diagnose. For aspergillus, our closest analogue to a fungal disease not adapted to humans, the test for fungal galactomannan (a component of the fungal cell wall) is 80% sensitive which means 20% of infections wouldn't be detected even if aspergillus is suspected (Brown et al, 2012:6).

Chronic pulmonary aspergillosis (CPA) which has high mortality within 5 years is often confused with tuberculosis, looking similar in medical images of the lungs, and also clinically. They can be distinguished by testing for antibodies to Aspergillus, but there was no standardised test for antibodies (as of 2012) (Brown et al, 2012:6). There are many marketed tests now, but still not 100% reliable. The ELISA IgG antibody tests for CPA vary in accuracy but on average is 93% reliable (sensitivity) but with 3% false positives (97% specificity) (as of 2020). One of the issues here is distinguishing between harmless colonization and the disease (Volpe Chaves et al., 2020).

Fungi are evolutionarily closer to humans than most microbes, which makes it harder to develop antifungals. The introduction of echinocandins and third-generation triazoles improved the options for antifungal therapy but they have had modest success in preventing death from fungi (Brown et al, 2012:6).

A fungal disease from Mars might be initially similarly hard to diagnose and confused with other diseases like tuberculosis and it might be similarly hard to develop antifungals to protect against it.

NEW: Our immune system responses are highly specific to each of the three genera of opportunistic human fungal pathogens – without the necessary pathogen associated molecular patterns (PAMPS) we might all be immunocompromised to a new genus of fungi from Mars

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Also, would our immune system be able to stop a fungal infection by an alien fungus from Mars?

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 genus (Kumar et al, 2018 : table 1)

Looking at the two fungal genera that infect via the lungs, I have shown in bold the patterns and the receptors shared in common between the two genera:

Aspergillus fumigatus

> **PAMPs*:*** β-1,3-glucan, chitin, galactomannan, DHN-melanin
> **PRRs: TLR2**, CLRs (dectin-1, **−2**, mincle, DC-SIGN), NLRs (NOD1, **NLRP3**), CR3, PTX3 MelLec

Cryptococcus neoformans

> **PAMPs:** Mannose,capsular polysaccharide, glucuronoxylomannan
> **PRRs:** TLRs (**−2**,-4), CLRs (**dectin-2**, MR), NLRs (**NLRP3**)

Suppose hypothetically that our human immune system only ever encountered Cryptococcus, and never encountered Candida or Aspergillus. It would have three pattern recognition receptors it could potentially use with Aspergillus,

PRRs: TLR2, Dectin-2 and NLRP3.

However, none of its acquired pathogen-associated molecular patterns would work with Aspergillus. It still wouldn't see it.

PAMPs: None

Similarly, our immune system might not have genus specific PAMPs for a martian fungus in a novel genus with a shared terrestrial biology. It's not at all likely to have PAMPs for a fungus with a totally alien biochemistry.

So it seems indeed, that there is some potential that we might all be immunocompromised against a fourth opportunistically pathogenic genus of fungi from Mars. We are likely even more immunocompromised if challenged by fungi with a totally alien biochemistry.

Warnings by some astrobiologists such as Sagan and Lederberg that in worst case we could be in effect immunocompromised to an entire exobiology from Mars

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Some astrobiologists say that there is a possibility that more generally, in a worst case scenario, we might all be in effect immunocompromised to an entire exobiology from Mars. Joshua Lederberg, a key figure in early work on planetary protection (Scharf, 2016) put it like this (Lederberg, 1999b):

> ***Joshua Lederberg:*** *Whether a microorganism from Mars exists and could attack us is more conjectural. If so, it might be a zoonosis [infectious disease that jumps to humans] to beat all others*

In that paper he is looking at the dilemma of a parasite that if it proliferates too fast it risks killing its host and few parasites benefit from the death of the host, but if it proliferates too slowly then it risks being overwhelmed by its host and may develop stealth tactics to continue to survive. So pathogens find a balance between the two. But a microorganism from Mars hasn't been through this process.

Lederberg goes on to argue 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. He concludes:

> ***Joshua Lederberg:*** *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."*

In another paper, he considers two possibilities, that martian life is mystified by us, or in the worst case, our immune system doesn't recognize the attackers as life, and does nothing to stop them (Lederberg, 1999a).

> ***Joshua Lederberg:*** *Many serious emerging infections are zoonotic transfers, including HIV, hantavirus, plague, and tickborne rickettsioses. In many of these cases human infection is incidental to the natural history of the microbe. Probably most inter-species transfers are totally innocuous, hence invisible. Many others will be neutral. We pay close attention to those where the microbe-host balance is disrupted by the change in genomic environment, has not yet reached new equilibrium, and manifests a rule-breaker.*
>
> *It likely takes as delicate fine-tuning for a microbe to moderate itself as it does to take on the defensive barriers of a new and strange host.*
>
> *New zoonoses are not alien encounters, as the microbe involved usually has a history of successful parasitosis in another species- even if that experience is as distant as transovarian propagation in a tick.*
>
> *These earthly encounters raise questions for those concerned about interplanetary travel and ensuing exposure to microbes that might be found on other celestial bodies. If Martian microorganisms ever make it here, will they be totally mystified and defeated by terrestrial metabolism, perhaps even before they challenge immune defenses? Or will they have a field day in light of our own total naivete in dealing with their "aggressins"?*

Carl Sagan, discussing the potential effect of Martian life on humans, put it like this (Sagan, 1973)

> ***Carl Sagan:*** *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.*

Perhaps microplastics would be a useful analog here. At 10 microns or less in diameter, they can potentially cross into the blood stream, for instance through the submicron barrier in the lungs, and access all organs and at 0.1 microns or less they can penetrate the skin through to the blood stream (Campanale et al, 2020). Our bodies are to some extent permeable to small particles that our immune system ignores.

So far, these ideas seem to have had no attention in the planetary protection literature since Lederberg's two papers. The paper (Lederberg, 1999a) has sixteen cites in Google Scholar, and the paper (Lederberg, 1999b) has seven cites in Google Scholar. None of these cites are to planetary protection discussions. The 2009 NRC Mars Sample Return study (SSB, 2009) doesn't cite or mention Lederberg's 2009 papers, see search results.

All this needs to be looked at by experts. This paper suggests that there is enough here to merit attention in a future Mars sample return study, as part of our assessment of what needs to be done to protect Earth.

The Mars sample return studies so far proceed almost entirely by analogy with specific examples of terrestrial pathogens, with little or no discussion of potential effects on terrestrial organisms of a totally different exobiology. It's natural for out studies to focus on terrestrial examples as we have no actual examples of extraterrestrial life. But the papers by Lederberg show that it is possible to discuss possible effects of exobiology without any example lifeforms.

I have found no further discussion of these ideas in the planetary protection literature. I hope at some point this will be covered.

Meanwhile, to try to sketch this out a little, it might help to look at some ways that our immune system would likely fail to recognize an alien exobiology as harmful, or indeed, overreact with an allergic response. That's the subject of the next couple of sections.

I have been unable to find any expert treatment of these topics, so this is preliminary. The main intention here is to suggest that this is a topic that can be studied and needs to be looked at.

If you wish to skip this section the next main section is:

- Sterilizing subcommittee's report agrees an invasive Martian species could potentially harm or displace terrestrial photosynthetic bacteria – but argues any microbe adapted to martian conditions wouldn't be viable on Earth – example of radiodurans, first found in irradiated cans of ham, can recover from 100 double strand breaks of DNA per chromosome – the similarly radioresistant blue-green algae chroococcidiopsis is one of our top candidates for a terrestrial microbe with potential to survive on Mars

NEW: How our body's first lines of defence could miss alien life – antimicrobial peptides might not work with an alien exobiology and dentritic cells might not recognize the need to split alien life into antigens to present to T-cells

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Our skin's first line of defence consists of sixteen broad spectrum antimicrobial peptides (Abdo et al, 2020 : table 1) and the second line of defence consists of T cell responses which lead to inflammatory cascades in the tissue below the epidermis, the surface of the skin (more generally subepithelial tissue) (Abdo et al, 2020).

With both these lines of defence, the issue is that the immune system has to protect its own cells, as well as beneficial microbes. It would be much easier if the peptides could just disrupt all cells. Similarly the immune system has to prevent an auto immune response and have minimal disruptive effect on beneficial microbes.

As a first precaution, the peptides are produced as inactive precursor proteins and need to be broken up to become active (Zeth, 2013)

> *All peptides are produced as inactive precursor proteins comprising presequences that are delivered in an inactive form to their destination, where they become activated through proteolysis*

An unfamiliar exobiology might break up these innocuous precursors further than for terrestrial life, until they are harmless. Or, after cleaving the harmless precursor peptides into the toxins the unfamiliar exobiology might extrude the toxins, or bind to them. Those are strategies used by terrestrial pathogens but an unfamiliar exobiology may do it for some other reason (Peschel et al., 2006).

Or an unfamiliar exobiology might ignore the precursor peptides, or might break them up in ways that produce different byproducts.

Some of the peptides are specific to particular genera of terrestrial life (Abdo et al, 2020 : table 1) so those would likely have no effect on an alien microbe.

The broad spectrum peptides interfere with specific cell processes of particular types of terrestrial microbes. This discrimination seems to be based on the composition of the cell membranes (Hancock et al., 2000)

> *The basis of discrimination for the relatively non-toxic peptides appears to be the lipid composition of the target membrane (selective peptides tend to prefer membranes that have a negatively charged surface and are free of cholesterol) and the possession, by the peptide-susceptible organism, of a large transmembrane electrical potential (oriented internal negative)*

An exobiology might not be affected if its cell membranes are less negatively charged than the target microbes, or if they have a lot of cholesterol naturally.

For instance Dermadectin, one of the most common and potent broad spectrum antimicrobials in the human skin imbeds itself in the membranes of microbes to form an ion channel which greatly increases the permeability of the cell wall for water and ions and can dissipate the transmembrane electrical potential (which protects the interior of the cell) in as little as a ten thousandth of a second (Song et al, 2013). This is also the general mode of action of these broad spectrum antimicrobials, relying on the negatively charged outermost acid groups of the cell walls (acid groups donate protons to water to become negatively charged) to attract a positively charged cation, and then using molecules that are water attracting at one end and water repelling at the other end to bridge into the center of the cell wall and then across it similarly to the lipid bilayer (Hancock et al., 2006)

Such a peptide could be very damaging to an alien microbe if its biology is similar enough to terrestrial pathogens. However these natural antimicrobials have to be limited in potency because of the risk of harming beneficial microbes as well as the host, and if the cell walls of the alien microbe more closely resemble the cells of the host or beneficial microbes or are just too different to be affected, the alien life might not be harmed.

For the second stage, the immune response, the alien microbe has to be recognized as a potential intruder. The T-cells respond only to pre-processed pieces of the intruder, the antigens, on antigen presenting cells. It's the dendritic cells that recognize the pathogens by using pattern recognition receptors. Once recognized they ingest them in a process called "Receptor mediated phagocytosis" (recognition and ingestion of large particles into a vesicle where it is broken up into smaller particles) and break them up and process them into antigens to present to the T-cells (Liu, 2016)..

As we saw with fungi above, if the dendritic cells don't have the receptors or patterns to recognize the alien organism, the dendritic cells will ignore it. They won't try to process it into antigens to show to the T-cells. See:

- NEW: Our immune system responses are highly specific to each of the three genera of opportunistic human fungal pathogens – without the necessary pathogen associated molecular patterns (PAMPS) we might all be immunocompromised to a new genus of fungi from Mars

So, in short, at the first line of defence, the alien organism might not be affected by the antimicrobials. At the second line of defence, if it doesn't match any of the standard patterns, as we saw could happen with a new genera of fungi, the dendritic cells might never notice the alien organism, and so not split it up into antigens. If the dendritic cells never notice it, then the T cells never see it.

So, one way that our immune system could be mystified by unfamiliar Martian life is that as with nanoplastics, very small microbes (up to 0.1 microns) might penetrate these barriers in our skin, without being noticed by our skin's antimicrobials or the immune system, and enter the underlying flesh and bloodstream. Larger microbes up to ten microns similarly might enter the blood stream via the lungs again as for the nanoplastics and microplastics (Campanale et al, 2020)..

NEW: Possibility of an allergic response to harmless alien life if it is recognized by the immune system but not by the inflammation dampening Treg cells

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In the last section we looked at the case where alien life isn't noticed by our immune system. There is another possible response we should look at.

It's also possible that the alien life is recognized. If the alien organism is detected, and processed into antigens by the dendritic cells, which are then taken up by T-cells, then, as with the peptides, the challenge for the immune system is to protect its own cells. The T-cells shouldn't respond to the body's own cells and they are also calibrated so they don't harm beneficial microbes either. These T cell responses are modulated by Treg cells that have an anti-inflammatory effect (Clark, 2010)

> *The immune system is faced with the difficult problem of mounting immune responses to dangerous pathogens while maintaining tolerance to the body's own tissues and to harmless or commensal organisms. Regulatory T cells (Tregs) are one of many mechanisms developed by the immune system to enforce tolerance to harmless and self antigens.*

These have an important role as they recognize various harmless microbes to dampen down allergic responses, for instance in the lungs the Treg cells prevent allergic responses to dust mites, Aspergillus fumigatus and plant pollen. Similarly in the gut and other barrier tissues the Treg cells help to dampen down responses to the many different species of microbes we are exposed to (Attias et al., 2019).

If the Dendritic cells do process and present the alien biology as antigens to the T-cells which then trigger an immune response, such as inflamation, the Treg cells could either misrecognise the alien life as familiar and dampen down the response when our body really needs protection, or the other way around Treg cells (perhaps more probably) might fail to dampen down an allergic reaction to a harmless alien microbe.

So in the case that the immune system does detect alien life, it has a delicate balance here with two responses that can lead to harm.

If the immune system misrecognizes an alien pathogen as harmless a pathogen can enter the blood stream and access all organs unopposed as in the previous section. While if it misrecognizes alien life as harmful with no dampening Treg response, it could lead to a possibly even dangerous allergic response to a harmless microbe, much as for allergies to aspergillus or peanuts.

NEW: Our antifungals and antibiotics might not work with unrelated Martian life and even related life might transfer accidental antibiotic resistance similarly to the way resistance to the new synthetic antibiotic quinolones seems to have originated in the Shewanella algae that never encountered it

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If a pathogen from Mars did evade our immune system we'd turn to our antibiotics and antifungals to treat it. But our antibiotics might not work with Martian life.

Let's take penicillin as an example. It binds to transpeptidase which is essential for cross linking in the final stage of cell wall synthesis to make rigid cell walls (Yocum et al, 1980). One way microbes develop resistance to penicillin is by using different enzymes for this cross-linking (Gordon et al, 2000). It is similar for our other antibotics. They target specific enzymes and processes within living cells based on Earth's biochemistry (Kapoor et al, 2017). An alien biochemistry might not have those enzymes or processes.

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

Eventually the greater difference between terrestrial and alien life might be a weakness for the martian pathogens, as we develop medicines that target an alien biochemistry or even a new genus of terrestrial fungi. But it takes a great deal of research to develop a new antibiotic or antifungal. It is easy to find substances that kill bacteria. The challenge is to find substances that kill bacteria, and also don't harm humans.

The process of developing a new antibiotic typically takes ten to 15 years and costs about $1 billion. For novel classes of antibiotic 1 in 30 completes the research process. (Welcome Foundation, n.d.). Also the search for a new antibiotic for humans usually starts from naturally occurring antibiotics in other organisms. With pathogens based on an alien biochemistry there might not be any naturally occurring antibiotic candidates for this research.

As for related life, a microbe based on terrestrial biology from Mars might also accidentally have antibiotic resistant genes that it can transfer to terrestrial life. Many of our 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. But 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 (Martínez, 2012).

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 (Martínez, 2012).

In the same way, with Shewanella algae as an example, even related Martian microbes such as a new genus of fungi could have antibiotic resistance through genes evolved for other purposes in the novel multiply extreme conditions on Mars, which might accidentally lead to their internal processes changing in ways that make even our synthetic antibiotics no longer effective. Then they might be able to transfer this resistance to terrestrial life.

NEW: Could the host of a martian pathogen on Mars be similar enough to protozoa to infect the white blood cells in our immune system as for Legionnaires' disease? This seems to be an open question.

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Here we return to Warmflash's comment based on the analogy of Legionnaires' disease which uses the same method to infect the white blood cells (phagocytes) of our immune system as it uses to infect protozoa in biofilms: (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.*
>
> *It is important to note the numerous biofilms observed aboard the Mir space station, which were found on surfaces and within water plumbing. These films were often multi-species and included bacteria, fungi, and protozoa.*
>
> *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.*

First, could there be protozoa on Mars, or more generally, larger bacterial grazers that play a similar role in biofilms?

Many protozoa can resist extreme conditions including dessication and are common in terrestrial soils (Stout, n.d.). Anaerobic protozoa can reach up to a quarter of the growth efficiency of aerobic protozoa (Priya et al, 2008). Also, Stamenković showed cold martian brines can in principle take up oxygen to a surprising degree so there may be aerobes in them too (Stamenković et al, 2018).

So it seems plausible that a martian ecology could include an analogue of protozoa.

If so they might well have pathogens. That then leads to the next question – could the Martian hosts of these pathogens be related to terrestrial protozoa? I haven't been able to find any panspermia studies for protozoa. Though they can be very resilient, it's a big ask for a large protozoan host to survive transfer from Mars to Earth on a meteorite.

If the Martian hosts are unrelated, including the possibility of a different exobiology altogether, we have a similar question to the one tackled by Sagan and Lederberg, extended to protozoa and phagocytes.

From what Warmflash said, this seems to be an open question for now. How well would the defences of a protozoan respond to a parasite of a protozoan analogue based on an unfamiliar exobiology? I include this as a topic that seems to need investigation, if there is any way to move it forward.

Sterilizing subcommittee's report agrees an invasive Martian species could potentially harm or displace terrestrial photosynthetic bacteria – but argues any microbe adapted to martian conditions wouldn't be viable on Earth – example of radiodurans, first found in irradiated cans of ham, can recover from 100 double strand breaks of DNA per chromosome – the similarly radioresistant blue-green algae chroococcidiopsis is one of our top candidates for a terrestrial microbe with potential to survive on Mars

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The sterilizing subcommittee report agrees that planetary protection must consider not just human health but the entire biota of Earth. It agrees that if there could be invasive Martian species, the effects could be serious, for instance by harming or even displacing photosynthetic bacteria (Craven et al., 2021:6-7):

> *Photosynthetic bacteria such as Prochlorococcus are among the most abundant organisms on Earth and intensely important for the health of oxygen-respiring organisms, such as humans and animals.*

They then give a list of ways that ecosystems can be damaged,

- Direct cellular infections (which they consider to be unlikely – but as we saw in the previous sections, it needs to be considered as a possibility even for humans)
- Competition for resources
- Production of biotoxic metabolites
- Displacement of organisms.

They conclude:

> *. Planetary protection must consider not just human health directly, but the entire biota of Earth.*

They then argue that Martian microbes wouldn't be able to survive on Earth. For this argument they use examples of extremophiles that can't live in our normal habitat to argue it's plausible any martian microbe would not be viable on Earth, and so, that martian life couldn't cause any environmental issues (Craven et al., 2021:6-7)

> *There are many described extremophiles that may survive in environments that are extreme to human or animal life (e.g. extremes of temperature or pressure) but do not survive under conditions in our normal habitat … Thus, it is plausible that any Martian microbe, after it arrives on Earth, would not be viable on Earth due to a lack of its required Martian nutritional and environmental conditions.*

This has a major omission, polyextremophiles that live in a wide range of extreme environments and can often also live in normal environments, such as the ionizing radiation resistant deinococcus radiodurans, which was first discovered in radiation sterilized cans of ham in 1956, which means it was living in the ham before sterilization (Seckbach et al., 2015) (Anderson, 1956) (Krisko et al, 2013). Radiodurans can repair 100 double strand breaks per chromosome without any loss of viability or mutation of its genome (Minton, 1994). Raidodurans has this capability without ever encountering radiation sterilization. This shows that a polyextremophile doesn't have to be pre-adapted to conditions it may later encounter and colonize. This is an example used by John Rummel, NASA's planetary protection officer at the time, in his response to Zubrin's op. ed, referring to radiodurans's ability to grow in nuclear plant environments (Rummel et al., 2000),

Strains of chroococcidiopsis, one of our top candidate for a terrestrial genus of microbes able to live on Mars also have very high levels of ionizing radiation resistance (Li et al, 2022). Chroococcidiopsis is able to survive and remain viable in the temperature and pressure conditions of Mars analogue chambers and in theory could grow on Mars if it had a source of water and nutrients (Billi et al., 2011).

These capabilities aren't unique to radiodurans and Chroococcidiopsis. There are many other radioresistant microbes. Also radioresistant microbes on Earth can evolve a similar level of ionizing radiation resistance to radiodurans rapidly, suggesting it's likely a wide range of species on Mars could be as radioresistant as radiodurans.

As a test of how fast cells can evolve to such exceptionally high levels of radioresistance as radiodurans, a radioresistant e. coli strain which could withstand 2,000 Gy initially was exposed 20 times to increasing doses of ionizing radiation, each time for long enough to kill more than 99% of the population. After 20 cycles it could resist 10,000 Gy, close to the radioresistance of radiodurans. The newly evolved radioresistant strain kept that radioresistance for 100 generations in normal conditions.

In this experiment their most radioresistant strain of e. coli evolved a 4,500-fold increase in survival at 3,000 Gy through the course of the experiment. See (Harris et al, 2009:fig 1) and the text following figure 1. Also in another experiment at 5,000 Gy, which destroyed all DNA in the founder strain, the repair happened too fast for the recovery to be due to normal replication. It must have done it through DNA repair.

So, radioresistance on Mars wouldn't rule out life capable of living on Earth. Multiple species of terrestrial biology can achieve high levels of radioresistance. In other ways also, Martian conditions would seem to favor polyextremophiles, for instance life that can survive both warm and extremely cold conditions, and dry and very humid conditions, as we'll see in the next section.

Microbes from near the surface in Jezero crater would withstand temperatures varying from below -70 °C to above 15 °C in a single day – and major changes in humidity and pressure – this is likely to favour polyextremophiles – and martian life would likely be able to resist higher levels of stresses like UV, low humidity, vacuum, dessication, and ionizing radiation – which seems likely to make it easier not harder for them to survive on Earth

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The extreme variability of the Martian surface conditions might encourage polyextremophiles adjusted to a wide range of temperatures and humidity, well below -70 C to well above 0 C, and near 0% to 100% humidity. As for the other factors it would encounter, any Martian life might well be better able than its terrestrial analogue to survive desiccation, UV, ionizing radiation, and low atmospheric pressure as a result of evolving in those conditions for billions of years. However there seems no reason why it would ***depend*** on any of those things. Let's look at this in more detail.

The sterilizing subcommittee report doesn't go into details. It only mentions temperature and pressure as environmental factors that extremophiles can be adjusted to that can make it impossible for them to live in less extreme conditions (Craven et al., 2021:6-7):

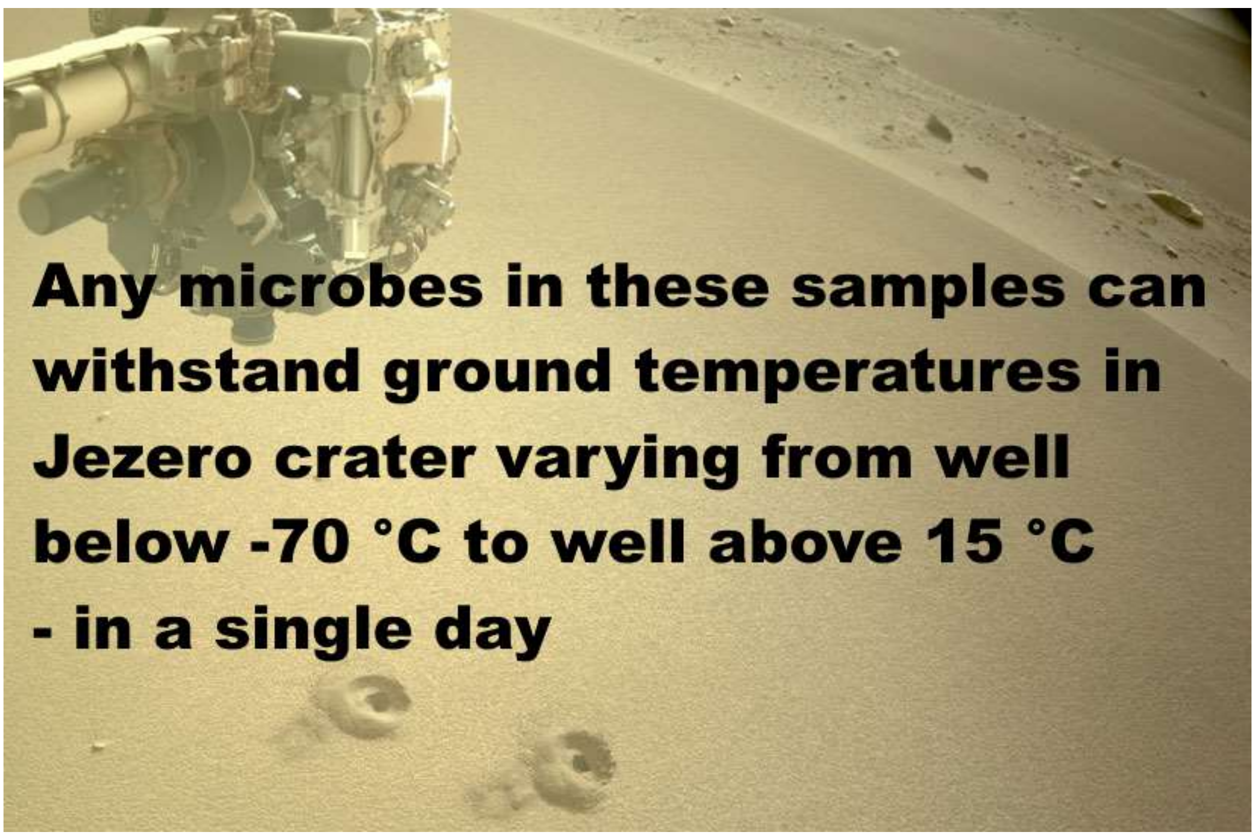
> *There are many described extremophiles that may survive in environments that are extreme to human or animal life* ***(e.g. extremes of temperature or pressure)*** *but do not survive under conditions in our normal habitat* (Merino et al. 2019). *extreme to human or animal life*
>
> *… Thus, it is plausible that any Martian microbe, after it arrives on Earth, would not be viable on Earth due to a lack of its required Martian nutritional and environmental conditions.*
>
> *[bolding added]*

They don’t say that those two examples apply to Mars particularly but it’s all we have to go on, so let’s start with pressure and temperature and then look at any other factors that may be relevant too.

Though low pressures on Mars can cause problems for some terrestrial life in the forward direction, it doesn’t seem likely that microbial life would ***depend*** on low pressure. Their cite doesn’t suggest that there would be extremophiles that depend on Mars like low pressure, and they don’t give any examples themselves. We would need all putative Martian life to depend on low pressure.

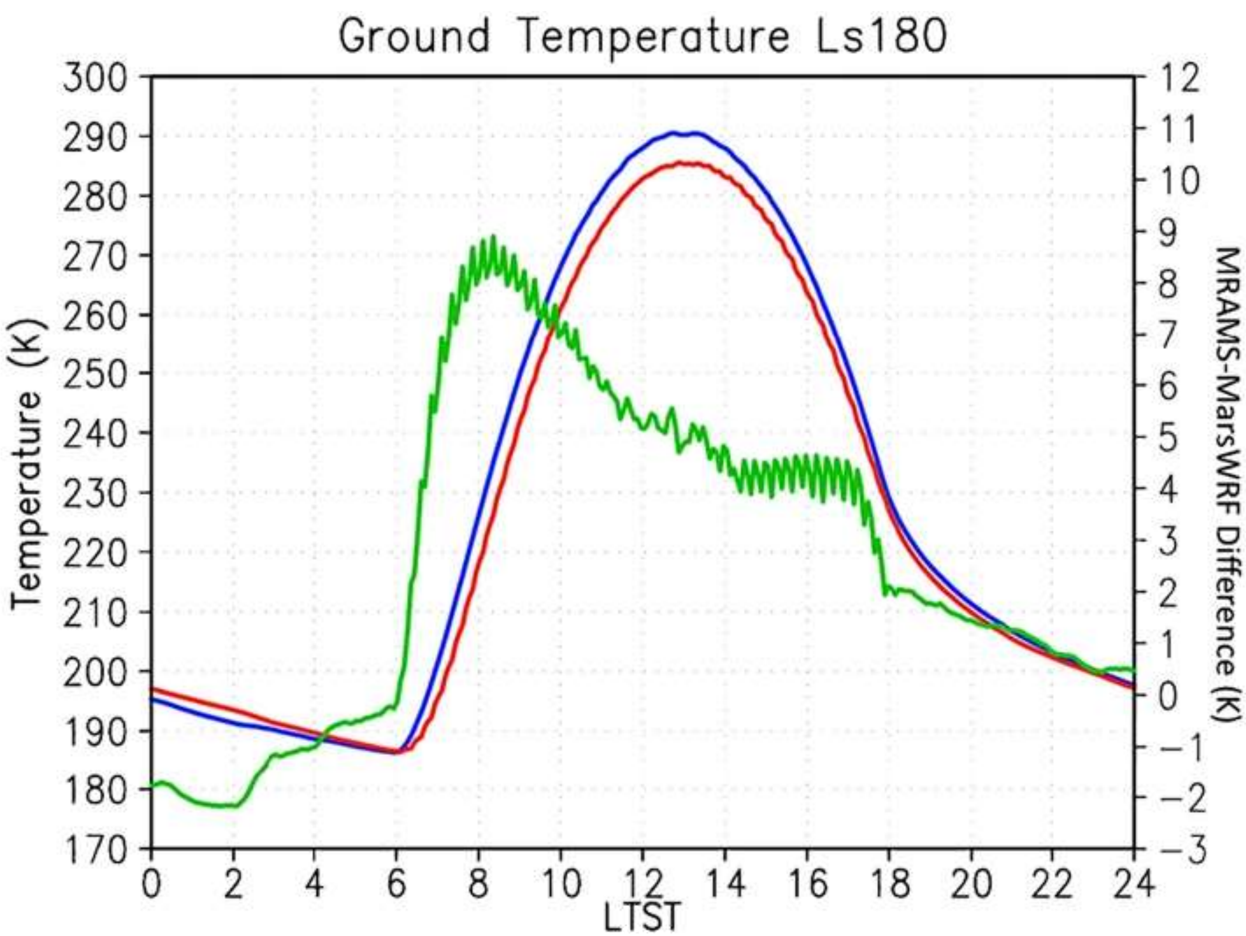
That leaves temperature of the two things they specifically mention. The cite they give does list many extremophiles that can only tolerate a narrow range of temperatures but others with a wider range. Merino et al’s example with the widest range is Planococcus Halocryphilus which is shown with a salinity range 0 to 19% and temperature range -15 to 37 °C (Merino et al, 2019: table 3)

Perseverance’s two regolith samples are from surface dirt (NASA, 2022). As measured by Perseverance the ground temperature in Jezero crater can vary from well below -70 °C to well above 15 °C in a single day (Afri et al, 2022: Figure 3).



Location photo of Perseverance’s two regolith samples (NASA, 2022).

The ground temperatures in Jezero Crater at the southern hemisphere autumn equinox (solar longitude Ls180) were predicted to be higher than at the summer solstice (solar longitude Ls90)



Predicted ground temperature for Jezero Crater for the autumn equinox at Perseverance's site (LS 180) using the Mars Regional Atmospheric Modeling System (red line shows the Mars Weather Research and Forecasting model and green line shows the differences between the two). From (Pla-García, 2020: Fig. 3)

The northern hemisphere autumn equinox, LS 180, was on Feb 24 2022 from the Mars Calendar (Planetary Society, n.d.). This is counted as mission sol 361 for Perseverance, as seen in the caption for an image as acquired on Feb. 24, 2022 (Sol 361) (NASA, 2022)

Perseverance's measured temperatures on mission sol 361 ranged from -74.33 °C to 16.96 °C (198.82 °K to to 290.11 °K). Later in the autumn Perseverance recorded even warmer days for instance mission sol 380 ranged from -65.05 °C to 18.84 °C (208.1°K to 291.99 °K). This is based on the calibrated data, details see: (Rodriguez-Manfredi et al, 2021). For the data files used see Supplementary Information

So, any martian life in the top few millimeters of the Martian surface needs to withstand extreme changes of temperature from below -70 °C to above 15 °C in a single day. It is also likely to be

UV resistant, ionizing resistant, radioresistant and able to tolerate low atmospheric pressures and low relative humidity in daytime.

Also though it is possible that Martian life in Jezero crater is incapable of adapting to higher temperatures than 15 C, it's also possible that the Martian surface life there has inherited capabilities to adapt to warmer conditions than it encounters on the surface in Jezero crater today. Mars had warmer conditions in the geologically relatively recent past. As recently as 210 million years ago, a volcanic eruption on the flanks of Arsia Mons melted enough ice for two lakes of 40 cubic kilometers each and a third one of 20 cubic kilometers of subsurface melt, which would have stayed melted for centuries to millenia insulated by surface ice (Scanlon et al, 2014). These would likely have subsurface high temperature habitable regions from volcano / ice interactions such as hydrothermal pools, and tuyas, a flat topped volcano beneath ice with liquid water forming around it (Glenister et al, 2021). More recently rootless cones (volcanic cones without a magma chamber below them) show evidence of steam explosions and hydrothermal systems that may have remained above 0 C for up to 1,300 years (Hamilton et al, 2010), with some of them possibly active as recently as less than 20 million years ago (Stacey, 2019), and there is evidence of explosive volcanism 53 to 210 thousand years ago by crater counting, which suggests some potential for present day subsurface hydrothermal activity (Horvath et al, 2021).

To guarantee no backwards contamination issues we need ALL possible life that could live on Mars and get into returned samples in Jezero crater to find it impossible to live on Earth or to adapt to commonly found terrestrial conditions after it escapes containment and spreads through our biosphere.

The gravity, atmospheric pressure and atmospheric composition for Mars is different, and it has temperatures that at times go below any temperatures recording on Earth. Almost everything else is duplicated somewhere on Earth in Mars analogue sites (Preston et al, 2013).

For completeness let's look at these other factors.

The lower gravity on Mars isn't likely to cause problems for terrestrial life. Microbes grow in the ISS in zero gravity, so there isn't any major obstacle to microbes adjusted to low gravity surviving on Earth.

The humidity on Mars is very variable and depending on location can reach 100% at night in winter and varies to close to 0% in spring to summer in daytime, and the pressure also varies greatly from day to night. This seems likely to encourage polyextremophiles that can tolerate any humidity level, rather than extremophiles adjusted to extreme low humidity.

The oxygen in Earth's atmosphere seems unlikely to cause problems. Mars may have photosynthetic life that produces oxygen when it converts CO_2 to organics. Curiosity discovered seasonal oxygen in Gale crater, 30% higher than expected in spring to summer. It could be produced abiotically or by low levels of present day life. (Trainer et al, 2019:3021) (Shekhtman, 2019)

Indeed, Martian surface conditions are superoxygenated. Martian salts include chlorides and sulfides as on Earth but also their oxygenated and superoxygenated forms, chlorates, sulfates and perchlorates, and we also find hydrogen peroxide on Mars. All this might make Martian life if anything better adjusted to oxygen stress than terrestrial life. Similarly to the situation for Martian life capabilities to cope with ionizing radiation, this doesn't mean it has to be dependent on perchlorates or hydrogen peroxide.

Mars life might use perchlorates as oxidants, as a source of energy. (Rummel et al , 2014). But again, at least if martian life is like terrestrial life, it's not likely that all martian life ***depends*** on perchlorates as a source of energy. Also, there are perchlorates in Mars analogue sites such as the hyperarid core of the Atacama desert.

It's possible to hypothesize a martian lifeform that depends on perchlorates and hydrogen peroxide for a faster metabolism in very cold conditions (Schulze-Makuch et al, 2010a). Their hypothetical organism couldn't survive for long above 10 °C with high humidity. (Houtkooper et al, 2006). Its biochemistry would limit its range on Earth to very cold dry Mars analogue conditions.

Martian life that depends on perchlorates represent a possible best case scenario for backwards contamination. But it doesn't mean that this is what we will find on Mars, it's just a scenario. Also if it can't survive on Earth it can't survive in warm wet conditions on Mars and especially not in hydrothermal vents in the past.

Then there's nitrogen, but it's hard to see why the extra nitrogen in Earth's atmosphere would cause problems. Microbes from Mars might not be able to fix nitrogen, but then many terrestrial microbes can't either. Also it's not impossible that there are nitrogen fixers on Mars. It's just on the border of possible. Experiments so far tested some cold tolerant microbes from Antarctica in air at normal atmospheric pressure but with nitrogen reduced to only 0.2 mbars similarly to Mars (Mancinelli, 1993) following (Klingler et al, 1989). These microbes could still fix nitrogen after simulating the temperature and UV flux of Mars (Sakon et al, 2005) (Sakon et al, 2006). More experiments are needed in Mars simulation chambers for 0.2 mbar nitrogen at a total pressure of 6 mbars similar to Mars (Sakon et al, 2006).

If there are nitrogen fixers on Mars they may be better able to fix nitrogen than terrestrial life. However, the abundant nitrogen on Earth would not be likely to be a problem for them.

In summary, there don't seem to be any major issues that would prevent life adapted to Mars surface conditions from living on Earth.

Microbes adjusted to Martian conditions can rapidly make small adaptations to terrestrial conditions if they establish a foothold such as higher growth rates, more efficient use of food and increased upper temperature limit for growth

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Microbes have such a short replication time that they can adapt rapidly allowing researchers to study evolution in action in the laboratory (McDonald, 2019). A martian microbe would only need to establish a foothold on Earth to start the process of adaptation to more terrestrial conditions. If the temperatures it encounters are within the range it is able to grow on Mars, it may be able to adapt quickly to higher growth rates and higher yields [how efficiently it uses the food it finds] at those temperatures. It may also be able to increase the upper temperature limit of growth by a degree or more, quite quickly.

As an example relevant to Mars, a cold adapted Antarctic bacterium with a growth range of −2.5°C to 29°C which shows signs of heat stress above 20 C was pre-adapted to 15 C. After a further 900 generations of evolution at gradually increasing temperatures it was able to grow at 30°C, one degree beyond the original temperature limit for growth. The ancestral strain could survive up to 30°C but not grow. The evolved strain could survive up to 31°C (Toll-Riera et al., 2022)

Another experiment looked at the yield [the mass of bacteria produced per mass of substrate consumed] of an e. coli clone. They started with a strain adapted to the same medium for 2000 generations at 37 C. Its yield near doubled at 42.2 C (1.94 fold increase) after 2000 generations in parallel in 115 separate cultures. This lead to 1331 mutations in total at 600 sites (Tenaillon et al., 2012).

Microbes can also increase their critical high temperature (CHT), the temperature limit for growth. In one experiment *Z. mobilis* TISTR 548's temperature limit for growth increased by 3°C from 38°C to 41°C. (Kosaka et al., 2019: table 1)

This suggests that a microbe from Mars that gradually spreads through the terrestrial biosphere might be able to change its temperature limits and its optimal temperature for growth relatively rapidly in numerous sub strains adapted to the different conditions it finds. These are relatively short term experiments with small numbers of microbes compared to the conditions a new microbe would find spreading for the first time through Earth's biosphere.

Several candidate terrestrial microbes and even higher organisms such as lichens may be able to survive on Mars, with promising results in Mars simulation chambers, suggesting a possibility that their Mars analogues may be able to live on Earth

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Also we now have a wide range of candidate microbes and even higher organisms such as lichens that could potentially survive and even grow in various candidate microhabitats on Mars with some tested in Mars simulation chambers and tested for their ability to survive in Mars simulation conditions complete with ionizing radiation using the BIOMEX experiment on the exterior of the ISS (Sielaff et al., 2019). From that 2019 review, their summary for each category tested is:

- **Methanogens:** The results from laboratory studies show it might be possible for some methanogens to inhabit the subsurface of Mars due to their tolerance to low pressure, desiccation, and perchlorate salts.
- **Bacteria:** The described studies show that the organisms with the highest potential for survival of Martian conditions are likely to be spore-forming bacteria which show resistance to multiple extreme physicochemical factors. It is important to determine if vegetative cells of spore-formers and nonspore-formers could withstand long-term simulated Martian conditions. The microorganisms isolated from various Earth environments show this potential, but more research is needed on studying the limits of life for bacteria in the context of Mars habitability.
- **Fungi:** Based on the study results, the fungi studies survived exposure to simulated Martian conditions in various capacities. Their resistance to radiation might be an important advantage over other microbial forms with regard to survival under Martian conditions.
- **Lichens:** Although Mars would present a harsh environment for sustaining life on its surface due in part to the high amount of radiation, … an environment protective against high levels of radiation could be present on the Martian surface, which may allow for the survival and proliferation of photosynthetic organisms [such as iron, salts, snows and crystalline rocks] These environments may allow for photosynthetic growth by lichens and other photosynthetic organisms on the surface, while allowing for a protective niche from the harsh environment.
- **Mosses:** These results showed that bryophytes have a high potential for survival in Martian conditions, although more research is needed. Even though UV exposure did not inhibit photosynthesis completely, it would be necessary to determine if bryophytes can conduct photosynthetic activity for extended periods under these conditions.

Some individual species or genera of special interest:

- Chroococcidiopsis – UV and radioresistant can form a single species ecosystem, and only requires CO_2, sunlight and trace elements to survive (Billi et al., 2011)
    - tested in BIOMEX (De Vera et al., 2019)
    - sometimes found in human microbiome (Ventero et al, 2022) (Lackey et al, 2019).
    - one strain produces a potential neurotoxin BMAA (Cox et al., 2005:fig 2),

- Methanogenic archaea such as *Methanosarcina soligelidi (*Maus et al, 2020) (Serrano et al., 2019)

- Alkalilimnicola ehrlichii MLHE-1 (Euryarchaeota)
  - able to use CO in Mars simulation conditions, in salty brines in conditions similar to those of the Recurrent Slope Linea for the water potential and temperature range, and could grow in oxygen free conditions if nitrates are present, and unaffected by magnesium perchlorate and low atmospheric pressure (10 mbar) (King, 2015)

- Halorubrum str. BV (Proteobacteria)
  - also did well in similar conditions to Alkalilimnicola ehrlichii, simulating the RSLs (King, 2015).

- rock inhabiting black fungi, Cryomyces antarcticus (an extremophile fungi, one of several from Antarctic dry deserts) and Knufia perforans,
  - adapted and recovered metabolic activity during exposure to a simulated Mars environment for 7 days using only night time humidity of the air; no chemical signs of stress (Pacelli et al, 2017)
  - Cryomyces antarcticus was tested in BIOMEX (De Vera et al., 2019)

- Rock inhabiting black fungus Exophiala jeanselmei MA 2853
  - also adapted and recovered metabolic activity during exposure to a simulated Mars environment for 7 days using only night time humidity of the air; no chemical signs of stress (Zakharova et al, 2014)
  - close relatives found in human microbiome (Urbaniakt al, 2019).
  - close relatives occasionally pathogenic (Urbaniakt al, 2019).

- Lichens such as Xanthoria elegans, Pleopsidium chlorophanum (de Vera et al, 2014) and Rhizocarpon geographicum
  - Xanthoria elegans and Rhizocarpon geographicum tested in BIOMEX (De Vera et al., 2019)
  - for more on Pleopsidium chlorophanum, see next section 2014: Example of an alpine lichen Pleopsidium chlorophanum found in places like California and the Alps that also grows in Mars analogue conditions in Antarctica and can survive and even grow in Mars simulation conditions – this shows even higher life from Mars could be adapted to live on Earth

- Mosses Grimmia sessitana collected in the alps (Huwe et al., 2019)
  - tested in BIOMEX (De Vera et al., 2019)

If terrestrial life can indeed grow on Mars, that would mean it is possible for the same species to survive on both planets which opens up the possibility for martian species able to survive on Earth.

2014: Example of an alpine lichen Pleopsidium chlorophanum found in places like California and the Alps that also grows in Mars analogue conditions in Antarctica and can survive and even grow in Mars simulation conditions – this shows even higher life from Mars could be adapted to live on Earth

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Not just microbes, even higher life from Mars could be adapted to live on Earth. One of our best candidates for a lichen to survive on Mars is Pleopsidium chlorophanum (gold cobblestone lichen), an alpine lichen that also grows in Antarctica in Mars analogue conditions and also grows in warmer alpine locations in places such as Europe and California.

> Pleopsidium chlorophanum showing its different growing habits.
>
> The photograph to the left shows its semi-endolithic growth in Antarctic conditions. You can see that it has fragmented the granite, and that pieces of the granite are partly covering it, possibly helping to protect from UV light. Photograph credit DLR
>
> The image to the left shows it at an altitude of 1492 m above sea level at "Black Ridge" in North Victoria Land, Antarctica (de Vera et al, 2014:figure 1). The image to the right shows it in California, above Lake Isabella, in the Kern River area (Sharnov, 1989)

Summarizing details about it from (de Vera et al, 2014), this lichen is able to cope with high UV, low temperatures and dryness in cracks, probably adaptive behaviour to protect it from UV light and desiccation. It remains metabolically active in temperatures down to -20 °C, and can absorb small amounts of liquid water from the atmosphere in an environment where it is only surrounded by ice and snow. The relative humidity in the lichen's niche microhabitat varies from

57% to 79% as the temperature varies from -6 °C to -8 °C and externally it varies from 23% to 46% as the external temperature varies from 8 °C to -8 °C.

In their 34 day Mars simulation chamber experiment the temperature varied between -50 °C and +21 °C, and the relative humidity varied between 0.1% and 75% (because warmer air has lower humidity for the same water content).

The atmosphere approximates conditions encountered in the equatorial and lower lattitude regions of Mars.

When exposed to full UV levels the fungus component of the lichen Pleopsidium chlorophanum died, and it wasn't clear if the algae component was still photosynthesizing,

However, when partially shaded from the UV light, as it is in its natural habitats in Antarctica, both fungus and algae survived, and the algae remained photosynthetically active throughout. Also new growth of the lichen was observed. Photosynthetic activity continued to increase for the duration of the experiment, showing that the lichen adapted to the Mars conditions.

This is remarkable as the fungus is an aerobe, growing in an atmosphere with no appreciable amount of oxygen and 95% CO_2. It seems that the algae provides it with enough oxygen to survive.

The lichen was grown in Sulfatic Mars Regolith Simulant, without ice. Photosynthetic activity was strongly correlated with the beginning and the end of the simulated Martian day, when atmospheric water vapour could condense on the soil and be absorbed by it, and could probably also form cold brines with the salts in the simulated Martian regolith.

The pressure used for the experiment was 700 – 800 Pa, above the triple point of pure water at 600 Pa and consistent with the conditions measured by Curiosity in Gale crater.

The experimenters concluded some lichens and cyanobacteria can probably adapt to Mars conditions, taking advantage of the night time humidity, and that it is possible that life from early Mars could have adapted to these conditions and still survive today in microniches on the surface (de Vera et al, 2014).

If lichens like Pleopsidium chlorophanum on Earth can grow in Mars simulation conditions, then it’s possible the other way around that any lichens on Mars may be able to grow on Earth.

So, not only microbial life, also higher life from Mars could potentially be able to colonize Earth.

2009, 2014: Possible future surprise discovery of large quantities of fresh water on Mars: most Antarctic meltwater is melted inside the ice through the solid state greenhouse effect when the surface is far below 0 °C – the same should happen on Mars if it has similarly optically transparent snow and ice – modelling shows its ice caps should have internal fresh meltwater at 0 °C, in summer, even with surface temperatures below -90 °C – with possibly also miniature melt ponds around sun warmed dust grains

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If Mars has sources for fresh liquid water, that greatly expands the range of terrestrial species that can live there and would give many new possibilities for indigenous martian life.

We already mentioned the potential for fresh liquid water in Jezero crater from melting frost.

- 2021: Potential for melting frost to form a "dew" of microns thick layers of fresh liquid water even in Jezero crater – as an example to show the potential for future surprise microhabitats

Another possible future surprise discovery proposed by some researchers could be large areas of liquid fresh water in the polar regions.

When researchers dig a half meter or so below the surface of the Antarctic snow and blue ice, they often find layers of fresh liquid water. They find this water even when surface temperatures are far too cold for ice to melt (Liston et al, 2005: p 1470 and fig 1).

This happens because the snow and ice is thermally insulating but optically transparent. Here optically transparent doesn't mean optically clear, it can be like frosted glass, you can't see through it, but it lets light through.

The covering insulating layer of snow or ice is thick enough at half a meter below the surface to hold in the heat, and enough sunlight gets through to warm up the snow or ice until it melts. This continues until the melt layer gets to a thickness of centimeters and even tens of centimeters. Actually, far more ice melts in the subsurface of Antarctica than on the surface. Researchers estimated that all except 46 out of 362.5 cubic kilometers of snow melted per year was subsurface melt in 1991–2000, while all except 2 out of 59.4 cubic kilometers of blue ice melted per year was subsurface melt. (Liston et al, 2005).

On a summer day on Mars in the polar regions it would take just one day to melt a layer 1mm thick at a depth of about 5 cm below the surface, even with surface temperatures on Mars as low as 180 °K (-93 °C). This thin layer should remain liquid through to the next day and gradually increase in depth to centimeters and tens of centimeters (Möhlmann et al, 2009) (Martinez et al., 2013:2.2.2) (Martinez et al., 2013:3.1.2).

This process should happen anywhere on Mars with sun facing optically transparent snow or ice, but it would not be easy to spot from orbit because of the ice cover and the thinness of the layers of water. It's essentially a cryptic habitat. All that's necessary is for the snow and ice to be optically transparent and thermally insulating, as it is on Earth and there is no particular reason why it wouldn't be.

There is another way to get fresh water inside the ice in Antarctica which should also work on Mars. Dust grains in the ice often produce tiny melt ponds around them in the heat of the summer sunshine. The dust grains absorb the heat (preferentially more of the heat is absorbed by the darker dust) and so heat up and melt the surrounding ice. The heat is trapped once more through the solid state greenhouse effect because ice traps heat radiation.

There is possible evidence for both these processes. The subsurface ice melt could explain the flow-like features in Richardson crater. These form in the debris of the Martian CO_2 geysers in early spring, extend down slopes in summer, and fade away in autumn, in a seasonal cycle not unlike the RSLs. (Martinez et al., 2013:3.1.2). These sometimes get confused with the similar looking Northern hemisphere flow-like features which are probably best explained by a dry formation mechanism involving dust and dry ice (Martinez et al., 2013:3.1.2). But the Richardson crater features seem to be formed by liquid water. Both current models explain these features as flowing brines and in one of them it is fed by fresh water fed by subsurface ice melt similarly to the Antarctica subsurface melt (Martinez et al., 2013:3.1.2).

As for the water melting about dust grains, in Antarctica a similar process around meteorites forms gypsum. There are large surface deposits of gypsum around the polar ice caps which are hard to explain because gypsum is soft and easily eroded (Fishbaugh, 2007) and this is one possible explanation for them. (Losiak et al., 2014). These could be surface melts that survive up to a few hours on warm windless days (Losiak et al., 2014), or they could be trapped in the snow with the ice freezing and keeping the water from evaporating similarly to the solid state greenhouse effect (Möhlmann, 2010), a process that should be effective between a few centimeters depth down to ten meters below the surface.

If Mars does have freshwater habitats in the polar regions, there may be a wide range of microbial species that exploit then. Similar Antarctic habitats have a diverse ecosystem of mcrobes, both in the fresh water and also in salty brines that form as the fresh water mixes with local salts (Doytchinov et al, 2022).

At the moment there is no direct evidence of fresh water on Mars, but these proposed microhabitats would be undetectable by the instruments and spacecraft we have sent to Mars so far. Mars has surprised us many times and it's not impossible that it surprises us again with fresh water.

The remarkable polyextremophile genus, the blue green algae chroococcidiopsis, one of our top candidate Mars analogue organisms, has strains in many terrestrial habitats, and sometimes in the human microbiome

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The remarkable ability of Radiodurans to repair multiple double strand breaks of DNA is also shared by one of our top candidates for a terrestrial microbe to survive on Mars, dessication resistant desert strains of the blue green algae chroococcidiopsis. The BIOMEX experiment on the exterior of the ISS tested Chroococcidiopsis sp. ASB-02, a species isolated from the Urad Middle Banner desert in inner Mongolia, and it remained viable after exposure to cosmic radiation in Mars simulation conditions (Li et al, 2022).

A microbe from Mars only needs to find a niche somewhere on Earth that it can survive in, then it can evolve and adapt and proliferate to other habitats. Species of chroococcidiopsis flourish 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, 2016:111). As a prime producer chroococcidiopsis survives on just rock, water, and light, fixing CO2 and nitrogen from the atmosphere.

Chroococcidiopsis is an ancient polyextremophile with numerous alternative metabolic pathways it can use, including nitrogen fixation, methanotrophy, sulfate reduction, nitrate reduction etc (KEGG, n.d.), with strains of chroococcidiopsis even able to grow in complete darkness with viable populations 750 meters below the Atlantic sea bed (Li et al, 2020). In this habitat chroococcidiopsis strains can get energy by oxidising hydrogen produced in the rocks by various abiotic processes (Puente-Sánchez et al., 2018).

Chroococcidiopsis like many bacteria reproduces asexually through cell division, making the distinction between a species and a strain rather fluid as they can’t interbreed, though they can share genes via horizontal gene transfer with other bacteria.

Species of chroococcidiopsis are also sometimes found in the human microbiome (Ventero et al, 2022) (Lackey et al, 2019).

NEW: Chroococcidiopsis indica produces an accidental neurotoxin, BMAA, which resembles serine and can cause protein misfolding, leading to questions about effects of amino acids from a novel exobiology

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One strain of Chroococcidiopsis, chroococcidiopsis indica produces BMAA (Cox et al., 2005:fig 2), which may be a contributing cause to neurodegenerative diseases such as ALS which

Steven Hawking suffered from, as it can bind to serine transfer RNA and so get misincorporated into proteins in place of serine. This leads to protein misfolding and these misfolded proteins have been found in nerve cells of people with ALS (Holtcamp, 2012).

This leads to interesting questions about what the effects might be of an extraterrestrial biology not based on terrestrial amino acids.

An extraterrestrial biology could use many more amino acids than the 20 encoded in RNA. There are 140 that occur naturally in terrestrial biology, but not in proteins (Ambrogelly et al., 2007). 52 amino acids have been identified in the Murchison meteorite (Cronin, 1983). A computer search turned up nearly 4,000 biologically reasonable amino acids (Meringer, 2013) (Doyle, 2014). Many of those won't occur in nature, but terrestrial biology also includes non natural amino acids. Meanwhile also many of the natural amino acids don't occur in terrestrial biology and might potentially be used in extraterrestrial biology.

Proteobacteria in our gut may provide some protection against BMAA 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.

NEW: Martian life could be better at photosynthesis than terrestrial life since terrestrial photosynthesis works at well below its theoretical peak efficiency and the lower light levels on Mars might favour evolution of more efficient photosynthesis

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Photosynthetic life on Earth operates at well below its theoretical peak efficiency for photosynthesis. Bains et al suggest this may be a many pathways event. Perhaps oxygenic photosynthesis could evolve in many ways, but with very low probability of achieving all the necessary steps so terrestrial life only happened to evolve it once. As a perhaps more plausible alternative, that it could be a "pulling up the ladder" event where once the niche was filled, a photosynthesizer not limited by the need for an electron donor such as sulfide, Fe(II) or hydrogen it was hard for a new photosynthesizer to evolve again (Bains et al, 2016).

Terrestrial photosynthesis rejects 50% of the incoming sunlight, mainly in the red part of the spectrum, leading to the distinctive "red edge". The purple bacteria and lichens don't have this "red edge" and Martian life would be likely to use red light like the purple bacteria, because of the high absorption of blue light by dust (Kiang, 2007).

Oxygenic photosynthesis goes through two photosystems, 1 and 2, and both use the same frequencies of light. The efficiency could be doubled by using red light for one of the two systems (Blankenship et al, 2011:808).

Martian life might also be able to use the full range of the spectrum. Terrestrial seaweeds are dark brown in colour because they use accessory pigments like fucoxanthin to gather the blue-green component of light rejected by chlorophyll. These then transfer the energy to the chlorophyll and so to the photosynthetic reaction centers. They do this so that they can use sunlight at only 1% of surface levels so it helps to use the blue-green light that passes through seawater (Caron et al, 2001).

A hypothetical Martian microbe with faster photosynthesis might find it useful to capture the full spectrum, especially in the low light levels on Mars. This would double its theoretical efficiency compared to terrestrial life.

According to Mellis, it would be possible to increase the typical 3% efficiency of green algae three fold, close to the theoretical maximum of 8 to 10% by truncating the light-harvesting chlorophyll antenna size (Mellis, 2009). Experiments back this up, though with smaller improvements (instead of tripling, they achieve modest increases of 55% to 60%) (Kirst, 2014). Terrestrial life likely uses a larger antenna than is needed to block out light from competitors, or because it allows it to capture more light at lower light levels with lower cell densities (Ort et al, 2015:8530) (Negi et al, 2020:15).

Although terrestrial life uses a fixed antenna size, cells have been designed that adjust the antenna size depending on the light intensity so that they achieve high efficiency both at low and high light levels compared to wild-type strains, doubling and even tripling the yields of the wild-type strains (Negi et al, 2020:15). A Martian photosynthetic organism would experience large changes in light levels with a need to capture light during dust storms if possible, and also to capture as much as possible during conditions of bright sunlight, so it might already have an adjustable antenna size and so have the advantages of both small and large antennas.

A Martian photoautotroph would only need a small improvement in efficiency compared to terrestrial life to be competitive with our photoautotrophs in the oceans, and there seem to be possibilities for major increases in efficiency. This Martian photoautotroph then might replace the natural species in our oceans.

In the best case, this could just be a drop-in replacement for terrestrial life, has minimal effect on the diversity of the terrestrial marine microbiota which survives but in smaller populations, and increases the productivity of the oceans. It might also have fewer or no exotoxins and might not form algal blooms. In the other direction though in the worst case it’s inedible, or produces many accidental toxins, is so competitive that the marine biota is almost a monoculture, and produces thick algal mats, any or all of those.

This leads to our new worst case scenario of mirror life. This could compete with terrestrial photoautotrophs even if it is no better or even less efficient at photosynthesis, and could be combined with better photosynthesis for a worst-worst case.

NEW: Example worst case scenario of a mirror life chroococcidiopsis analogue from Mars which could gradually convert organics in ecosystems into indigestible mirror organics

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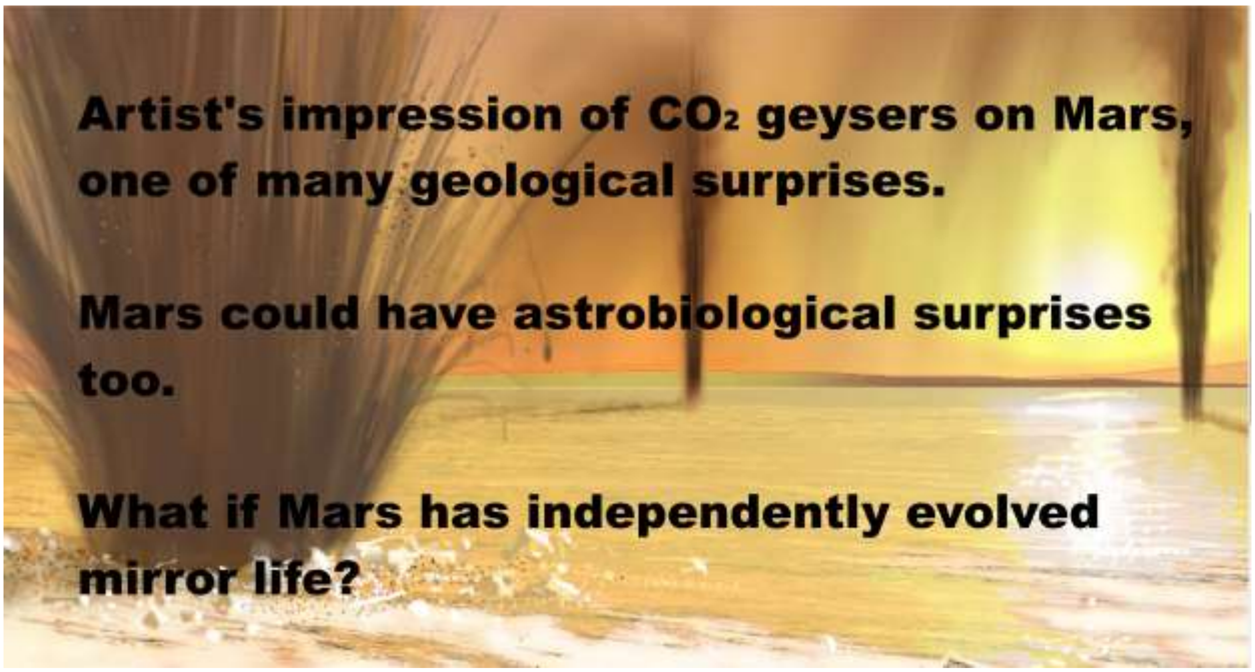
The planetary protection literature doesn't cover many scenarios in depth. In a search for new scenarios, I found many ways life from Mars can harm humans, our crops or ecosystems – as well as many ways it can be harmless or beneficial. These are for a future paper (Walker, 2022b). However, it may help to briefly mention one detailed worst case scenario, which as far as I know is new to the topic, to encourage space agencies to treat planetary protection more rigorously.

Mars has had many geological surprises for us. CO_2 geysers, evidence of a shallow northern sea in the past, deltas like the one in Jezero crater, the lake in Gale crater, the perchlorates, the droplets on the Phoenix lander, the lakes below polar ice. It may have astrobiological surprises too.



Artist's impression by Ron Miller of the martial CO_2 geysers that form in spring in the polar regions (Miller, 2006) (JPL, 2006).

This worst case but very interesting scenario is independently evolved mirror life, an algae like chroococcidiopsis able to survive on just rock, water, CO_2, nitrogen and sunlight, but with the DNA flipped the other way, everything flipped as in a mirror.

Nearly all terrestrial DNA spirals the same way. Most of the organics that make up terrestrial life are asymmetrical and so can have two mirror forms, like your left and right hand – but they nearly all are only found in one form in terrestrial life. That's because the molecules fit together a bit like an intricate mechanism, and the enzymes, and translation machinery and ribosomes which construct proteins and so on wouldn't work as intended if some of the pieces were flipped as in a mirror.

When a molecule is asymmetrical and has two mirror forms, like your left and right hand, it's called chiral. Terrestrial life is homochiral, which means that nearly all of its asymmetrical molecules occur in only one of its two mirror forms.

We don't know how terrestrial life became homochiral, with many proposed mechanisms (Blackmond, 2019). Some experts say it is *"luck of the draw"* (Brazil, 2015).

The theory of punctuated chirality suggests that early on as life was just starting to evolve, there were patches of chemicals that worked together with each other in chiral networks which expand and flip all the chemicals around them to be of compatible chirality (Gleiser et al, 2008).

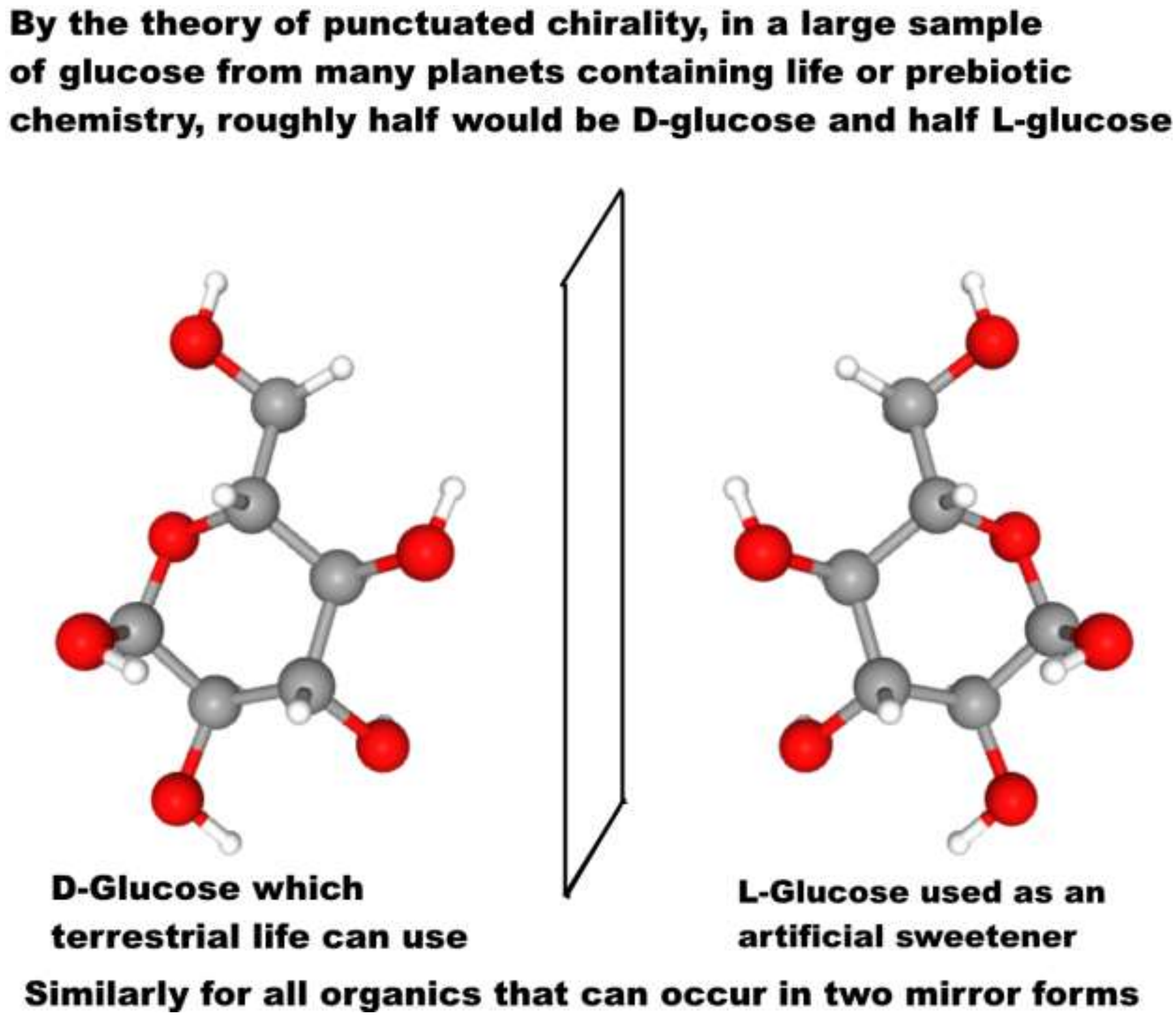
However there would be many such patches, some of one chirality some the opposite, and they would expand and flip each other back and forth in chirality on an environmental scale, with these flips perhaps frequent in Early Earth, until one of them got established as the basis for the evolution of life. If so, depending on how the flips went on Mars, life could easily have evolved from chemicals with the opposite chiral bias to Earth life (Gleiser et al, 2008):

> *Our analysis predicts that other planetary platforms in this solar system and elsewhere could have developed an opposite chiral bias.*

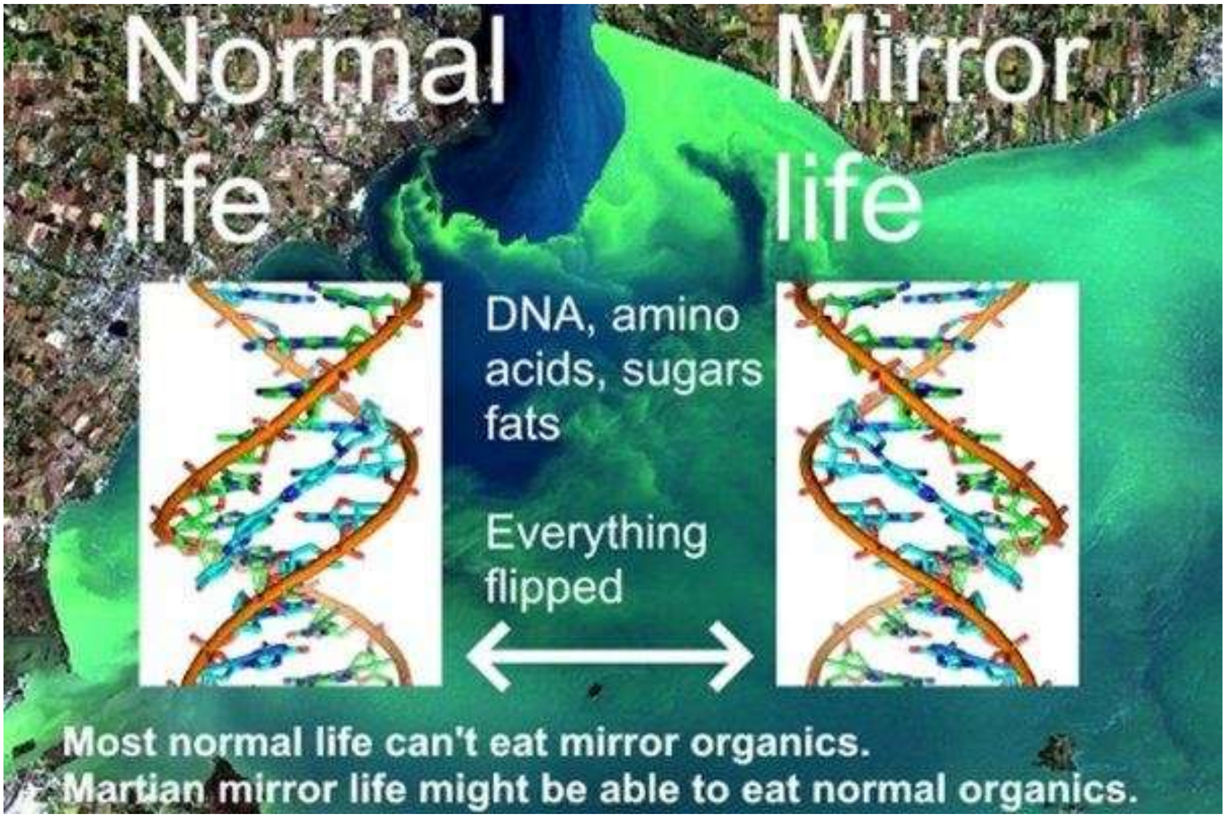
They predict that in the universe as a whole if an organic is found in a large sample of independently evolved forms of life it should occur in both forms. E.g. if there are many independently evolved forms of life that all use glucose, then there should be roughly the same amount of D and L glucose in the universe.

> *As a consequence, a statistically large sampling of extraterrestrial stereochemistry would be necessarily racemic on average*

Summarizing this as a graphic:



Graphic for L-glucose from (NCBI, n.d.) and D-glucose by reflecting the graphic horizontally. Grey: Carbon, Red: oxygen, white: Hydrogen.



Background image from (NOAA, n.d.), DNA spiral from (Pusey, 2012)

If we could flip a cake in 3D, like reflecting it in a mirror, we might be able to eat it, like artificial sweeteners, but our metabolism couldn't do anything with the flipped starches or proteins, and many fats would also be inaccessible (Dinan et al, 2007)

Synthetic biologists plan to gradually flip ordinary to mirror life over a period of a decade or so – and will make sure synthetic mirror life is engineered to depend on chemicals only available in the laboratory. They warn escape of mirror life could cause major transformations of the terrestrial biosphere by locking up organics in unusable mirror forms (Bohannon, 2010).

The biggest risk here is if mirror life gets enzymes (isomerases) that transform ordinary organic molecules into their mirror form. A few rare terrestrial microbes already use this in reverse to eat mirror organics (Pikuta et al, 2016). In the worst case scenario, mirror life has the enzymes to let it consume ordinary organics, but terrestrial life can't make anything of the mirror organics (Bohannon, 2010)..

> ***Kasting:*** *"It would quickly consume all the available nutrients," he says. "This would leave fewer or perhaps no nutrients for normal organisms." …. As the CO_2 in the ocean was incorporated into inedible mirror cells, they would "draw down" CO_2 from the atmosphere … in about 300 years the bugs would suck down half of Earth's atmospheric CO_2. Photosynthesis of most land plants would fail. "All agricultural crops other than corn and sugar cane would die," … "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..

Any life on Mars, mirror or normal, likely has those isomerases because it needs to be able to eat both types of organics. That's because nearly all martian organic is infall from comets, asteroids and interplanetary dust or made locally abiotic. Non biotic organics nearly always come in both normal and mirror forms.

Eventually many terrestrial microbes are likely to develop the necessary isomerases to eat it, but higher life couldn't evolve so quickly. The outcome is a mix of normal and mirror organics. Kasting and Church's worst case scenario is that mirror life retains the edge over normal life in this evolutionary race.

I think we would survive. We have already designed almost self-sustaining space habitats like the early Russian BIOS-3 based on plants grown for food, and oxygen, which in turn take up carbon dioxide and water from humans which should work in space, a more challenging situation (Salisbury et al, 1997) (Johansson, 2006) .

We could enclose large areas of Earth with its tropical jungles, coral reefs etc, like Biosphere 2 (UA, n.d.). But it would be a severely diminished world to leave to the next generation.

NEW: Closely related worst case scenario of a shadow biosphere of mirror life nanobes that produce indigestible mirror life biofilms on Earth

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For a closely related scenario, Earth and Mars exchange normal life, but Mars has a shadow biosphere with a different biochemistry that never got here like the hypothesis of a terrestrial shadow biosphere of nanobes (Cleland, 2019, pp 213 - 214) which could co-exist with modern life. Earth doesn't seem to have one (yet) but small cells have an advantage in an environment with low nutrient concentrations, as they have a larger surface to volume ratio, and so take up nutrients more efficiently. They would also avoid protozoan grazing (Ghuneim et al, 2018).

In this second mirror life scenario, Martian mirror life cells have a less sophisticated biology, but compete in a shadow biosphere on Earth because of their small size, with the extra advantage that they form mirror organics biofilms. These shadow biosphere biofilms are inedible to most terrestrial life and expand.

The mirror nanobes could have evolved in regions separated by physical barriers, for instance after a volcanic eruption such as volcanic eruption on the flanks of Arsia Mons 210 million years ago, which likely lead to 100 cubic kilometers of subsurface melt in three lakes, which would have stayed melted for centuries to millenia insulated by surface ice (Scanlon et al, 2014).

We could devise other examples such as mirror life with similar capabilities to normal life so it has a mix of normal and mirror life analogues of chroococcidiopsis to take most advantage of the infall of achiral organics. Also, more speculatively, chirality indifferent life using enzymes such as Joyce's RNA enzyme which can replicate RNA of opposite chirality including its own mirror version (Joyce, 2007) (Sczepanski, 2014) (Singer, 2014)

In these worst case mirror life scenarios, we may be able to reduce impacts, perhaps with engineered normal life predators of mirror life, or if Mars has both forms of life, we may be able to import these predators from Mars. However, these are scenarios to avoid, with consequences hard to predict.

NEW: Claudius Gros's worst case scenario for forward contamination – in this scenario nearly all higher life eventually goes extinct outside habitats, though it takes a long period of time and humans can survive by "paraterraforming Earth" with large enclosed habitats and preserve nearly all our biodiversity

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For completeness perhaps I should mention this scenario too. It is an extension of the ideas of Sagan and Lederberg about the potential that we might have no protection against an exobiology from Mars in the worst case.

- Warnings by some astrobiologists such as Sagan and Lederberg that in worst case we could be in effect immunocompromised to an entire exobiology from Mars

If Sagan and Lederberg's alternative scenario was true of terrestrial life mystified by an alien pathogen, it wouldn't just apply to humans but potentially to all terrestrial life. So, what would the outcome be if we returned life that none of Earth's organisms has immunity to or has natural defences against? It seems we should look at this possibility, if it is a possibility.

As with Sagan and Lederberg's warnings, it is impossible to go to the expert reviews for comment on this because it isn't covered in the backward contamination studies for a Mars sample return. We have some experts saying it is possible, but there is no dialog in the published scientific literature on the topic.

In the forward direction, 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' "

Is this argument valid, and can we apply this argument in reverse for backwards contamination?

If it can be applied in reverse, then in the worst case scenario,. terrestrial life is naive and offers no resistance when eaten by Martian life. The worst case would be that almost all multicellular organisms on Earth could be eradicated. All that would be left would be some small rapidly evolving organisms.

Humans wouldn't go extinct in such a scenario, as we saw in the last section.

- NEW: Example worst case scenario of a mirror life chroococcidiopsis analogue from Mars which could gradually convert organics in ecosystems into indigestible mirror organics

It would happen slowly enough, maybe decades to generations, that we would have time to recognize what is happening and build habitats to survive in. Also, we would be able to preserve much of the Earth's biodiversity including all the plants with preservable seeds (which is most of them). However such a paraterraformed Earth would severely diminish life prospects for several generations.

Eventually life outside the habitats would reach an equilibrium, with small microscopic single cell and multicellular terrestrial lifeforms able to evolve fast enough to take advantage of the new microbial environments. Over millions of years, perhaps faster with assistance from humans, there would be higher life forms again able to survive in an environment with both kinds of biology. Perhaps humans also could artificially adapt our progeny to survive outside the habitats or find ways to supplement their own immune systems so that they are protected from extraterrestrial microbes that our naive immune systems don't recognize as life. But essentially this process would turn Earth into an alien planet for macroscopic terrestrial biology in its current (original) form.

Although we have technology we could use to survive this scenario today, it would have been much harder with the early technology of the 1960s. The first “bubble boy” David Vetter who lived his life in an isolation room was born in 1971 (Gannon, 2012). Without experience of such technology, it would be that much harder for 1960s humans to survive back contamination of Earth’s biosphere with life that our biology is not able to protect itself against naturally.

As far as I know, none of the planetary protection studies have looked at such a scenario, or at the scenarios of Lederbeg and Sagan mentioned above:

- Warnings by some astrobiologists such as Sagan and Lederberg that in worst case we could be in effect immunocompromised to an entire exobiology from Mars

So there is no expert comment to refer to. All I can do is to draw attention to the scenarios.

I suggest future planetary protection studies of the effects of a Mars sample return use Lederberg’s papers as a starting point, and discuss the potential impacts on human health and more generally on Earth’s biosphere of a totally alien unrelated exobiology such as mirror life or life not based on DNA.

The backward contamination studies so far proceed almost entirely by analogy with known effects of terrestrial life and only briefly mention the potential impact of returning samples of life based on a different biochemistry or don’t discuss it at all.

NEW: Enhanced Gaia – ways that introduced Martian life could be beneficial to humans, ecosystems and Earth’s biosphere

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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. We need to focus on them for the same reason that a designer of a smoke detector has to focus on house fires.

We should also recognize that the introduction of extraterrestrial life to our biosphere could be harmless and also be beneficial, as Rummel mentioned in his 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!"

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 CO_2 in the sea and on land, improve soil organic content, and perhaps help with reduction of CO_2 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
- Martian microbes with more efficient photosynthesis 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

Introduced martian life could also have mixed effects, beneficial for some organisms and in some ecosytems and harmful in other contexts.

It can also be harmless. We could return a "drop in replacement" for terrestrial life. Just return another slightly different strain of chroococcidiopsis say not much different from returning life from another terrestrial desert. Or it could be life that has no chance of competing on Earth, an example might be Woese's early life transformable cells with "all the cellular componentry altered and/or displaced through *HGT [Horizontal Gene Transfer]" (*Woese, 2002)

But here, let's continue to focus on the best-best case scenario of enhanced Gaia.

Our planet is not necessarily optimal for global biomass (Kleidon, 2002). Perhaps extraterrestrial life with additional capabilities could enhance the productivity of the terrestrial Gaia.

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 could add a new domain of life with almost entirely beneficial interactions similarly to the Archaea
- It could add to biodiversity with 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 which only enhances the diversity of our biosphere.

NEW: Amongst a million extra-terrestrial civilizations that return unsterilized unstudied life – how many would find they harmed the biosphere of their home world? We don't know

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Amongst a million extra-terrestrial civilizations that return an unsterilized sample of life from a nearby biosphere without studying it first, and limited technological capabilities to contain it, we don't know how many would find they have harmed the biosphere of their home world.

It could be anywhere in the range from no effect or beneficial to frequently harmful.

- it is never seriously harmful, it usually leads to an enhanced Gaia, or has no effect, and is almost always a beneficial process or harmless,
- [many other possibilities], all the way to
- most civilization's biospheres are seriously degraded after they return unstudied unsterilized life.

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

If NASA or another space agency accepts the NRC study's assessment that the risk of large scale effects on human health or the environment is not demonstrably zero – this has major legal ramifications domestically, with agencies such as the DoA, CDC, NOAA etc involved and also internationally and through international treaties with the FAO, WHO etc involved as well as potentially domestic laws of other countries

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There are numerous legal ramifications if a space agency such as NASA takes on board the assessment of the National Research Council's study in 2009, that the risk from martian life of minor or major global harm to humans or the environment can't be assessed, and though likely low is not demonstrably zero (SSB, 2009: 48).

In the US, NASA itself, as a federal agency, is mandated to consider such matters as (NASA, 2012):

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

Uhran et al mention many other agencies likely to declare an interest including (Uhran et al, 2019) (Meltzer, 2012:454) (Race, 1996).

- 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 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

Although they don't mention this, if the mission is seen as having potential for transboundary effects, it seems likely that European countries such as the UK and the EU would get involved at some point since it is a joint ESA / NASA mission. The Directive 2001/42/EC might apply (EU, 2001), and the Espoo convention (UNECE, n.d.).

It seems unlikely that 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.

No matter which country is involved in planning a Mars sample return mission, at some stage, international agencies like the Food and Agriculture Organization may get involved, 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.

International treaties would be triggered and domestic laws of other countries are also likely to be triggered. Race and Urhan et al summarize some of these potential legal ramification see: (Uhran et al, 2019) (Race, 1996).

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

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.

In short, great care is taken to make sure that Earth is kept safe.

In the other direction, if a country such as the US passes their space agency's sample return mission as safe based on a claim of no potential for large scale harm to other countries, this may make it hard to object should another country approve a riskier sample return from Mars based only on their internal legislation and a claim that they also assess that there is no risk of large scale harm.

NASA's draft EIS fails NEPA requirement for a valid Environmental Impact Statement to ensure scientific integrity – with missing cites and cites that overturn the sentences they are cited to

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NASA's draft fails several of NEPA's central requirements for a valid EIS.

> *Agencies shall* ensure *the professional integrity, including scientific integrity, of the discussions and analyses in environmental impact statements*
> § 1502.23

The EIS has major issues, mainly

- **Currency:** uses out of date research, with major omissions of later studies that overturn results it relies on.
- **Accuracy:** sentences in the EIS are contradicted by the cites attached to those sentences, and the reader isn't alerted to this discrepancy
- **Accuracy:** doesn't mention views opposed to their conclusions in their own sources or other sources with views that contradict the agency's conclusions in the EIS.

A credible scientific report needs to be reviewed carefully to eliminate or minimize such errors (Blakeslee, 2004) (Tripp, n.d.) (Nausman, n.d.). For a list of the main issues found in the draft EIS see:

- Questions for NASA
- Reasons for these questions: controversial or mistaken statements in NASA's draft EIS and the report of the sterilizing subcommittee

On the last point of omissions of opposing views (Feldman et al., n.d.)

> *An agency must address in an EIS "responsible opposing view[s]." Courts have interpreted this regulation as requiring agencies to address opposing scientific viewpoints. In recent years, courts have given an agency's response to opposing scientific viewpoints deferential treatment, so long as the agency addressed the opposing statements and differing opinions in a meaningful way during the decision-making process.*

So, for instance on the topic of environmental effects, it seems the courts would be able to pass it as a valid Environmental Impact Statement under NEPA based on NASA's own statement that in their view there is no significant risk of environmental effects, so long as NASA alert the reader to the opposing views in sources such as the

- the NRC Mars sample return study in 2009
- the ESF Mars sample return study in 2012

and so long as NASA address these differences of view in a meaningful way in the EIS. Presumably NASA would need to discuss the reasons the ESF and the NRC gave for their views, and explain why they came to a different view.

However, the views in the ESF and NRC studies on environmental effects are simply not mentioned. So, it would seem to fail this requirement for a valid EIS. For a discussion of the views they omitted see:

- The planetary protection literature warns the potential for even large scale harm to human health and the environment isn't demonstrably zero – NASA's draft EIS conclusion that there is no significant risk of environmental effects seems a minority view amongst microbiologist and they don't alert the reader to the existence of any other view on the topic

For a list of the main issues found in the draft EIS see:

- Questions for NASA
- Reasons for these questions: controversial or mistaken statements in NASA's draft EIS and the report of the sterilizing subcommittee

NASA's draft EIS fails NEPA requirement to consider reasonable alternatives in detail so that reviewers may evaluate their comparative merits – doesn't examine the reasonable alternatives to sterilize samples in space first or to delay the mission until it can be done safely

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Another of NEPA's central requirements for a valid EIS.

> *(a) Evaluate reasonable alternatives to the proposed action, and, for alternatives that the agency eliminated from detailed study, briefly discuss the reasons for their elimination.*
> *(b) Discuss each alternative considered in detail, including the proposed action, so that reviewers may evaluate their comparative merits.*
> § 1502.14

NASA's EIS doesn't have rigorous analysis of ANY alternative except "no action". Reasonable alternatives include sterilizing samples in space before they approach humans or our biosphere or delaying the mission until it can be done safely.

NASA's draft EIS fails NEPA requirement to use an interdisciplinary approach including the social sciences by failing to involve the public early on, not just in the USA but through fora open to representatives from all countries globally as recommended in sample return studies – and the public weren't given the opportunity to comment on a scientifically valid draft EIS

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Another of NEPA's central requirements for a valid EIS.

> *Agencies shall prepare environmental impact statements using an interdisciplinary approach that will ensure the integrated use of the natural and social sciences and the environmental design arts*
> § 1507.2

Mars sample return studies emphasize the need to involve the public early on, not just in the USA, but through fora open to representatives from all countries globally because negative impacts could affect countries beyond the ones involved directly in the mission (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*

The public weren't involved early on in that way. Not only that, the public weren't given the opportunity to comment on a scientifically valid EIS.

I hope NASA can ensure a mishap like this never happens again

Other commentators raised significant issues – including one of the principle authors of NASA's probabilistic risk assessment guide who said the it should include the possibility of delaying the return until the risks are better understood

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Several other commentators raised significant issues including some of the ones already mentioned as well as new ones (Dehel, 2022) (DiGregorio, 2022) (Everline, 2022) .

Everline, a JPL employee and a principal author of NASA's probabilistic risk assessment guide (Stamatelatos, 2011), made a detailed public comment which said (Everline, 2022)

> ***Chester Everline:*** *A better statement of options should include the possibility of delaying the return of Mars samples until the risks associated with their return are better understood*

NEPA say to contact the agency to resolve issues, however NASA is not responding to attempts to contact them on this topic

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NEPA say the first step is to contact the agency to resolve issues (COEQ, 2007:28):

> *Your first line of recourse should be with the individual that the agency has identified as being in charge of this particular process.*

The natural point of contact is NASA's planetary protection office. They haven't responded to my email about the issues I raised after the draft EIS was published.

The comments section of the draft EIS didn't include responses to substantial issues I raised in May (Walker, 2022a)

NEPA don't mention the many significant issues I or anyone else raised with the draft EIS in their final letter to the public comments page on the last day of the public comments period, December 7th (EPA, 2022).

It's also not appropriate to try to work with other employees of NASA to resolve this issue when NASA's planetary protection office aren't responding.

There seems no way forward by way of dialog with NASA at this point in time. I encourage the reader of this paper to try asking NASA the questions raised here themselves.

Questions for NASA

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2012 ESF Mars Sample Return size limit review:

- Are you aware of the ESF Mars Sample Return study in 2012 which reduced the size limit from 0.2 microns to 0.01 microns for the 1 in a million threshold and required 100% containment at 0.05 microns? If so, why doesn't the EIS cite it?
- Are you aware that the ESF study recommended their size limit is reviewed regularly and that a BSL-4 doesn't even comply with the size limit they recommended in 2012? If so, why doesn't the EIS say so?

2015 MEPAG review:

- Are you aware of the 2015 MEPAG review that overturned all the findings you rely on to say that life couldn't get to Gale crater? If so, why doesn't the EIS cite it?
- Are you aware that the JAXA cite you use says on page 2 not to use their meteorite argument for samples returned from the Mars surface? If so, why isn't the reader alerted to this discrepancy?

- Are you aware of the debate about whether Curiosity should approach RSLs in Gale crater because of risk of forward contamination by terrestrial life? If so, why does the EIS say that existing credible evidence says Mars has been uninhabitable for millions of years

Scoping:

- What is your reason for requiring “safety testing” when all the samples will test positive and go to hold and critical review?
- Why wasn’t the option considered to sterilize samples before they return?

Large scale effects

- Are you aware of warnings about large scale effects in the NRC study in 2009? If so, why isn’t this mentioned in the EIS?
- Are you aware of the warnings about the potential that we have no defences against alien life by Joshua Lederberg and others? If so, why doesn’t the EIS discuss them?

Mars microbes surviving on Earth or as pathogens of humans:

- Are you aware that Legionella pneumophila is a disease of biofilms that also opportunistically infects humans as Legionnaire’s disease, sometimes lethal, and is not adapted to multicellular life? If so why is this not included in the discussion of whether pathogens have to coexist with humans to harm us?
- Are you aware that the mold Aspergillus fumigatus is not particularly adapted to a human host and causes an estimated 200,000 life threatening cases a year, mainly in immunocompromised people, with 30% to 95% mortality rate? If so, why isn’t this mentioned in the discussion of Candidas yeast’s adaptations to humans?
- Are you aware of the example from the NRC sample return report of an independently evolved hydrothermal vent organism that shares many virulence genes with a human pathogen? If so why isn’t this included in the discussion of Shiga’s toxin?
- Are you aware that the toxin produced by Clostridium tetani is not a result of adaptation to humans and neonatal tetanus kills thousands of unvaccinated children every year? If so, why isn’t this mentioned in the discussion of Shiga’s toxin?
- Did your sterilizing subcommittee have any examples of extreme conditions microbes face on Mars that could mean they can’t survive on Earth? If they don’t have any specific examples, why doesn’t the report mention this issue?
- Was your sterilizing subcommittee aware that there are many Mars analogue terrestrial organisms such as chroococcidiopsis that are thought to have some potential for living on Mars? If so, why isn’t the reader informed of this?

Procedure:

- Why aren’t you responding to attempts to ask these questions?

- Do you know how the EIS come to have so many citing errors of central importance to your arguments and can NASA ensure this won't happen again?

The simplest answer is that it is all a big mistake, and they weren't aware of any of those things. If so fine, we all make mistakes! But that means we need to start again with a scientifically credible EIS starting with a new size limit review etc.

At some point they are going to have to look at these questions. I don't think they can continue to ignore them all the way through until the samples are on their way back from Mars.

Reasons for these questions: controversial or mistaken statements in NASA's draft EIS and the report of the sterilizing subcommittee

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Here is a list of the most controversial or mistaken statements found in the EIS or the report of the sterilizing subcommittee which that list of questions is based on, with links to the sections of this paper that discuss them:

2012 ESF Mars Sample Return size limit review:

Are you aware of the ESF Mars Sample Return study in 2012 which reduced the size limit from 0.2 microns to 0.01 microns for the 1 in a million threshold and required 100% containment at 0.05 microns? If so, why doesn't the EIS cite it?

See:

- Draft EIS does mention a 0.05 micron limit – but not for the BSL-4, only for the return capsule – and without mentioning the ESF study

Are you aware that the ESF study recommended their size limit is reviewed regularly and that a BSL-4 doesn't even comply with the size limit they recommended in 2012? If so, why doesn't the EIS say so?

The draft EIS says they would use many of the basic principles of a Biosafety level 4 facility (BSL-4): (NASA, 2022: S-4):

> *"The material would remain contained until examined and confirmed safe or sterilized for distribution to terrestrial science laboratories. NASA and its partners would use many of the basic principles that Biosafety Level 4 (BSL-4) laboratories use today to contain, handle, and study materials that are known or suspected to be hazardous."*

See:

2012: The European Space Foundation study reduced the size of particle to contain at 1 in a million from 0.2 microns to 0.01 microns, well beyond the capability of a BSL-4 and NASA's recommendation is based on the science of 1999

- and following sections

2015 MEPAG review:

Are you aware that the JAXA cite says on page 2 not to use their meteorite argument in the way you use it, for samples returned from the Mars surface? If so, why isn't the reader alerted to this discrepancy?

(NASA, 2022: 3-3):

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

See:

- The meteorite argument can't be used for potential life in surface dust, salts and dirt [and following sections]

Are you aware of the 2015 MEPAG review that overturned all the findings you rely on to say that life couldn't get to Gale crater? If so, why doesn't the EIS cite it?

(NASA, 2022: 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."*

See:

- Jezero crater seems uninhabited from orbit – but so do terrestrial Mars analogue deserts – the 2015 MEPAG review which the EIS doesn't cite overturned all the conclusions relevant to Jezero crater that NASA's EIS relies on
[And following sections]

Are you aware of the debate about whether Curiosity should approach RSLs in Gale crater because of risk of forward contamination by terrestrial life? If so, why does the EIS say that existing credible evidence says Mars has been uninhabitable for millions of years

(NASA, 2022: 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."

See:

- NASA's draft EIS argues that existing credible evidence suggests Mars has not been habitable to Earth life for millions of years — yet their cite for this sentence is about a search for current localized habitable regions on Mars – another conclusion reached through a citing error

Large scale effects:

Are you aware of the warnings about large scale effects in the NRC study in 2009? If so, why isn't this mentioned in the EIS?

(NASA, 2022: 3-3):

"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."***

See:

- The planetary protection literature warns of not demonstrably zero potential for even large scale harm to human health and the environment – NASA's draft EIS conclusion of no significant risk of environmental effects seems a minority view amongst microbiologist and they don't alert the reader to this discrepancy

Are you aware of the warnings about the potential that we have no defences against alien life by Joshua Lederberg and others? If so, why doesn't the EIS discuss them?
(Craven et al., 2021:6)

"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."***

See:

- Warnings by some astrobiologists such as Sagan and Lederberg that in worst case we could be in effect immunocompromised to an entire exobiology from Mars
  [And previous sections]

Mars microbes surviving on Earth or as pathogens of humans: zzz

Are you aware that Legionella pneumophila is a disease of biofilms that also opportunistically infects humans as Legionnaire's disease, sometimes lethal, and is not

adapted to multicellular life? If so why is this not included in the discussion of whether pathogens have to coexist with humans to harm us?
(Craven et al., 2021:6)

> *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.***

See:

- Argument by sterilizing subcommittee that martian pathogens wouldn't be adapted to humans or other Earth hosts has a major omission – legionnaire's disease, a disease of biofilms that opportunistically infects human lungs

Are you aware that the mold Aspergillus fumigatus is not particularly adapted to a human host and causes an estimated 200,000 life threatening cases a year, mainly in immunocompromised people, with 30% to 95% mortality rate? If so, why isn't this mentioned in the discussion of Candidas yeast's adaptations to humans?
(Craven et al., 2021:6):

> *Existing microorganisms that coexist with humans over long periods of time can also …*
>
> *opportunistically infect a host with a weakened or compromised immune system such as candidiasis yeast infections*

See:

- NEW: Opportunistic fungal pathogens are sometimes deadly especially for immunocompromised people – Aspergillus is not adapted to humans – there are an estimated 200,000 life threatening Aspergillus infections a year with mortality 30% to 95% – and we might all be immunocompromised to a new genus of fungi from Mars with likely no effective antifungals available initially or for some time, and issues with testing for presence of the new fungus, or recognizing the disease through clinical diagnosis or imaging

Are you aware of the example from the NRC sample return report of an independently evolved hydrothermal vent organism that shares many virulence genes with a human pathogen? If so why isn't this included in the discussion of Shiga's toxin?
(Craven et al., 2021:6).

> *Existing microorganisms that coexist with humans over long periods of time can also cause new diseases when the organism takes on new pathogenicity, such as the Escherichia coli strain 0157:H7 that acquired a gene for Shiga toxin, …*

See:

- The sterilizing subcommittee's report mentions a strain of e. coli that they hypothesize became toxic by coexisting with humans – however the NRC sample return report gave an example of an independently evolved hydrothermal vent organism that shares many virulence genes with a human pathogen – martian microbes would continue to evolve on Earth – and this omits the suggestion by Łoś et al that e. coli developed Shiga's toxin to deter protozoan grazing in biofilms and only uses it opportunistically in humans

Are you aware that the toxin produced by Clostridium tetani is not a result of adaptation to humans and neonatal tetanus kills thousands of unvaccinated children every year? If so, why isn't this mentioned in the discussion of Shiga's toxin?

(Craven et al., 2021:6).

> *Existing microorganisms that coexist with humans over long periods of time can also cause new diseases when the organism takes on new pathogenicity, such as the Escherichia coli strain 0157:H7 that acquired a gene for Shiga toxin, …*

See:

- Sterilizing subcommittee's report doesn't mention clear examples of microbes which express accidental toxins without coevolution with humans or higher life, such as neonatal tetanus which kills thousands of unvaccinated children every year – and even the internal chemistry of an unfamiliar exobiology could be accidentally toxic

Did your sterilizing subcommittee have any examples of extreme conditions microbes face on Mars that could mean they can't survive on Earth? If they don't have any specific examples, why doesn't the report mention this issue?

(Craven et al., 2021:6-7)

> *"There are many described extremophiles that may survive in environments that are extreme to human or animal life (e.g. extremes of temperature or pressure) but do not survive under conditions in our normal habitat … Thus, it is plausible that any Martian microbe, after it arrives on Earth, would not be viable on Earth due to a lack of its required Martian nutritional and environmental conditions."*

See:

- Sterilizing subcommittee agrees an invasive Martian species could potentially harm or displace terrestrial photosynthetic bacteria – then argues this is not a concern on the basis that plausibly any microbe adapted to martian conditions wouldn't be viable on Earth – this omits polyextreomophiles such as radiodurans, first found in cans of corned beef, yet able to withstand 100 double strand breaks of DNA per chromosome

- Microbes from near the surface in Jezero crater would withstand temperatures varying from below -70 °C to above 15 °C in a single day – and major changes in humidity and pressure – this is likely to favour polyextremophiles – while microbes able to resist stresses like UV, low humidity, vacuum, and ionizing radiation do not require a non-terrestrial biology and there is no reason for them to be dependent on these conditions to survive
  [And following sections]

Was your sterilizing subcommittee aware that there are many Mars analogue terrestrial organisms such as chroococcidiopsis that are thought to have some potential for living on Mars? If so, why isn't the reader informed of this?

See:

- Several candidate terrestrial microbes and even higher organisms such as lichens may be able to survive on Mars, with promising results in Mars simulation chambers, suggesting a possibility that their Mars analogues may be able to live on Earth

What is your reason for requiring "safety testing" when all the samples will test positive and go to hold and critical review?

(NASA, 2022: 3-3)

> *These same principles regarding the importance of using terrestrial laboratories to enable the best scientific return also apply to the care and attention to detail that would be required to conduct a proper and comprehensive sample safety assessment in a proposed SRF.*

See:

- So sterilization preserves virtually all geological interest with minimal impact on astrobiological impact – but NASA's EIS doesn't permit it due to a requirement for "safety testing"
  [And previous and following sections]

Why wasn't the option considered to sterilize samples before they return?

See:

- We can forestall all these issues and make the mission 100% safe by sterilizing samples before they reach Earth – NEW

We can't actually assess the level of risk until we know more about Mars – it could be zero or it could be far higher than expected

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This mission raises many novel ethical and legislative questions. First, as the NRC observed, we can't actually assess the current level of risk (Space Studies Board, 2009: 48).

> … **it is not possible to assess past or future negative impacts caused by the delivery of putative extraterrestrial life**, based on current evidence.

If later we find only prebiotic synthesis on Mars, or slowly and imperfectly reproducing life with a biochemistry compatible with terrestrial predators, our risk from an unsterilized sample return is zero. Our main risk is in the forward direction that we might lose the chance to discover and investigate early life or prebiotic synthesis on Mars.

However, if later we discover a mirror life analogue of chroococcidiopsis on Mars, our risk from an unsterilized sample return of even large scale harm is far higher than we currently assess it to be.

Worst case scenarios introduce novel ethical and legal questions – is a 1 in a million level of risk acceptable?

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The very worst case scenarios for martian life such as mirror life also introduce novel ethical and legal questions about the level of risk we are prepared to take.

Kelly has traced the 1 in a million figure back to a 1 in 100 million figure in a 1961 article, introduced by Mantel et al for the purpose of discussion (Mantel et al, 1961). When asked why he chose this figure he replied ***"We just pulled it out of a hat"*** (Kelly, 1991). The FDA adopted this in 1973 but it became 1 in a million when the final rule was issued. Graham (Graham, 1993) says in practice, EPA's air office tries to reduce the risk to as many people as possible to 1 in a million and the maximally exposed individual to 1 in 10,000. In other situations, EPA recommends a range of risk levels from 1 in 100,000 to 1 in 10 million, and sometimes approves at a level of 1 in 10,000.

This is an ad hoc ethical decision by regulators about levels of acceptable risk, which got accepted more widely by legislators and the general public.

It also doesn't take account of human error. There are many examples, such as a SARS outbreak in 2003 in Taiwan which happened because a technician skipped the standard procedure after a spill, because it would make him late for a conference (Demaneuf, 2020).

Other escapes could happen from equipment failure. During the Apollo sample returns, two technicians had to go into isolation after a leak was found in a sample handling glove for Apollo 11 (Meltzer, 2012:485), and 11 technicians in a similar incident for Apollo 12 (Meltzer, 2012:241).

All this needs especially close scrutiny once there's potential for novel and even unprecedented larges scale harm – including other issues such as accidents, a fire at the facility or criminal actions.

Synthetic biologists suggest a safety mechanism for synthetic life should be many orders of magnitude safer than a BSL-4

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Synthetic biology already permits the creation of inheritable synthetic life such as life with hachimoji DNA (Hoshika et al, 2019). They make sure that this is safe by designing nucleotides that depend on chemicals only available in the laboratory.

Synthetic biologists have suggested that a safety mechanism to contain synthetic life should be many orders of magnitude safer than any contemporary biosafety device. Schmidt puts it like this (Schmidt, 2010)

> *The ultimate goal would be a safety device with a probability to fail below 10^{-40}, which equals approximately the number of cells that ever lived on earth (and never produced a non-DNA non-RNA life form). Of course, 10^{-40} sounds utterly dystopic (and we could never test it in a life time), maybe 10^{-20} is more than enough. The probability also needs to reflect the potential impact, in our case the establishment of an XNA ecosystem in the environment, and how threatening we believe this is.*
>
> *The most important aspect, however, is that the new safety mechanism should be several orders of magnitude safer than any contemporary biosafety mechanism.*

We can't rely on the same risk-benefit calculus for release of SARS and for release of mirror life, without legislative / executive / public involvement to decide if this is what we should do.

NEW: Society places very high value on the environment and given the potential for large scale effects, we might require Earth is kept 100% safe for this mission – i.e. use the prohibitory precautionary principle

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This mission also leads to novel questions about variations on the precautionary principle – principles to do with how we need to handle situations where the level of risk can't currently be assessed because the science is incomplete.

The ESF study considered variations on the precautionary principle (Ammann et al, 2012:25) based an analysis of the principle by Stewart (Stewart, 2002), including:

- **Best Available Technology Precautionary Principle**: Activities that present an uncertain potential for significant harm should be subject to best technology available requirements to minimise the risk of harm unless the proponent of the activity shows that they present no appreciable risk of harm.
- **Prohibitory Precautionary Principle**: Activities that present an uncertain potential for significant harm should be prohibited unless the proponent of the activity shows that they present no appreciable risk of harm

The ESF ruled out the Prohibitory Precautionary Principle on the basis that it would simply lead to cancellation of the mission (Ammann et al, 2012:25):

> *It is not possible to demonstrate that the return of a Mars sample presents no appreciable risk of harm. Therefore, if applied, the Prohibitory Precautionary Principle approach would simply lead to the cancellation of the MSR mission.*

They did this as experts mandated to find the safest way to conduct the mission.

However Stewart, elsewhere in that same paper, suggests there may be situations where prohibition may be needed, since society places very high value on the environment and its protection (Stewart, 2002:15).

Carl Sagan and others warning we can't take even a small risk with a billion lives – this could be formalized into law as a requirement to use the prohibitory precautionary principle whenever there is any appreciable risk for harm unprecedented in human history

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Chester Everline in his comment said (Everline, 2022):

> *A possible consequence of unsuccessful containment is an ecological catastrophe. Although such an occurrence is unlikely, NASA should at least be clear regarding what level of risk it is willing to assume (for the biosphere of the entire planet)*

Is a sample return mission one where we should consider the prohibitory version of the principle?

Carl Sagan said we can't take even a small risk – that's the prohibitory version (Sagan, 1973):

> ***Carl Sagan:*** *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, 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.*** *.*

Gill Levin, who died shortly before the EIS, said the same, as recorded on video by Dehel and mentioned in his public comment (Dehel, 2022).

> ***Gill Levin:*** *I believe people will realize, especially after the Covid-19 catastrophe, that even if there's only a small chance that something could be contagious and pathogenic, coming from a foreign planet, I don't think it's worth taking that chance….you don't take unnecessary chances where the risk-to-benefit ratio is almost infinite."*

DiGreggorio in his public comment quotes from an interview he did with Dr Carl Woese who also expressed a similar sentiment (DiGregorio, 2022)

> ***Carl Woese:*** *Unless you can rule out the chance that it might do harm, you should not embark on such a course*

One possible outcome of public debate on this topic is to formalize Woese, Levin and Sagan's ethical views on this topic into legislation. The general public, and legislators, could decide that if an action has potential for unprecedented levels of harm to human health or the environment, the prohibitory version of the principle should always be used.

Perhaps it might be formulated something like this (for illustrative purposes only not a proposal):

> *If it is impossible to show that there is no appreciable risk of unprecedented levels of harm to public health or the environment, the Prohibitory version of the Precautionary Principle must always be used*

Unprecedented here means unprecedented in human history (e.g. mass extinction level events).

The decision about acceptable levels of risk for large scale harm is an ethical decision and can't be decided on the basis of science or engineering

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This decision is something that needs global public debate.

NASA are likely to set a higher priority to completing the mission assigned to them than the general public, but we are all potentially affected in the worst case. It needs to be opened out to larger debate.

This is something we can't decide on the basis of science or engineering. It is an ethical and legislative choice. 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.***

Public comments on the EIS show that many members of the public have similar views to Carl Sagan that this is a qualitatively different situation from a human pathogen in a BSL-4 and that shouldn't take even a low level of risk

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The public comments aren't a poll, but they do show that many members of the public have similar views to Carl Sagan, Gill Levin, Carl Woese and others, that this is a qualitatively different situation from a known pathogen in a BSL-4 lab and that we shouldn't take even a low level of risk.

Many specifically mention potential for unprecedented harm in one way or another. I think it is also reasonable to assume that all or nearly all the ones that say, test first, sterilize first or stop mission would support Carl Sagan's quote (Sagan, 1973):

> ***"The likelihood that such pathogens exist is probably small, but we cannot take even a small risk with a billion lives."***

Here are the comments summarized, and I've shown in bold the ones that likely support Carl Sagan's statement that we can't take even a small risk with a billion lives

As a rough estimate, 51 supporting some variation on Carl Sagan's view out of a total of 63 separate people commenting (selected one only for duplicate entries). Some were anonymous and it's not possible to know for sure if some of those were also duplicate. At any rate, several dozen distinct members of the public expressed views that suggest they would be in support of Sagan's quote, on a not very widely publicised EIS.

- **stop mission, unprecedented harm – test first – protect Earth – test first**
- **stop mission – stop mission – test first – test first, unprecedented harm – keep Earth 100% safe**

- **test first – stop mission** – need clarity about security measures – off topic – alternative design
- **keep Earth 100% safe – unprecedented harm – stop mission, unprecedented harm** – alternative design – **test first**

- **test first – test first – unprecedented harm – test first – Test first**
- **Don't return unless 100% safe – or sterilize first – Don't return – don't return until 100% safe – test first – test first**

- **test first – ISS first – test first – test first – unknown risk, test first**
- **sterilize first** – extra precautions for EES reentry – **sterilize first – sterilize first – sterilize first**

- **sterilize first – do not return – do not return – do not return** – send to Russia first
- **test first** – support EIS – **sterilize first** – fully support, suggests more samples – off topic

- multiple cautious measures – support EIS – support EIS – **test or sterilize first – sterilize first**
- **test in situ or don't return – EIS shouldn't be allowed – unprecedented harm, test first – unprecedented harm, return to space station**

- - and the four comments already mentioned by name **(Walker, 2022a) (Dehel, 2022) (DiGregorio, 2022) (Everline, 2022)**

Also notice that 12 said sterilize first, even though it's not listed as an alternative action in the EIS.

- sterilize first – sterilize first – sterilize first – sterilize first – sterilize first – sterilize first – sterilize first – ensure safe or sterilize first – study in situ or space lab or sterilize first
- Plus (Walker, 2022a) (Dehel, 2022) (DiGregorio, 2022)

EPA's letter on last day of public discussion says they didn't identify significant environmental concerns in their review of the EIS – with no mention of the public comments raising concerns similar to Carl Sagan

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EPA posted on the last day of public comments. Their letter says it didn't identify significant environmental concerns in its review of the EIS. It doesn't say anything about a need for NASA to respond to new issues raised in these comments by the general public. (EPA, 2022):

> *We appreciate NASA addressing EPA's concerns regarding water resources, unplanned releases and cultural/biological resources identified in the letter.*
>
> *Based on the review of the draft PEIS, EPA did not identify significant environmental concerns to be addressed in the Final EIS.*

If Carl Sagan was still alive today he would surely have commented on the EIS raising the same concerns as many of the general public made.

This doesn't look like broad acceptance which is essential for success of this mission – if NASA continues this action is vulnerable to being stopped in the future

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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 doesn't look like broad acceptance of NASA's proposed action. It may be stopped at various points.

First NASA could withdraw the EIS, do the size limit review, do a scientifically rigorous EIS.

This seems far the best outcome for NASA. Not forced to do anything by a court decision. Not responding to public panic. They can decide in their own time how to proceed. For instance they can do a 100% safe mission using sterilize first, or they can work on other ideas, but it's all done in coordination with the general public, legal experts, ethicists, social scientists etc.

Even a last minute conversion to a 100% safe mission could cause problems if NASA do it in response to panic from a distrustful public. Far better to get the public involved from the outset.

Assuming NASA continue with the EIS, it could be stopped by other agencies but this is unlikely as the draft EIS says that there are no significant environmental effects, so they'd have no reason to look at it closely.

The next point it can be stopped is by a court case. There is no provision for this within NEPA, so it is done through judicial review, usually on the basis that: (Congressional Research Service, 2021).

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

They can only be taken to the courts by someone with "standing". For this, they need to take part in the public comments or debate in the NEPA process, and need to be directly affected by the proposed action.

There you have to show that you are particularly affected by it, which is normally understood to mean more so than by others. If the petitioner claims NASA overlooked a worst case risk of global effects NASA could try to block it on the basis that in their hypothetical scenario they wouldn't be affected more than anyone else in the world and so don't have standing.

In the past, environmental cases have gone either way based on subtle legal arguments about whether environmental effects give the petitioner "standing" for the case (Birnbach, 1997).

If it does get as far as the courts, the case is usually (Congressional Research Service, 2021)

- referred back to the agency (such as NASA) for further proceedings HOWEVER
- the court can order the agency to stop the project going ahead or issue some other action (in this case perhaps order to sterilize the samples first?).

So if a case is taken out and it's successful, that could lead to a justice asking NASA to either stop the mission or to sterilize the samples first.

If nobody takes them to court or NASA successfully block the case, the next step is the presidential directive NSC-25, which requires a review of large scale effects that could be reasonably expected to result in allegations of major or protracted effects. It has to be done even if the agency feels confident such allegations are false (Whitehouse, 1977):. This happens after the NEPA process is completed (Race, 1996).

If it gets past all those hurdles with little public awareness, it could be stopped at the last minute with samples already on their way back to Earth.

Mounting global public concern could lead to Congress and the president acting to tell NASA to divert the mission away from Earth. A worst case here might be an infodemic about Mars life similar to the COVID infodemic, junk science, problems for NASA's credibility, and issues with eventual return of even 100% safe sterilized samples.

NEW: We can forestall all these issues and make the mission 100% safe by sterilizing samples before they reach Earth

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There is a way to make the mission 100% safe from the outset. That is to sterilize the samples before they reach Earth. What's more we'll see that sterilization done carefully has virtually no impact on either the geological or the astrobiological interest of the mission.

The sterilizing subcommittee said "… it is impossible to remove all risk without ceasing space exploration". (Craven et al., 2021:4).

> *"While it is impossible to remove all risk without ceasing space exploration, … There is always some level of risk associated with exploration into the unknown, and it was the goal of the SWG to help manage the risks of possible adverse effects to the Earth's biosphere while maintaining the science integrity of the returned samples."*

However, though there is no way to remove all risk of robotic spacecraft crashing or malfunctioning, or accidents for humans in space during space exploration, it is possible to completely eliminate any risk of harm to Earth's biosphere or to the health of humans on Earth.

There is no risk to Earth's biosphere from in situ robotic exploration of Mars, or human exploration by telepresence from orbit around Mars, or if we sterilize samples before they return them to Earth. The only possibility of risk to Earth's biosphere is from an unsterilized sample return specifically, not from space exploration. The safest approach here is to eliminate all risk to Earth's biosphere and human health while retaining the science interest of the returned samples as far as possible.

First for the geological interest. One way to sterilize the samples with minimal impact on geological studies is to duplicate the Martian surface ionizing radiation. Even the equivalent of 500 million years of surface radiation would have virtually no impact on geological interest, as rock samples from the ancient delta are have had the same sterilizing radiation for 3 billion years, and Perseverance can't drill to ancient layers that were protected from surface ionizing radiation all that time.

This solution can make the whole process far simpler, with none of the legal complexities of an unsterilized return. However if we do this, it is still important to keep the public fully involved, to coordinate and respond to questions and concerns, and liaise with the help of ethicists, legal experts, representatives of other countries and so on. It is not enough to ensure that the samples are 100% safe. We also need to make sure everyone agrees and understands they are 100% safe.

NEW: Sterilization with 500 million years equivalent of surface ionizing radiation will have virtually no effect on geological studies

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Allen et al tested the effect of sterilization of simulated Mars samples (Allen et al, 1999) with a gamma ray dose equivalent to 3 megagrays. There was no effect on radiometric dating, rock composition, crystal structure, no dehydration of gypsum, no changes in the spectra of the components of the Mars soil simulant.

There was no effect on the basal spacing of montmorillonite, which is extremely sensitive to temperature and degree of hydration. The only change they found was a change in the colour of quartz (clear to deep brown) and halite crystals (to blue) and a change in their thermoluminescence properties.

If the rocks have already had 3 billion years of ionizing radiation, these changes have likely already happened to Mars surface deposits.

500 million years of ionizing radiation would reduce a gram of amino acids to a milligram

By (Kminek et al, 2006:4) 500 million years at 200 milligrays per year reduces many amino acids to a millionth of the original concentration, however, that's more than double the yearly dose measured by Curiosity of 76 milligrays a year on the Martian surface (Hassler, 2014). Since it's logarithmic, half the dose means a 1000 fold instead of a million fold reduction in 500 million years.

So the result is that 500 million years of surface radiation at 100 milligrays a year, or 50 megagrays total, reduces many amino acids 1000 fold, with only one milligram left of every original gram of amino acids.

More generally, the dose x in megagrays for an n-fold reduction is

$x = 50 * \log(n) / \log(1000) = 50 * \log(n) / 3.$

For example, a 4-fold reduction in amino acids needs around 10 megagrays or around 100 million years of surface radiation. Halving the amino acids would need only 5 megagrays. That might be more than enough since Radiodurans can only survive 0.14 megagrays when dessicated and frozen (Horne et al, 2022) which works out as an approximately 1.02 fold reduction in amino acids, destroying around 2% of the amino acids.

It would be for experts to consider what level of dose is needed to sterilize even unknown exobiology and there doesn't seem to be a thorough study of this in the literature. For the purposes of this paper we'll use a value of 5 megagrays to halve the amounts of amino acids, but that's not intended as a recommendation, it's just for purposes of illustration.

Using this level of sterilization for the samples would reduce the amounts of past organics, but there is likely so little left of past organics after 3 billion years of surface ionizing radiation that even a small amount of forward contamination will overwhelm it.

NOTE: We can also calculate the n-fold reduction from the dose as

n = 10^(3*x/50)

and the % destroyed for the dose x in megagrays as

100 – 100/ n.

This also gives a way to calculate the figure for the JAXA samples mentioned earlier. Any that got to Phobos over 18.5 million years ago had a dose of over 1.85 megagrays, so at least 22.5% of many of their amino acids have been destroyed since then. See:

- It is safe for Japan to return unsterilized samples from Phobos without any special precautions because any life in samples from the most recent impact already experienced ejection from Mars – then on the surface of Phobos it accumulated similar amounts of ionizing radiation to any life in the meteorites from the same impact arriving on Earth today

Amino acids exposed to 3 billion years of surface radiation have been reduced from grams to attograms, a billionth of a billionth of a gram – meanwhile infall from space adds about 60 micrograms per gram but is constantly destroyed by surface processes

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Using the same result that the amino acids are reduced a thousand fold every 500 million years, amino acids that have been on the surface for 3 billion years got reduced by 10^{18} leaving only one attogram for every original gram of amino acids (a billionth of a nanogram, which in turn is a billionth of a gram).

These minute traces of past organics may also be mixed with infall from space (Frantseva et al, 2018). Many processes degrade the surface organics, but without them, Mars would have around 60 ppm or 60 micrograms per gram of organics infall, averaged over its entire surface to a depth of a hundred meters (Goetz et al, 2016:247) as well as indigenous abiotic synthesis.

Even if Mars had abundant life in the past, those attograms that remain will be completely swamped by infall from meteorites, comets, interplanetary dust and in situ abiotic processes .(Mulkidjanian, 2015) (Westall et al, 2015) (Franz et al, 2020).

This is why astrobiologists devised ultra sensitive instruments such as astrobionibbler able to detect just a single amino acid in a gram (Schirber, 2013) (Noell et al, 2016).

As for present day life, one microgram per gram is enough for ten million ultramicrobacteria at a tenth of a picogram each (see next section). Even if there are thousands of ultramicrobacteria they might be easily overwhelmed by organic infall in searches for biosignatures. But the situation is far worse because of the issue of forward contamination in the Perseverance samples.

NEW: Sadly Perseverance's permitted levels of 0.7 nanograms per gram for their most abundant biosignatures would overwhelm any faint signature of biosignatures from past life or a few cells per gram of present day life even if viable

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Given these figures, sadly, Perseverance's permitted levels of forward contamination are too high for the samples to be likely to be of much astrobiological interest, Perseverance's engineers believe they achieved a maximum of 0.7 ppb or 0.7 nanograms per gram for their most abundant biosignatures (Boeder et al, 2020: table 6)

By definition an ultramicrobacteria has a volume of at most 0.1 cubic microns. A micron is a millionth of a meter or a 10,000th of a centimeter. So a cubic micron has a volume of a trillionth of a cubic centimeter, or a mass of a trillionth of a gram, or a picogram. So ten ultramicrobacteria weigh in at at most a picogram so the 0.7 nanograms is equivalent to 7,000 ultramicrobacteria.

Sample tube photo from (NASA, n.d.pst)

That’s well above the attograms that might remain for past life if lucky enough to sample it. Meanwhile present day life might not be present at more than a few ultramicrobacteria per gram, so a few picograms (thousandths of a nanogram) per gram unless Perseverance serendipitously samples a biofilm.

Perseverance seems unlikely to be able to detect martian life, past or present in its sample tubes, even if by chance it returns it.

Attendees to the 2020 conference “Mars extant life: what's next?” (Carrier et al, 2020: *801*) agreed that we would be able to detect extant life resembling terrestrial life if it has been returned without getting contaminated. It’s not so clear that we’d be able to detect unfamiliar life even without contamination. But if returned to Earth we can use a much wider range of instruments to search for it than we can send to Mars in situ.

So there is some value to astrobiology of returning samples to Earth if we think there is a chance they contain extant life, but have been unable to detect it in situ on Mars – however we need to ensure that they aren’t contaminated. With the current level of contamination we have little chance of extant life detection even if it’s there. As for study of present day habitability and so on, the sterilization would likely have little effect on that, again because of the high level of forward contamination.

So sterilization preserves virtually all geological interest, and because of the forward contamination would have minimal impact on astrobiological interest – but NASA's EIS doesn't permit it due to a requirement for "safety testing"

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NASA's draft EIS doesn't permit sterilization as an alternative because it says unsterilized samples need to be returned to Earth for "safety testing" in its Purpose and Need (NASA, 2022: 3-3)

> ***These same principles regarding the importance of using terrestrial laboratories to enable the best scientific return also apply to the care and attention to detail that would be required to conduct a proper and comprehensive sample safety assessment in a proposed SRF.***

However, there is no need for safety testing for samples that are sterilized before they reach Earth as they are made safe by sterilization.

Also this is a reasonable alternative since virtually all the geological interest is preserved by sterilization and the forward contamination is so high for astrobiology that sterilization would have minimal impact on the astrobiology interest too.

So, this requirement seems to improperly exclude a reasonable alternative. By the U.S. Court of Appeals for the Seventh Circuit in Simmons v. U.S. Army Corps of Engineers (7th Circuit, 1997), it is contrary to NEPA for agencies to

> ***"contrive a purpose so slender as to define competing `reasonable alternatives' out of consideration (and even out of existence)."***

NEW: Even if samples are returned unsterilized this "safety testing" seems to serve no useful purpose – all the samples would be guaranteed false positives – with no biosignature to distinguish terrestrial from potential martian life – and we can't test for martian life by trying to cultivate it as we can't reliably cultivate even terrestrial life in a lab

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Meanwhile if samples are returned unsterilized, NASA's "safety testing" seems to serve no useful purpose. By their own cite (Kminek et al., 2022) it is practically impossible to assess the environmental impact if life is found, so the only testing they can do is for presence of life or not.

> ***During the Working Group's deliberations, it became clear that a comprehensive assessment to predict the effects of introducing life in new environments or ecologies is***

difficult and practically impossible, even for terrestrial life and certainly more so for unknown extraterrestrial life.

This cite goes on to discuss how to test for life by checking for biosignatures. However, by Perseverance's permitted levels of forward contamination, they are guaranteed to generate false positives for all the samples tested. The next stage is that the samples all go to "hold and critical review".

This cite doesn't say what would happen next, but we currently have no way to reliably distinguish terrestrial from potential martian biosignatures.

Sequencing won't work.

- Swabs of Perseverance's clean room found many microbes only detected through their 16S RNA ribosome subunit, with four not closely resembling any known terrestrial life (Hendrickson et al., 2021). It would be impossible to use sequencing to prove they aren't martian after taking them to Mars and back again.

Also we can't reliably test for viable martian life by attempting to cultivate it.

- Even terrestrial life is often impossible to cultivate in laboratory conditions. Microbes may need a nutrient poor medium, have generation times of 6 months or more, or depend on other microbes in biofilms for amino acids or even require other microbes for their nucleotides, yet be widespread outside the laboratory (Solden et al, 2015). The vast majority of microbial species haven't been characterized or sequenced or cultivated in the laboratory. This is the problem of "microbial dark matter" (Dance,2020).

NEW: Too early for any form of safety testing at the level of assurance needed for potential large scale harm – after destructively testing 10,000 grains of dust the 10,001th grain could have a viable microbe in it

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It's actually too early to do ***any*** form of safety testing, even with samples returned in sterile containers, at least, not at the level of assurance needed when there is potential for large scale harm.

Even if we have a test guaranteed to identify Martian life, which we don't have, we could destructively test 10,000 grains of uncontaminated dust individually – and perhaps the 10,001th grain has a viable microbe which we can't detect without destroying it. We could destructively test 10 grams for biosignatures, and the next milligram contains a viable microbe.

From simulated wind blown Martian dust, microbes can indeed get attached to a dust particle and blown in the winds (van Heereveld et al, 2017) (Osman et al 2008). A viable microorganism could be imbedded in a dust grain (Sagan et al, 1968). The iron oxides shield the microbe from UV but also hide a microbe from non destructive tests, such as Raman spectroscopy or autofluorescence which in any case are also less reliable.

Then there's the issue that we don't know for sure what to look for by way of biochemistry. We can't construct a test that is guaranteed to detect Martian life until we have a better idea what we are looking for.

NEW: So "safety testing" is not feasible at present, and sterilization keeps Earth 100% safe with likely virtually no difference to the science return

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In short, this "safety testing" for unsterilized samples" can't be used to keep Earth safe at our current level of knowledge. It may be possible later once we know more about Mars, how to identify the life and what harmful capabilities it has if any.

Meanwhile sterilization keeps Earth 100% safe with virtually no difference to the geological or astrobiological science return.

That leaves the practical question, how can we sterilize samples before they reach Earth, while still maintaining effectively zero risk of harm? We have to sterilize the samples in a way that has no risk of the unsterilized samples entering into a chain of contact with Earth's biosphere.

NEW: Samples can be sterilized in a satellite similar to those for Geostationary orbit, but above GEO – and the spacecraft can be returned through low energy ballistic transfer without aerobraking or even flybys of the Earth or Moon

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The simplest solution might be to use nanoscale x-ray emitters on the return journey. In one experiment the tube operates at 50 kV with an emission beam current of 140 μA, or a power output of 7 watts which would suggest power consumption of 10s of watts, far less than a conventional X-ray. The dose at 3 cm is 8.19 Gy per minute (Kim et al, 2016). Depending on the dose, for our illustrative example of 5 megagrays, to halve the amino acids, this would require 1.15 years of continuous operation, so, several of them would be needed per sample for a six month journey back, which might then run into issues of available power. Lower doses would be easier. Also, they were blocked by just 3 mm of a copper collimeter and may be blocked by the walls of the tungsten sample tubes.

If we don't have sufficient solar power available for that, we can return it to a larger satellite similar to a geostationary satellite for sterilization.

We need to avoid aerobraking and we can do that using “ballistic capture”, also known as “weak stability boundary transfers” (Topputo et al, 2015), the low delta v, fuel efficient, three or four body transfer orbits first used for the Japanese Hiten mission in 1990 (Belbruno, 2018). The ESA Earth Return Orbiter will use continuous low thrust transfer (Huesing et al, 2019), ideal for such an orbit.

One way to avoid aerobraking is to return the sample via a lunar retrograde orbit (actually a prograde orbit around Earth but retrograde around the Moon). This involves a close flyby of Earth followed by a flyby of the Moon to get into that orbit (Lock et al, 2014).

However, an especially promising low energy trajectory avoids even flybys of Earth or the Moon. This is the reverse of the trajectory in figure 13 from (Kakoi et al, 2014). It uses a halo orbit manifold to spiral from ballistic capture to a halo orbit around Sun Earth L2, the unstable gravitational point of balance between Earth and sun, which is on the far side of Earth from the sun as well outside the orbit of the Moon.

This then dovetails to another manifold to spiral down to a halo orbit around Earth Moon L2 point of gravitational equilibrium above the far side of the Moon, which dovetails to a low energy transfer to an Earth Moon L1 halo above the near side, and then the spacecraft slowly reduces the size of its orbit around Earth and circularize it in an orbit well above GEO.

Whatever method we use to bring the spacecraft back to above GEO,ing the spacecraft back to above GEO, we can then target the Laplace plane inclined at approximately 7.2° from the equatorial plane. This is a proposed “graveyard orbit” for GEO satellites at end of lifetime as even large light fragments of cladding from the satellites stay trapped well away from GEO, through the balance of the light pressure from the sun and gravity (Rosengren et al, 2013). It’s where ring particles would orbit if Earth had a ring system.

The sterilizing sample could be placed, say, 50,000 km or 100,000 km above this proposed GEO disposal orbit. This is very safe as the delta V is over 1 km / second to both Earth and the Moon and it would also be safe for GEO and far from the proposed Laplace plane GEO graveyard orbit.

NEW: This keeps Earth 100% safe with virtually no loss to science and little change in NASA’s budget – since they save the cost of a Sample Receiving Facility – estimated at $471 million in 2015 US dollars for the 1999 technology specifications

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Launch costs to above GEO wouldn’t be prohibitive for NASA as the Falcon Heavy can already deliver over 25 tons to GEO at a cost of $150 million and launch costs are sure to go down.

NASA add the cost of the sterilizing satellite, but they save other costs including the cost of a sample receiving facility on Earth, estimated at $471 million in 2015 dollars (Mattingly, 2010:20) based on the 1999 size limit. There are no designs available or costs for the 2012 ESF size limit review but it would likely cost more than that estimate, if it is feasible at all. They also save on the mass of the aeroshell, and the fuel budget to take it to Mars and back again.

For the cost of the sample receiving facility it would be possible to send a large satellite to geostationary orbit. Also universities might well be interested to join in on the cost. It could also be a special item for a budget in Congress if there is enough public interest.

In this way we keep Earth 100% safe, with virtually no loss to science and little change in overall budget.

Cobalt 60 sources can be very heavy, with much of the mass for the shielding. X-rays might be better as they can be switched on and off and adjusted. A satellite above GEO can potentially have a significant power supply for generating X-rays. The Inmarsat 5 F1 has a power supply of 15 kilowatts on launch (Inmarsat, 2013).

With more ambition but little change in the budget we can make this a far more interesting mission for astrobiology – though we can't expect samples returned to answer central questions in astrobiology except with extraordinary luck

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With more ambition, with bonus samples collected in a sterile container, and not much change in the budget, we could transform this into a much more interesting mission for astrobiology.

However we need to be clear first, that it is unrealistic to suppose we can answer central questions in astrobiology even with extra bonus samples.

A white paper submitted to the decadal review by astrobiologists emphasized the need to be able to detect life in situ before we can intelligently decide which samples to return demonstration (Bada et al, 2009:7):

> ***We feel that organic detection efforts over the next two decades via investment into advanced in situ robotic instrumentation are fundamental in support of a future intelligent MSR mission.***
>
> *Currently, MSR is regarded by much of the scientific community as largely weighted towards a technology demonstration as the rationale for good astrobiology will not be apparent until we discover more about our neighboring planet.*

Other studies came to the same conclusion (Paige, 2000) (Davila et al, 2010). Most recently in 2020 (Carrier et al, 2020: *802*)

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

Perseverance's geology focus dates back to an oversight present from the mission's inception a decade ago. The decadal review in its summing up said (Space Studies Board, 2012:17).

> ***Mars science has reached a level of sophistication such that fundamental advances in addressing the important questions above will come only from analysis of returned samples.***

They relied on a 2002 paper, Safe on Mars from a time with a much simpler understanding of Mars and less capable instruments for in situ studies (Space Studies Board, 2002a, chapter 5:38) and even then it said that:

> ***"If such capabilities were to become available, one advantage is that the experiment would not be limited by the small amount of material that a Mars sample return mission would provide. What is more, with the use of rovers, an in situ experiment could be conducted over a wide range of locations."***

The instruments were already far smaller and more capable just 7 years later at the time of the paper by Bada et al. Since then astrobiological instruments continued to get smaller and more capable, while our understanding of past and present day habitability of Mars gets more complex. The now overwhelming case for in situ study for astrobiology continues to get stronger.

That is for future missions. But though we have no possibility of in situ searches at present there are some samples we could return of great interest to astrobiology without these in situ searches, the salts, dirt, dust and atmosphere.

NEW: We could transform this into a much more interesting mission for astrobiology with little change in the overall budget by adding bonus samples collected in a STERILE container sent on the ESF fetch rover of dust, dirt, ideally salts, atmosphere and then some pebbles for a technology demo of a rock sample return without forward contamination

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One thing astrobiologists are sure to do once they can send life detection instruments to Mars is to study the dirt, dust and salts in situ on Mars. The dust is like a collection of tiny rocks from the rest of Mars. If we are lucky it might snag some life along with the dust. The dirt can help us understand conditions on the surface of Mars. We already were surprised by the perchlorates in the dirt, and can't know what other surprises we might find that may lead to new research directions (David, 2015). Meanwhile, the salts may be inhabited by microbes or may preserve evidence of life processes or trapped microbes from the past.

These studies could also resolve the puzzle of the Viking labelled release. Did it find life or complex chemistry? Miller's discovery that the Viking evolved gases were offset 2 hours after the temperature maximum (Miller et al, 2002) raises intriguing questions that need answers.

We can only replicate the Viking experiments on Mars because the active agent, whatever it was, was inactivated by several months of darkness (Levin et al, 2016). So by the time it returns to above GEO after a minimum of six months in a sample tube, it will be inactivated.

However, if Viking found life, these samples have a high chance to return viable or dead propagules in the dust or dirt, and if instead we find products of complex chemistry, we can use that to refine the chemical explanations of the Viking results. This would make Mars surface simulation experiments and studies more accurate – and perhaps be of interest for prebiotic synthesis?

Astrobiologists are especially interested to study the salts. In the summary of the 2020 conference "Mars extant life: what's next?" salts are singled out as of interest for a sample return, indeed it is the only suggestion they make for a near future sample return. Salts are of great interest because (Carrier et al, 2020: *797*)

- We might find viable or at least identifiable microbes in fluid inclusions in the salts
- Spectroscopic analysis could uncover biochemicals synthesized by life or resulting from breakdown of life organics
- Salts and brines could contain dissolved solutions and pockets of gas, such as perchlorates, nitrates, sulfates, organics and methane that life could exploit
- The salts could also give access to sunlight for life that can make use of it.

They say salts (including gypsum as well as the halite salts) are of interest because (Carrier et al, 2020: *797*)

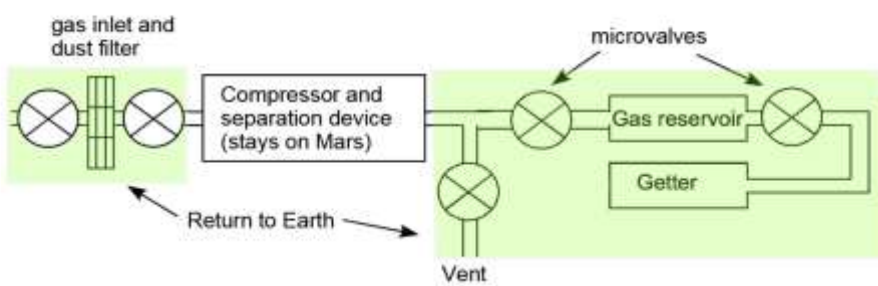
- There is a lot of life in hypersaline environments on Earth
- Microbes are often preserved intact along with easily detectable carotenoid pigment biosignatures
- Salts attenuate UV which would help protect life on Mars
- Salts deliquesce protecting life from long term deliquescence
- Deliquescence of salts can provide potential liquid resources for life.
- Salts could also preserve ongoing evolutionary processes
  [That could include cases where life has gone extinct but then evolves anew in uninhabited habitats Mars (Cockell, 2014) – perhaps it could evolve anew even when there is extant life elsewhere on Mars]
- Salts extend the temperature range of liquid brines

They don't discuss NASA's sample return mission specifically. But a bonus sample of salts would be sure to get the interest and attention of astrobiologists interested in the possibility of extant life on Mars, if collected in sterile containers.

Another suggestion, we might also find evidence in uncontaminated samples of subtle abiotic processes such as abiotic photosynthesis, or abiotic nitrogen fixation. Mars could have an abiotic nitrogen cycle with photochemically produced HNO_3 fixed in thin (0.2 to 5 nm) pure water metastable interfacial films, potentially supporting up to one kilogram of fixed nitrogen per square meter (Boxe et al, 2012)

To make a start on these questions, we could add a STERILE container to send to Mars on the ESF fetch rover to return bonus samples of dirt, dust, and if possible salts, and atmosphere without forward contamination.

We have already sent an atmospheric compressor to Mars on Perseverance but it is used for Moxie, an independent experiment to test options for creating fuel from the atmosphere, not connected with the sampling experiments. Jakosky et al propose sending a similar experiment to Mars in a small sample tube sized container or alternatively a larger container to return 100 cc of atmosphere. This is how it works.



First it uses the getter to remove evolved gases from the container wall. Then it closes one microvalve and opens another to get an atmospheric sample. Finally it closes both microvalves to the gas container and opens the vent to run more atmosphere through the compressor to collect dust in the filter (Jakosky et al, 2021)

Assuming a volume of, say, 50 cc of dust, and a dust density of 0.5 grams per cc, it could return up to 25 grams of dust.

This is enough to detect life at around one cell per gram or less. This is also a useful first upper bound of the amount of life in the dust if none is returned.

For astrobiology, it might be useful to do the air collection at night or in the early morning to detect the composition of the air at times of high humidity. Ideally two air collectors, one for daytime sampling and one for night / early morning.

We can then add a scoop of dirt and return all these samples of dust, dirt and atmosphere in a separate small sealed sterile container which goes to Mars on the ESF fetch rover.

The active agent in the Viking experiment was de-activated after storage of the dirt in darkness for several months (Levin et al, 2016). So it's possible that the active agent, whatever it was, chemistry, prebiotic synthesis, or life, could be de-activated during the return journey.

If practical in terms of engineering, it may help to add a window to the container with a neutral filter, similarly to the Mars simulation chamber in the BIOMEX experiment. This would let sunlight in to illuminate part of the sample, duplicating surface conditions on Mars (ideally with day / night changes) which might possibly preserve the active agent whatever it was. The rest of the sample would be protected from UV in darkness, perhaps just kept dark by the shading of the dirt or dust itself.

We could also make Perseverance into a far better rock sample return technology demo for astrobiology by returning CLEAN rock samples. The ESA fetch rover could use a sterilized scoop and pick up a sample of dirt along with a few small pebbles / rocks. This would demonstrate the capability to return rock samples without forwards contamination, and by returning a clean rock, could help with fine details of surface chemical / abiotic processes.

NEW: These clean samples can be studied above geostationary orbit in Mars simulation conditions with a Martian gravity centrifuge – not for safety testing, humans never go near the satellite

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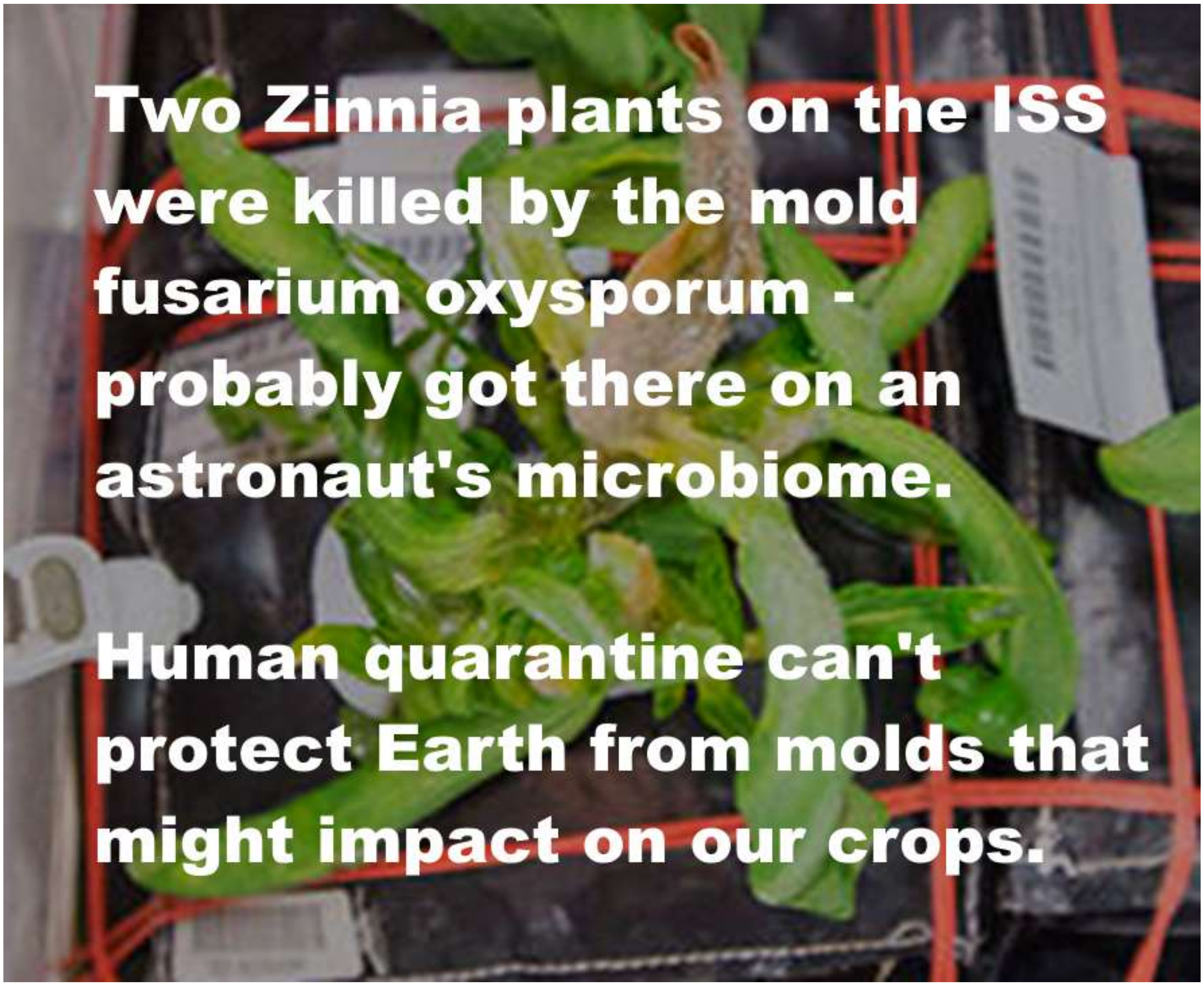
These clean samples could be studied above geostationary orbit, in Mars simulation conditions with a centrifuge for artificial martian gravity – which would make it unique as a facility, as we can't simulate martian gravity accurately on Earth.

These samples would be returned to a small robotic satellite and NOT a human occupied space station like the ISS.

In the backwards direction, quarantine can't protect Earth from mirror life or indeed fungal diseases. Two zinnia plants on the ISS died of a fungal disease fusarium oxysporum (NASA, 2016) probably brought there on an astronaut's microbiome (Urbaniak et al, 2018).



Mold growing on a Zinnia plant in the ISS. The mold fusarium oxysporum is thought to have got to the ISS in the microbiome of an astronaut (Urbaniak et al, 2018). Two of the four infected plants died (NASA, 2016). It would be impossible to keep a pathogen of terrestrial plants out of the terrestrial biosphere reliably with quarantine of technicians or astronauts, at least until we know if there is life on Mars and what its capabilities are.

This fungal disease disease fusarium oxysporum is also an occasional opportunistic pathogen of humans (Urbaniakt al, 2019).

As another example, Aspergillus Flavus and Aspergillus niger are amongst the most common fungal spores in the HEPA filters on the ISS and found at relatively high concentrations compared to US homes (Vesper et al, 2008). Human quarantine couldn't keep these out either as they can lead to harmless colonization of healthy humans but harmful pathogens of immunocompromised. See (above):

- NEW: Opportunistic fungal pathogens are sometimes deadly especially for immunocompromised people – Aspergillus is not adapted to humans – there are an estimated 200,000 life threatening Aspergillus infections a year with mortality 30% to 95% – and we might all be immunocompromised to a new genus of fungi from Mars with likely no effective antifungals available initially or for some time, and issues with testing for presence of the new fungus, or recognizing the disease through clinical diagnosis or imaging

As we mentioned, chroococcidiopsis species are sometimes found in the human microbiome including in the nasopharyngeal microbiota (Ventero et al, 2022), and in human milk from Gambia (Lackey et al, 2019), so it's unlikely a mirror life chroococcidiopsis analogue could be reliably kept out of Earth's biosphere by human quarantine of technicians or astronauts. See (above):

- NEW: Example worst case scenario of a mirror life chroococcidiopsis analogue from Mars which could gradually convert organics in ecosystems into indigestible mirror organics

The Apollo astronauts had 3 weeks quarantine to protect Earth's biosphere. But this was never subject to legal review or public scrutiny (Meltzer, 2012:452). Carl Sagan gave the example of leprosy for the "vexing question of the latency period" (Sagan, 1973:130)

> *There is also the vexing question of the latency period. If we expose terrestrial organisms to* Martian *pathogens, how long must we wait before we can be convinced that the pathogen-host relationship is understood? For example, the latency period for leprosy is more than a decade.*

We now know that leprosy can take 20 years or more to show symptoms (WHO, 2019). However the planetary protection literature so far doesn't seem to cover the topic of a lifelong symptomless superspeader similar to Typhoid Mary (Korr, 2020). The Occupational Safety and Health Administration in the USA is sure to declare an interest for questions of quarantine. Then the WHO is likely to declare an interest at an international level (Uhran et al, 2019). At this point the issue of symptomless spreaders would be sure to be raised if not before and there would be no solution to this issue.

So, quarantine can't be used to protect Earth from putative martian organisms with unknown capabilities. It may be useful in the future in some scenarios once we know what we have to protect Earth against (not all as the example of mirror life shows, and fungal disease would also be likely to be challenging to keep out using quarantine).

In the forward direction, an unmanned satellite let's us study martian life in far cleaner conditions than a human occupied space station, as ultramicrobacteria can get through HEPA filters both ways.

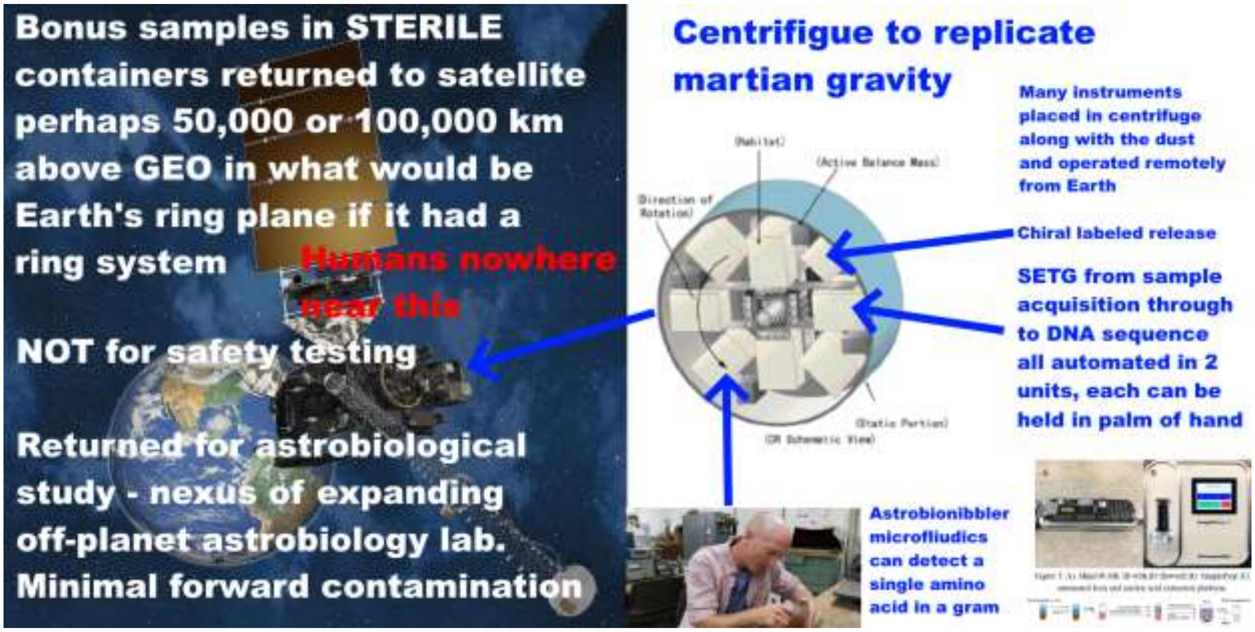
This orbital lab is still not for “safety testing”. Suppose we successfully cultivate life from the sample, and detect familiar life, a novel strain of a familiar microbe such as chroococcidiopsis. Even then, this could bring new capabilities to Earth acquired from billions of years of evolution in Martian conditions. For instance it is reasonably likely to be better adapted to cold, to rapid fluctuations in temperature, to ionizing radiation, to desiccation and amongst its many metabolic pathways, it may have the ability to metabolize mirror organics because much of the organics it encounters on Mars is achiral. These changes may be harmless but amongst them all there may be some adaptation that causes problems when returned to Earth.

Also if we find familiar life, it would be hard to prove that there is no unfamiliar life in the sample as we saw in the example of a mirror-life nanoboes. As with terrestrial life much of it could be uncultivable in laboratory conditions yet do fine in nutrient poor and more challenging situations outside the orbital lab. Also if there was a mix of some life with similar biology to terrestrial life and some with unfamiliar biology we might detect the familiar biology first.

This dust and dirt samples are just the first step in Sagan’s “exhaustive program of unmanned biological exploration of Mars”– and the first try out for the supersensitive instruments that astrobiologists have developed to send to Mars to find life in situ so that we know what to return

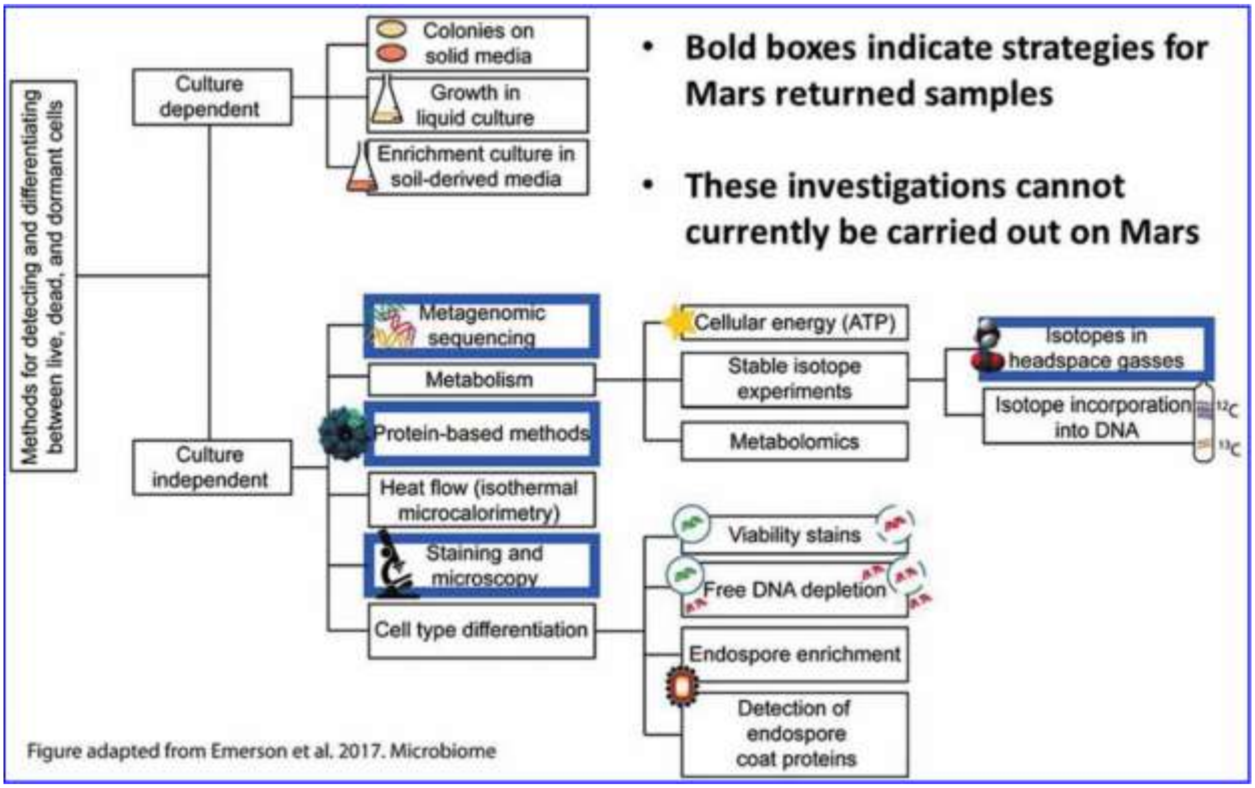
The dust and dirt samples are just a start. There is likely no shortcut alternative to Sagan’s “*exhaustive program of unmanned biological exploration of Mars”.*

This orbiting astrobiology lab is the equivalent of one geostationary satellite far above GEO. Humans can study the dust, dirt and atmosphere as they would on Mars using exquisitely sensitive in situ instruments designed for end to end sample preparation to analysis – these already exist such as the Life Marker Chip LDChip300 (antibodies) almost sent on Exomars but descoped (Parro et al, 2011) target mass of less than 1 kg (ESA, n.d.), the gene sequencer SETG (Mojarro et al, 2016)., astrobionibbler able to detect a single amino acid in a gram (Schirber, 2013) (Noell et al, 2016), a chiral version of the Viking labelled release experiment (Anbar et al, 2012), and many others.



Graphic shows:
GEOS17 ([Clark,, 2018]) just to have an image of a geostationary satellite, not that it would be a $2.5 billion dollar satellite.
SETG from [(Mojarro et al, 2016)]
Astrobionibbler from ([Elleman, 2014])
ISS centrifugal motor for plant experiments, dialable to any level from microgravity to 2 g [(NASA, n.d.)]

Most of the studies astrobiologists want to do can be done in situ on Mars and so could be done in the orbiting satellite too. Only the ones in bold boxes in this graphic currently need to be done on Earth [(Carrier et al, 2020]::802)



From (Carrier et al, 2020: fig 10) adapted by Mackelprang from (Emerson et al, 2017: fig 1)

Also, if we return samples to a satellite above GEO we can send many more instruments to study the samples than we could send to Mars, and we can also build new instruments to send there based on discoveries made previously.

We need a wide range of instruments in order to have the best chance of detecting life as often multiple biosignatures are required simultaneously to detect life with confidence (Westall et al, 2015).

Take the example of the chiral labelled release. Although an excellent experiment combined with others, on its own it can generate a false positive or a false negative.

- False positive – it could detect complex prebiotic chemistry, for instance if the active agent consists of chiral organics on Mars, a prebiotic chiral network as for the punctuated chirality hypothesis. This would of course be very interesting, but not life (Gleiser et al, 2008).

- False negative – the active agent could be chirality indifferent if it is either
    1. A mix of life of both chiralities, see NEW: Closely related worst case scenario of a shadow biosphere of mirror life nanobes that produce indigestible mirror life biofilms on Earth

OR

2. Chirality indifferent life (“ambidextrous”), for instance Joyce’s RNA enzyme in its D or right hand form can make copies of L RNA and its L or left handed version can copy D RNA. (Joyce, 2007) (Sczepanski, 2014) (Singer, 2014)

There are many other instruments have been devised to send to Mars and such a satellite would stimulate work to develop many more light weight in situ life detectors. Here are some of the others, some not so well known as the ones already listed:

- check for redox reactions directly by measuring the electrons and protons they liberate. This is sensitive to small numbers of microbes and has the advantage that it could detect life even if not based on carbon or any form of conventional chemistry we know of (Abrevaya et al, 2010).
- Aromatic amino acids (incorporating a ring of six carbons) fluoresce when stimulated with deep UV at wavelengths less than 250 nm. Chlorophyll and some other biological organics also autofluoresce. We could also use fluorescent dyes that bond to specific macromolecules such as lipids, proteins and nucleic acids (Head et al, 2017),
- We can also use this autofluorescence to directly search for the activity of swimming microbes (Head et al, 2017).
- An off-axis holographic microscope to let the focus be adjusted after the image is taken making it easier to image individual microbes in a liquid medium (Lindensmith et al, 2016),
- Raman microspectroscopy synchronized with visible light can do a chemical analysis of the microbes directly (Head et al, 2017),
- superresolution optical microscopy can go beyond the usual optical resolution limit of 200 nm to observe nanobacteria (Head et al, 2017).
- a miniature variable pressure electron microscope that combines imaging with in situ chemical analysis (Gaskin et al, 2012)

These are all very small instruments, miniaturized to be light enough to send to Mars, at up to a few kilograms each. However for the suggestion of a receiving satellite above GEO, at over 25 tons per payload and probably more by the 2030s we could send massive instruments up there too, and maybe some of the ones that can’t yet be done in situ on Mars could be done above GEO by the 2030s.

This approach has similar advantages to returning to Earth without the risk of forward contamination of the samples and without the risk of backwards contamination of Earth. This is especially useful if we find unfamiliar life. For instance if we find mirror life, and perhaps not even based on DNA we may need to devise new instruments to study it. That will be far easier with a returned sample of it above GEO without the risk to Earth of returning it to the surface. (Carrier et al, 2020: *801*)

> *Meanwhile, it is important to note that if the life-form were based on another biochemistry, modern techniques might be too specific. We determined that using a well-*

> *designed suite of multiple advanced detection techniques, which provide complementary information for life detection, would be especially important. The advantage of a return mission would thus be in the ability to access a multitude of more sensitive instruments and wide-ranging laboratory techniques than is possible for in situ missions. We agreed that life-detection instrument development programs should be a priority and that more research should be conducted to understand what signals of life may be universal and how to best detect those.*

In the other direction, if we find familiar life on Mars, we shouldn't jump to the conclusion that all life on Mars is familiar without more study. It could co-exist with unfamiliar life as with the idea of the mirror nanobes shadow biosphere.

The Moon may seem a better place to return the samples if we have a continuous human presence in a base on the Moon by the 2030s. Humans close by could reduce latency for teleoperation and might make it easier to add or remove equipment and supplies.

However, latency for telerobotics from Earth would remain reasonably low above GEO, we can send multiple ton missions up there at low cost, and COSPAR guidelines for category 5 (sample return) missions currently say that (COSPAR, 2011) (Debus, 2004)

> *"(The Moon must be protected from back contamination to retain freedom from planetary protection requirements on Earth-Moon travel)".*

NEW: With yet more ambition we can search for past organics using a Marscopter to return pebbles excavated by a recent crater to a depth of 2 meters or more – as a technology demo and first look at organics from 3 billion years ago though unlikely to return life with the first sample found

Next section – all sections – previous section

With yet more ambition, we could search for past organics. It might be enough to sterilize a separate sample collector which is lifted by the Marscopter and used to acquire a rock sample.

We could search for a crater recently excavated to 2 meters and the marscopter could search for an exposed pebble which by the geological context was exposed from 2 meters depth, and with minimally degraded organics. This is not likely to return recognizable past life unless the marscopter has in situ multiple biosignature detection, but this could be a start towards investigating organics from 3 billion years ago in Jezero crater, and how it's been chemically altered since then, to use to help plan future in situ studies and later sample returns.

A typical small crater of 16 to 32 meters in diameter can excavate the surface of Mars to more than 2 meters. An observational study by Daubar et al found that this size of crater excavated the surface to depths of between 2 and 9 meters (based on seven newly formed craters at this size in Figure 4 of Daubar et al, 2014).

This is deep enough to find organics not significantly damaged by cosmic radiation even after three billion years. It may also be able to penetrate below the average depth of impact gardening by meteorites. The fines dominated regolith in Jezero crater is estimated as 2 to 5 meters thick (Schuyler et al, 2020). In the size range 16 to 32 meters the crater rate is about 2.57 craters per square kilometer every ten million years (1.9 + 0.67 for first two rows in table 1 of Hartmann et al, 2017). For more on this and some of the other suggestions here see my draft for a future paper (Walker, 2022b).

NEW: With even more ambition we could make a 100% sterile marscopter by specifying components able to resist heating at 300 °C for several hours – which can be flown to sensitive locations with no risk of forward contamination and retrieve samples with no risk of backwards contamination

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We don't sterilize our spacecraft as much as for the Viking lander, relying on the harsh conditions there to take the place of the hours the Viking landers spent in ovens. This saved about $100 million per mission (Chang, 2015).

That made sense in the late 20th century, but since then the conditions aren't as harsh as were thought. Still, our rovers likely shed only a few occasional spores and viable microbes which would then find it hard to survive on Mars given the harsh conditions there. Hopefully the chance per mission still very low. But the more missions we have the more the risk of forward contamination.

Technology has advanced so much since Viking that it's now feasible to have a 100% sterile lander or rover on Mars. To work towards that, with even more ambition, we could make a 100% sterile marscopter by specifying components that are not affected by preheating to a few hours at 300 °C.

The sterilized copter could be flown to nearby RSLs or other sensitive locations with no risk of forward contamination, and it could be used to return contamination free rock samples such as pebbles or rock fragments from crater rims or crater floors. Our technology has advanced since the Viking landers which were baked for 112 °C for 30 hours, enough for a million-fold reduction of the originally low population (Beauchamp, 2012).

We now have high temperature microprocessors and memory devices for oil wells, aviation and electric cars. Their heat resilience means they don't have to be cooled, and they can be placed closer to heat sources such as engines. This helps with cost, weight and most important, reliability (Watson et al, 2012).

- At 250 °C the half life of the RNA bases under hydrolysis is between 1 and 35 minutes, and at 350 °C the half-lives are between 2 and 15 seconds (Levy et al, 1998). Eight of the 20 amino acids have been proven to not just evaporate or liquify but to decompose at temperatures between 185 for Q (Glutamine) to 280 for H (Histamine) (Weiss et al, 2018) There might be other more recalcitrant organics remaining but it seems that this should be sufficient to eliminate both forwards and backwards contamination.

The Venus lander teams sketched out a design for a largely mechanical rover with minimal onboard electronics capable of functioning at Venus surface temperatures of around 500 °C, as part of Venus Rover studies. The researchers proposed that the same approach could be useful for planetary protection (Sauder et al, 2017, section 6.2).

Since then, temperature technology continued to improve. NASA's HOTTECH program has developed sensors, imagers, solar arrays, batteries, electric motors, actuators, and other technologies that work even at 500 C for at least 60 days for a Venus surface lander (NASA, n.d.). The Long-lived in-Situ solar system explorer (LLISSE) is a design for a complete in situ Venus surface probe that can meet this specification with no active cooling (Kremic et al, 2021).

By using some of that technology plus commercial off the shelf components we could achieve specifications for a Marscopter that is essentially the same machine, except that it can be heated to 300 °C for a few hours, finally perhaps cleaned with carbon dioxide snow – and shipped to Mars in a container only opened on the surface – which could then be flown to nearby sensitive sites such as the RSLs with no risk of forwards contamination, and be used to collect samples for return for analysis in the automated lab above GEO.

This could be the first of many 100% sterile rovers we could use in the future to explore sensitive areas of Mars. The 100% sterile Marscopter would incur one off cost for the research effort, but after that the extra costs of using those components, or sterilizing it or packing it in a suitable container to remain sterile to Mars is not likely to be a significant % of the total mission cost per copter for future missions, especially in proportion to the science value of contamination free exploration of special regions and vulnerable microhabitats. As we develop experience we can use similar methods for more and more complex 100% sterile rovers, landers, aerobots etc on Mars.

NEW: The satellite above GEO could include a Mars simulation chamber, similar to BIOMEX but much greater fidelity, simulating Mars gravity, variation of temperature, pressure and humidity between day and night, seasons, UV levels for dust storms etc

Next section – all sections – previous section

First, to prevent forward contamination with terrestrial life to eliminate the risk of false positives, the satellite would need to be presterilized of all terrestrial life, perhaps by heating to 300 C and CO_2 snow, similarly to the Marscopter. Then as for Viking it would be covered in a shroud until it reaches space. This is necessary for the best science anyway.

Reagents could be sterilized using ionizing radiation or whatever method is appropriate to the reagent and then kept sterile in a separate container. The result would be a clean facility at levels hard to achieve on Earth.

Then the satellite could become a basis for a far more sophisticated Mars simulation chamber than BIOMEX. It could have adjustable filters and blinds simulating day night cycles on Mars, and even seasonal cycles with UV levels as for Mars by simply filtering down the sunlight to half terrestrial intensity and then filtering out more of it to simulate the effect of the Martina atmosphere. Then more filters could simulate the shading and UV shading in dust storms.

The simulation chamber could be shielded from cosmic radiation and solar storms to a level needed to approximate the ionizing radiation levels on the surface of Mars. Water vapour could be added / removed to help approximate the seasonal cycles on Mars and the day night pressure and humidity cycles would happen to an extent automatically by the warming and cooling. Perhaps even the Martian frosts could be simulated and the pressure / temperature gradients over the near Martian surface?

In this way it could become a facility also for studies of terrestrial analogues similarly to BIOMEX but dedicated to Mars simulation chamber studies 24/7. The simulated Mars gravity, the natural cosmic and solar ionizing radiation and the natural sunlight would make it far more straightforward to simulate Mars surface conditions to far greater fidelity than we can ever do on Earth.

In this way we can study the returned samples including any martian salts, dirt and dust in close to the natural conditions on Mars. We can also use those to construct more accurate regolith analogues and test terrestrial life in the regolith analogues in separate chambers again in very close to Mars surface conditions.

NEW: The satellite above GEO could expand to a receiving station for samples throughout the solar system including Ceres, and eventually Europa and Enceladus

Next section – all sections – previous section

The new satellite would also be an investment for the future as it could be expanded to a receiving station for samples from everywhere in the solar system including Ceres, and Enceladus, with simulation chambers that can be adjusted to any gravity and any light intensity. NASA may return samples from Ceres in the late 2030s with the mission already recommended in the Decadal review (Carter, 2022) (Castillo-Rogez, 2022).

There are proposals to return samples from Enceladus captured by flying through the plumes. The return to Earth risks the samples getting further altered through reentry heating, landing shock and heating, and forward contamination from terrestrial life (Neveu et al, 2020).

They comment:

> *Organisms in Enceladus' ocean would be highly unlikely to survive ejection to space, sample capture, exposure to radiation during the back cruise, Earth reentry, and/or any exposure to the relatively oxidizing conditions of Earth's surface*

Return to a sample above GEO eliminates two of those four challenges, the Earth reentry, and the oxidizing conditions of Earth's surface.

For returned samples they give four advantages

1. More modern instruments (spacecraft can only have instruments available at the time of launch and usually selected long before launch)
   - Same applies above GEO

2. Instruments that cannot be miniaturized
   – partially fulfilled – much larger instruments can be sent to above GEO

3. Complex wet chemistry protocols or sample preparation steps
   – more difficult but with artificial gravity and low latency telepresence may be some options here

4. A much more diverse suite of techniques than could be accommodated on any spacecraft.
   – Same applies above GEO.

The satellite above GEO meets two of those four challenges.

The other two then can be used with pre-sterilized samples returned to Earth.

For a Europa sample return, any surface ice samples would be sterilized already by the high levels of ionizing radiation, and don't have planetary protection issues unless there is liquid water near the surface.

However, there is some evidence suggests the chaos terrain in Thera Macula may be forming over a rising layer of liquid water heated from below (rather similar to the way that magma plumes form on Earth) (Schmidt et al, 2011) and it could be shielded by an insulating layer a few centimeters thick from thermal imaging (Abramov et al, 2013). It also may have water plumes as for Enceladus (Lesage et al, 2022). NASA's Europa Clipper will help resolve this question when it gets there in 2030 (NASA, 2022). If it does have near surface liquid water, it could potentially have indigenous near surface life even with the high levels of surface ionizing radiation (NASA, 2011).

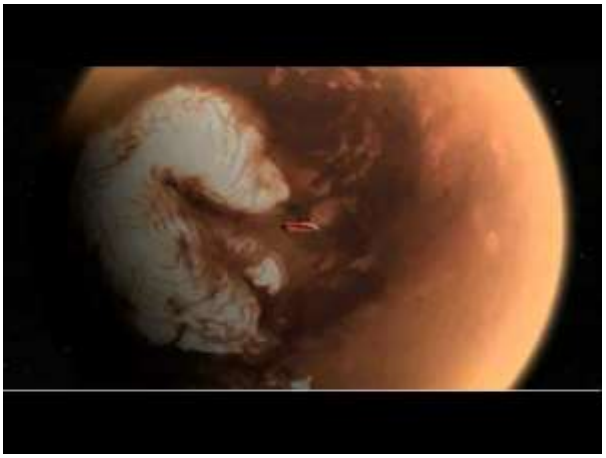
If so, we may be able to return samples from Europa's near subsurface too in the near future. They could be handled in similar ways to Ceres and Enceladus samples.

NEW: NASA have an opportunity to set precedent for future missions to keep Earth 100% safe – and if we find life on Mars that can never be returned safely it may stimulate rather than discourage space exploration and settlement

Next section – all sections – previous section

NASA have an opportunity to set a precedent to keep Earth safe. Other countries are likely to follow its example, or indeed, collaborate in a multi-national astrobiology sample handling and pre-processing lab above GEO – in a similar spirit to the ISS but far lower cost.

If we do find life on Mars that can never be returned safely, this may stimulate rather than discourage vigorous space exploration and settlement. The first astronauts to Mars might study the surface remotely in a spectacular orbit that flies near to both poles twice a day and skims in close over a different part of Mars on the opposite sides of the planet twice a day.



Video: One Orbit Flyby, Time 100x: Mars Molniya Orbit Telerobotic Exploration in HERRO Mission

Early astronaut explorers would likely use two spacecraft joined via tethers for artificial gravity to stay healthy, simulating mars gravity perhaps, and then operate surface marscopters, rovers and other surface assets, similarly to avatars in a computer game.

In the scenario where Mars has mirror life or other life that can never be returned to Earth, settlers in orbital settlements or on the Martian moons could make 100% sterile rovers in surface factories controlled as in the game of civilization. It would be similar to exploring the Venus surface, or the Jupiter cloud decks or other parts of the solar system where humans can’t go safely.

Main image: NASA, 2012 "*Safely tucked inside orbiting habitat, space explorers use telepresence to operate machinery on Mars, even lobbing a sample of the Red Planet to the outpost for detailed study."*

Inset image of a tele-operated Centaur as an insert. Carter Emmart / NASA Ames research center (Mann, 2012)

NEW: We might later be able to return even mirror life to Earth – sketch for a potentially 100% safe lab even with the prohibitory version of the precautionary principle

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Even if we use the prohibitory version of the precautionary principle and find mirror life on Mars, it might be possible to return it to a future sample receiving laboratory on Earth with appropriate precautions. I sketch out an idea for a way this could be done in my preprint (Walker, 2022b) using a titanium sphere surrounded by a Whipple shield for containment during re-entry, black box flight recorder technology for protection during transport, then final analysis in a telerobotic facility accessed via a sump filled with vacuum stable light oil sterilized with ionizing radiation and heated at high temperatures, and the whole thing inside a large externally maintained oven in a nuclear fallout shelter for end of life sterilization. This is just a sketch of basic scientific ideas not an engineering proposal.

There might be simpler ways to do it but this sketch may be enough to establish the possibility to achieve the high standard of “no appreciable risk” for the prohibitory version of the precautionary principle.

However, by then it may be preferrable to return it to a laboratory on the Moon or indeed on Phobos, for telerobotic study, if we have a scientific human outpost there.

NEW: We can explore and exploit Mars without humans on the surface, as part of a vigorous program of exploration and perhaps settlement throughout the solar system

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We can explore and exploit Mars without humans on the surface, settling the Martian moons and orbital space habitats, as part of vigorous exploration and perhaps settlement throughout the solar system. Humans and robots work together each doing what it does best. Torrence Johnson, Galileo Chief Scientist, put it like this in the foreword to Meltzer’s “Mission to Jupiter” (Meltzer, 2007)

> ***Torrence Johnson:*** *What we call robotic exploration is in fact human exploration. The crews sitting in the control room at Jet Propulsion Laboratory as well as everyone out there who can log on to the Internet can take a look at what’s going on. So, in effect, we are all standing on the bridge of Starship Enterprise*

My aim with this review is to do everything I can to help make sure voices and concerns of the public are heard. My wish is that this will encourage space agencies to do a rigorous scientific review with full public involvement. I am sure somehow, the public will get their say, though I don’t yet see clearly how exactly it will happen.

Method and limitations

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This paper is written as a review for the general public to use, legislators, ethicists, decision makers and scientists of other disciplines, so is designed to be maximally accessible.

This paper has to consider research published after the last comprehensive review in 2009 or it would be 13 years out of date. A new comprehensive review is needed but this is not that review.

Instead the cites here are selected as illustrative examples to answer the main mistakes in NASA’s draft EIS which are treated as representative of mistakes other space agencies are likely to make.

This paper includes new worst case scenarios. These shouldn't be seen as likely. We need to look at those is for the same reason that we look at the scenario of a house fire when we design or install a smoke detector.

This paper covers some options in depth such as sterilizing samples before they reach Earth, and some views in depth such as Carl Sagan's view that we cannot take even a small risk with a billion lives. It does this because NASA's draft EIS doesn't mention them, so there is a clear need to alert space agencies to these options and views.

This paper argues that the public and decision makers need to know this information, and that by the NEPA regulations, a valid EIS should cover these options or views. However this paper is not the place to advocate for or against any of those views. That is for public debate and for legislators and decision makers to look into.

This paper comes to the conclusion that it is possible to keep Earth 100% safe with minimal or no impact on science and even a major increase in science return with minimal effect on the budget with the bonus samples. This thesis needs to be looked at carefully, and if it is indeed true, this also is something the public need to know when making their decision.

However, readers should be aware that any paper like this is part of a dialog. We need to see what others say in response to that reasoning.

In more detail on these points:

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

Next section – all sections – previous section

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:

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

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

- Million instead of 10^6
- "Didymo" instead of Didymosphenia geminate

Where there is no ordinary language equivalent, 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.

As for the choice to make this paper maximally accessible to autistic people – as part of my voluntary work helping scared people over the internet, I am used to working with scared people, many of them autistic. I 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. It also seems to make the text easier for everyone to parse quickly. You may not notice much difference. The most obvious change may be that sentences and clauses within sentences tend to be a little shorter than for most academic papers.

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.)

This paper frequently covers recent research findings – because if it didn’t it would be 13 years out of date – however it is not itself a comprehensive review and shouldn’t be used as such

Next section – all sections – previous section

It would be a major omission to write this paper and not mention the Curiosity brines, but they were discovered 6 years after the major National Research Council sample return study in 2009 (SSB, 2009), and three years after the European Space Foundation major revision in 2012 which focused mainly on the size limit (Ammann et al, 2012). There have been numerous other major advances in our understanding of many topics relevant to this mission since those two studies, as we’ll see.

Similarly it would be a major omission not to mention the work on transport of biofilms in dust storms or the transport of viable b. subtilis spores in saltation bounces and many other topics mentioned here.

It would also be a major omission not to include the material on the potential for subsurface ice melt in the polar regions which gives potential for a present day fresh water habitat on Mars and the same for may other topics mentioned here.

This paper needs to refer to some of that newer research or it would be 13 years out of date. However it’s not in any way comprehensive. It just draws attention to some of the most major findings of the last decade or so that NASA’s draft EIS omits.

I hope that this can encourage NASA and ESA to commission a new sample return study to look into these and many other many major new developments of the last decade thoroughly.

Scope of this review – material likely to be of especial interest to space agencies, based on mistakes in NASA's draft EIS – rather than any attempt at a comprehensive review

Next section – all sections – previous section

It is clearly impossible to attempt a comprehensive review to survey all the most important research since 2009, as it would need book length treatment and the participation of many experts. So, the cites here are selected in response to mistakes made in NASA's draft EIS. These are treated as likely to be representative of mistakes that other space agencies might make in similar Mars Sample Return environmental impact statements.

- Questions for NASA
- Reasons for these questions: controversial or mistaken statements in NASA's draft EIS and the report of the sterilizing subcommittee

This review doesn't attempt to be comprehensive in its responses to those questions either. Instead, the aim is simply to draw attention to some of the more important results of the last decade omitted from NASA's draft Environmental Impact Statement that decision makers need to be aware of. The studies mentioned should be seen only as a few illustrative examples drawn from a much large literature which a comprehensive review would need to look at.

This review also focuses on Jezero crater, however this can be thought of as a representative illustration, as the planetary protection concerns are relevant to just about any site likely to be selected for a near future Mars sample return study.

In the near future at least, samples are likely to be returned from equatorial regions since our spacecraft are not sterilized sufficiently for the polar regions, and likely to be returned from low altitude sites since the need for aerobraking makes a high altitude landing challenging.

Pretty much the entire surface of Mars is within reach of the global dust storms. Frosts are likely over much of the equatorial regions and high night-time humidity and the Curiosity brines are also likely to be widespread.

This paper includes new worst case scenarios – they shouldn't be considered likely – they are considered in detail for the same reason you consider the worst case scenario of a house fire when installing or designing a smoke alarm

Next section – all sections – previous section

This paper includes several new worst-case scenarios which it covers in some detail. These are included to encourage space agencies to treat planetary protection more rigorously. There are many other scenarios where no life is returned or the life returned is easily contained or managed or is beneficial or harmless. Beneficial scenarios are covered only briefly in one section.

- NEW: Enhanced Gaia – ways that introduced Martian life could be beneficial to humans, ecosystems and Earth's biosphere

This doesn't mean that the worst case scenarios are more likely. It is just that they are ones that we need to consider carefully for planetary protection issues. Margaret Race's analogy of a smoke detector may help (Rummel et al., 2000)..

When you install a smoke detector in a house, you need to consider the worst case scenario of a house fire and install it correctly so that it will detect a fire. This doesn't mean that you consider the house fire likely.

This paper covers several options and views not mentioned in NASA's draft EIS such as the option to sterilize samples before they reach Earth, and Carl Sagan's view that "we cannot take even a small risk with a billion lives" – the public and legislators need to know about them when making their decisions – but this paper shouldn't be taken as advocating for or against these options or views

Next section – all sections – previous section

This paper covers the option of sterilizing the samples before they are returned to Earth in considerable detail. The aim is to help ensure that this option is considered carefully and thoroughly and the public and legislators have this information available when they make their decisions.

It's similar for the discussion of the ethics of when and whether we should require Earth to be 100% safe, based on a discussion of the prohibitory version of the precautionary principle in a discussion of Carl Sagan's quote (Sagan, 1973):

> ***“The likelihood that such pathogens exist is probably small, but we cannot take even a small risk with a billion lives.”***

It’s important that the public and legislators know about this view and the reasoning that Carl Sagan and others give for it.

This paper shouldn’t be seen as advocating for or against these or any of the views or options discussed here. It’s written carefully to abstract away from any views the author may have.

It’s the same for the analysis at the end. This paper reasons that:

- we can keep Earth 100% safe by sterilizing samples before they are returned to Earth,
- this can be done with virtually no impact on geological or astrobiological science return, and
- the astrobiological sample return can be greatly boosted with minimal impact on cost with bonus samples of salt, dirt, atmosphere and dust.

This is part of a dialog. We need to see what conclusions others come to with their reasonings.

All this then can become input to the discussions by public and legislators which then leads to the final decision.

The main thing is that, as NEPA requires, we need to make these decisions based on a scientifically credible, clear and open process, where the full range of views can be expressed. It is also important that the public are part of this process, and not ignored or excluded.

Factors for space agencies to look out for that may lead to them assigning planetary protection of Earth much less significance and attention than the general public

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It seems worth drawing attention of space agencies to some factors that could lead to them weighting planetary protection differently from the general public. This might also help the general public to understand the point of view of NASA engineers and counteract any infodemic in advance. It is hard to see how NASA could make so many mistakes except through inattention as many of the mistakes are obvious and easy to spot, such as not noticing that their cite for the meteorite argument says on page 5 that it shouldn’t be used to support this argument.

- The meteorite argument can’t be used for potential life in surface dust, salts and dirt as these materials can’t mechanically survive ejection from Mars

For the other mistakes:

- Questions for NASA
- Reasons for these questions: controversial or mistaken statements in NASA's draft EIS and the report of the sterilizing subcommittee

There is no way to know why NASA’s EIS has so many mistakes or if any of these factors apply to this draft EIS. That would need an investigation to find out. But these are factors space agencies could consider to try to ensure they weigh planetary protection higher in their environmental impact statements to match the expectations of the general public.

1. Engineering focus – NASA engineers have been tasked with returning samples from Mars to Earth

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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 handling 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.

For the engineers, and scientists directly involved in the mission it is "above their pay grade" to change it to a mission that sterilizes the samples first or to delay the sample return. Their focus is on completing the mission as specified for them, within budget and on time.

2. The new fast track NEPA process means their EIS won't get the close scrutiny by regulators that an EIS had before when the process would take years

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With the new fast track NEPA process, they are likely to feel there is less need to spend much time on checking the statement, especially since they have feedback from EPA that their draft EIS is already adequate.

See above:

- EPA's letter on last day of public discussion says they didn't identify significant environmental concerns in their review of the EIS – with no mention of the public comments raising concerns similar to Carl Sagan

3. The example of Apollo – the plan closely parallels the procedures for Apollo – they may not be aware that those had no scientific peer review and even by the standard of the science of the time were not adequate

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Their plan closely parallels what the Apollo missions did. There are two natural questions one might have here. The first one:

3 (a) If it is good enough for Apollo – why wouldn't it be good enough today, updated a little to take account of modern science? – because the Apollo plans never had public scrutiny and failed internal peer review

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– because, amongst other things, the Apollo plans never had public scrutiny in 1969 and other agencies told NASA at the time that their plans were inadequate.

The NASA planetary protection plans for Apollo predate NEPA which was published the year after the Apollo landings. So the plans weren't actually open to public scrutiny and there was no external review process. It was done by private interagency debate.

The plans were made available to the public for the first time on the day of launch of Apollo 11 giving no time for the public to scrutinize or comment on them (one of the things fixed by NEPA).

NASA did make an effort to protect Earth from the possibility of life in the lunar samples in 1969. However this attempt was largely symbolic. Sadly, even at the time their plans were not considered adequate.

Amongst many lapses, the astronauts opened the door of the Apollo 11 capsule after splashdown in the open sea, letting out air previously exposed to lunar dust from the landing module. There was dust in the astronauts' clothes. The astronauts donned biological isolation garments and exited into a life-raft bobbing in a heavy sea, and quickly swabbed the isolation garments with a bleach solution. They then weighted those swabs and dropped them into the sea. Finally they disinfected the raft with an iodine solution (Meltzer, 2012:404) and sank the raft (Meltzer, 2012:205).

These procedures wouldn't be enough to protect the sea from microbial spores even with the scientific understanding of the 1960s. The view of Vishniac of the National Academy of Sciences is summarized by Meltzer as : (Meltzer, 2012:203).

> *Opening and venting the spacecraft to Earth's atmosphere after splashdown would, in his view, make the rest of Apollo's elaborate quarantine program pointless.*

The chairman of the Interagency Committee, David Sencer, from Public Health Service said these plans violated the concept of biological containment (Meltzer, 2012:203).

However, NASA set up the internal Interagency Committee with a requirement that all parties had to agree on any change to its plans. This consensus had to include NASA itself (Meltzer, 2012:129). This gave NASA the authority to block any objections. It used this power in 1969 to block requests for more stringent precautions on the basis that there wasn't enough time left before the launch of Apollo 11 to add the precautions required by interagency experts.

It is impossible to know what scientists and the general public would have decided at the time if NEPA had predated Apollo 11. But we could have made the Apollo missions completely safe with robotic sample returns similarly to the Soviet missions. It wouldn't have impacted on the science return much to sterilize the first robotic samples. The chance of life in the lunar samples or dust were already considered to be extremely low. NASA could have done a sterilized robotic sample return, or maybe several, to confirm that the Moon was as uninhabitable as it seemed from other observations;

Once NASA had a high level of confidence that the surface of the Moon was sterile they could then have dropped all planetary protection protocols and sent humans.

So, even then NASA had the possibility to keep Earth 100% safe. But they didn't even consider it. There was much less public awareness of the importance to protect ecosystems and Earth's biosphere in those times.

The second natural question one might have:

3 (b) There was no harm to Earth from the Apollo samples, so why would there be any harm to Earth from Mars samples? – Mars has a very different history and we may have been lucky for the Moon

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– the difference is that the potential for life on Mars as we understand it today is far higher than it was for the Moon even as it was understood in the 1960s – and we might have been lucky with the Moon anyway.

Indeed if we had done as much robotic exploration of the Moon by 1969 as we have done for Mars today - we would already know by 1969 that the Moon was lifeless.

However, there are differences that make Mars a better candidate for surface native life today than the Moon seemed to be with the understanding of the 1960s.

In summary:

- **We have clear evidence today, that early Mars had conditions favourable for evolution of life, with lakes and even seas .** Though it's possible early Mars was ice covered much of the time (Vago et al, 2017) even an ice covered ocean would have habitable hydrothermal vents. Then there's evidence of at least two tsunamis, likely the result of impacts (Rodriguez et al, 2019) which suggest at least a temporary largely liquid ocean (Turbet et al, 2019), as recently as 3.4 billion years ago (Rodriguez et al, 2019)**.**

    - In the 1960s we had no clear evidence for a past habitable Moon. There was weak evidence suggesting the Mares were ancient sea beds (Gilvarry, 1964a) (Gilvarry, 1964b), but this evidence was not persuasive (and of course soon turned out to be false)

- **Curiosity has detected ultra cold salty brines on and near the surface of sand dunes just before dawn / after dusk and below the surface just after sun rises and just before the sun sets** (Martin-Torres et al, 2015).
    - There was no detection of liquid water on the Moon, just a hypothetical layer that could exist at a depth of tens of meters enriched with organics, far from the surface.

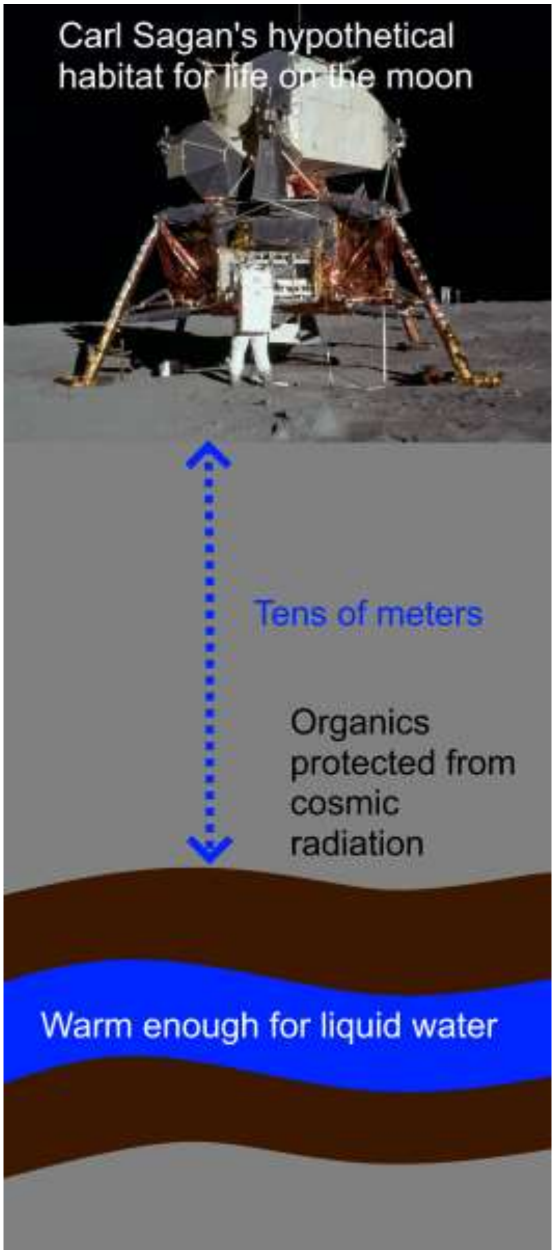

Figure 3: Sagan's hypothetical liquid water layer on the Moon (Sagan, 1961), photograph is of Buzz Aldrin and the Eagle lunar landing module, 1969 (NASA, 1995).

- **Mars has a sparse atmosphere humid enough for thin layers of frost to form at night in many regions.**
  See: 2021: Potential for melting frost to form a "dew" of microns thick layers of fresh liquid water even in Jezero crater – as an example to show the potential for future surprise microhabitats
  Also, some terrestrial blue green algae and lichen have been able to grow in Mars simulation conditions using just the night time humidity in partial shade.

See: Example of a lichen which is found all the way from Antarctica to mountains in California which can survive and even grow in Mars simulation conditions to show that even higher life from Mars could be adapted to live on Earth

- The Moon has no atmosphere, only an exosphere. Frost can't form there at night, and by 1969 it was already clear no life could grow on the surface of the Moon. (Sagan, 1961:25) (Stern, 1999),.

- **The Martian dust storms may be able to transport spores from distant regions of Mars**. See: 2015: MEPAG2 review draws attention to potential for viable life transported through the atmosphere (for instance in dust storms) and following sections.

  - There is no way for life to be transported from distant parts of the Moon.

- **A small minority of scientists believe that the Viking landers may have detected life in the 1970s and the data from the Viking landers is not yet fully explained by either a biological or a chemical hypothesis.**

  See: The Viking landers in the 1970s remain our only attempt to search for life on Mars – a few astrobiologists think its labelled release may have already detected life in the 1970s – while others say the data can be explained by complex chemistry – we haven't sent the follow up experiments needed to finally resolve this debate and we can't deduce anything about whether Perseverance might return life even if the Viking experiment did find complex chemistry

  - There were no puzzling observations from lunar experiments that anyone interpreted as possibly due to life.

So, Mars has greater potential for life with our current understanding of Mars than the Moon ever did even with our understanding of the 1960s before Apollo.

Also, we may have been lucky with the Moon. Sagan's deep subsurface layer was not totally implausible, at the time. Then for a similar Moon but with that deep subsurface layer, there could be various processes that could move life from such a layer to the surface and for it to still be viable.

Another civilization in another star system might return life from a moon closely resembling ours superficially as we understood it at the time of Apollo, perhaps with a different accretion history.

As an example, Ceres is superficially somewhat similar to the Moon as we understood it in 1969. However the patches of ice in regions of permanent shade suggest it may have a subsurface liquid water or mud ocean, not unlike Sagan's hypothesis for a deep subsurface habitable layer for the Moon. We don't know if there is life on Ceres. We may return samples from Ceres in the late 2030s. We will need to take precautions to protect Earth in case there is life in those samples (Carter, 2022) (Castillo-Rogez, 2022).

4. Inspiration of science fiction

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Many who work for NASA have a deep interest in space exploration and space technology, so they would be likely to be inspired by science fiction stories about astronaut and space explorers.

Just about all the science fiction greats set stories on Mars. Examples include Arthur C. Clarke, Isaac Asimov, C.S. Lewis, Edgar Rice Burroughs, Robert Heinlein, H.G. Wells, Gregory Benford, Kurt Vennegut, Frederick Pohl, Ben Bova, Greg Bear, Ray Bradbury, Phillip K. Dick, Kim Stanley Robinson, Andy Weir, Larry Niven amongst others.

None of these stories feature Martian life that harms Earth's biosphere. Indeed, as a science fiction enthusiast myself, I can’t think of even a short story that features native life returned from Mars that causes large scale harm to Earth’s biosphere or human health. If anyone reading this knows of any, do say.

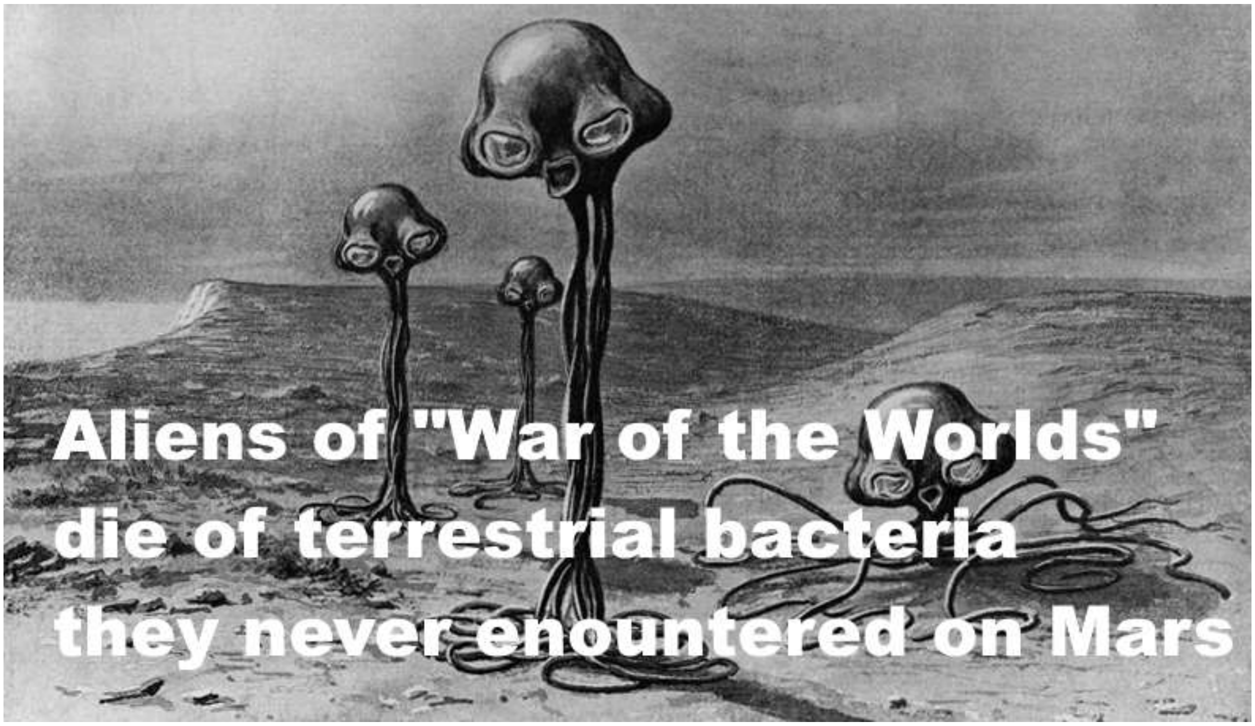
That's understandable because it isn't a plot twist to appeal to a human reader and it wouldn't advance their story line in any way.

In many stories Mars is lifeless. In others, especially from the early 20th century, when Mars was thought to be more habitable, Mars has life but it’s compatible with terrestrial life. Examples include the Edgar Rice Burroughs and C.S. Lewis stories. In these stories, Mars is seen as like another terrestrial continent like Australia perhaps, or Africa or the Americas with unusual creatures influenced by the low gravity.

Amongst these early famous Martian science fiction stories, one of the more notable fictional martians is Stanley G. Weinbaum's "Tweel", a cute intelligent creature in between a plant and an animal, the last relic of an ancient Martian civilization (Weinbaum, 1945). Harmless to Earth.

Jarvis meeting Tweel. Painting in the Smithsonian's Mars gallery (Rowland, 2010)

The only major story involving Mars with a plot twist of large scale harm to living organisms, is H.G. Wells's "War of the World". But there it is resolved in human favour and the creatures harmed are the Martians.



M. Dudouyt's vision of the Martians from the 1917 edition of *War of the Worlds (*Dickson, M. n.d.)

In H.G. Wells' story, Martians had never encountered bacteria on their planet (Wells, 1898 : book 2 section VIII).

> *And scattered about it, some in their overturned war-machines, some in the now rigid handling-machines, and a dozen of them stark and silent and laid in a row, were the Martians—dead!—slain by the putrefactive and disease bacteria against which their systems were unprepared; slain as the red weed was being slain; slain, after all man's devices had failed, by the humblest things that God, in his wisdom, has put upon this earth.*
>
> *For so it had come about, as indeed I and many men might have foreseen had not terror and disaster blinded our minds. These germs of disease have taken toll of humanity since the beginning of things—taken toll of our prehuman ancestors since life began here. But by virtue of this natural selection of our kind we have developed resisting power; to no germs do we succumb without a struggle, and to many—those that cause putrefaction in dead matter, for instance—our living frames are altogether immune. But there are no bacteria in Mars, and directly these invaders arrived, directly they drank and fed, our microscopic allies began to work their overthrow. Already when I watched them they were irrevocably doomed, dying and rotting even as they went to and fro. It was inevitable. By the toll of a billion deaths man has bought his birthright of the earth, and it*

is his against all comers; it would still be his were the Martians ten times as mighty as they are. For neither do men live nor die in vain.

This was the only example Carl Sagan had available to him when he referred to the backwards contamination risk as the plot twist of War of the Worlds, but in reverse:

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. … The chance of such an infection may be very small, but the hazards, if it occurs, are certainly very high.*

The difference here is humans don't get to write the story line for a real-life return of microbes from Mars.

Perhaps it is time for a science fiction writer to write a ground-breaking work of science fiction where there is a risk of harm to Earth? But Carl Sagan's plot twist of the War of the Worlds in reverse wouldn't work as an inspiring science fiction work for humans. It likely needs to be some other plot.

5. Space colonization enthusiasts who see parallels between themselves and the settlers of the American west

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This is likely to be at least a factor indirectly. The only previous article I found that resembles the report of the sterilizing subcommittee is Robert Zubrin's op. ed. for the Planetary report. They don't refer to Zubrin, and didn't go as far as he did in their conclusions, and if there is any connection it is likely to be indirect.

However, the inspiration may be similar as the sterilizing subcommittee report is a more scientific version of that. It doesn't include any of the examples from the planetary protection literature that count against his arguments and doesn't mention the major Mars sample return studies that ALL came to the opposite conclusion to his arguments. See:

- The argument about harmlessness of martian life was previously only presented in a non peer reviewed op ed by Zubrin along with the meteorite argument, with an immediate response from planetary protection experts that his recommendation to take no precautions with Mars sample returns is like building a house without smoke detectors

Robert Zubrin's motive at least is clear as he describes it eloquently himself. He sees Mars as the frontier that the US no longer has in the American West, now that the entire continent has been explored by Europeans (Zubrin, 1996).

> *Without a frontier from which to breathe life, the spirit that gave rise to the progressive humanistic culture that America has offered to the world for the past several centuries is fading. The issue is not just one of national loss — human progress needs a vanguard, and no replacement is in sight.*

He wants humans to colonize Mars and sees Mars colonists as essential for the vitality of Earth's civilizations. He is focused on the engineering and practicalities of this. He has arguments that seem plausible to him that there is no risk of harm to Earth. His views are very influential amongst space colonization enthusiasts. This in turn influences science fiction writers and may reinforce the tendency to envision Martian life as harmless to Earth in their fiction
.

This isn't the place to try to decide whether our civilization does need a physical frontier for vitality. Or to try to settle the long running debate in the US about Turner's "Frontier thesis" or to ask if it really was a frontier given that the Americas were already colonized (Aron, 2016). Perhaps it's best looked at on the level of individual inspiration?

There are many in our civilization who find inspiration in frontiers in art, music, literature, in science and technology, in pushing the physical limits of their bodies in sports challenges, and so on. Others are inspired by the need to learn to live sustainably, or to protect species and stop and reverse biodiversity loss, looking for undiscovered species, or in horticulture, saving old varieties of apples (Brown, n.d.), or striving to breed a blue rose (Nanjaraj et al., 2018) or C-4 rice for higher yields with global warming, which they describe as one of the scientific "Grand Challenges" of the 21st century (C4 Rice, n.d.). Others want to push terrestrial physical limits of colonization such as living sustainably in deserts with saltwater greenhouses such as the Sahara forest project (SFP, 2020), or in floating cities (UNHabitat, 2022) and Buckminster Fuller and Osker worked on engineering details for a future with permanent dwellings in the air (Lippincott, n.d.). For others, advances in medicine or human rights may be what they see as important to the vitality of a civilization.

But there are those like Zubrin who do see physical exploration in space and settling too, as vital to our civilization. For those of us who see things like that, it may help to look at a larger picture, Space can still be a vibrant frontier without humans colonizing the surface of Mars.

If Mars has life such as mirror life that can never be returned safely to Earth – this forever unattainable frontier could invigorate space exploration – with many other attainable frontiers for humans on the Moon, moons of Mars, asteroids, independently orbiting space settlements, aerostats above Venus clouds, Jupiter's moon Callisto, Saturn's moon Titan and beyond

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The example of mirror life shows that it's not a foregone conclusion that Martian life is eventually proven safe for Earth. If we do find alien life on Mars which can never be returned safely to Earth, initially perhaps this is disappointing for aspiring space settlers, but it could invigorate space exploration and settlement. The public interest and the scientific and practical benefits to humans could be enormous from a completely alien biology such as mirror life.

A Mars with mirror life, perhaps, becomes a forever unattainable frontier, a world you can never actually land on, but can still explore with high fidelity telepresence. We have never had a frontier like that.

We have places on Earth we can't settle currently such as the deep ocean, even the shallow ocean is hard for us to inhabit, for now the atmosphere is hard to inhabit, we have only made a start on sea steading, and some places are well beyond current technology. We can't explore down to the core of our planet as Jules Verne envisioned in his "Journey to the center of the Earth" (Verne, 1897). But we have never had a frontier due to biology.

Might that "untravell'd world" (Tennyson, 1842) also lead to stimulus and challenges and invigorate our culture?

> *Yet all experience is an arch wherethro'*
> *Gleams that untravell'd world whose margin fades*
> *For ever and forever when I move.*
> *…*
> *And this gray spirit yearning in desire*
> *To follow knowledge like a sinking star,*
> *Beyond the utmost bound of human thought.*

Mars isn't such a prize as a planet for humans to inhabit as it seems to be visually from the attractive reddish looking terrain. There is no soil there, of course, it's just desert. And Mars is not habitable to humans in any ordinary sense of the word. The atmospheric pressure of 0.6 to 0.7% is well below the Armstrong limit of 6.3% where water and body fluids boil at body temperature (Murray et al, 2013). The atmospheric pressure is too low for our lungs to function, so we couldn't breathe even with bottled oxygen. We would go unconscious in seconds, and require a full body pressure suit to breathe.

Suppose we had a plateau on Earth, at a height of 45 kilometers (NASA, n.d.), five times higher than Mount Everest at 8,848.86 km (Dwyer, 2020). The pressure would be typical for Mars, but it would be far more habitable and it would be far easier to colonize such a high plateau than Mars. Yet if such a plateau occurred on Earth, we would likely have few living there permanently except to extract resources or for scientific study. We don't colonize most deserts, or the shallow continental shelves, which are far more habitable.

Lunar dust isn't laced with perchlorates like the Martian dust, but it was still a major issue for lunar astronauts. They all reported difficulties with the dust (Stubbs et al, 2007). Astronaut Eugene Cernan, the last man to walk on the Moon to date (NASA, 2017), described the lunar dust as one of the greatest inhibitors to a nominal operation on the Moon (Levine, 2020).

Mars has worse dust problems than the Moon. For instance, the Moon doesn't have dust storms. Several times a decade dust storms on Mars block out the sunlight for weeks making surface conditions as dark as night. On the Moon we can clear the dust from around a settlement, or melt it into paving slabs, and on any landing sites for our rockets, and eliminate the issue at least locally as well as along roads and tracks (Taylor et al., 2005). That would be only a temporary solution on Mars as the paved surface would get covered by dust again with the next dust storm and the dust would still be suspended in the air.

Lunar dust falls back in ballistic arcs, in an atmosphere so thin it is classified as an exosphere rather than an atmosphere.

However, on Mars, some of the finest dust kicked up by astronauts or rovers would linger in the air for some time even in calm weather without winds.

Inhaling a few milligrams of Martian dust could exceed the recommended maximum daily dose for perchlorates (Reference dose or RfD) (Davila et al, 2013). When the perchlorates are activated by ionizing radiation they may change to the more deadly chlorates and chlorites with some potential for more serious and immediate effects such as respiratory difficulties, headaches, skin burns, loss of consciousness and vomiting (Davila et al, 2013).

There are methods for dealing with this, used for dust suppression when mining uranium, lead or other heavy metal contaminated areas. But it adds to the complexity of Mars colonization (Davila et al, 2013).

The challenges to keep the dust out impact on most aspects of a mission to the surface (Rucker, 2017). It can be done. The suitport may be a solution for the problem of dust inside habitats (Boyle et al, 2013).

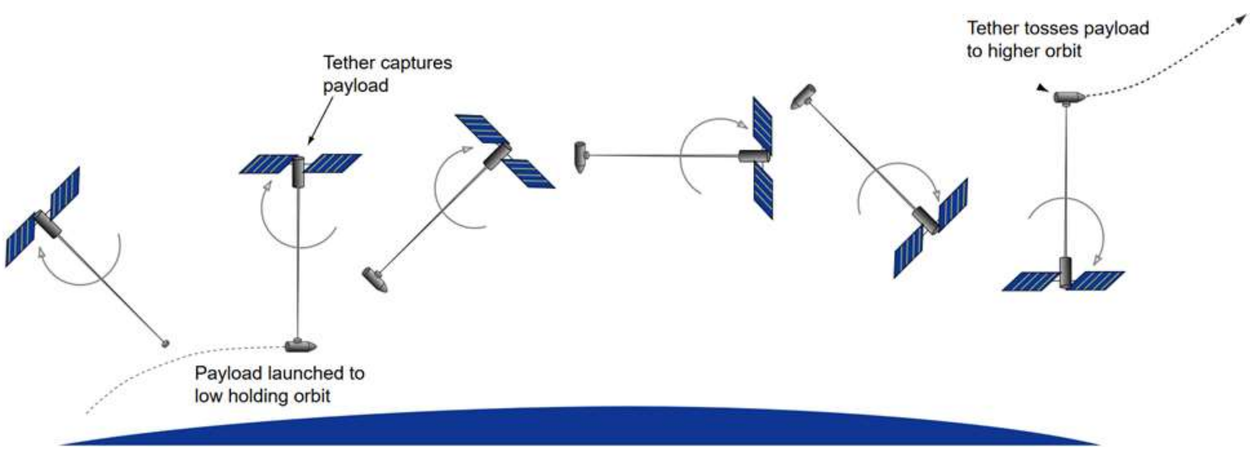
Suitport illustration from (Gernhard et al, 2008) reduces loss of air when exiting or entering the rover and greatly reduces the dust problem.

None of this makes Mars settlement impossible, but Mars does not seem to be an optimal place to colonize for its own sake. There has to be another reason to take the tremendous efforts needed to colonize a place like this. If we find instead that we have to explore Mars from orbit, actually it would be safer and easier to explore Mars in that way.

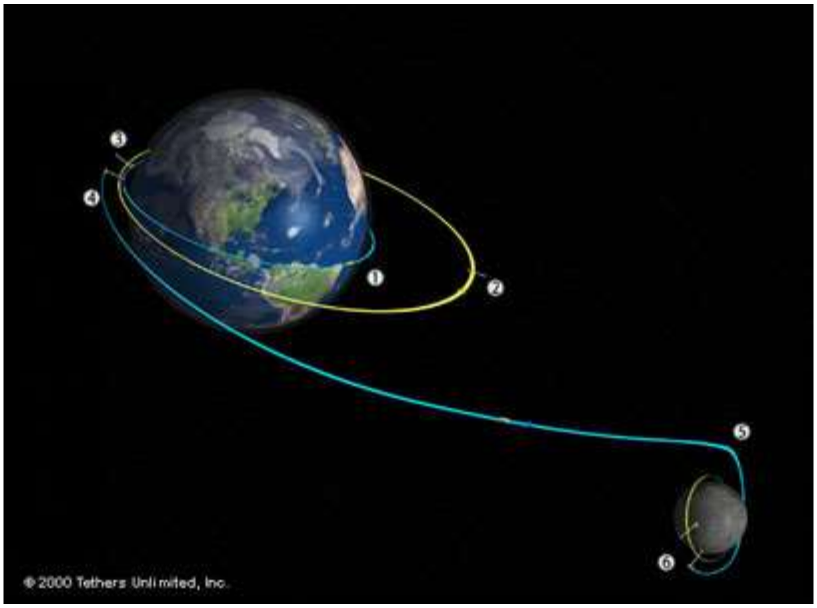
There are many other places pioneering humans can explore and perhaps settle. Including the moons of Mars. The Moon itself. There are resources on the Moon that may be valuable if the cost of returning them is low enough.

Hoyt's cislunar tether system is an ingenious way of using the gravitational potential difference between the Moon and Earth much like the way a syphon can lift water over the lip of a container to a lower place. It uses two spinning tethers, one lifts materials out of the lunar gravitational well, the other receives them in orbit around Earth. The system is powered by the flow of materials from the Moon to Earth which in turn lets astronauts and their provisions and tourists travel from Earth to the Moon again with no extra fuel except whatever is needed to fly to the lower tip of the tether in Earth's orbit in a hypersonic space plane (Hoyt et al., 2000).

This is a brief outline to show it works, first, it captures a payload launched to a low holding orbit and boosts it into a lunar transfer orbit to the Moon.



The complete cislunar transport system looks like this:



"The Cislunar Tether Transport System. (1) A payload is launched into a LEO holding orbit; (2) A Tether Boost Facility in elliptical, equatorial Earth orbit picks up the payload (3) and tosses it (4) into a lunar transfer trajectory. When it nears the Moon, (5), a Lunavator Tether (6) captures it and delivers it to the lunar surface."

At the Moon end of the transport system, the tether can spin at just the right speed to be stationary relative to the Moon's surface when it is closest to the Moon make it easier to transfer passengers and materials to / from the Moon. The material flowing from Earth to the Moon is used to keep the lunavator tether spinning and to boost it.

Then in the other direction, material flowing from the Moon to Earth is used to boost the tether in Earth orbit to prevent it de-orbiting and to keep it turning. In this way both tethers are continually boosted and kept turning, and it turns out that the gravitational potential difference between the Moon and the Earth can be exploited to keep the system going with almost no extra fuel so long as more mass is transferred from the Moon to Earth than in the other direction. (Hoyt et al., 2000).

"By balancing the flow of mass to and from the Moon, the orbital momentum and energy of the system can be conserved, eliminating the need to expend large quantities of propellant to move the payloads back and forth"

Both can be made with materials we have already. The Earth-orbit tether boost facility needs a mass 10.5 times the payloads it can handle and the lunar tether facility needs a mass less than 17 times the payload it can handle. So, once we have frequent payloads back and forth between the Earth and the Moon it will pay for itself in terms of payload mass quickly.

There are many details to the idea – that's just the outline of how it works (Hoyt et al., 2000).

The Moon is also a natural destination for space tourism; for our first science outposts. Once we have an industrial base on the Moon, it is a natural place to build spacecraft and rockets, to send to explore further afield. We will be able to launch spacecraft, mirrors, and space habitats of any size and shape from the surface. There is no need for a faring to make the payload aerodynamic and the low gravity makes it easy to launch heavy payloads (Metzger et al, 2013). The vacuum conditions also make it a natural place for chip manufacturing plants, for making some types of solar panels and so on.

On the Moon, we can even convert the surface into solar panels with an autonomous solar panel paving rover taking advantage of the vacuum conditions to make solar panels in situ (CAM, n.d.).

The moon is also far more suitable for human settlement than was thought at the time of Apollo. New results include the evidence of vast lunar lava tube caves useful for shelter from surface ionizing radiation. The largest signal from the Grail gravity data suggests a cave 3.5 km wide and 550 meters high, and these lunar caves can be tens of kilometers long (Chappaz et al., 2017). That's far larger than a Stanford torus space habitat. E.g. a ten kilometer long habitat 3.5 km wide has a living area of 35 square kilometers or 35 million square meters. The Stanford torus has various sizes, the simplest has an area of 2.7 million square meters (Johnson et al, 1977:table 4.1, page 45)

Some of the lunar caves probably have an internal steady temperature of around -20 °C, potentially useful as a constant heat sink for a settlement (Daga et al., 2009). The challenge of providing energy during the lunar night is a similar challenge to providing energy during Martian dust storms. Then there are the peaks of almost eternal light at the poles with solar power 24/7 nearly year round (Foing, 2005), the polar ice and so on (NASA, 2019). The Moon is a place where we can make our first steps in sustainable living in space, within easy access of Earth for repairs, supplies, and emergency medvac back to Earth in only two days.

Further afield, as another frontier, it's likely humans explore Venus from the clouds, teleoperating assets on the surface. First they would use airships as with the HAVOC design (NASA, n.d.) and then more permanent aerostats. We need to be sure first that there is no life already in the Venusian clouds, or that if there is, that the sulfuric acid in the clouds makes it so alien there is no risk of forward or backward contamination.

Some enthusiasts think that humans could eventually settle in aerostats living above the clouds of Venus at a height where the temperature and pressure is similar to Earth normal and the skies are clear (Landis, 2003). The idea of colonizing the Venusian skies was first explored by the Soviets in the 1970s (Moskalenko, 1981). Venusian cloud settlers could mine a lot of their resources from the atmosphere and perhaps get other resources from the surface using grapples. What is harder to explain is a reason for living there apart from scientific study of the Venus surface. However, sky living does seem to be possible at least in terms of engineering. Buckminster Fuller's kilometer diameter "Cloud Nine" would float in Earth's atmosphere like a hot air balloon using the waste heat from the settlement and using his lightweight robust dome construction to make a habitat that is safe from even the strongest hurricanes. This was later elaborated in engineering detail to Osker's proposal for a one mile diameter Solar Thermal Atmospheric Research Station (STARS) (Lippincott, n.d.)

A Venusian space settlement, if it is possible, would be similar, but in the Venusian atmosphere using oxygen and nitrogen for buoyancy. A balloon filled with these gases will float in the CO2 atmosphere much in the same way that a helium balloon floats in ours. Another idea is to suspend the habitat from a balloon filled with a mix of water and ammonia which would evaporate or condense depending on the external temperature, keeping the balloon stable at a particular temperature, and so, height, in the atmosphere (Moskalenko, 1981).

Whether we settle the Venus clouds or not, our exploratory outposts in the Venusian clouds would require far less mass launched from Earth for the same habitable volume than any other space habitats, except perhaps habitats in lunar caves. It would also require less technological sophistication for protection against acid than to contain the atmosphere in a vacuum and protect against cosmic radiation.

As our spacecraft get more capable (Adams et al, 2003) , humans can also explore and even colonize Callisto, outermost of the Galilean moons of Jupiter (McGuire et al, 2003) .

In the Jupiter system, Europa is very unsuitable for humans. It is well within Jupiter's very hazardous radiation belt where humans would die quickly even protected by a spacesuit. They couldn't survive long on or near the surface without very thick shielding. Then there's the forward and backward contamination risk if there is any native life there, especially if it has rising plumes of liquid water in places like Thera Macula (Schmidt et al, 2011).

However, Jupiter's Callisto has lower radiation levels than Mars and the same planetary protection classification as the Moon (Kerwick 2012). It is outside of Jupiter's ionizing radiation belt and also shielded by Jupiter's magnetic field from solar storms (Kerwick 2012) . Callisto also has rock and water ice in abundance. It may have a deep subsurface ocean but the surface is unaltered for likely billions of years so there is no forward or backward planetary protection issue. Also humans would have a scientific motive to live there, to study Jupiter's moons and the planet itself from close up and as a base for remote exploration of Europa particularly, with sterilized robotic explorers. As we explore the solar system Callisto is a natural place for a human base and eventually settlement. (Kerwick 2012)

NASA's Human Outer Planet Exploration (HOPE) program in 2003 selected Callisto as the optimal destination for humans beyond the orbit of Mars suitable for human surface exploration and with features of scientific interest (Callisto has a deep subsurface ocean). This summarizes their reasons for selecting Callisto (Adams et al, 2003:4)

- *Europa—Jupiter's smallest Galilean satellite and the second closest to the planet. Scientific interest is largely prompted by the likely presence of a submerged ocean with tidal heating, which could offer conditions conducive to the development of life.*

Europa's location within the Jovian radiation belts poses significant design problems, particularly when contemplating human surface exploration.

- *Callisto—the second-largest Galilean satellite and the most distant from Jupiter. Scientific interest is prompted by the possibility of subsurface water. Callisto's distance from Jupiter places it in a significantly less hazardous radiation environment than Europa, potentially permitting human surface operations.*

…

The Jovian moon Callisto was selected because of the balance that it offers concerning scientific interest, design challenge severity, and the level of hazard to human operations posed by the local environment

.

Elon Musk's artist's impression of his spacecraft for a crew of 100, the Interplanetary Transport System. He said his spacecraft would use Europa as a refueling stop in the outer solar system. Callisto is a far better refueling stop because of the lethal ionizing radiation around Europa which is within Jupiter's radiation belts. The artist's impression actually more closely resembles Callisto as the surface of Europa is probably broken up and rough on the meter scale, at least with current understanding (SpaceX, 2016).

Inset shows artist's impression of an exploration base on Callisto (NASA, 2004:22)

Then there's Titan in Saturn's system, which like the Venusian clouds, has an atmospheric pressure similar to Earth, indeed greater. It is far easier to protect against cold than against vacuum or acid. Explorers only need a thick insulating high tech version of a diver's dry suit with insulation only 7.5 cm thick, with batteries to heat a visor and gloves, and they could explore

Titan's surface using a closed circuit oxygen or air rebreather system (without bubbles) much like military and deep sea divers use (Nott 2009). The winds just a few kilometers above the surface can be a source of energy for a settlement, while surface winds are so light as to cause no issues (Hendrix et al, 2017). It also has organics for making plastics, a stable environment and complete protection from ionizing radiation and the smaller meteorites (as for Earth) . (Wohlforth et al, 2016a) (Wohlforth et al, 2016b). Unless there is cryovolcanism, "volcanoes" of liquid water instead of lava, there is no risk of forward contamination. As for backwards contamination, we'd need to find out what is there but it's plausible that Titan life if it exists would not be able to survive terrestrial temperatures

Whether any of these are easy places to live long term may depend also on the gravity requirements for human health which are not yet known. However it is not yet known if the gravity on the Moon or Mars is suitable for human health long term either. It's possible that they all need to be supplemented with the use of slow centrifuges spinning for artificial gravity during sleep, exercise etc.

If our aim is space settlement, planets may not necessarily be the obvious choice they seem to be. This is an observation that goes back to O'Neil in 1969 when he was teaching freshman physics, (Heppenheimer, 1977:chapter 2)

> ***The first answers they came up with indicated there was more than a thousand times the land area of Earth as the potential room for expansion. They concluded that the surface of a planet was not the best place for a technical civilization. The best places looked like new, artificial bodies in space, or inside-out planets.***
>
> ***The classical science-fiction idea, of course, is to settle on the surface of the moon or Mars, changing the conditions there as desired. It turned out that there were several things wrong with this, however. First, the solar system doesn't really provide all that much area on the planets—a few times the surface area of Earth, at most. And in almost all cases the conditions on these planets are very hard to work with.***

The asteroid belt has resources sufficient to make habitats with a thousand times the land area of Earth, slowly spinning for artificial gravity. Settlers can choose the climate and even atmospheric pressure and composition, and gravity level for the habitats.

Finally over the centuries, and millennia, with space habitats slowly spinning for artificial gravity and large thin film mirrors to focus sunlight, we could explore and settle the entire solar system to Pluto and beyond (Johnson et al, 1977:175)

> ***"At all distances out to the orbit of Pluto and beyond, it is possible to obtain Earth-normal solar intensity with a concentrating mirror whose mass is small compared to that of the habitat."***

Once we have fusion power we have the entire Oort cloud to explore / settle / colonize. Once we can live in any ice dwarf in the Oort cloud, humans can live almost anywhere around any star in the galaxy as nearly all stars have clouds of comets and ice dwarfs surrounding them.

By being careful now to protect Earth and other planets in a solar system we prepare for a future where we learn to live sustainably in the galaxy just as we currently need to live sustainably on Earth. This protects our solar system from our distant descendants throughout the galaxy and protects other life and civilizations from ourselves. I explore that in my “Pale Blue Dot” preprint (Walker, n.d.).

So if Mars turns out to have native life we can never return to Earth, there is no shortage of frontiers in the solar system, and new places humans can explore. Perhaps we eventually settle in some of those places.

However, wherever we go, Earth will remain the planet we evolved on and the one we are adapted to. Jeff Bezos put it like this (Boyle, 2016):

> *We have looked at this solar system. We have sent probes to every planet in this solar system. And believe me, this is the best planet. There is no doubt this is the one that you want to protect. This is the jewel. We evolved here, we're kind of made for this planet. It's gorgeous, and we can use space to protect it.*

As we explore in space, many would say that we do need to protect Earth as our top priority, and indeed use space to protect it. As Carl Sagan put it (Sagan,1997)

> ***...There is nowhere else, at least in the near future, to which our species could migrate. Visit, yes. Settle, not yet. Like it or not, for the moment, the Earth is where we make our stand.***

All sections – for an outline of this paper

Titles of sections are like mini-abstracts and summarize the details of the section. For a first overview of this paper read the section titles.

Contents

Supplementary Information

Start – all sections – end

Perseverance ground temperature

The ground temperature data for Perseverance mission sols 361 and 380 uses the data page; Welcome to the Mars 2020 Perseverance archive which you can browse online by clicking on the Calibrated Data Link or the Derived Data where appropriate, for ground temperature you use the calibrated data.

- ground temperature from the TIRS data for sol 361 range 198.82 °K to to 290.11 °K.
- ground temperature from the TIRS data for sol 380 range 208.1°K to 291.99 °K

Data files used:

Perseverance TIRS data for sol 361.
Hyperlink:
https://atmos.nmsu.edu/PDS/data/PDS4/Mars2020/mars2020_meda/data_calibrated_env/sol_0300_0419/sol_0361/WE__0361___________CAL_TIRS_______________P02.CSV
All the calibrated data for sol 361
Hyperlink:
https://atmos.nmsu.edu/PDS/data/PDS4/Mars2020/mars2020_meda/data_calibrated_env/sol_0300_0419/sol_0380/

Perseverance TIRS data for sol 380.
Hyperlink:
https://atmos.nmsu.edu/PDS/data/PDS4/Mars2020/mars2020_meda/data_calibrated_env/sol_0300_0419/sol_0361/WE__0361___________CAL_TIRS_______________P02.CSV
All the data for sol 380
Hyperlink:
https://atmos.nmsu.edu/PDS/data/PDS4/Mars2020/mars2020_meda/data_calibrated_env/sol_0300_0419/sol_0380/

New: extending the JAXA analysis to photosynthetic life on or near the surface of any Martian meteorites

There may be a slight omission in JAXA's discussion of the fireball of re-entry as their 10% figure is based on life that inhabits the interior rather than just the surface of rocks. (SSB, 2019 : 40). The astrobiologist Charles Cockell tested the blue-green algae chroococcidiopsis. When he attached it at a typical growing depth on a re-entry aeroshell, he found that not only the algae, but all its associated organics were destroyed. He concluded (Cockell, 2008)

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

So, could photosynthetic life get to Phobos on the surface of a rock, and still be viable there, living within a layer that would be destroyed during the re-entry fireball on Earth?

First, it helps that some photosynthetic life near the surface of the ejected rocks may be sterilized or destroyed already by the fireball of exit from Mars on its way to Phobos. However the details here are uncertain because the total mass ejected by the Zumil impact was about 30% of the mass of the atmosphere above it, which could be enough to shield some of the ejecta from aerodynamic heating (SSB, 2019 : 27).

Second, all our martian meteorites come from locations where photosynthesis is unlikely. They were thrown into space by glancing collisions into the high altitude southern uplands

(Tornabene et al, 2006), where the thin atmosphere makes ejection to Earth easier. They also come from at least 3 meters below the surface (Head et al, 2002:1355), and anywhere below 12 centimetres has a uniform temperature of around -73°C (Möhlmann, 2005:figure 2).

Third, there is yet another twist here to look at if we want to be as thorough as possible. The remarkable blue-green algae chroococcidiopsis can use alternative metabolic pathways to grow underground without any light (Li et al, 2020) (Puente-Sánchez et al., 2018). So, martian life capable of photosynthesis is not impossible deep underground, but even then it would be using other metabolic pathways with no reason to live near the surface of a rock, in complete darkness.

Fourth, there is another possible exception, life can use thermal radiation from a deep sea hydrothermal vent for photosynthesis (Beatty et al, 2005). The most recent possibility for a hydrothermal system in the southern uplands on Mars might be the rootless cones (volcanic cones without a magma chamber below them) which may have had hydrothermal systems above 0 C for up to 1,300 years (Hamilton et al, 2010) possibly active as recently as less than 20 million years ago (Stacey, 2019). However, these are very different conditions from a deep sea hydrothermal vent. This may need expert review but it seems unlikely that photosynthetic life using thermal radiation for photosynthesis could be found in the rootless cones in the last 20 million years and then be ejected to Earth, with the photosynthetic life only found near the surface of the ejected rocks, as is needed for it to be sterilized in Earth's atmosphere but potentially survive impact on Phobos.

Then we don't need to go further back than 20 million years, because any life from earlier impacts that might get into the surface samples from Phobos has had over 22.5% of many of its amino acids destroyed (calculated in discussion of sterilization dose below).

So, the JAXA analysis seems correct with this minor tweak to account for photosynthetic life. The more eyes that look at these studies the better given how important it is to protect Earth's biosphere.

By a similar argument it may be safe to send astronauts to Phobos so long as they sterilize any materials before contact from deep below the surface. There may be viable life on Phobos buried deep after ancient larger impacts on Mars which can't get to Earth currently.

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>
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>
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stratospheric exposure, samples were assayed for survival and metabolic changes. Spores from the fungus A. niger and cells from the Gram-(–) bacterium S. shabanensis were the most resistant with a 2- and 4-log reduction, respectively.

The fungus Aspergillus niger and the bacterium Staphylococcus capitis subsp. capitis were included in this study because they are human-associated and opportunistic pathogens, and have both been previously detected on the International Space Station (ISS). Thus, they are likely to travel to Mars in crewed space missions …. Moreover, spores from A. niger might resist space travel on the outside of a spacecraft; therefore, understanding their survival potential in a Mars-like environment is of interest to planetary protection.

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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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>
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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 µm, 0.1 - 0.2 µm or 0.2 - 0.3 µm.*

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>
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> *QUOTE One single colony isolate was taken from each of these populations, generating purified strains designated CB2000, CB3000, and CB4000. The founder, like other E. coli K-12 strains, is quite sensitive to IR; exposure to 3,000 Gy gamma radiation (60Co; 19 Gy/min) reduced the viability 4 orders of magnitude compared to that of the unirradiated culture (Fig. (Fig.2).2). The D37 value [37% survival] for CB1000 was 1,100 Gy, whereas the D37 value for CB2000 and CB3000 was 2,000 Gy—approximately threefold less than the D37 value measured for actively growing cultures of Deinococcus radiodurans R1 (41). The D37 value for the founder was 730 Gy. Higher doses of IR revealed a major improvement in resistance. CB1000, CB2000, and CB3000 exhibited 1,500- to 4,500-fold increases in survival relative to the founder after exposure to 3,000 Gy (Fig. (Fig.2).2). CB4000 was approximately 10-fold less radioresistant than the other isolates.*

> *QUOTE 20 iterative cycles of irradiation and outgrowth. The length of each exposure was adjusted to kill >99% of the population, with this dose increasing from 2,000 Gy for the first cycle to 10,000 Gy on the last cycle*

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…
Dike-induced melting of ground ice and hydrothermal circulation could generate favorable conditions for recent or even extant habitable environments in the subsurface. These environments would be analogous to locations on Earth where volcanic activity occurs in glacial environments such as Iceland, where chemotrophic and psychrophilic (i.e., cryophilic) bacteria thrive (Cousins & Crawford, 2011).

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Aflatoxins, ochratoxins, trichothecenes, zearelenone, fumonisins, tremorgenic toxins, and ergot alkaloids are the mycotoxins of greatest agro-economic importance. …
Mycotoxins have various acute and chronic effects on humans and animals (especially monogastrics) depending on species and susceptibility of an animal within a species.
…

Mycotoxins are secondary metabolites that have no biochemical significance in fungal growth and development

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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-UV-irradiated 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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the near ultraviolet. ... A terrestrial microorganism imbedded in such a particle can be shielded from ultraviolet light and still be transported about the planet."

…

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>
> *July, 1976, the month that VL1 [Viking Lander 1] landed on the Martian surface, was also the month of the outbreak of Legionnaires' disease at the American Legion convention in Philadelphia.*
>
> *The cause, Legionella pneumophila, is a facultative, Gram-negative rod that is one of several human pathogens now known to be carried in the intracellular environments of protozoan hosts. L. pneumophila can also persist, even outside of any host, as part of biofilms.*
>
> *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. It is important to note the numerous biofilms observed aboard the Mir space station, which were found on surfaces and within water plumbing. These films were often multi-species and included bacteria, fungi, and protozoa.*
>
> *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.*

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community as a whole, the ecosystem, which evolves. The individual cell designs that evolved in this way are nevertheless fundamentally distinct, because the initial conditions in each case are somewhat different. As a cell design becomes more complex and interconnected a critical point is reached where a more integrated cellular organization emerges, and vertically generated novelty can and does assume greater importance."

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