

On the ability of the Viking gas chromatograph–mass spectrometer to detect organic matter

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A recent paper by Navarro-Gonzalez *et al.* [Navarro-Gonzalez R, Navarro KF, de la Rosa J, Iniguez E, Molina P, Miranda LD, Morales P, Cienfuegos E, Coll P, Raulin F, *et al.* (2006) *Proc Natl Acad Sci USA* 103:16089–16094] claims to show that the Viking GCMS (gas chromatograph–mass spectrometer) experiment, which carried out a search for organic matter at the surface of Mars in 1976, “may have been blind to low levels of organics.” To rebut this assertion, the Viking experiment, test data, and results on Mars are reviewed, and the fallacies in the design, execution, and interpretation of the new experiments presented by Navarro-Gonzalez *et al.* are critically examined.

In 1976, two spacecraft, Viking Lander 1 and 2, arrived at the surface of Mars and carried out a number of experiments, among them the search for organic matter in two soil samples at each landing site. Somewhat surprisingly, no organic molecules were found at the parts per billion (ppb) level,[†] not even those expected from meteoritic infall. Many performance data, particularly the signals for trace contaminants remaining from the preflight cleaning procedure and the successful analysis of the Martian atmosphere, clearly indicated that the instrument had worked perfectly as designed (1, 2).

As the first opportunity to learn in detail about the chemical environment prevailing on the surface of another planet, the experiment was designed to provide broad coverage of a wide range of organic substances that may be present rather than to look for specific compound types. A mass spectrometer was the clear choice, because of its broad applicability and high sensitivity. To provide for the possible presence of complex mixtures, it was interfaced with a gas chromatograph, preceded by a sample oven, which permitted the release of volatile organic material by vaporization and finally thermal decomposition of more refractory substances through heating to various temperatures (50°C, 200°C, 350°C, and 500°C).

At the time, it was understood clearly that a few organic materials might not be detected in this way (3). Highly polymerized and refractory substances, such as densely cross-linked kerogens, or molecules that decompose to products indistinguishable from the background, such as carbon suboxide polymer (C₃O₂)_n that pyrolyzes to CO and CO₂, fall into this category. However, if kerogen were present, one would expect it to be accompanied by less highly polymerized aliphatic/aromatic molecules; carbon suboxide was of interest only because it had once been proposed as the source of the Red Planet's color (4).

The Viking 1976 Molecular Analysis Instrument

The instrument had been carefully designed to maximize performance while minimizing weight and power requirements: the three tubular sample ovens, after filling with up to 100 mg of finely ground (<300 μm) soil, were tightly sealed into the gas line to assure quantitative transfer of the products; the gas chromatographic column was specifically designed (5) to tolerate water and carbon dioxide while transmitting a wide range of organic compounds; and the electrically scanning magnetic sector mass spectrometer had a scan range from *m/z* 12 to 220. A test instrument (engineering breadboard) that corresponded exactly to the flight units (except that the sample oven was

reusable) was used, before and after the Viking mission, to evaluate its performance. In early tests, most of the compounds previously found in the organic-rich Murchison carbonaceous meteorite (6) had been correctly identified (7). A number of well defined and documented Antarctic soils (8, 9) that at the time served as the closest terrestrial analogues of Martian material were tested before launch and during the postmission period (2, 3). For five of these soil samples, the complete gas chromatograms of the products evolving at 500°C were shown, along with the list of 20–30 compounds (ranging from acetonitrile, MW 41, to dibenzofuran, MW 168) identified at levels of 1–2,000 ppb. One of these data sets is reproduced here (Fig. 1 and Table 1). Two samples from the interior of a sandstone rock collected in the Beacon Valley (Antarctica) (10), which at the time had aroused the interest of biologists, was also analyzed with similar results (3).

In contrast to more recent popular opinion, the Viking Molecular Analysis [commonly referred to as the Viking GCMS (gas chromatograph–mass spectrometer)] experiment was not a “life detection experiment.” This was clearly stated in all published descriptions of the instrument and of the results obtained during the mission (1–3, 11). The search for microbial life was the subject of three separate Viking biology experiments (12–14). The misconception presently prevailing in the astrobiology community and literature is probably caused by the effect the results from the Viking GCMS had on the interpretation of the data from these biological experiments. On the basis of their extensive work on biologically active as well as sterile Antarctic soils, the Viking Biology Team as a group felt it difficult to imagine an active biology in the absence of organic matter (15).

Recent Criticism of the Viking GCMS Experiment

Over the past decade, reports occasionally appeared suggesting that the Viking GCMS experiment may not have been able to detect certain organic compounds or low levels of microorganisms. For example, in a widely cited paper, Benner *et al.* (16) proposed generic oxidation pathways (involving UV-generated hydroxyl radicals) by which highly cross-linked kerogen yields benzenepolycarboxylic acids. They also showed that the salt of mellitic acid (benzenehexacarboxylic acid) does not decompose into volatile fragments at 400°C. However, salts of benzene-1,2-dicarboxylic acid and acetic acid (the ultimate oxidation product of aliphatic compounds) did produce phthalic anhydride and acetone, respectively, which would have been detected by the Viking GCMS. Another frequently cited paper from Bada's group (17) simply confirmed that the Viking GCMS could not have detected the pyrolysis products of <10⁶ *Escherichia coli*

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Abbreviations: GCMS, gas chromatograph–mass spectrometer; ppb, parts per billion; TOM, total organic matter.

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[†]For molecules containing only one or two carbon atoms and coeluting with water or carbon dioxide, the detection limit was in the ppm range.

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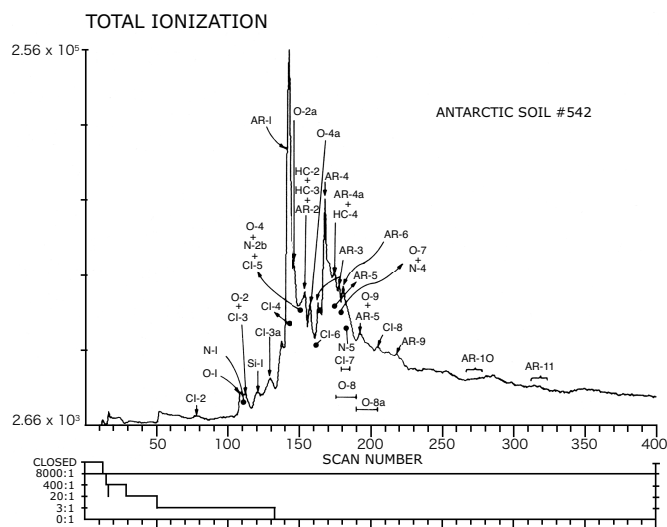


Fig. 1. Gas chromatogram (as total ion current) obtained upon heating Antarctic soil #542 (8) to 500°C for 30 sec. The vertical axis is in arbitrary units. The section below indicates the status of the effluent divider, which protects the mass spectrometer's ion pump from overloading. It indicates the proportional reduction of the gas chromatograph's effluent entering the mass spectrometer. Components Cl-2 to Cl-8 are traces of contaminants from the PVC lining of the sample bags. Adapted from ref. 3.

bacteria (see footnote 19 in ref. 1). As outlined above, both of these cases had been pointed out before and after the 1976 mission and thus needed no further response.

However, recently there appeared a paper by Navarro-Gonzalez *et al.* (18) suggesting that “the Martian surface could have several orders of magnitude more organics than the stated Viking detection limit.” This conclusion is reached on the basis

Table 1. Compounds identified (in ppb) by the Viking GCMS upon heating Antarctic soil #542 (8) to 500°C

Code in Fig. 1	Compound name	ppb
AR-1	Benzene	90
AR-2	Toluene	20
AR-3	Phenyl-C ₂	90
AR-4	Styrene	100
AR-4a	Methylstyrene	4
AR-5	Phenyl-C ₃	20
AR-6	Phenyl-C ₄	10
AR-9	Naphthalene	10
AR-10	C ₁ -naphthalene	2
AR-11	Biphenyl	10
HC-2	Cyclooctane	100
HC-3	Hexane	70
HC-4	Heptane	70
N-1	Acetonitrile	100
N-2b	Vinylacetonitrile	40
N-4	Benzonitrile	20
N-5	Methylbenzonitrile	4
O-1	Furan	20
O-2	Acetone	200
O-2a	Methylmethacrylate	90
O-4	Methylvinylketone	200
O-7	Benzofuran	2
O-8	Phenol	20
O-8a	Cresol	10
O-9	C ₁ -benzofuran	1

Data are from ref. 3.

of a misunderstanding of the design concept, operation, and extensive tests of the Viking GCMS summarized above. Some of the experiments reported by Navarro-Gonzalez *et al.* (18) are poorly conceived, executed, and interpreted, whereas the others are not relevant to the questions at hand. For these reasons and because the acknowledgment thanks “Prof. Klaus Biemann for comments on an earlier version of the manuscript,”[‡] which may give the impression that I agree with the content of the now published paper, it must be responded to in print.

Navarro-Gonzalez *et al.* (18) claim (on page 16092) to have shown “two limitations of the Viking TV [thermal volatilization]-GC-MS for the detection of organic material”: (i) that 500°C may be inadequate to release the organic compounds and/or (ii) that these compounds were oxidized during the heating to 500°C by the iron oxides present in the sample.

The first of these statements is contradicted by the results of the extensive tests of the Viking GCMS instrument reiterated above. Although Navarro-Gonzalez *et al.* (18) cite our paper (3) on the Antarctic soils (their reference 21), they apparently have not read it carefully. Their conclusions are based on data obtained from 14 terrestrial soil samples. They report total organic matter (TOM) in micrograms of carbon per gram of soil and benzene (also in micrograms per gram) evolved upon heating the sample for 60 sec to 500°C and 750°C, respectively, as measured by gas chromatography-mass spectrometry. Also determined were the isotope ratios of carbon (as $\delta^{13}\text{C}$) and the C/N ratio. These isotopic and elemental ratios have no relation to the performance of the Viking GCMS but seem to have been measured as part of an unrelated characterization of these soils.

Only for 3 of the 14 soils was benzene detected upon heating of the sample to 500°C (the temperature used in the Viking protocol), and 8 produced benzene at 750°C, resulting in the conclusion that the Viking GCMS conditions are inadequate and that a higher temperature should have been used. The remarkable fact of these measurements and their interpretation is that the lowest level of detection at either temperature is 1 $\mu\text{g/g}$ (1 ppm), i.e., a 1,000-fold poorer sensitivity than the 1 ng/g sample (1 ppb) demonstrated with the Viking engineering breadboard instrument (see above and Fig. 1 and Table 1). The lack of sensitivity seems to be due to the experimental design. The investigators combined three commercially available laboratory instruments, a pyrolyzer, a gas chromatograph (using a column suitable only for the separation of low-polarity organic compounds containing seven or fewer carbon atoms), and a quadrupole mass spectrometer scanning from m/z 12–100 or 45–200. For some reason, only benzene was reported, rather than all of the compounds evolved upon heating the sample.[§]

The three sets of numerical data presented (TOM and benzene evolved at 500°C and 750°C) also do not make logical sense. The amount of benzene evolved at 750°C from some of the samples seems extremely high compared with the corresponding TOM value: over 10% from NASA Mars-1 Martian soil simulant, up to 38.5% from Mojave Desert sample DV02-10, and up to 45.5% from Atacama Desert sample AT02-22. Such high yields are highly implausible but were not explained.

These results may be due not only to the low sensitivity of the instrument combination used but also to the ways the TOM is measured and found to range from 10 to 1,500 $\mu\text{g/g}$ of soil. Two methods are described in the on-line supporting materials and methods of ref. 18: a titration with potassium permanganate and a batch-oxidation, also with potassium permanganate followed

[‡]Most of the more significant comments and questions received no response and are thus raised herein.

[§]The assumption that benzene is always the major pyrolysis product is naïve. It would have been more convincing to present the entire chromatogram, including amounts detected, of at least a few representative experiments as it was done for the Viking GCMS tests (2, 3).

by measurement of the resulting CO_2 using gas chromatography–mass spectrometry. A close reading of the first procedure reveals that it only measures the organic material soluble at room temperature in 10 ml of 30% aqueous sulfuric acid per gram of soil. The second method is described only very briefly in the materials and methods section without any references to its source and applicability. In essence, 1 g of soil is acidified with 30% sulfuric acid, and the CO_2 produced from carbonates is removed by evacuation. The organic carbon was then oxidized by the addition of 10 ml of 0.001 M KMnO_4 at 70°C in a closed system. The resulting CO_2 (and much water?) was frozen out in a liquid nitrogen trap and quantified by gas chromatography–mass spectrometry to calculate TOM. Not only is it difficult to visualize the apparatus and manipulations involved, but more importantly, even if the oxidant attacked only organic material and reacted with it quantitatively, that amount of permanganate could oxidize only 150 μg of carbon ($<1/10$ of the maximum reported) and even less of aromatic or aliphatic material. Furthermore, the method is also subject to similar solubility restriction as the titration mentioned above.[†] Thus, both procedures used to determine TOM in these soils will have led to a considerable underestimation of the true value. This problem should also have been recognized when interpreting the results of the elemental analysis, which measures not only the reported C/N ratio but also total carbon.

The comment in the abstract of the paper that “the release of 50–700 ppm of CO_2 [in the Viking analyses] . . . may indicate that an oxidation of organic material took place” reveals the unwarranted assumption that evidently underlies this work. If this were the case, the soil at the two landing sites would have had to contain from ≈ 15 to 200 ppm of organic matter.[‡] The notion that substantial amounts of organic material could be present in the Martian soil but then quantitatively destroyed by oxidation during the heating of the sample to 500°C for 30 sec is not supported by the experimental data presented by Navarro-Gonzalez *et al.* (18), as explained below.

As a sample is heated, the processes of oxidation and volatilization compete. Navarro-Gonzalez *et al.* (18) examine this in model experiments using stearic acid on silica with various concentrations of ferric iron as the oxidant. Their results (figure 5 in ref. 18) show clearly that only a portion of the organic material is oxidized. The efficiency of the oxidation depends on the concentration of ferric iron. The Viking x-ray fluorescence analyses found $<15\%$ total Fe at the two landing sites (19). More recently, Mössbauer spectra recorded by the Mars exploration rovers Spirit and Opportunity have found Fe(III)/Fe(total) ratios of 0.25–0.4 for Martian soils (20). Applying these findings, the results obtained by Navarro-Gonzalez *et al.* indicate that even at 750°C ,^{**} only 20–40% of the organic material would have been destroyed.^{††} This would shift the Viking detection

limit from 1 ppb to at most 2 ppb, a difference completely irrelevant in the search for organic matter on another planet.

Another experiment briefly mentioned in their text in support of the oxidation argument is described in more detail in the legend of the corresponding figure (figure 2 in ref. 18). Small samples of Rio Tinto (Spain) sediment were heated to various temperatures ranging from $\approx 250^\circ\text{C}$ to 750°C while monitoring the CO_2 evolved by gas chromatography–mass spectrometry. The results are interpreted as to “demonstrate that the oxidation of the organic matter to carbon dioxide is catalyzed by the iron species present in the inorganic matrix and goes to completion at temperatures of $\geq 350^\circ\text{C}$.” However, there is no information presented about the amount, oxidation state, or nature of the iron species nor any reason for the assumption that the CO_2 is indeed formed by oxidation of organic carbon rather than from carbonates. It is also of interest to note that the amount of CO_2 evolved at temperatures $>400^\circ\text{C}$ exceeds 100% of the $\approx 1,200$ ppm of TOM, which the authors attribute to inhomogeneity of the soil sample. Most likely, this is due to the inadequacy of the TOM measurement outlined above, and the extent of oxidation is actually much less than complete, rendering the entire experiment and its interpretation meaningless. Therefore, the second “limitation” of the Viking GCMS experiment suggested by Navarro-Gonzalez *et al.* is also not supported but indeed contradicted by their own experiments.

At this point it is useful to recount the origin of the concept of an oxidizing environment at the surface of Mars. It was based on two surprising but unrelated findings of the Viking mission: first, the evolution of O_2 upon exposing a surface sample to water vapor in one of the three biology instruments, the gas exchange experiment (13); and second, the apparent absence of organic matter (even that expected from meteoritic infall) as determined by the GCMS experiments. For the evolution of oxygen in the gas exchange experiment, a number of processes have been proposed (21). The most plausible explanation for the second finding was the destruction of organic molecules residing on or within dust particles while suspended in the atmosphere for a long time, blown there by the periodic sand storms. Hydroxyl radicals formed by UV irradiation of water molecules adsorbed on the dust particle can oxidize and destroy the organic matter. Alternatively, the $\text{HO}\cdot$ radicals could combine to H_2O_2 , which would serve the same purpose, catalyzed by heavy metal ions. Thus, neither of the two Viking findings require the presence within the Martian soil of a mineral capable of oxidizing organic molecules of the type present in meteorites by heating to 500°C for 30 sec.

The question of oxidation of the organic matter upon heating in the Viking GCMS sample oven had, of course, been considered in the course of the interpretation of the data obtained at the two landing sites (2). It was ruled out because the traces of the solvents remaining from the cleaning procedure and detected during interplanetary flight still appeared as well defined chromatographic peaks. They must, therefore, have been at least in part present in the ovens and thus survived the heating with the samples. The oxidants, whatever they are (21), causing the evolution of O_2 in the gas exchange experiment upon exposure to water vapor at ambient temperature surely would not survive the exposure to the water evolved when heating the Martian soil samples to 200°C and higher (1, 2).

Conclusion

None of the data presented by Navarro-Gonzalez *et al.* (18) support the assertion that the capability of the Viking Molecular Analysis Experiment to detect organic material in the surface soil of Mars was inadequate. The two Viking missions flawlessly executed more than 30 years ago represented a quantum leap in our knowledge about the Red Planet and should not now be

[†]The chemical equation for the oxidation of HCOOH with KMnO_4 shown in the on-line supporting materials and methods of ref. 18 is not balanced, i.e., incorrect; the use of formic acid, in which the carbon is already highly oxidized, is also not appropriate in the context of a TOM analysis.

[‡]This was pointed out to Dr. Navarro-Gonzalez in the comments on the earlier version of his manuscript, but the statement remained in the abstract.

^{**}The text of ref. 18 states that a “similar result was obtained at 500°C ,” but the extent of oxidation would undoubtedly be lower, and it would have been more appropriate to report these results instead. Furthermore, stearic acid is not a realistic surrogate for the polycyclic aromatic hydrocarbons one expected on Mars from meteorites, the only organic compounds one would expect to find there with any certainty.

^{††}The model experiment (figure 5 of ref. 18) indicates that 1% and 10% Fe_2O_3 causes oxidation of $\approx 10\%$ and 40% , respectively, of the organic matter; for $\text{Fe}_2(\text{SO}_4)_3$, the values are 35% and 45%. Sulfur represents only $3.1 \pm 0.5\%$ of the soil at both Viking landing sites (19). Therefore, iron sulfate must be a minor component, compared with oxide. The presence of free sulfuric acid in the Martian soil is not supported by any data, and it is not realistic to include it in any experiment modeling the Martian soil.

unjustly criticized on the basis of poorly conceived, executed, and, at least in part, incorrectly interpreted experiments.

Future missions to Mars will sooner or later answer the question of organic matter at the surface or in the near subsur-

face of that planet. It will require carefully designed instrumentation to carry out well planned experiments and thoughtful interpretation of the resulting data. This holds even more for the vastly more difficult task of detecting extant or extinct life there.

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