TANPOPO: Astrobiology Exposure and Micrometeoroid Capture Experiments

By Akihiko YAMAGISHI¹⁾, Hajime YANO^{2, 3, 4)}, Kyoko OKUDAIRA⁵⁾, Kensei KOBAYASHI⁶⁾, Shin-ichi YOKOBORI¹⁾, Makoto TABATA5^{, 7)}, Hideyuki KAWAI⁸⁾, Masamichi YAMASHITA⁹⁾, Hirofumi HASHIMOTO⁹⁾, Hiroshi NARAOKA¹⁰⁾, Hajime MITA¹¹⁾

Department of Life Sciences, Tokyo University of Pharmacy and Life Sciences, 1432-1Horinouchi, Hachioji-shi, Tokyo 192-0392, Japan
Department of Planetary Science, ISAS/JAXA, 3-1-1 Yoshinodai, Sagamihara-shi, Kanagawa 229-8510, Japan
Program Office & Research and Development Office, JAXA Space Exploration Center, JSPEC/JAXA, 3-1-1 Yoshinodai, Sagamihara-shi, Kanagawa 229-8510, Japan
Department of Space and Astronautical Science, Graduate University for AdvancedStudies, 3-1-1 Yoshinodai, Sagamihara-shi, Kanagawa 229-8510, Japan
Department of Space Information and Energy, ISAS/JAXA, 3-1-1 Yoshinodai, Sagamihara-shi, Kanagawa 229-8510, Japan
Department of Chemistry and Biotechnology, Yokohama National University, Hodogaya-ku, Yokohama 240-8501, Japan
Graduate School of Science and Technology, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba-shi, Chiba 263-8522, Japan
Department of Physics, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba-shi, Chiba 263-8522, Japan
Department of Earth Sciences, Okayama University, Tsushima-Naka 3-1-1, Okayama, 700-8530 Japan
Department of Earth Science, Gukuyamu University, Tsushima-Naka 3-1-1, Okayama, 700-8530 Japan
Department Life Scince, Fukuoka Institute of Technology, 3-30-1 Wahakutou, Higasihiku, Hukuoka, 811-0295 Japan

There is a long history of the microbe-collection experiments at high altitude. Microbes have been collected using balloons, aircraft and meteorological rockets. Spore forming fungi and Bacilli, and Micrococci have been isolated in these experiments. It is not clear how high do microbes go up. If the microbes might have been present even at higher altitudes, the fact would endorse the possibility of interplanetary migration of life.

TANPOPO, dandelion, is the name of a grass whose seeds with floss are spread by the wind. We propose the analyses of interplanetary migration of microbes, organic compounds and meteoroids on Japan Experimental Module (JEM) of the International Space Station (ISS). Ultra low-density aerogel will be used to capture micrometeoroid and debris. Particles captured by aerogel will be used for several analyses after the initial inspection of the gel and tracks. Careful analysis of the tracks in the aerogel will provide the size and velocity dependence of debris flux. The particles will be analyzed for mineralogical, organic and microbiological characteristics. Aerogels are ready for production in Japan. Aerogels and trays are space proven. All the analytical techniques are ready. The TANPOPO mission was accepted as a candidate experiments on Exposed Facility of ISS-JEM.

Key Words: Astrobiology, micrometeoroid capture experiments, interplanetary migration of microbes, ISS-JEM, organic compounds

1. Introduction

There has been a hypothesis on the origin of life called "panspermia" ([1], [2]). According to this hypothesis, life has migrated between the Earth and other extra terrestrial objects. The finding of life-like structure in a meteorite originated from Mars recalled the possibility. There is also a possibility that the life on the Earth may have ejected from the Earth by volcanic eruption or by meteorite impact. We have been analyzing the presence of microbes at high atmosphere by aircraft and balloons. Microbes have been captured by these experiments. The microbe-sampling experiments could be extended to lower Earth orbit by using ISS. It is also important to test if the microbe ejected from the Earth may survive during the voyage to other planets. We also propose the survival test of microbes on ISS.

Another important subject on the origin of life is related to the pre-biotic production of organic compounds. The extra-terrestrial and outer-solar area can be the place for the pre-biotic organic compound synthesis. To test the possible pre-biotic organic compound synthesis, simulation has been conducted. However, more direct evidence could be obtained by the intact meteoroid capture experiment. It is also important to know what kind and degree of denaturation could occur on the complex organic compounds which are expected to be formed in extra-terrestrial area. To test the kind and degree of denaturation process, simulated complex organic compounds are proposed to be exposed on ISS.

The development of extra-low density aerogel is an important subject for the micrometeoroid capture experiments. Silica aerogel is made of SiO_2 and is transparent solid. Aerogel have been used for the collection of artificial debris and interplanetary dust. For the proposed ISS project, we developed extra-low density aerogel and will test the aerogel on ISS. The developed extra-low density aerogel will provide proof of the applicability of the aerogel, which can be used for the next generation sample return mission in the Solar system.

Our debris capture mission will collect many types of debris. They will include debris of artificial objects, exhaust form ISS, micrometeoroid, and micro particles from the Earth. Much important information will be obtained from the analysis of the many types of particles collected on ISS.

2. Collection of Microbes in Space

There is a long history of the microbe-collection experiments at high altitude. Microbes have been collected at the altitude from 3 to 58 km, using balloons, aircraft and meteorological rockets from 1936 to 1976 ([28]-[30]). Spore forming fungi and Bacilli, and Micrococci have been isolated in these experiments. However, the experiments have been done before the development of modern molecular biology and only the taxonomic affiliation has been analyzed on the isolates. It is not clear how high do microbes go up. If the microbes might have been present even at higher altitudes, the fact would endorse the possibility of interplanetary migration of life.

Previously, we have conducted microbe-sampling experiments using aircraft. Microbes were isolated from the particles collected at the altitudes form 0.8 to 12 km. The genes of the isolated microbes were analyzed. The analysis revealed that the isolates belong to spore formers (Streptomices, Bacillus and Paenibacillus) and *Deinococcus* related species. Previously *Deinococcus radiodurans* has been classified to be *Micrococcus*. Accordingly, the *Micrococcus* strains isolated at high altitude in previous experiments may be or may include *Deinococcus* species.

Deinococcus radiodurans is the species that is known to be most radio-resistant. Then, we have analyzed the UV resistance of high altitude isolates. Two of the high atmosphere isolates showed the UV resistance similar to or higher than *Deinococcus radiodurans*. The flux of UV light at high atmosphere is expected to be much higher than ground surface. Accordingly, it is reasonable that the microbes isolated at high atmosphere shows high UV resistance.

We have also conducted microbe-sampling experiments using balloons. The sampling device consists of vacuum pump and filtration system. The air was incorporated by a vacuum pump and passed through an ultra-membrane filter. The air intake was controlled by a valve placed in front of the filter. About 10 m³ (corresponding mass at SPT) air was sampled at the altitude from 20 to 35 km. Four strains were isolated from the balloon sampling experiments. Analysis of the isolates is now on progress.

To extend the sampling altitude we propose the microbe collection experiment on ISS-JEM. The microbe/particle collection on ISS needs totally different strategy. We are going to use ultra low-density aerogel for the sampling experiments. If there are microbes at ISS altitude, they have to have the earth orbit velocity. The expected mutual velocity of the microbes against aerogel is up to 16 km/s depending on the direction of the microbe relative to the ISS movement. We have tested the possibility of microbe sampling using a two-stage light-gas gun.

Another point which has to be considered is the survival of microbes in the environment where the UV dose is high. The single cell of microbe is not expected to survive under the high UV dose. However, if the microbes are present in the mineral particles, there will be much higher possibility of survival. We have tested the possible survival of microbes using the montmorillonite particles containing microbial cells. The particles were accelerated to 4 km/s by a two-stage light-gas gun. Microbes were pre-stained with fluorescent pigment. The particles were targeted to aerogel. The aerogel was inspected by fluorescence microscopes. The fluorescent particles were detected in the aerogel. Now, we are trying to assure that the fluorescent particles are really microbial cells we have used.

3. Survival of Microbes in Space

To evaluate the possibility of migration between planets including the Earth and Mars, the survivability of microbes in space must be tested. As a part of this project, we plan to test the survivability of microbes in space by direct exposure experiment.

The exposure experiment of microbes in space has been performed (e.g. [31], [32]). Most of experiments were not direct exposure experiments: windows that shield EUV were used to cover microbes. Therefore, these experiments might underestimate the effects of light on the microbial cells.

In such exposure experiments, Bacillus sp., which is spore-forming bacteria, was used ([31]). Spore is a stage of cells that is most tolerant to extreme environments. On the other hand, bacterial species such as *Deinococcus radiodurans* have been known to show extreme tolerance to the UV-light and gamma radiation. Recently, we have isolated several bacterial species from the high-altitudes (ca. 10 km) (Itahashi, Yang, Yokobori, & Yamagishi, manuscript in preparation). They showed higher tolerance to the UV-light than *D. radiodurans*. Such extreme UV-tolerant bacteria might be able to survive at higher altitudes.

The microbes that are directly exposed to the space have lower possibility of survival. However, some microbial cells may survive, if the cells were shielded by other microbe cells and/or clay minerals. UV light will not reach several tens to hundreds of micrometers in depth (cf. [31]).

In our project, various microbial cells including *D. radiodurans* and our newly isolated UV-resistant bacteria will be used for exposure experiments. Cells will be freeze-dried with/without clay mineral. In the space environment, the cells are expected to be freeze-dried (dehydrated, in another term). The cells will be dehydrated in a hole of a metal plate. The dehydrated cells will be tightly fixed to the metal plate without any covers that might shield. The cell will be exposed to the space at least for 1 year. The cells in the metal holder of the exposure apparatus of TANPOPO will be retrieved and will be returned for the analyses.

The returned samples will be used for various tests for checking the survival of microbes. Most direct test is the cultivation of exposed microbes. The possible contamination of microbial cells during the operation will be tested by PCR analysis of genes. The method will tell whether the recovered bacterial colonies are those of original species or not.

4. Collection of Organic Compounds in Space

A wide variety of organic compounds have been found in such extraterrestrial bodies as meteorites (carbonaceous

chondrites) and comets. Chyba and Sagan estimated that more than 100 kt of carbon had been delivered to the Earth on extraterrestrial bodies [17]. It could be an important source of carbon for the first biosphere on the Earth. Bioorganic compounds like amino acids and nucleic acid bases were detected in the hot water extracts from carbonaceous chondrites. Cronin and Pizzarello reported that Murchison meteorite contains more L-isomers of some amino acids than D-isomers [18], and such enantiomeric excesses could be a seed for homochirality of bioorganic compounds in our world. Nakamura-Messenger et al. have found proto-cell-like organic globules in Tagish Lake Meteorite [19]. Complex organic compounds were also found in comets by the direct analysis of the dust from Comet Halley with mass spectrometer on spacecraft [20]. Preliminary organic-compound analysis of cometary dusts returned by Stardust mission also showed the presence of complex organic compounds in Comet Wild 2 [21].

There is the possibility that organic compounds in interplanetary dusts (IDPs) have contributed more for the generation of terrestrial life than those in meteorites and comets because of the following reasons: (i) much more organic compounds could be delivered by IDPs than by comets and meteorites, (ii) organic compounds in IDPs could be delivered to the Earth less destructive while those in comets and meteorites could be destroyed on their impacts. Though many IDPs have been collected in deep-sea and in Antarctica, there is high probability of terrestrial contamination. We propose the collection of IDPs for the analysis of organic compounds as a part of TANPOPO project.

4.1 Simulation Experiment of High-Velocity Impact

For the analysis of organic compounds on IDPs, the major problem is how to collect IDPs with ultra high velocity. We are going to use aerogel with ultra low density. We have performed simulation experiments by using the two-stage light-gas gun at ISAS / JAXA. The following samples were used for the analysis. (i) Porous silica gel (PSG) used as a blank, (ii) powder of R-2-aminobutyric acid (AABA) with sizes from 220 to 350 μ m, (iii) AABA adsorbed to PSG. AABA was chosen due to its low possibility of accidental detection from contamination, since it is non-proteinous amino acid and scarcely found in living organisms. Each sample was placed in a "sabot" made of polycarbonate, and targeted to aerogel at 4 km/s. The aerogel with a track made by the impact was digested with 5 M HF - 0.1 M HCl mixed acid at 383 K for 24 h, hydrolyzed with 6 M HCl at 383 K for 24 h, desalted with AG-50X-X8 cation exchange resin, and then analyzed with an amino acid analyzer (Shimadzu LC-10A). AABA detected in (iii) was much more than that in blank (i). AABA was not detected, however, in (ii). The results suggest that amino acid itself is not stable against impact even if aerogel is used, but amino acid associated with inorganic matrix is more stable.

4.2 Alteration of Organic Compounds in Space Environments

Where did organic compounds in meteorites, comets and

IDPs come from? Greenberg [22] proposed the following scenario. The Solar System is formed in a dense cloud (molecular cloud). Since temperature in dense clouds is as low as 10-20 K, most molecules are frozen onto surface of interstellar dusts (ISDs). Such "ice mantle" of ISDs contains such molecules as water, carbon monoxide, methanol and ammonia. When frozen mixtures simulating the ice mantle were irradiated with high-energy particles or ultraviolet light, amino acid precursors (molecules which give amino acids after hydrolysis) were formed ([23]-[25]). Organic molecules containing amino acid precursors formed in dense clouds would be altered by cosmic rays and ultraviolet light before they were incorporated in parent bodies of meteorites and/or comets. They were again altered in the Solar System small bodies. IDPs seem to have been made from the small bodies, and organic compounds in IDPs were irradiated with cosmic ravs and strong solar ultraviolet light before fallen into the Earth

It is of interest to examine how organic compounds alter in actual space environments. There have been a great number of experiments on radiochemical and photochemical alteration of organic compounds. In these experiments, either a light source or a radiation source was used on ground. In addition, extreme ultraviolet light (EUV) has never been used on ground, since there are no appropriate windows to pass EUV. ESA has conducted several exposure experiments in space, e.g., LDEF and BIOPAN, but target samples were still covered with windows and space EUV did not reach to the samples [26].

We are planning to expose organic compounds on ISS-JEM Exposure Facility. The exposure unit is set next to the aerogel unit. Here, samples such as amino acids and "simulated interstellar organic compounds" will be adsorbed onto a metal substrate and there will be no covering. The simulated interstellar organic compounds will be made from possible interstellar molecules like carbon monoxide, ammonia and water by proton irradiation [27]. The proposed setup could make it possible to irradiate samples with cosmic radiation and ultraviolet light including EUV simultaneously, and could give information how cometary / meteoritic organic compounds alter in IDPs. These samples will be made of isotopic atoms to avoid the faulty identification of the compounds for the exposure experiments as organic compounds of ISD.

5. Micrometeoroid Intact Capture

Meteoroid observation and collection have been conducted to study their parent bodies in planetary science. Several types of analyses have been conducted on meteoroids. Zodiacal dust cloud could be observed from the Earth surface. Cosmic spherules have been found in Antarctic ice core. Stratospheric interstellar dust particles have been captured by aircraft. Meteoroid impacts have been noted on the surface of LEO spacecrafts. Analysis of these particles has provided information on their origin and the parental bodies. However, intact and contamination-less collection of micrometeoroids is desired. Measurement and modeling of debris flux have another importance: It is important to know the debris flux to evaluate the risk of LEO spacecraft. Debris with relatively lager sizes has been monitored by ground observation. Debris with smaller sizes have been detected and analyzed on the surface of retrieved parts of spacecraft. Retrieved spacecraft have been analyzed in the following missions: LDEF (84-90, NASA/ESA), EuReCa (92-93, ESA), HST (89-93, NASA/ESA), SFU (95-96, JAXA) ([3]-[11]). Passive particle collection apparatus have been also deployed and analyzed: Euro-Mir (96-97, ESA), ODC (97-98, NASA), MPAC-SEED (02-05, JAXA). However, the number of debris is increasing and continuous monitoring is needed.

The key technology for TANPOPO is the particle intact captures using aerogel. The aerogel is amorphous SiO2 with low bulk density (below 0.03 g/cm3), is optically transparent and thus most suitable for hypervelocity particle capture experiments. The aerogel is also an excellent thermal insulator: thermal conductivity is about 0.017 W/mK. Accordingly, aerogel is suitable for space utilization. The aerogel tiles have been used for EuReCa, Euro-Mir, ODC, MPAC-SEED, Stardust, etc, and thus can be said space proven.

For example, the aerogel has been used in Stardust project ([12], [13]). In the Stardust project, cometary and interstellar dust were captured intact and the samples were returned for analysis. The spacecraft was launched on 1999. Interstellar dust was collected on 2002. The cometary particles were collected from the Comet Wild-2 coma during the fly-by on 2004. The samples were returned on January 2006. The particles with hypervelocity, 6.1 km/s, were successfully captured. The initial analysis of the overwhelming success has been reported.

Japanese aerogel has been also used in ISS-MPAC-SEED. In the mission, sampling devices were launched on 2001 placed on the Russian Service Module of ISS in LEO of about 400 km. Sampling devices were retrieved sequentially on 2002, 2004 and 2005. However, the sampling device was exposed only on one face of ISS. It is known that the type of the particles collected depend on the face of the exposure relative to the direction of ISS movement. East face, which is the direction of ISS orbital, has the highest possibility of capturing debris of man-made origin. West face and space face are most suitable for collecting interplanetary dust.

One of the TANPOPO teams has already tested the possibility of collecting hypervelocity meteoroid by aerogel. CM2 Murchison powder was accelerated to be 6.2 km/s by a two-stage light-gas gun and targeted on aerogel [14]. The track of the particle in the aerogel was inspected and the particle was found at the tip of the track. The thin section of the particle was inspected by an electron microscope. The peripheral area of the particle consisted of amorphous nodules that are typical to melting phenomena. However, crystal structures were retained at the central part representing the original mineral structures of the Murchison powder. The result shows that it is possible to capture hypervelocity meteoroid partially intact.

The aerogel tiles are going to be attached on several faces of integrated experimental rack that will be placed on ISS-JEM Exposure Facility. No signal-connection is required during the whole exposure period. After more than 1-year exposure in LEO, the trays will be retrieved manually by EVA crew and sealed in the ISS pressurized module for the Soyuz Earth return.

Interface has been designed based on the discussion with EVA director.

6. Aerogel for Microparticle Collection

Silica aerogel is an amorphous solid with a void volume up to 99.5%. Aerogel has been widely used as optical radiators for PID (particle identification) devices in high energy and nuclear physics experiments, since it is optically transparent and has very low refractive index among solids. One of the advantages of our aerogel is that it is possible to make aerogel hydrophobic, which makes the handling easier. We have been developing aerogel in cooperation with the High Energy Accelerator Research Organization (KEK) and Matsushita Electric Works, Ltd. in Japan [15]. At present, we are able to produce aerogel at a wide range of densities i. e. 0.01-1.2 g/cm3 [16]. The aerogel that were made with our production method were actually used in MPAC-SEED which was a Japanese contributory experiment exposed at ISS Russian Service Module. Because of the extremely low bulk densities, transparencies and thermal insulation properties, aerogel is the most suitable medium for the nondestructive capture of hypervelocity particles in space.

In TANPOPO mission, it is important to reduce the thermal metamorphism of micro particles captured in Earth orbit upon impact. The key to the successful scientific analysis is the performance of aerogel with extremely low densities. For this purpose, we developed an aerogel with the lowest density (0.01 g/cm³ or less) in our current production method. Until now, typical density of aerogel employed in space is 0.03 g/cm³. The density is suitable for easy mounting and handling. However, for micro particles with relative incident velocities over 6 km/s, the density would not be low enough for nondestructive collection. We developed the way of installing the ultra low-density aerogel in a module. The layer of the extremely low-density aerogel for micro particle capture was cast on a base layer of higher density. We have already succeeded in developing a monolithic aerogel block consisting of multiple layers with different densities.

Our primary purpose is to obtain information not only on meteoroids and space debris but also on migration of organic compounds and microbes through space. In addition, we aim to demonstrate the ability of the aerogel-based micro particle collecting apparatus in the space environment. Aerogel units with different density structures will be tested on ISS Japanese Experiment Module, which will extend a reach of the micro particle capture operation. Extra low-density aerogel used in TANPOPO will provide an innovative technique for planetary expedition in the future.

3. Conclusions

The group member of Chiba University in TANPOPO

mission has the ability to produce ultra low-density aerogel. The aerogel made in the method has been used on ISS Russian Service Module for particle sampling experiment. The basic design of aerogel tray has been also tested in the JAXA mission MPAC-SEED. The TANPOPO mission needs neither signals nor mechanism during the whole exposure period. The TANPOPO mission is selected as one of the second stage experiment of ISS-JEM EF. After more than 1-year exposure in LEO, the trays will be retrieved manually by EVA crew and sealed in ISS.

The TANPOPO mission teams already have long experiences in bacterial analysis, organic compound analysis and micrometeoroid analysis. Accordingly, all the analytical techniques are ready.

Interface between TANPOPO apparatus and ISS-JEM EF have been basically designed based on the coordination of EF designers and EVA directors. This coordination must be completed within the development time frame of ISS-JEM EF coordination experiment project. However, all of above requirements are feasible.

References

- S. Arrhenius, Worlds in the Making-the Evolution of the Universe (translation to English by H. Borns) (1908) Harper and Brothers Publishers, New York.
- 2) F. Crick, *Life Itself* (1981) Simon & Schuster, New York.
- M. J. Burchell, R. Thomson and H. Yano, Planet. & Space Sci., 47 (1999) 189.
- Y. Kitazawa, A. Fujiwara, T. Kadono, K. Imagawa, Y. Okada and K. Uematsu, J. Geophys. Res., 104 (E9) (1999) 22035.
- 5) H. Yano et al., Adv. in Space Res., 25 (2000) 293.
- 6) H. Yano, Earth, Planets & Space, 51 (1999) 1233.
- H. Yano: Meteoroid and space debris impacts on telescopes in space, in "Preserving the Astronomical Windows", (Eds. S. Isobe and Y. Hirayama), Astro. Soc. of the Pacific, p65-86, (1998).
- H. Yano and Y. Kitazawa, Proc. the 21st Int'l Symp. on Space Tech. and Sci.(ISTS), Hakushinsha., Tokyo, Japan, (1998) 1819.
- 9) H. Yano et al., Adv. in Space Res. 20 (1997) 1489.
- H. Yano, Proc. the 19th Int'l Symp. on Space Tech. and Sci.(ISTS), (Eds. M. Hinada, et al.), Hakushinsha., Tokyo, Japan, (1994) 1017.
- H. Yano, H.J. Fitzgerald and W.G. Tanner, Planet. & Space Sci., 42 (1994) 793.
- 12) D. Brownlee et al., Science 314 (2006) 1711.
- 13) M. E. Zolensky et al., Science 314 (2006) 1735.
- 14) T. Noguchi et al., Meteorit. Planet. Sci., Vol. 42, No. 3. (2007) 357.
- 15) I. Adachi et al., Nucl. Instr. and Meth. A355 (1995) 390.
- 16) M. Tabata et al., 2005 IEEE Nucl. Sci. Symp. Conference Record, Puerto Rico, (2005) 816.
- 17) C. Chyba, and C. Sagan, Nature 355 (1992) 125.
- 18) J. R. Cronin, and S. Pizzarello, Science 275 (1997) 951.

- 19) K. Nakamura-Messenger et al., Science 314 (2006) 1439.
- 20) J. Kissel, and F. R. Krueger, Nature 326 (1987) 755.
- 21) S. A. Standford, et al., Science 314 (2006) 1720.
- 22) J. M. Greenberg, and A. Li, Adv. Space Res. 19 (1997) 981.
- 23) T. Kasamatsu, et al., Bull. Chem. Soc. Jpn. 70 (1997) 1021.
- 24) G. M. Munoz Caro, et al., Nature 416 (2002) 403.
- 25) M. Bernstein, et al., Nature 416 (2002) 401.
- M. Hegedüsa, et al., J. Photochem. Photobiol. B, 82 (2006) 94.
- 27) Y. Takano et al., Earth Planet. Sci. Lett. 254 (2007) 106.
- 28) L. A. Rogers and F. C. Meier, Natio. Geographic Soc. Stratosphere Series. 2 (1936) 146.
- 29) C. W. Bruch, In: Airborne microbes: symposium of the society of general microbiology (Gregory, P. A. and Monteith, J. L. Eds.), Vol. 17 (1967) 385. Cambridge University Press.
- A. A. Imshenetsky et al., On microorganisms of the stratosphere. Life Scie. Space Res. 14 (1976) 359.
- W.L. Nicholson et al., Microbiol. Mol. Biol. Rev. 64 (2000) 548.
- 32) G. Horneck et al., Orig. Life Evol. Biosph. 31 (2001) 527.