Aerospace Toxicology and Microbiology

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All substances are poisons: There is none which is not a poison. The right dose differentiates a poison and a remedy.

Paracelsus (Philippus Aureolus Theophrastus Bombastus von Hohenheim, 1493-1541)

INTRODUCTION TO TOXICOLOGY

Toxicology dates to the very earliest history of humanity with various poisons and venom being recognized as a method of hunting or waging war with the earliest documentation in the Evers papyrus (circa 1500 BCE). The Greeks identified specific poisons such as hemlock, a method of state execution, and the Greek word *toxos* (arrow) became the root of our modern science. The first scientific approach to the understanding of poisons and toxicology was the work during the late middle ages of Paracelsus. He formulated what were then revolutionary views that a specific toxic agent or "toxicon" caused specific dose-related effects. His principles have established the basis of modern pharmacology and toxicology. In 1700, Bernardo Ramazzini published the book *De Morbis Artificum Diatriba* (The Diseases of Workers) describing specific illnesses associated with certain labor, particularly metal workers exposed to mercury, lead, arsenic, and rock dust. Modern toxicology dates from development of the modern industrial chemical processes, the earliest involving an analytical method for arsenic by Marsh in 1836. Industrial organic chemicals were synthesized in the late 1800's along with anesthetics and disinfectants (1). In 1908, Hamilton began the long study of occupational toxicology issues, and by WW I the scientific use of toxicants saw Haber creating war gases and defining time-dosage relationships that are used even today (2).

Aviation

The advent of aviation in WW I also saw the first use of toxins affecting pilots and ground crew.

In particular, the use of castor oil as a lubricant required pilots in open cockpit aircraft to wear goggles for eye protection, and scarves around their mouths to reduce ingestion and inhalation, but the nauseating effects of this compound could not be avoided (3). Early ground support also involved hazardous chemicals causing “airplane dope poisoning” due to butyl acetate, ethyl acetate and isopropyl alcohol inhalation (4). The modern industrial complex that supports aviation requires the use of complex and often dangerous chemicals and industrial processes which can adversely affect the health of workers in the production, maintenance and basic operations of an aircraft. The industrial base employs many times more people than are occupied as aircrew. Aircrew themselves may be exposed to toxicants in the course of routine operations, but are at greater risk during mishaps. The need to supply a portable form of energy to propel aircraft and spacecraft necessitates the use of high-energy fuels and hazardous oxidizers which in the course of handling and use creates certain unique toxic hazards to the humans and the environment (5).

Space flight

The conduct of space flight in sealed capsules and the use of reactive compounds for propulsion have caused toxicological concerns from the earliest days of human spaceflight (6). Before the first Apollo moon landing, the National Academy of Sciences (NAS) released a long report recommending that submarine air quality standards for 90 days of continuous exposure be adopted for spacecraft, and that proposed limits for 60 minutes and 1000 days of exposure be considered provisional for selected compounds (7). In 1972, the National Research Council (NRC) published limits on 52 compounds for exposure durations from 60 minutes to 6 months, with a few limits set for 10 minutes of exposure (8). The National Aeronautics and Space Administration (NASA) continued to set and to revise its spacecraft maximum allowable concentrations (SMACs) independently of any outside expert panel; however, by 1989 it was apparent that the NRC should be engaged again to formally review and set air quality guidelines for the planned space station. Since then, NASA and the NRC have maintained a partnership to set and fully document exposure guidelines for air and water quality. Most recently, these limits are being extended once again to 1000 days as planning begins for long-duration missions to the moon and Mars.

The Apollo 1 (originally numbered 204) fire in January 1967 on the pad at the Kennedy Space Center resulted in the death of the 3 crewmembers in part due to toxic exposures to combustion products that were thought to include carbon monoxide, carbon dioxide, and irritant gases (9). A few years later, an Apollo crew was exposed to nitrogen tetroxide fumes as the capsule descended through the Earth's atmosphere after a successful low-Earth-orbit rendezvous with a

Soyuz capsule in 1975 (10). Other dramatic events in the toxicological history of space flight include the refrigerator motor failure aboard STS-40 in which copious amounts of formaldehyde were generated from pyrolyzed Delrin® polymer (11); the solid fuel oxygen generator (SFOG) fire aboard Mir in 1997 that produced little carbon monoxide but did generate organic compounds including benzene; the pyrolysis event one year later in the micro-impurity filter that produced hundreds of parts per million (ppm) of carbon monoxide and elicited delayed headache and nausea in the crew; the persistent leaks of ethylene glycol from the thermal loops of the Mir; and the ill-fated regeneration of the metal oxide canisters used during extravehicular activity aboard the International Space Station (ISS) that caused the crew to take refuge in the Russian segment of the ISS for 30 hours to avoid the noxious vapors from the canisters regenerated in the U.S. segment (12).

Toxicological risks associated with space flight have been a challenge to manage because of the diversity of sources, the risks from pyrolysis of polymeric material, and the limited options available to deal with such events. Despite a concerted effort toward space flight safety, it is reasonable to anticipate that such events will continue to occur as we endeavor to fly a complex ISS with an expanded number of crew, fly sorties and long duration flights to the moon, and begin Mars exploration by humans.

BASIC PRINCIPLES OF TOXICOLOGY

Potency

A poison may be defined as an agent capable of producing an adverse effect. Virtually every

chemical, including water, can produce an adverse effect in sufficient amounts. Toxic agents can be classified by the potency or relative dose required to elicit a specific adverse effect. This creates a spectrum of poisons with potencies differing by many orders of magnitude. A relative degree of toxicity can be expressed by comparing lethal doses in 6 categories. For example, super toxic agents require a dose of less than 5 mg/kg of body mass to produce death in half of those exposed, a concept expressed as the LD_{50}. Examples of super-toxic chemicals are botulism toxin and nicotine. An extremely toxic poison requires between 5-10 mg/kg for an LD_{50}, with an example being the organophosphate pesticide malathion. A very toxic chemical has an LD_{50} from 50-500 mg/kg, with an example being phenobarbital. A moderately toxic agent has an LD_{50} in the 500-5000 mg/kg range. An example would be table salt, sodium chloride. A slightly toxic chemical with an LD_{50} of 5000-15000 mg/kg is ethyl alcohol. A nontoxic agent, such as water, requires more than 15000 mg/kg to produce an LD_{50} (13).

Route of Exposure and Target Organs

Except for contact toxicants, the initial step in exposure is absorption, which is the process by which a toxicant enters the body. Absorption can occur through various pathways. Inhalation is the most common pathway seen in the aerospace environment with vapors (gaseous component), fumes (oxides of metals), and solid particles entering the respiratory system. Water solubility determines the depth of penetration into the respiratory system. The highly-soluble gas, sulphur dioxide (SO_2), is readily absorbed in the nose, whereas the poorly–soluble gas, nitrogen dioxide (NO_2), penetrates deeply into the lung where serious injury can ensue. For fumes and dust particles, aerodynamic size determines the depth of penetration. Under conditions of nasal breathing, particles larger than 5 μm are typically captured in the nasopharyngeal region, those in

the size range of 1 to 5 μm in the tracheobronchial region, and those less than 1 μm in the alveolar region. These particles may be removed from the lungs by the mucociliary escalator, by dissolution, or by the lymphatic drainage system. Smaller particles may penetrate to the alveoli and be absorbed through epithelial membranes into the body (14). Special forms of particles, such as fibers (asbestos, carbon fibers), are measured by their cross section so that a long, hair-like particle traveling lengthwise acts like a small particle based upon its cross sectional area and not its length.

Chemicals enter the body via routes other than the pulmonary system. The eyes and nasal mucosa are nonkeratinized epithelium and readily absorb water-soluble particles and respond to acids and bases. The skin, however, is waterproof and lipid proof, and highly resistant to absorption of most chemicals. Only chemicals with unique polarity, such as dimethyl sulfoxide, can penetrate intact skin, although burns and infections may break down the keratinized layer and permit significantly increased amounts of chemicals to cross the dermal barrier and enter the body. Ingestion through the gastrointestinal tract provides opportunities for chemicals requiring acidic environments (the stomach) or alkaline environments (esophagus and duodenum) to be solubilized and absorbed. Finally, some chemicals may be injected intentionally across the skin in a parenteral fashion through accidents or medical procedures.

The next phase of exposure is called distribution. Chemicals will be dispersed throughout the body, often redistributing on the basis of their pH-based solubility or solubility in fat. Those chemicals that tend to dissociate in an acid environment such as aspirin will concentrate in low pH areas such as the stomach or joint spaces even when absorbed by another mechanism. Thus, aspirin taken as an enteric-coated capsule and absorbed in the small intestine will eventually

distribute back into the stomach. Other chemicals will distribute by the nature of their binding capacity for blood proteins, especially albumin. Protein-bound toxicants are not available to disperse into other tissues until they have been freed by dissociation. Metals such as lead will be carried on specific proteins designed as carriers for normal metabolic components. In the case of lead, erythrocyte protoporphyrin, normally used to transport zinc, will carry the chemically-similar lead where it will bind in place of zinc and calcium.

Once in the body, chemicals are distributed and will contact the target organ. Highly perfused organs (e.g. liver) will receive more of a toxicant than those less perfused. Special transport proteins concentrate selected toxicants into certain organs; for example iodine is concentrated into the thyroid by a protein in thyroid cell membranes. Most toxicants have a specific binding site where the effect takes place. For lead, it may substitute for zinc in intracellular processes affecting the synthesis of heme or it may substitute for calcium in bone. Other chemicals, particularly strong acids or alkalies such as hydrazine are nonspecific, and their effects are to denature proteins of all kinds causing a very nonspecific response at the binding site.

Metabolism will also affect the outcome of chemical exposure. Enzymes in the nasal mucosa and stomach will often metabolize chemicals before they enter the body. Gastric dehydrogenases break down ethyl alcohol within the stomach, and differing amounts of the enzyme such as larger amounts present in males than females, will affect the relative toxicity. Chemicals absorbed through the gastrointestinal tract undergo first-pass metabolism in the liver through the portal system. Thus, chemicals absorbed from the stomach and intestine will travel to the liver before entering the general distribution. The importance of metabolism is illustrated in this example: cocaine ingested as a form of tea, commonly done in South America, is

metabolized to benzylecgonine, a nonpsychoactive agent, and thus very little of cocaine taken as an ingested liquid enters the system and causes psychogenic effects. When cocaine enters the body via the lungs or nasal mucosa, this first-pass metabolism is not present and there is no transformation of the chemical and psychotic effects are observed (15). Biotransformation may also adversely change a chemical, as demonstrated by aflatoxin, which is transformed by cytochrome P450 in the liver into a carcinogen through its first-pass metabolism (16).

Ultimately, a toxicant will be eliminated through any one of a number of excretion systems. Many chemicals are excreted through the kidneys via urine. Others may be excreted through the bile, although these toxicants may be subject to enterohepatic recirculation. An example is mercury which is excreted in the bile by methylation in the liver. However, in the colon, intestinal bacteria will break down the methyl mercury and allow reabsorption and return to the system (17). This can prolong the effective half-life of the chemical. Other chemicals such as solvents can be excreted as a vapor through the lungs, an example being ethyl alcohol. Ethanol itself, being a carbohydrate, is also metabolized to acetaldehyde, acetic acid, and carbon dioxide. Other chemicals may be excreted as deposits in the hair, skin, and nails. Examples are arsenic and cannabinoids. Some chemicals may be stored for long periods of time, particularly those soluble in fats or in bone. All these reservoirs can serve to affect the half-life of a chemical in the body and can possibly lead to toxicity if the reservoir is suddenly "emptied". For example, as bone is mobilized during space flight, the release of other compounds stored in bone, such as lead, could cause toxicity if the amount stored in bone were sufficiently high before flight (18).

Extrapolations in Exposure Times and Species

Judgments must be made concerning the dose that a human can experience without succumbing to toxicological effects. Those judgments are formed *a priori* and given as exposure standards for specific times of exposure to avoid specific adverse effects. The data to set such standards is often in a non-human species and has not been generated for the times of exposure for which a standard is needed. In order to set the human exposure standard, a paradigm for extrapolation from one species to another is needed and another paradigm is needed to extrapolate from one time of exposure to another. There is by no means a consensus on how each of these extrapolations should be done. Some of the options are discussed below.

The most accurate method for extrapolation of animal data to the human condition is through physiologically-based pharmacokinetic (PBPK) modeling. By this approach, the concentration of the ultimate toxicant (the one causing the injury) is modeled and measured where possible to estimate the differences between the tested species and the human. Physiological and metabolic parameters must be derived or assumed for each species in this approach, and the modeling must account for changing routes of metabolism as the exposure concentration increases and metabolic pathways are overwhelmed. For example, a PBPK model has been used to compare the relative blood concentrations of n-butanol (a common spacecraft pollutant) in rats and make a prediction in humans if they were exposed to a precursor of the alcohol (n-butyl acetate) (19). The target endpoint to be avoided is central nervous system depression, which can be observed in the exposed rats as reduced voluntary activity.

When extrapolating from one species to another the toxicologist must ask whether the toxic effect observed in the animal species is relevant to human exposures. Examples where rodents are an inappropriate model for humans include the calcium mobilization response of guinea pigs

to elevated carbon dioxide, the neoplastic response of rats to thyroid carcinogens, and the hyaline-droplet accumulation in the male rat kidney during solvent exposures (20). Another example of an inappropriate endpoint is the neoplastic response of rat liver to ingestion of di (2-ethylhexyl) phthalate in water. Rodents not only absorb much less of the toxicant than humans after ingestion, but the peroxisome proliferative response in their livers, which is mediated by peroxisome proliferator–activated receptor-alpha, is nearly absent in primates (21). The toxicologist must consistently question the relevance of the modeled response to a toxicant to the expected human response.

Low Dose Extrapolation

One of the classic problems in toxicology is extrapolation of the toxic responses of an animal model at high doses to the anticipated response at much lower doses, well below any of those used in the animal study. The typical approach to this problem is to derive a dose-response curve that expresses the severity or incidence of a response to toxicant exposures. In the last decade, the benchmark dose approach has been promulgated by the NRC and others as a means of estimating low dose responses from studies performed at higher doses (22). As the Johnson Space Center Toxicology Group has attempted to apply this method to setting of exposure guidelines in cooperation with the NRC, 3 issues with its application have emerged. The first problem is the random variability in the data when the number of test animals per dose is in the vicinity of 10, which is a typical number for many studies. The second problem is how to select the model or models to fit the data, and then how to combine their predictions into a single parameter. The third problem is which statistical endpoint to use as the predictor. Should it be the

lower confidence limit or the maximum likelihood value, and should the predicted risk level be 1, 5, or 10 %? There is not a consensus on how benchmark dosing should be applied; however, it is deemed to be an improvement on the default approach of using a no-observed-adverse-effect level as a starting place for low dose predictions.

Combined Exposures

In any actual situation, people are exposed to mixtures of compounds in the air and water. Often the toxic risk is primarily due to a single pollutant; however, at other times the combined effects of several compounds must be considered. For example, the initial step to addressing the problem of multiple-compound exposures is to calculate a toxicity index (T value) for each space mission. The calculation is as follows:

$$\text{T-value} = \sum_{i=1}^{n} C_i/\text{SMAC}_i$$

Where there are "n" compounds found at concentrations C_i and the exposure standard for the i^{th} compound is the SMAC_i, which is the limit for the time the crew has been exposed. For example, for short Shuttle missions the 7-day SMACs are typically used, whereas for a prolonged stay aboard the ISS, the 180-day SMACs are used. The air is considered acceptable if $T<1$; however, this is often not the case. When $T>1$ is found, the compounds are sorted into groups according to their toxic mechanism or target organ. For example, irritants, carcinogens, hematotoxicants, immunotoxicants, neurotoxicants, cardiotoxicants, etc. constitute separate groups. Then a T_{group} is calculated for each group of toxicants. The air is considered safe if each T_{group} is less than 1 unit. Note that some compounds with multiple toxic effects, such as carbon monoxide, can contribute to more than 1 group. To apply this method one must know *a priori* the target organs of each compound, and that is sometimes not well established.

Immediate vs. Delayed Toxic Effects

It is important that flight surgeons and biomedical engineers supporting a flight be aware that certain toxicants do not elicit their maximum effects immediately, or the nature of the effect may change with time. A good example of this is the delayed pulmonary edema caused by nitrogen tetroxide exposures in the Apollo capsule as the oxidizer was aspirated into the capsule from thrusters. The estimated time of exposure was 4 minutes and 40 seconds at an average concentration of 250 ppm, with a peak at 700 ppm. One crewmember was unconscious when the capsule was opened. The crew experienced immediate symptoms of respiratory irritation, but the pulmonary edema (infiltrate) was delayed for about a day (10). The crew symptoms included chest tightness, a retrosternal burning sensation, and a cough upon deep breathing. Chest X-rays taken the following day were suggestive of chemical pneumonitis; however, these returned to normal 5 days after landing. The crew was treated with oral steroids. To our knowledge, no long-term health consequences have been reported in the 3 crewmembers.

Another example of delayed toxic effects during space flight was the carbon monoxide exposure after the micro-impurity filter pyrolysis aboard Mir. When crewmembers were exposed to several hundred ppm of carbon monoxide, several hours were required for the carboxyhemoglobin to accumulate in the blood to its maximum level. Thus, it was not until approximately 8 hours after their initial exposure that any crewmembers reported headache and nausea after the filter burn.

Adaptive Responses vs. Adverse Effects

When assessing the health significance of a toxic exposure one must clearly delineate *adaptive responses* of an organism from *adverse effects* caused by a toxicant. One example that directly applies to space flight is the anthropogenic compound carbon dioxide (CO_2). The normal outdoor concentration of carbon dioxide is about 0.05%, thus it is relatively easy for people to discharge this metabolic product from the body. Hyperventilation (an adaptive response) can be demonstrated at exposure concentrations of 1% or more, but the effect goes unnoticed by the person exposed. The increased respiratory rate is mediated by chemoreceptors in the carotid and brain; however, if the exposure is prolonged, it appears that the hyperventilation fades as the person acclimates to the high CO_2 during weeks of exposure (see chapter 2). Substantially higher concentrations (>3 %) are generally required to elicit clearly adverse effects such as headaches, dyspnea, or intercostal pain (23). Spacecraft are operated to maintain the concentration below 0.7% (7,000 ppm), but there may be sensitive crewmembers that are susceptible to headaches even at this low concentration.

Individual Sensitivities (Genetic Factors)

Individuals can vary in their response to toxic insult because of age, health status, previous exposure, or genetic differences. People who are very young or very old are generally considered to be more susceptible to environmental toxicants. Persons with respiratory disease are likewise more susceptible to air pollutants and are often warned to remain inside when air quality in urban areas is poor. Previous exposures can either increase a person's sensitivity to subsequent exposure or decrease it. In occupational asthma and reactive airways disease, once an allergen

has sensitized an individual, subsequent re-exposure will evoke a symptomatic response at a much lower level (24). On the other hand, smokers, human test subjects, and even experimental animals that experience multiple exposures to carbon monoxide and the many component carcinogens, combustion products, and irritants, have adapted to it, and therefore are less susceptible to some of the adverse effects of carbon monoxide than naïve organisms (25).

Our understanding of the genetic basis for differences in susceptibility of individuals to toxic insults is being revolutionized by the new field of toxicogenomics, which is the study of all genes of a cell or tissue at the DNA, mRNA (messenger ribonucleic acid), or protein level (26). Before the era of toxicogenomics, there had been long-standing recognition that certain persons were much more susceptible to adverse effects when exposed to certain toxicants. An example that applies to aviation and to space flight is that of ethanol. Ingestion of ethanol is discouraged in both settings, yet we must recognize that certain individuals are extremely prone to the "alcohol sensitivity syndrome", because of genetic polymorphisms in the enzymes that catalyze the removal of ethanol's metabolite, acetaldehyde. In sensitive individuals, this compound causes an unpleasant flushing response, which includes facial redness, increased pulse rate, headache, nausea, and drowsiness, even with a single ingestion of a small amount of ethanol (27). Toxicogenomics facilitates our understanding at the molecular level of the effect of a toxicant on the genome as well as our understanding of individual variability in susceptibility because of their genetic predisposition to be adversely affected by exposure to a compound. In principle, each person could be screened for genetic markers that suggest their level of susceptibility to the adverse effects of a drug or toxicant.

SPECIFIC CHEMICALS IN THE AEROSPACE ENVIRONMENT

Aviation Fuels and Compounds

Liquid aviation fuels are primarily petroleum-based compounds. In general, all aviation propellants consist of mixtures of alkanes, cycloalkanes, and other hydrocarbons in varying ratios. All are volatile and flammable. Petroleum-based fuels fall into two categories: aviation gasoline or jet fuel. In most locations throughout the world, aviation gasoline or avgas no longer contains tetraethyl lead which was removed for environmental protection. Most jet fuel is a blend of kerosene with specific additives to produce characteristic performance. Each manufacturer of avgas and jet fuel will blend approximately 300 hydrocarbon compounds to produce the energy requirements, controlled oxidation, and inhibition of corrosion and freezing necessary for specific applications. Blends are adjusted for seasonal changes in weather and to control "weathering" of stored fuels in specific locations (28).

Fuels can cause skin irritation, and the vapors will cause nausea and sedation. Long-term inhalation can lead to neuropathies and are suspect for hepatotoxicity and carcinogenicity (29). However, most fuels are now handled by a single-point system. This means that the fuel line must be locked and sealed between the tankers and aircraft so that no fuel will flow until the system is closed. This produces very minimal exposure to fuel vapors in the occupational setting (30). Combustion products of fuels include carbon monoxide, carbon dioxide, incompletely oxidized hydrocarbons, and oxides of nitrogen generated by heating of atmospheric nitrogen. The exhaust of internal combustion engines using aviation gas tend to be rich in carbon monoxide while jet-fuel-burning gas turbine engines are typically low in carbon monoxide, but may be high in oxides of nitrogen.

Other petroleum products encountered in the aviation environment include lubricants which are typically heavier oils and hydraulic fluids. Some hydraulic fluids have phosphate components, and under certain circumstances such as combustion, highly toxic organophosphates may be generated. Despite this concern, there is no consistent proof that such events have occurred; nonetheless, such fluids have generally had the phosphate esters substituted (31). De-icing fluids may contain ethylene glycol or propylene glycol which have the potential to be nephrotoxic and must be recovered to prevent environmental contamination (32).

Rocket Fuels

Rocket fuels may consist of kerosene (RP1) used in the Atlas missile, liquid hydrogen which is used for the main engines of the space shuttle and upper stages of other missiles, or hydrazine (33). Kerosene's toxicological properties are similar to jet fuels. Hydrogen is a non-toxic gas that is a cryogenic liquid at -255 ºC. Hydrazines are used as the fuel in hypergolic engines. This fuel when mixed with an oxidizer, will spontaneously combust. Hydrazines are available as hydrazine (H_2N-NH_2), monomethyl hydrazine (H_2N-$NHCH_3$) [MMH], and unsymmetrical dimethyl hydrazine (H_2N-$N(CH_3)_2$) [UDMH]. Hydrazines are used as the fuel for the space shuttle orbiter maneuvering system and reaction control systems and as the power supply for the auxiliary power unit on F-16 fighters. In the F-16, MMH is passed through a catalytic converter which decomposes the hydrazine to ammonia and steam (34). Hydrazines are very stable, clear, colorless liquids with an ammonia-like odor. However, hydrazine is much more toxic than ammonia. Ammonia has a 24-hr SMAC of 20 ppm and can be smelled at 5 ppm, whereas, hydrazine is also smelled at 5 ppm, but its 24-hr SMAC is only 0.3 ppm (NASA/JSC 20584,

March 2001). As highly alkaline chemicals, they rapidly penetrate intact skin and coagulate proteins. Extreme toxicity to the eyes and respiratory tract occur, and workers should have complete isolation in the form of respiratory and skin protection. Rapid decontamination with copious amounts of water is necessary, followed by symptomatic treatment of skin, eye and airway injury. Seizures may occur following MMH absorbtion and are resistant to standard therapy. Pyroxidine treatment at 25 mg/kg has been suggested from experimental studies. (35).

Nitrogen tetroxide and nitric acid are the oxidizer components in hypergolic engines and spontaneously ignite when mixed with hydrazines. These oxidizers are used in the space shuttle on-board maneuvering system as well as the upper stage for other rocket engines (36). They are highly acidic, forming nitric acid on contact with water or the human body, and will rapidly cause burns to the eyes, skin, and respiratory tract. Complete protection must occur if these oxidizers are present in the environment. Rapid decontamination with copious amounts of water is necessary, followed by symptomatic treatment of skin, eye and airway injury. Inhalation injury may include pulmonary edema which may be fatal or result in residual bronchiolitis obliterans. (37)

Solid fuels contain a mixture of ammonium perchlorate, the oxidizer, and a metal, usually aluminum or magnesium, which is the fuel. Additionally, a binding agent such as a plastic will be included in the mixture to create a stable solid. Most air-to-air missiles and the solid rocket boosters for Space Shuttle use this composition. This mixture is extremely stable until ignited and the combustion products produced are hydrochloric acid and a metal oxide (38). The exhaust gases are irritating to eyes and the respiratory tract (39).

Toxic Space Systems Chemicals

Ammonia

This water-soluble gas accumulates slowly in spacecraft atmospheres as a result of human metabolism, but it is effectively removed by filters and by co-condensation with water into the humidity condensate. Other sources of ammonia contamination on the ISS include external and internal thermal loops. Anhydrous ammonia, used in the external thermal loop of the US segment of the ISS, presents a small risk of suit contamination when the loop is serviced. In addition, there is a remote risk of ammonia penetrating into the inner thermal loops, and subsequently into the ISS internal environment. Flight rules, based on data from ammonia monitoring devices, have been formulated to manage ammonia present in the airlock after an EVA, and to manage a potentially catastrophic leak into the ISS from the thermal loops. The primary toxic effect of ammonia is respiratory system and eye irritation but formation of ammonium hydroxide in tissues from exposure to high concentrations of ammonia can lead to alkali-like chemical burns. Typically, ammonia can be smelled before harmful exposures occur; however, adaptation to slow increases in its concentration could result in undetected higher exposures (40).

Glycols

When mixed with water, glycols make good heat-exchange fluids. An ethylene glycol solution was used on Mir as the heat exchanger, and this has been replaced in ISS with a fluid called "triol," a mixture of glycerol and water. These fluids typically require trace amounts of anticorrosion and antimicrobial compounds that do not contribute significantly to the toxicity

risk. Ethylene glycol vapor or fine aerosol can cause immediate irritation of mucosal surfaces, whereas ingestion of the liquid results primarily in intoxication and renal damage (41). The primary experience with this glycol was aboard the Mir space station in the late 1990s when frequent leaks from the thermal exchange system occurred. Because of the low volatility of the fluid, the airborne concentrations remained highest in the module where the initial leak occurred. The vapor spread into other modules over a period of weeks, but adjacent modules never reached as high a contamination level as the module where the leak first occurred based on the information acquired from the tracking of a single event with available analytical measurements. Exposed crewmembers reported mild mucosal irritation, and there were anecdotal reports of unpleasant encounters with floating "blebs" of the coolant fluid that resulted in moderate eye irritation. In contrast to ethylene glycol, the glycerol solution used in the Russian segments of the ISS is much less toxic. Furthermore, after 7 years of ISS operation, no leaks of the fluid have been detected.

Freons

This class of compounds also makes excellent heat exchange fluids. Freon 21® and Fluorinert ® are used in the space shuttle, Freon 218 is used in the Russian air conditioner in the ISS service module, and various Freons have been proposed for use in payload experiments. Of the Freons used during space flight, Freon 21 is the most toxic with a 180-day exposure limit of only 2 ppm because of hepatotoxicity. Freon 218 is essentially devoid of toxicity, and its release into the ISS is of no toxicological consequence. There are 2 important questions when assessing the toxicity of a Freon: 1) are there impurities in the as-used formulation that could be highly toxic, and 2) can any compounds in the Freon be decomposed within a spacecraft environmental control

system to a more toxic compound? Answering either of these questions can present a challenge because of proprietary issues and because the precise behavior of the compound inside an environmental control system is seldom well characterized.

Fire Extinguishants

There are 3 major classes of fire extinguishants that have been used aboard spacecraft in recent years: aqueous-based foams, carbon dioxide, and Halon® (42-44). The first type is the kind that was used in an attempt to extinguish the solid fuel oxygen generator (SFOG) fire aboard Mir in 1997. The result was that the fire persisted because of the oxygen being generated and the aqueous base became a fine aerosol of corrosive droplets (based on the appearance of air samplers returned after the incident). The current fire extinguishant used in the Russian segment of the ISS is similar in composition. Carbon dioxide is the extinguishant available in the U.S. segment of the ISS. There is some concern that the use of this in large quantities could increase the ambient carbon dioxide levels to potentially toxic levels and overwhelm the scrubbing capability. A supplemental carbon dioxide scrubber based on lithium hydroxide beds is flown aboard the ISS to deal specifically with excess carbon dioxide. Halon ($CBrF_3$) is used as the extinguishant aboard the Shuttle. This material is highly effective and low in toxicity. However, in the past some concerns have been voiced about thermal decomposition products if this were to be used. In a modest fire this would not be an issue, but in a large hot fire, hydrogen bromide (HBr) and hydrogen fluoride (HF) products could be produced in significant quantities. For example, Halon would not be an appropriate extinguishant to use on an SFOG fire.

Volatile Organic Compounds

Under nominal conditions the major trace pollutants are organic compounds such as alcohols, ketones, aldehydes, and aromatics. The most toxic of the alcohols is methanol, which is seldom measured at concentrations above 1 ppm, but is generally present above 0.1 ppm. This compound can cause visual disturbances if long-term (months) exposures exceed 7 ppm (45). The most noxious of the small alcohols is n-butanol, which is typically present at about 0.1 ppm or less, but during the Metox regeneration contingency was found at concentrations up to 2.5 ppm (T value = 0.19) (12). The most toxic aldehyde is formaldehyde, which can cause mucosal irritation at concentrations well below 1 ppm. In fact, the 7 to 180 day limit for this compound was 0.04 ppm until it was recently increased to 0.1 ppm based on re-evaluation of irritancy data (46). Under conditions of reduced intermodular ventilation in the ISS, it had been a challenge to maintain formaldehyde concentrations below 0.04 ppm; however, the higher standard can readily be met. The only aromatic compound that presents a significant toxicity risk on the ISS is benzene which is a well known immunotoxicant and carcinogen. Normally, this compound is below detection limits in air samples but it is occasionally generated during an accident [e.g. SFOG fire or Elektron (see Chapter 2)] from overheating and briefly reaches concentrations of a few ppm. Other aromatic compounds, such as toluene, and the xylenes are consistently present in spacecraft air at harmless concentrations.

Toxic Fires and Other Unpredictable Toxic Sources in Spacecraft

Carbon Monoxide

Carbon monoxide is the most ubiquitous toxicant in most combustion or pyrolysis processes. CO

is rapidly absorbed by inhalation and bonds to hemoglobin at a much higher rate than oxygen. Caution should be used in monitoring oxygen levels through pulse oximeters, as these devices will measure carboxyhemoglobin and report it as oxygenated hemoglobin. Cyanide should always be suspected in any carbon monoxide exposure (47). Carbon monoxide is expected to significantly increase during ordinary combustion events that occur in space. Because convection does not occur to any significant extent in near-zero gravity, a small fire can quickly become oxygen depleted and thereby produce more CO than would be produced in an identical fire on Earth. The accidental burn within the regenerable micro-impurity filtering system aboard Mir in 1998 demonstrated how much CO can be produced from what appeared to be a minor event. Detector tubes and an electrochemical sensor are flown aboard ISS to help manage CO in the event of a fire. An electrochemical sensor is also available on Shuttle and an ambient temperature catalytic oxidizer filter is available to remove excess CO in the event of a fire. For treatment of CO exposure see chapter 2.

Acid Gasses

When polymeric materials are subjected to thermal degradation a "soup" of compounds is produced. Of the compounds in that mixture, the acid gasses are expected to be the most hazardous based on the inherent toxicity of this class of compounds and the relative amounts expected in a fire. The compounds of most concern are hydrogen chloride (HCl), hydrogen cyanide (HCN), and hydrogen fluoride (HF). These originate from polymeric materials containing chlorine, nitrogen, or fluorine, respectively. Typical wiring insulation used in U.S. spacecraft consists of Kapton® (a nitrogenous polymer) and Teflon® (a fluorine-containing polymer). The short-term exposure limits for acid gases are in the few-ppm range, because of the

ability to irritate mucosal surfaces (HCl and HF) or cause depression of the central nervous system (HCN). U.S. crews are provided with an instrument capable of quantifying HCN and HCl; however, an accurate sensor for HF has proven elusive. More information on the toxicity of these compounds and monitoring strategies can be found on the JSC Toxicology Group website: http://hefd.jsc.nasa.gov/tox.htm.

Exposure Standards for Spacecraft Air Quality

Setting defensible exposure standards requires a broad range of expertise that cannot be found in single individuals. Therefore, such standards are typically set by a panel of experts selected for their knowledge of toxic effects, metabolism, epidemiology, statistics, pathology, and exposure methods. Since the earliest days of human space flight, with some gaps, the NRC Committee on Toxicology has provided a subcommittee with the needed expertise to advise NASA on appropriate environmental standards and documentation. According to the current paradigm, NASA toxicologists prepare a document for each compound containing a survey of the literature and how the data from that survey can be used to set exposure standards. After careful review, in an iterative process, adjustments are made to the document and proposed standards until all parties are satisfied that the approach and standards are defensible. The resulting documents and standards can be accessed through the website of the NRC (http://newton.nap.edu/books/NI000062/html/R15.html or http://www.nap.edu/books/0309091667/html) or through the JSC Toxicology Group website.

FAA Standards for Air Quality in Commercial Aircraft

Cabin “altitude” is currently regulated to a maximum of 8,000 feet in commercial airliners. Carbon dioxide levels are recommended not to exceed 1500 ppm by the American Society of Heating Refrigeration and Air-conditioning Engineers (ASHRAE) Cabin Air Quality Technical Committee (48).The reason for this limit is not based upon carbon dioxide physiology, which does not measurably change until levels of 3000 ppm (3%) are exceeded (49). Instead, the 1500 ppm standard is used to signify static air and potential for unpleasant aromas. In the commercial aircraft cabin, with many sources of carbon dioxide from human respiration and carbonated beverages, this has proven to be a difficult limit to meet. Control of trace gases in aircraft cabins to ensure the comfort of passengers and crew has been debated for decades. Disallowing smoking on such aircraft has greatly reduced the impetus for specifying levels of air pollution beyond the ones given above; however, ASHRAE has had a draft set of standards out for review and the review period has closed. Recommended air quality standards may soon be available for commercial aircraft.

DUST

Dust Originating within the Spacecraft or Habitat

Floating particulate matter continues to be an issue for spacecraft operations and crew health. For example, flight rules now require that a crewmember must wear eye and respiratory protection when entering a new module attached to the ISS. This rule was developed because the debris that settled during ground preparations of the module floats once it reaches zero gravity. Other dust is not nearly so innocuous. For example, lithium hydroxide dust can escape from Shuttle CO2 scrubbing canisters and may come in contact with the eyes potentially causing lasting damage to

the cornea due to its corrosive nature. Recently, there has been concern about cadmium (Cd) dust in the ISS originating from corroded bayonet pins that were plated with Cd. After lengthy analyses and inspections of the pins and ISS air filters, it was concluded that any crew exposures to Cd dust were well below toxic levels.

External Sources of Toxic Dust in Celestial Habitats

During the Apollo missions it was obvious that lunar dust could be a problem for the crew (50). This dust adhered tenaciously to surfaces such as spacesuits and accumulated in the Lunar Lander in large quantities. When the vehicle re-entered the micro-gravity environment during lunar rendezvous the dust floated into the air and at times presented a challenge for the crew to manage. Although there were several reports of the crew being annoyed by the dust, there was no unequivocal evidence that it was toxic. Exposures were brief and the crewmembers often replaced their helmets when the airborne dust was at its worst. When humans return to the moon's surface for long stays and surface vehicles are used for exploration, the potential for lunar dust to affect crew health is a concern. The processes that activate the surface of lunar dust particles and give it a large surface area (Swiss-cheese appearance) are unique to the moon, and it is possible that this reactivity and huge surface area could render the dust much more toxic than comparable Earth analogs (51). Efforts are underway to understand and mimic the activation processes found on the lunar surface, and determine how much these processes increase the toxicity of lunar dust. This problem is made more interesting and challenging because there are various types of dust, highland, mare, mature, and immature, to include in this endeavor.

PURGE GAS "TOXICITY"

Even gases that are viewed as totally non-toxic can be lethal if they are present in sufficient concentrations to displace oxygen. Coolants and purge agents come to mind in this regard. Nitrogen and helium are often used to create an inert environment over a fuel. These gases may be used to pressurize a fuel tank or to prevent air from entering the tank resulting in a fuel-tank explosion. The presence of air inside the main fuel tank of an airliner can lead to catastrophic results such as the fuel tank explosion that destroyed TWA Flight 800 in 1996 (52). In that case, only a small amount of fuel remained in the aircraft's main tank, allowing the upper explosive limit to be reached. New regulations requiring nitrogen pressurization in aircraft fuel tanks can prevent such an event in the future and were ordered by FAA in 2007. Nitrogen pressurized fuel tanks have been common practice in military transports for many years. The problem with the use of nitrogen or helium is the displacement of oxygen; if an individual enters the confined space, asphyxiation will result.

The mechanism of asphyxiation unfolds rapidly when an individual enters a closed environment where there is little oxygen; loss of consciousness occurs within 10 to 15 seconds. This situation unfolds rapidly because the diffusion gradient within the lung is reversed. Pulmonary arterial blood with an approximate PO_2 of 40 mmHg typically is carrying less oxygen than the alveoli which typically have a PO_2 of 100 mmHg. However, in the presence of a pure nitrogen or helium atmosphere, the alveolar oxygen content would be 0, and oxygen would diffuse *from* the blood stream into the alveoli, resulting in virtually no oxygen being available in pulmonary venous blood, and within seconds the brain will have exhausted its reserves (53). These accidents may occur when workers enter fuel tanks that have been purged of fuel using an inert

gas. Such an accident occurred on March 19, 1981 at the Kennedy Space Center when the Space Shuttle Columbia was being processed and a compartment had been purged with nitrogen gas. Two workers entered the compartment and rapidly lost consciousness. Five rescuers also lost consciousness and were themselves not rescued until other workers had donned self-contained breathing apparatus and were able to extract the rescuers, but the original two victims died (54).

DRUG AND ALCOHOL TOXICOLOGY

Aircrew and safety-sensitive personnel must comply with drug and alcohol rules of the United States Department of Transportation and the Federal Aviation Administration (FAA) in the United States and the Joint Aviation Administration in Europe. These rules are very similar and prohibit the use of alcohol at work and the use of banned drugs at work or at any other time. Drug testing is articulated in the Omnibus Transportation Employee Testing Act of 1991 and implemented by the FAA's own regulations. Drug testing is performed pre-employment and after accidents as well as for reasonable suspicion and on a random basis. These are governed by Department of Transportation 49 CFR Part 40 and FAA 14 CFR with parts governing each section of flight operations. Safety-sensitive positions include flight crew, flight attendants, mechanics, aircraft dispatchers, ground security, flight instruction, air traffic control, and security personnel. The FAA and agency rules govern urine testing for specifically banned substances which include amphetamines, opiates (morphine, codeine, and heroin but not semisynthetic opiates), phencyclidine, marijuana (cannabinoids), and cocaine.

Alcohol use is banned on duty. The FAA limits specifically ban the performance of safety-sensitive duties if alcohol is detected by an evidential breath alcohol tester (EBAT) at an

equivalent blood alcohol concentration of 0.040%. Levels at or above this value are considered positive. Alcohol concentrations between 0.020% and 0.039% are not considered positive, but individuals must be removed from safety-sensitive functions for at least eight hours and retested and found to have a level below 0.020%. Levels below 0.02% are considered negative and represent the lowest reasonable level that can be tested with accuracy.

Alcohol use by astronauts, although discouraged, is not as rigorously controlled as it is for pilots and other safety-sensitive commercial airline personnel. In 2007, NASA chartered an astronaut health care system review committee; in that report it was stated that on at least 2 occasions "astronauts had been so intoxicated prior to flight that flight surgeons and/or fellow astronauts raised concerns to local on-scene leadership regarding flight safety (55)." From this observation, 3 recommendations were formulated by the NASA external review committee. These can be summarized as follows; 1) policies, educational efforts, and discipline must target individual and supervisory accountability for responsible use of alcohol, 2) an alcohol-free period must be established prior to flight, and 3) a mechanism must be available to address concerns raised by responsible persons. These proposals are against a backdrop of certain cosmonauts proposing that a certain amount of alcohol consumption be allowed aboard the ISS (56).

Other drugs may result in significant effects upon the ability to perform safety-sensitive functions, even if they are not prescription drugs, and the results of FAA aircraft accident toxicology findings suggest that over-the-counter antihistamines may have a greater impact on aviation safety than do recreational drugs or alcohol. US aircraft accident investigations of fatalities have demonstrated that unapproved drugs often contribute to, or are a causal factor for an accident. In a study of recent accident victims, alcohol was present in 5.6%, controlled substances (including opiates, cocaine, methamphetamine and marijuana) in 8.6%, and over-the-

counter medications in 14.9% of 1683 fatalities (57).

TOXICOLOGY CONCLUSIONS

Aerospace activities present unique problems to the practice of toxicology. Reactive, toxic compounds are integral parts of aviation and space exploration, thus opportunities for exposure are commonplace. These are most often via inhalation; however, ocular and dermal exposures can be injurious as well. Medical personnel must recognize that some compounds elicit delayed effects, and that even trace contaminants can adversely affect health if the exposures are continuous and prolonged. Exposures are invariably to a group of compounds, and certain individuals may be unusually susceptible to trace pollutants. Astronauts must be considered a susceptible population for many toxicants. Risks in space flight originate from predictable sources and also from unpredictable sources, such as combustion events. The safety-sensitive personnel associated with commercial aviation require stringent control to ensure that drugs and alcohol do not increase the risk of accidents.

INTRODUCTION TO MICROBIOLOGY

The environment is an important element of human existence on Earth. Similarly, the closed environmental microcosm of spacecraft/space stations plays a crucial role in human survival in space. Favorable physical characteristics such as gas composition and temperature of the internal environment are essential for human habitation whereas unacceptable biological and chemical contamination levels of the habitable space environment can make continued habitation impossible. Establishment and maintenance of a comfortable, safe, and productive environment is a top priority. Generally, we think of infectious diseases as the major microbiological-related concern, but other adverse effects as shown in Figure 1 may also affect the safety and performance of astronauts. In addition to infectious diseases, allergies, volatile chemicals, and microbial toxins may cause crew discomfort and reduced productivity. Plant pathogens may endanger food supplies, microbial contamination may result in food spoilage and degraded water quality, and severe accumulation may lead to performance degradation of critical spacecraft systems (e.g., life support system). In addition to being inherent contaminants of our environment, microbes release a wide array of chemical contaminants into the environment.

Microbial risks to astronauts generally do not include those associated with high risk public health diseases such as *Mycobacterium tuberculosis* and hepatitis viruses. This is because the crewmembers are screened for such diseases prior to flight and no credible exposure route is available for such microorganisms during spaceflight. Crewmembers are a major source of microorganisms on spacecraft, and most of these microbes released into the space environment are generally harmless along with some opportunistic pathogens such as *Staphylococcus aureus*. Microbial contaminants may also originate from payloads and experiments, equipment, water and food, consumables, and the environment prior to launch. These contaminants along with

those of human origin contaminate the ISS and may cause concerns related to function of critical spacecraft systems (e.g., life support system). Our approach to mitigating these risks is discussed below.

Approach to Risk Mitigation aboard the ISS

Acceptability limits

To ensure optimum crew productivity aboard the ISS, acceptability limits for microbial contamination of breathing air, spacecraft surfaces, drinking water, and food have been established (Table 1). These standards were created for human space flight by utilizing existing industry standards (e.g., Environmental Protection Agency guidelines for drinking water) and expert panels. Preflight monitoring of resupply spacecraft, new ISS modules, water, food, equipment, and materials, and periodic monitoring of the ISS environment assesses conformance with the acceptability limits.

Air

Numerous diseases are disseminated through the air. Many respiratory viruses, such as influenza, varicella-zoster virus, respiratory viruses, bacterial diseases including tuberculosis, and fungal diseases such as aspergillosis are commonly spread by airborne routes. Gravity is an effective means of limiting the spread of airborne infectious diseases since larger droplets fall rapidly to Earth. In normal gravity on Earth, aerosol particles of 40 micrometers and larger settle to the floor within 60 seconds (58). The longer airborne infectious agents stay in the breathing air, the greater the risk of infecting a crewmember. In the reduced gravity environment of spaceflight, generation of bioaerosols (aerosols of microbes or microbial products) is particularly problematic because aerosolized droplets are more easily generated and remain suspended in the air until they collide with a surface or captured on an air filter.

The breathing air is monitored on a quarterly basis with a small, hand-held, battery operated air impaction sampler (Burkard) as shown in Figure 2. Spaceflight limitations and constraints require in-flight monitoring to utilize small, portable devices that are battery powered, easy to operate, low maintenance, and easily calibrated. Accuracy and reliability are additional essential factors. Eighty-five liters of air are impacted on culture medium plate for bacteria and fungi. After incubation, the bacteria and fungi can be visualized and quantified by visual count. Low levels of airborne bacteria and fungi are found onboard the ISS. The most commonly recovered bacterial genera from the air on the ISS are *Staphylococcus*, *Micrococcus*, and *Bacillus*. These bacteria are commonly associated with humans (except *Bacillus*, a common spore-forming environmental bacterium). *Aspergillus* and *Penicillium* are the prevalent fungal (mold and yeast) genera found (59). These are common environmental molds. Levels of bacteria and mold aboard the ISS have been consistently below acceptability limits and far below levels found in typical homes, offices, and previous spacecraft. These low levels of contaminants are attributed to the inclusion of high efficiency particulate air (HEPA) filters in the original ISS design.

Surfaces

Many diseases, such as influenza and tuberculosis, can be transmitted to others through human contact or contacting inanimate objects known as fomites (e.g., doorknobs). Accumulation of microorganisms within the spacecraft can lead to other undesirable effects (Figure 1) including degradation of performance of critical spacecraft systems such as the environmental control system. Growth media-filled slides are used quarterly for the collection of bacteria and fungi from 25 cm^2 of selected surfaces. After suitable incubation, these samples can be analyzed and quantified on-orbit (similar to air samples) providing crewmembers data on current environmental conditions. Results from the ISS indicate that bacteria of human origin (e.g.,

Staphylococcus) are the most commonly recovered bacterial genera (60). *Penicillium*, *Aspergillus*, and *Cladosporium* are the prevalent genera of mold. Surface contamination levels on the ISS are consistently low and below acceptability limits. Over six years of data from the ISS verify the effectiveness of a rigorous housekeeping schedule, monitoring, and constant vigilance by the crews. However, infrequently, excessive fungal growth has occurred in most spacecraft, including the ISS (Figure 3). When surfaces exceed the acceptability limits for bacteria or fungi (Table 1), the surface is cleaned by using either a Russian supplied disinfectant wipe (hydrogen peroxide and quaternary ammonium compound) or a U.S. supplied wipe (quaternary ammonium disinfecting compound).

Water and Food

More than 200 diseases are transmitted through food (61). The Centers for Disease Control and Prevention (CDC) estimate that 76 million cases of food borne illnesses and 5000 deaths occur in the United States yearly (62). Norwalk-like viruses, Campylobacter, and Salmonella are major causes of foodborne illnesses (62).

Food is analyzed prior to flight to ensure the microbiological safety according to Table 1. Water is tested on orbit for bacterial content as shown in Figure 4. A measured volume of water is passed through a filter trapping the suspended bacteria. After addition of growth media and incubation, the bacteria can be visually quantified. Typically, the bacterial load is low and within acceptability limits (Table 1). The high cost of transporting drinking water (potable) to the ISS requires the reclamation and recycling of humidity condensate on the ISS to reduce the volume and mass of potable water that must be resupplied from the ground. The reclaimed and processed humidity condensate is supplemented by water provided by the space shuttle and by

ground-supplied water. Potable water aboard the ISS is available from 3 water ports, including a dispenser of ground or shuttle supplied water and two ports from the humidity condensate recovery system. A detailed description of these systems (63) and analysis hardware has been described previously (64).

Postflight analysis of water samples have identified the predominant genera recovered included *Sphingomonas*, *Ralstonia, Pseudomonas*, and *Methylobacterium* species. These species are commonly found in water supplies. Although not uncommon in water, the opportunistic pathogens, *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa,* were recovered from the potable water systems a few isolated times (60, 65). All three ISS potable water sources are routinely analyzed for coliform bacteria, a common indicator of fecal contamination and the potential for disease causing microorganisms (e.g., hepatitis B or gastrointestinal bacterial pathogens). It is important to note that no indication of coliforms or major waterborne pathogens has been detected in ISS potable water. The closed ISS environment and occupation by exceptionally healthy astronauts precludes most pathogens associated with waterborne diseases. Thus, no medically significant bacterial contamination of the potable water system aboard the ISS has occurred.

Payloads

Payloads may contaminate the environment of spacecraft. Astronauts are exposed to many biological materials, a small number of which may be hazardous. Most risks associated with biohazardous materials are microbiological.

Microbiological risks associated with biohazards are assessed specifically for each spacecraft, space station, or space habitat depending on a number of factors. These include: the specific

microorganism identified, the infectious dose, pathogenicity, disease associated with agent, total number of microbes used in the investigation (allowing for growth during the mission), the biosafety level, and availability of vaccine and treatment options.

Flight payloads are proposed by payload organizations from countries around the world. All payloads undergo a rigorous safety evaluation by the Payload Safety Review Panel (PSRP) at NASA's Johnson Space Center in Houston. The evaluation requires the preparation and submission of a safety data package by the payload organization. One aspect of the overall safety review is biosafety. The JSC Biosafety Review Board reviews all payloads that contain biological materials. Some payloads may not inherently be classified as biohazardous, but they may harbor hazardous microorganisms. For instance, animals included in the payload for investigational purposes may harbor microorganisms that are hazardous. Animals must meet the requirements on microbial agents defined by the Johnson Space Center's Committee for the Protection of Human Subjects document. Other payloads may similarly harbor microbes requiring evaluation. For example, plants, soil (or soil stimulants) may harbor fungi and bacteria that may present a hazard to the crew and/or the spacecraft. In essentially all cases, the microbial hazards can be contained.

Risk Mitigation Countermeasures

Risk reduction should begin in the design phase of spacecraft and space habitats. Experience has shown that early identification of risks followed by design and implementation of effective risk mitigation countermeasures is the most cost-effective approach. Many microbiological risks can be reduced or eliminated by this approach. For example, the risk of airborne

infectious/allergenic agents and nuisance particulates can be greatly reduced by placing safeguards into the air conditioning and distribution system. Inclusion of High Efficiency Particulate Air (HEPA) filters into the air circulation system has proven to be very effective in maintaining air with very low concentrations of bacteria, fungi, viruses, and particulates aboard the ISS. Various allergens including fungal spores, pollen, and dust mites are effectively removed as well. HEPA filters are 99.997% effective in removing particulates greater than 0.3 micrometers in diameter. HEPA filtration is the most effective, proven technology to provide breathing air with very low levels of microbial contaminants. When possible, engineering and design solutions should be sought to eliminate environmental contaminants associated with adverse health effects or contamination of essential systems (e.g., life-support system). This is much more cost effective and often eliminates the problem instead of pursuing only monitoring approaches.

The selection of materials used to construct and outfit spacecraft is highly important in discouraging inappropriate microbial growth. Non-porous surfaces are more easily cleaned and disinfected than porous surfaces (e.g., fabric). Surfaces containing antimicrobial substances should be considered. Vigilance with emphasis on water leaks, spills, and condensate is very effective in early detection of conditions that eventually lead to microbial growth. Generally, routine cleaning of exposed surfaces with cleaners containing surfactants to loosen and emulsify contaminants is sufficient to meet established acceptability limits for bacteria and fungi on spacecraft internal surfaces. However, disinfectant wipes are available for use when indicated. All cleaning and disinfecting substances must be compatible for use in closed environments that recycle air and water for crew consumption.

Maintaining environmental conditions unfavorable for sustained growth of microbial contaminants is essential to prevent or control environmental microbial contaminants. Spacecraft characteristically provide a shirt-sleeve environment for crew comfort. Unfortunately, such temperatures promote microbial growth. However, controlling availability of water (e.g., humidity and surface condensate) is essential in controlling microbial growth. Prevention of water condensation on surfaces, water leaks and spills, and holding relative humidity to 60% and below are effective controls.

Approaches for Mars Missions

Lessons have been learned from the ISS and earlier programs and must be applied to spacecraft and space habitats for Mars missions. Inclusion of HEPA filters in the ISS resulted from lessons learned from the space shuttle and the Russian Mir experiences. Microbiological risks can be identified and levels of acceptable risks must be defined. These risks can be mitigated by early development and implementation of effective countermeasures beginning with the spacecraft design phase. Monitoring must be independent of the Earth and limited. Experience on a lunar habitat may demonstrate that no routine monitoring is necessary. Instead, preventive routine cleaning of critical surfaces or systems and crew vigilance of environmental conditions (e.g., odors, leaks) with the capability to remediate (e.g., disinfect) heavily contaminated areas may be the approach. Careful preflight and inflight vigilance of crew health should limit infectious agents to opportunistic pathogens that are manageable in astronauts with normal immunity. Clinically significant decreases in immunity (66, 67) and/or increased virulence of microorganisms (68) could present medical challenges.

MICROBIOLOGY CONCLUSIONS

Microbiological agents can adversely affect the health, safety, and performance of astronauts. In addition to direct effects on crewmembers, microorganisms can degrade the environment and the performance of critical spacecraft systems, ultimately jeopardizing mission objectives. Over seven years of inflight and postflight environmental data clearly demonstrate that the ISS environment is microbiologically safe and consistent with a clean, healthy human habitat.

A Mars mission can be undertaken successfully, but lessons learned from previous spaceflight programs, and especially the lessons to be learned from years of human occupation of lunar habitats must be applied to the mission. Intervention early in the design phase of Mars vehicles and surface habitats can provide microbial countermeasures such as HEPA filters for air, use of antimicrobial materials in areas prone to microbial growth such as internal components of the ECLSS (e.g., water coolant loops), advanced disinfection techniques for drinking water, and others. Prevention must still be the hallmark, and many risks can be mitigated prior to flight. A healthy and fit crew is essential, and sustaining healthy immunity is essential. All environmental microbiological planning (acceptability limits, etc.) assume a normal immune response. Medically significant diminishment of the immune response will increase the risk considerably.

Table 1. Microbiology Acceptability Limits for International Space Station

Parameter	Preflight	In-flight
Air	Total Bacteria: 300 CFU/m^3 Total Fungi: 50 CFU/m^3	Total Bacteria: 1000 CFU/m^3 Total Fungi: 100 CFU/m^3
Surfaces	Total Bacteria: 500 CFU/100 cm^2 Total Fungi: 10 CFU/100 cm^2	Total Bacteria: 10,000 CFU/100 cm^2 Total Fungi: 100 CFU/100 cm^2
Water	Total Count: 50 CFU/1 ml Total Coliforms: Non-detectable/100 ml	
Food	Total aerobic count: <=20,000 CFU/g Escherichia coli: <=1 CFU/g Coagulase positive Staphylococci: <=1 CFU/5g Salmonella: <=1 CFU/25g Clostridium perfringens: <100 CFU/g Yeasts and Molds: <100 CFU/g	

Figure 1: Adverse Effects of Microorganisms in Space Environment

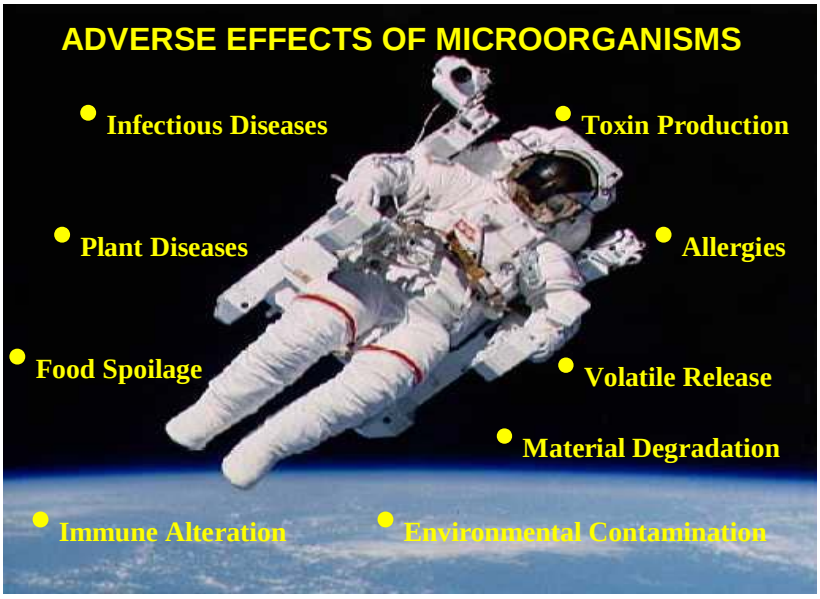

Figure 2: Inflight Monitoring of ISS Breathing Air

Figure 3: Fungal contamination on International Space Station.

Figure 4: Analyzing bacterial content of drinking water on the International Space Station

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