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ACCUMULATION AND EFFECT OF VOLATILE ORGANIC COMPOUNDS IN CLOSED LIFE SUPPORT SYSTEMS

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ABSTRACT

Bioregenerative life support systems (BLSS) being considered for long duration space missions will operate with limited resupply and utilize biological systems to revitalize the atmosphere, purify water, and produce food. The presence of man-made materials, plant and microbial communities, and human activities will result in the production of volatile organic compounds (VOCs). A database of VOC production from potential BLSS crops is being developed by the Breadboard Project at Kennedy Space Center. Most research to date has focused on the development of air revitalization systems that minimize the concentration of atmospheric contaminants in a closed environment. Similar approaches are being pursued in the design of atmospheric revitalization systems in bioregenerative life support systems. In a BLSS one must consider the effect of VOC concentration on the performance of plants being used for water and atmospheric purification processes. In addition to phytotoxic responses, the impact of removing biogenic compounds from the atmosphere on BLSS function needs to be assessed. This paper provides a synopsis of criteria for setting exposure limits, gives an overview of existing information, and discusses production of biogenic compounds from plants grown in the Biomass Production Chamber at Kennedy Space Center.

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INTRODUCTION

Bioregenerative life support systems (BLSS) for long duration space missions are being designed to operate with limited resupply, utilizing biological systems to revitalize the atmosphere, purify water, and produce food. The diversity of material used in construction of such a facility, the incorporation of plant and microbial processes for life support, and daily human activities suggest that volatile organic compounds will be produced and accumulate in the atmosphere. NASA has traditionally taken an industrial hygiene approach to the monitoring and control of organic constituents in the atmosphere and has established exposure limits and monitoring protocols (NASA, 1990; NASA, 1991).

As NASA develops atmospheric control strategies for long-duration space missions, the role of volatile compounds on human physiology, human psychology, and plant physiology will need to be understood. Crew health and safety has been, and will continue to be, the overriding criterion when establishing standards for components used during space flight (NASA, 1991). Although standards have been established to maintain crew health and safety for long-duration space missions, no analogous standards have

been established for non-human biological components of a BLSS. It is generally accepted that volatiles play a vital role in the normal growth and development of plants (Abeles et al., 1992; Sharkey et al., 1991), but the threshold levels of phytotoxicity have not been established for most known biogenic compounds.

HISTORICAL OVERVIEW

Atmospheric monitoring and sampling of the atmosphere from shuttle missions has identified over 250 organic compounds in the cabin atmosphere of the Space Shuttle (Buoni et al., 1989). Most of these compounds are found in only trace amounts and are well below their spacecraft maximum allowable concentration (SMAC) limits (NASA, 1990). In addition, a number of unknown compounds have been detected at or near the detection limit of the GC/MS. Although indicative of the types of volatile compounds present, these data are of limited value for designing a BLSS since atmospheric revitalization systems are used to remove all organic compounds from the atmosphere. These data indicate that air revitalization systems are generally effective, but do not allow an assessment of potential toxicity or interactions between humans and plants in a BLSS.

Volatiles reported by Zlotopolsk'ii and Smolenskaja (1996) in the atmosphere of closed chambers used for plant growth included acetone, ethanol, methanol, toluene, acetaldehyde, ethylacetate, methyethylketone, and cyclohexane. Charron et al. (1996) reported on the production of hexenal, hexenol, and hexenyl-acetate from lettuce grown in a closed chamber. Wheeler et al. (1996) detailed the developmental production of ethylene by soybean, lettuce, wheat, and potato in the BPC.

One of the first long-duration human-rated bioregenerative testbed studies using higher plants was the BIOS-3 study conducted in 1977 by the Department of Biophysics, L.V. Kirenskii Institute of Physics, in Krasnoyorsk, Russia. (Terskov et al., 1981; Gitelson and Okladnikov, 1994). This study documented VOCs in a human habitat in an atmosphere being regenerated continuously for four months by higher plants. The primary volatile constituents observed in the human habitat are summarized in Table 1.

Table 1: Estimates of the concentration of classes of volatile organic compounds in the living compartment of the "BIOS-3" Experiment during a 4-month test conducted in 1977 [z].

Substances	*With Catalytic Furnace*	*Without Catalytic Furnace*
Aldehydes, (mg m^{-3})	0.56	0.51
Alcohols, (mg m^{-3})	2.5	3.6[y]
Mercaptans, (mg m^{-3})	0	0
Acetic Acid, (mg m^{-3})	1.15	0.92
Carbon Monoxide, (mg m^{-3})	6.8	0
Readily oxidizable, total, (mg O_2 m^{-3})	5.0	7.8[y]
Difficult to oxidize, total, (mg O_2 m^{-3})	34.3	30.1

[z]Data derived from Terskov et al., 1981. NASA TM-76452
[y]Increased concentrations are associated with use of ethanol for microbial monitoring.

The volatiles detected during BIOS-3 were primarily associated with man-made materials and not of biological origin. During the four-month test, the classes of compounds reported fluctuated around a low, consistent concentration (Terskov et al., 1981; Gitelson and Okladnikov, 1994). This observation is interesting since the facility had no physical or chemical atmospheric regeneration system incorporated during the initial stages of the experiments, suggesting an interaction with the bioregenerative component

(Gitelson and Okladnikov, 1994). Incorporation of a catalytic furnace during the final stages of the experiment reduced the concentration of CO, but had limited effect on the other compounds (Terskov et al., 1981).

More recently, trace contaminants of the atmosphere were monitored twice a week during the Early Human Testing Initiative: Phase I Experiment at NASA's Johnson Space Center (NASA, 1996; Edeen et al., 1996). A number of compounds were reported to be present in all samples at trace concentrations. The background compounds included solvents, Freon 113, siloxanes and silenes. Tetrahydrofuran and ethylbenzene were detected as transients in the chamber. Although a number of VOCs were detected, all were at concentrations < 0.1 part per million with the exception of methanal and isopropyl alcohol. The only plant produced volatile reported was ethylene, which reached concentrations of 0.9 mg m^{-3}, which is high enough to affect growth and development of plants (Abeles et al., 1992), but does not pose any health risk to the crew (NASA, 1990).

CLASSIFICATION OF VOCS

Within a closed environment, volatile organic compounds can be classified based on whether the compounds are anthrogenic or biogenic. Anthrogenic compounds originate from man-made sources, such as construction materials, solvents, or physical/chemical processes. Biogenic compounds originate from biological systems including humans, animals, plants and microbes. This categorization is useful in distinguishing between volatiles that can be partially controlled through pre-treatment (off-gassing of materials) and those expected to be produced by the plant or human subsystems. In addition to identifying the source of the volatiles, this classification provides a means of estimating the relative impact of the physical and biological systems on total atmospheric VOC load.

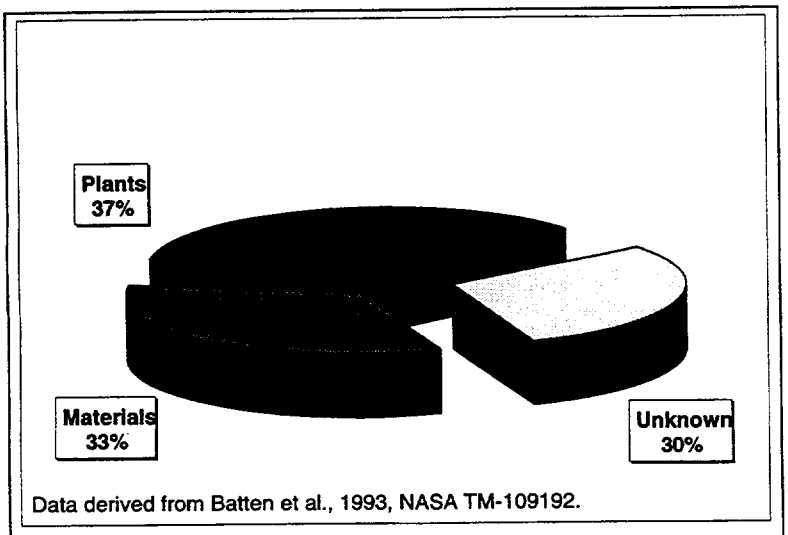

Figure 1: Relative percentage of volatile organic compounds detected in the Biomass Production Chamber headspace (based on total number of compounds) during an 84-day experiment with wheat cv. Yecoro Rojo.

In one study of wheat grown in the Biomass Production Chamber (BPC) at KSC, a total of 89 VOCs were identified from 94 samples of air analyzed during the growout (Batten et al., 1993). The results revealed that approximately equal numbers of compounds were derived from biogenic and anthrogenic sources. (Figure 1). Analysis of the atmosphere of all experiments in the BPC have resulted in similar distributions of compounds (unpublished results). However, the total mass of VOC exposure was primarily due to compounds off-gassing from materials. (Figure 2). These results have been confirmed with other crops grown in the BPC. Materials accounted for 87% of total VOC mass in a tomato study and 92% of the total mass in a soybean study (Stutte, 1996). Siloxanes and siloxones have been the primary anthrogenic VOC constituents in the atmosphere of the BPC in all cases.

The data reported from many experiments are based on a functional classification of the compounds. This type of classification assumes that compounds with similar chemical structures will behave similarly within the environment.

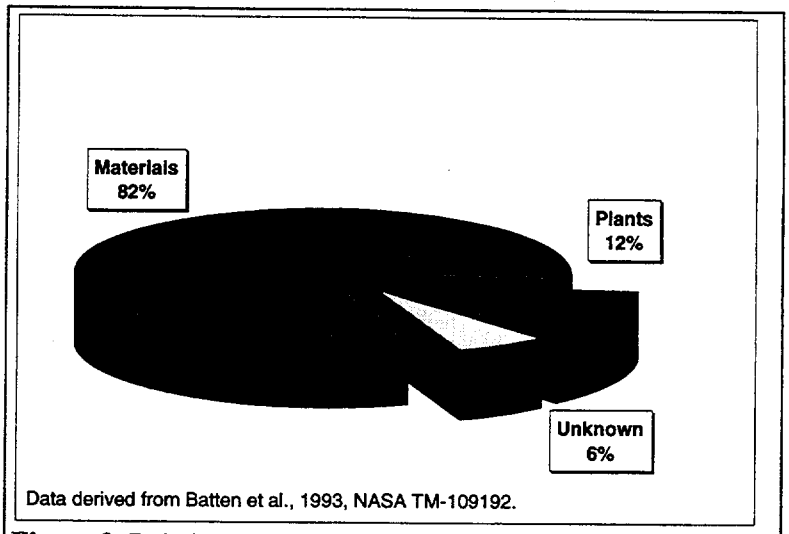

Figure 2: Relative percentage of volatile organic compounds, by source, detected in the Biomass Production Chamber headspace during an 84-day experiment with wheat cv. Yecoroa Rojo.

Leban and Wagner (1989) listed 219 potential atmospheric contaminants used to develop trace contaminant load models for space station. This classification was used by Wydenven and Golub (1990) to describe the waste streams in a typical space habitat and to establish the spacecraft maximum allowable concentrations (SMAC) for volatile compounds (NASA, 1991).

Limits of human exposure for many of the compounds listed in the functional classification discribed in Table 2 have been established for industrial and space habitat applications. The SMAC limits have been established using the best available data for over 200 volatile compounds. The SMAC limits are "those atmospheric concentrations that would be unlikely to cause discomfort, impairment, illness or injury to crew members upon continuous exposure for a 7-day period". These limits also serve as the interim 30-day SMAC levels for extended orbiter missions. For compounds without SMACs, or where insufficient data are available to establish a SMAC, a default value of 0.1 mg m^{-3} has been assigned. The sources of toxicological limits are the American Conference of Governmental Industrial Hygienists (ACGIH) and U.S. Occupation Safety and Health Administration (OSHA). OSHA has the power to enforce maintenance of exposure limits in workplaces in the USA. The ACGIH has no enforcement power, but their recommendations for threshold limit values (TLV) for chemical substances are widely followed.

Table 2: Functional classification of potential VOCs in the atmosphere of space habitats with characteristic examples of the class[z]

Category	*Examples*
Alcohols	Butanol, Isopropyl alcohol, Ethanol, Methanol
Aldehydes	Acetaldehyde, Butanal, Ethanal
Esters	Butyl Acetate, Ethyl acetate, Furans
Halocarbons	Freons, Tetrachloroethylene
Hydrocarbons, Aliphatic	Ethylene, Isoprene, Methane,
Hydrocarbons, Aromatic	Benzene, Toluene, Xylene
Ketones	Acetone, Methylethylketone
Organic Nitrogens	Indole, Acetonitrile
Nitrogen Oxides	Nitric oxide, Nitrogen dioxide, Nitrous oxide
Organic Acids	Acetic acid, Butyric acid, Propionic acid
Silanes and Siloxanes	Tetradecamethylcycloheptisoloxane, etc.
Sulfides	Carbon disulfide, Dimethyl sulfide

[z]Data excerpted from NASA, 1990

In a closed environment with several known sources of VOCs, it is probable that a mixture of compounds will be present. The SMAC assumes that compounds with a similar toxicological category will have an

additive effect. If the ratio of the concentration of detected compounds to their SMAC is < 1.0, then the toxicological exposure within this category is considered acceptable.

Space station trace contaminant control is being designed around threshold values (T-values) and SMACs as outlined in NASA-NHB 8060.1C (1991). The exposure limit is established so that the cumulative T-value for all compounds, without separation into toxicological categories is less than 0.5. This assumes that no compound exceeds its SMAC threshold. JSC 20584 establishes exposures limits based on total concentration of a compound within a toxicological category. This means that a compound can be "counted" more than once in the exposure limit in JSC 20584 if it has multiple toxicological classifications (e.g. an irritant and aphixicant).

VOCS IN THE BIOMASS PRODUCTION CHAMBER AT KSC

In order to establish the relative abundance and potential biological impact of VOCs within the biomass production component of a BLSS, a database of VOC production by candidate crops is being compiled by the Breadboard Project at Kennedy Space Center. Data on VOC concentration in the BPC throughout development has been reported for wheat (Batten et al., 1993; Batten et al., 1995), lettuce (Batten et al., 1996), potato (Batten et al., 1996), soybean (Stutte, 1996) and tomato (Stutte, 1996).

Table 3. Primary biogenic compounds detected from wheat cv. *Yecoro Rojo* in the atmosphere of the BPC at different stages of crop development.

Vegetative	*Anthesis*	*Grain Fill*	*Senescence*
2-Ethyl-1-hexanol	Benzaldehyde	2-Methyl furan	Tetramethyl urea
	Nonanal	3-Methyl furan	Tetramethyl thiourea

Data derived from Batten et al., 1995, Phytochemistry 39: 1351-1357

In one study with wheat, the effect of crop development on the production of biogenic volatiles was studied (Batten et al., 1995). Air samples were collected on a weekly basis approximately 2 hours after the lamps came on. The experimental conditions used to quantify VOCs in the Biomass Production Chamber utilized modifications of standard methodologies. The samples (16-liter) were collected in passivated stainless steel canisters (SUMMA® polished stainless steel). The canisters and pumping system were checked for cleanliness and background levels by filling the canisters with ultra-pure nitrogen and analyzing for any contaminants. The air was analyzed according to a modification of a standard method (EPA method TO14). The analyses were carried out by concentrating 1.1-liter samples onto a multibed graphitized carbon adsorbent trap. The adsorbent trap was interfaced through a cryofocusing unit to a gas chromatograph-mass spectrometer (GC-MS) equipped with a 30 m x 0.596 mm DB™ 5.625 bonded phase column and a jet separator. During this study, using these methods, 23 compounds were determined to be of biogenic origin. Of those compounds, eight were found to differ significantly in apparent concentration throughout the development cycle of the plant. The compounds that were characteristic of each stage of development are noted in Table 3. Batten et al. (1995) suggested that these compounds may provide a readily monitored, non-destructive developmental marker for assessing crop development and physiology in a closed environment.

Table 4: Relative abundance of presumed biogenic volatile organic compounds detected in the Biomass Production Chamber at Kennedy Space Center over the growth cycle of crops being evaluated for bioregenerative life support systems.

Compound [z]	*Soybean*	*Wheat*	*Potato*	*Lettuce*	*Tomato*
Benzaldehyde	+++[y]	++	+	+	++
Butanal				+	
Butanol, 1-		+			
Butanone, 2-	+++	+		+	
Carbon disulfide	+	+	+	+	+
Carene					++
Cymene, p-	++				
Decanol		+			
Dimethyl sulfide			+	+	
Ethylene	+	+	+	+	+++
Ethyl-1-hexanol, 2-		+	++	+	+
Ethylfuran, 2-	++				+
Fenchene, α- and cyclo-					++
Furan		+		+	
Heptadecane					+
Heptanal	+(+++)[w]	+			+++
Hexadecanol, 1	+(+++)[w]				
Hexanal	+(+++)[w]	+			+++
Hexen-1-ol acetate, 2-	+(+++)[w]				+++
Isoprene				+	++
Limonene	+				++
Methyl-2-propamine, 2-			++		
Methylfuran, 2-		+		+	++
Methylfuran, 3-		+		+	
Nonanal	+(+++)[w]	+		+	+++
Ocimene	+(+++)[w]				+
Pentylfuran, 2-		+			
Phenol		+			
Pinene, α-	++			+	+++
Pinene, β-	++			+	++
Propanone, 2-			+		
Propenenitrile, 2-			+		
Terpinene, α-	+(+++)[w]				+++
Terpinene, γ-	+				++
Tetrahydrofuran	++				+++
Tetramethylthiorea		+	+		
Tetramethylurea		+	++	+	
Thiobismethane		+	+	+	
Thiourea		++			
Thujone, α-					++

[z] **Compounds were detected and identified by either GS/MS standards or spectral matches with Wiley Spectral Library on at least two dates during a growout.**

[y] **Relative abundance where: + = <10 μg m^{-3}; ++ = 10-100 μg m^{-3}; +++ = > 100 μg m^{-3}.**

[w] **Relative abundance following horticultural practice of removing senescent leaves.**

In over six years of monitoring, a number of different compounds have been identified in the atmosphere of the BPC which may be of biogenic origin. The criteria for determining whether a compound was biogenic included direct determination of the compound from plant tissue (Bertis et al., 1993), detection in BPC only when plants were present (Batten et al., 1995), or previous citation in the literature (Duke, 1992; Buttery et al., 1993). It is difficult to rigorously quantify the concentration of a particular compound because losses associated with surface adsorption, sample transfer, and GC/MS analysis are only partially accounted for with the analytical methods used for the analysis. However, these data do provide a series of "snapshots" of the concentration of compounds, and suggest what compounds may be associated with particular developmental events or horticultural practices. A summary of the compounds tentatively identified as being biogenic that have been detected from crops grown in the BPC is presented in Table 4. This table also displays the relative abundance of the compounds. It should be noted that these VOCs are at trace concentrations: the (+++) abundance of >100 $\mu g\ m^{-3}$ is approximately 10 ppb for a compound with a molecular weight of 250 at 20° C.

IMPACT OF VOCS ON BLSS FUNCTION

The biological functions of most VOCs at low concentrations are not known. Terpenes were detected from all species, with tomato having relatively large concentrations during flowering and ripening periods. Terpenes are compounds that are derived from isoprene skeletons. Of the known terpenes, only pinene and isoprene, the building block compounds, have been studied in any detail. Species appear to produce either one or the other in a significant amount. Isoprene is a characteristic compound of deciduous forests and pinene of coniferous forests. High concentrations of terpenes were detected in soybean following the horticultural practice of removing of senescent leaves from the chamber. It is hypothesized that the process of crushing the leaves released the volatile terpenes. This suggests that modifing horticultural practices may reduce the overall VOC load.

Only a few of the compounds identified in the BPC, benzaldehyde, carbon disulfide, and 2-ethyl-1-hexanol, were detected in atmosphere of all the crops. The other compounds were detected in only a few of the crops. Although the biological functions of these compounds are not known, terpenes have been reported to have antifungal activity by several authors (Sharkey et al., 1992 and references therein). In addition, isoprene may have some role in the development of high temperature tolerance for some species (Sharkey and Singsaas, 1995). Several of the other compounds detected also have been reported to have ecological functions related to pest mitigation. Among the compounds reported to have antifungal properties are aldehydes (Aharoni and Stadelbacher, 1973), nonanol (Vaughn, 1995), and hexanal (Song et al., 1996). Undoubtedly, the biological role of these compounds will become clearer with increased research activity.

As previously noted, over 80% of the total concentration of volatiles have been from non-biological sources for every experiment conducted in the BPC, with the primary contaminants being siloxanes, which are the outgassing products of sealants used to maintain chamber integrity. Activated carbon filters (Heath and Manukian, 1992) have been used to reduced the concentration of these anthrogenic compounds. The GC/MS analyse have indicated that concentrations of alkanes, furans, and aromatic hydrocarbons are significantly reduced in the BPC with passive filtering through the activated charcoal filters, but these filters were not very effective for removing halocarbons. In addition, the filters were ineffective at reducing ethylene concentrations in the BPC. However, the activated charcoal filters were effective at reducing the concentration of sulfides, resulting in an enhancement of the olfactory quality of the atmosphere (Stutte et al., 1995).

Although NASA's primary approach has been the removal of volatiles from closed atmospheres, there is mounting evidence for the age-old belief that contact with plants fosters psychological well-being and helps

reduce physiological stress (Ulrich and Parsons, 1992). The physiological benefits to stress reduction appear to be achieved when the individual perceives that plants are present, even when direct physical or visual contact is not maintained (Heerwagen and Orians, 1986). The strong psychological benefit to having plants in the closed environments has been recognized by Russian scientists (Nechitailo and Mashinsky, 1993) who have routinely maintained plants on the MIR space station.

The roles of volatiles in inducing positive psychological and physiological responses is not known. However, it is known that the olfactory system is especially sensitive and can trigger both positive and negative psychological responses. For example, the aroma of fresh cut grass is typically pleasing, and flavor is enhanced when terpenes are added to "bland" food products. As a consequence, the strategy to remove all volatiles from the atmosphere should be approached cautiously, since their biological activity may have positive effects on human health as well as the growth and development of plants.

CONCLUSIONS

The most abundant VOCs in the closed environment are of man-made origin and threshhold exposure limits for humans have been established. There is significantly less known of the effects of VOCs on the performance of plants within a BLSS. The limited available information suggests that biogenic compounds will constitute a minor fraction of the total VOC component. Of the individual biogenic VOCs, the biological activities are largely unknown, with the notable of exception of ethylene. There may be potential positive benefits to maintaining low concentrations of biogenic compounds in the atmosphere to obtain normal growth and development practices and to provide some protection against potential pathogens. Each species appears to have a unique VOC production profile, which changes during the development cycle of the plant. Additional work is required to determine the production rates and impact of anthrogenic and biogenic compounds on BLSS performance.

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