ASSESSMENT OF ELECTRON BEAM IRRADIATION AS A LIPID DECONTAMINATION TECHNIQUE FOR LIFE DETECTION INSTRUMENTS. D. K. Buckner[1,2,3], M. B. Wilhelm[2], A. J. Ricco[2] L. Jahnke[2], T. W. Evans[4], M. Abraham[2,5]; [1]Blue Marble Space Institute of Science (bucknerd@ufl.edu), [2]University of Florida, [3]NASA Ames Research Center, [4]Massachusetts Institute of Technology, [5]The Aerospace Corporation

Introduction: In the search for extraterrestrial life, lipids are molecular biosignatures of special interest. Lipids, which are organics broadly defined by their solubility in organic solvents, make up the cell membranes universal to all life as we know it, can be synthesized biotically and abiotically, bear diagnostic molecular features indicating origin, and have high geologic preservation potential (~Gyr) [1]. To search for extraterrestrial lipids *in situ*, we are building the Extractor for Chemical Analysis of Lipid Biomarkers in Regolith (ExCALiBR), a life detection tool that will accept a planetary sample, extract and purify lipids, and send the aliquot to a gas chromatography-mass spectrometer (GC-MS) for analysis. Candidate flight GC-MS units have pico- to femtomole limits of detection [2], so stringent decontamination of sample handling hardware is essential to prevent false positives.

COSPAR-approved contamination control (CC) techniques for planetary protection (PP) compliance are tuned to sterilize and do not remove lipid contaminants [3]. CC techniques used in spacecraft cleanrooms reduce biological and lipid contamination, but studies show up to ~10^5 viable cells/m^2 and ~10^8 total cells/m^2 on hardware surfaces post-cleaning [3], (~500 picomole fatty acids/m^2). Laboratory CC techniques effectively remove lipids but are often incompatible with life detection instrument materials.

We report here the investigation of electron beam irradiation (EBI) as a CC solution for ExCALiBR. EBI is used to sterilize foods and medical devices [4], as well as spaceflight payloads [5]; it is proposed for PP applications [6], but effects on lipid contaminants are untested. Five representative astrobiologically-relevant lipid standards were subjected to EBI at materials-compatible doses of 50 and 100 kGy [6]; GC-MS was used to measure their breakdown. We hypothesized EBI would degrade the lipids; however, no significant degradation was observed at these doses, suggesting that EBI should not be used to remove lipid contaminants from life detection instruments.

Methods: Five lipid standards were selected based on astrobiological relevance and contamination potential (Fig. 1), including: palmitic acid (*n*-$C_{16:0}$ FA), oleic acid ($C_{18:1}$ FA), heneicosane, 5α-cholestan-3β-ol, and 5-α-cholestane. Using combusted sample handling tools and organically clean techniques, standards were partitioned into individual borosilicate glass vials under pure N_2, sealed with PTFE caps, and stored at -20 °C (except during irradiation, ~1-2 h).

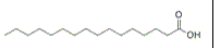
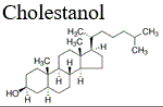
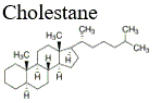
Standard & structure	Astrobiological relevance	Contamination potential
C16:0 FA 	Membrane lipid subcomponent, abundant in biology	High (cell membranes)
C18:1 FA 	Membrane lipid subcomponent, abundant in biology	High (cell membranes)
Heneicosane	Fatty acid subcomponent	High (machine oils)
Cholestanol 	Steroid alcohol, selected as a hopanoid proxy	Moderate (uncommon lab contaminant)
Cholestane 	Degraded sterol, hopanoid proxy	Moderate (uncommon lab contaminant)

Figure 1. Standards, relevance, and contamination potential

Irradiation was performed at Steri-tek Expert Sterilization Services (Fremont, CA) with a 10 MeV, 20 kW linear accelerator; each set of standards received a dose of either 0, 50, or 100 kGy. Post-EBI, samples were solubilized with dichloromethane or anhydrous pyridine. FAs and cholestanol were silylated with N,O-bis(trimethylsilyl) trifluoroacetamide + 1% trimethylchlorosilane. Internal standards were added to each sample prior to injection.

GC-MS used an Agilent 6890 system equipped with an Agilent DB-5MS column (60 m x 250 µm x 0.25 cm, Agilent) with high-purity He as the carrier gas at 1 mL/min. Injection volumes were 1µL each. Inlet temperature was 280 °C; initial oven temperature of 50 °C was ramped to 120 °C at 10 °C/min, then increased from 120 °C to 320 °C at 3 °C/min and held at this temperature for 5 min. The MS source temperature was 300 °C.

Molecules were quantified relative to internal standards and identified based on retention time and fragmentation pattern. Percent reduction in lipid standards was determined by comparing absolute abundances (relative to the internal standard) of the irradiated standards to the controls.

Results: No substantial concentration changes were observed for the 50 or 100 kGy-irradiated lipids (Fig. 2). Two individual sets showed a decrease relative to the

control: the *n*-$C_{16:0}$ FA 100 kGy set reduced to 79% (±2%), and the cholestanol 50 kGy set reduced to 84% (±10%). However, no corresponding lipid loss/reduction was observed for either the *n*-$C_{16:0}$ FA 50 kGy set or the cholestanol 100 kGy set.

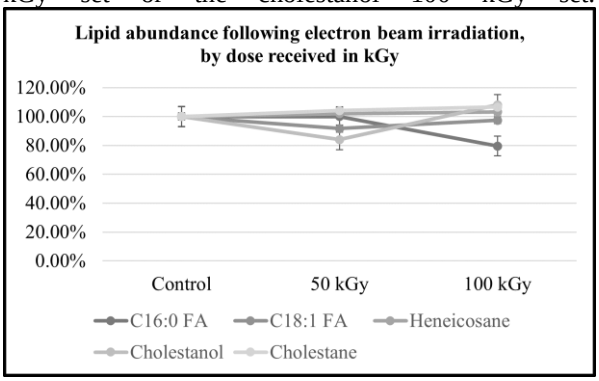


Figure 2. Lipid abundance (% remaining) following EBI (by kGy received). No substantial degradation was observed.

Numerous low-abundance (<1% of total signal) radiolytic products were observed for all irradiated aliphatic lipids, each with chain lengths shorter than the starting compounds. *n*-$C_{16:0}$ FA products include a homologous series of unsaturated FAs and *n*-alkanes; several dicarboxylic acids, monounsaturated FAs, oxo FAs, and oxo dicarboxylic acids; and one furanone. $C_{18:1}$ FA products include a homologous series of unsaturated fatty acids and *n*-alkanes; several alkenes, dicarboxylic acids, oxo FAs, oxo dicarboxylic acids; and one dihydroxy acid. Heneicosane products include a homologous series of *n*-alkanes. No radiolytic products were observed for the polycyclic lipids.

Discussion & Conclusion: We anticipated that irradiation of 50 and 100 kGy EBI doses would effectively degrade *n*-$C_{16:0}$ FA, $C_{18:1}$ FA, heneicosane, cholestanol, and cholestane standards, but no consistent reduction was observed at these irradiation doses for any lipids tested. Although two sets (*n*-$C_{16:0}$ FA 100 kGy, cholestanol 50 kGy) showed a decrease, corresponding degradation was not observed for the other EBI doses applied to these lipids. Additionally, no substantial quantities of breakdown products were formed, so we interpret observed reductions as anomalous and due to either GC-MS response or to molecules being degraded into smaller volatile fragments; the latter seems unlikely, as no corresponding reduction was observed in other sets.

Minor breakdown products were identified for all aliphatic compounds following irradiation, including both degraded compounds and recombination products, but none in substantial quantities. Degradation products include FAs (*n*-$C_{16:0}$ FA, $C_{18:1}$ FA) and *n*-alkanes (*n*-$C_{16:0}$ FA, $C_{18:1}$ FA, heneicosane) of every chain length shorter than the starting compounds. This indicates that EBI cleaved the carboxyl group and broke C-C bonds in the hydrocarbon backbone and suggests that irradiation induces breakdown at random points wherever the electron happens to strike, as opposed to preferentially attacking specific bonds [7]. Recombination products were observed for FAs: dicarboxylic acids, oxoacids, hydroxy oxoacids, and a furanone. Since the standards were monocarboxylic, we believe these products were produced from FA fragments that were cleaved and recombined during or after irradiation. A similar phenomenon was observed in a study of electron-irradiated volatile FAs, supporting our interpretation [7]. We also observed a furanone in the irradiated *n*-$C_{16:1}$ FA, indicating cyclization of the hydrocarbon tail around the carboxyl group occurred. A similar phenomenon is observed in food studies; irradiated triglycerides yield 2-alkylcyclobutanones [8].

Our results show that EBI at doses up to 100 kGy is not effective at removing lipids under our experimental conditions. Doses higher than 100 kGy could induce more degradation but are likely to damage some space hardware materials [6]; therefore, we cannot recommend using EBI as a lipid CC technique. EBI can effectively sterilize and might remove other organics, but it is beyond the scope of this study.

Future Work: Future work includes finding and verifying a materials-compatible lipid CC technique for ExCALiBR; we plan to explore thermal and chemical degradation. We also note that the studied lipid biomarkers were surprisingly robust against electron-induced degradation, so we plan to explore implications for lipid-based life detection on planetary bodies subject to high radiation dose rates (e.g., Europa, Mars), but under relevant environmental conditions (i.e., temperature, pressure, mineral/ice composition, etc.).

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References: [1] Eigenbrode, J. L. (2008) *Strategies of Life Detection*, 161-185. [2] Wilhelm, M.B. et al. (2019) *AGU Fall Meeting 2019*, Abstract #P41C-3447. [3] Ghosh, S. et al. (2010) *Astrobio., 10(3)*, 325-335. [4] Pillai, S. D. and Shayanfar, S. (2017) *App. of Rad. Chem. In the Fields of Industry, Biotech. and Enviro.*, 249-268. [5] Ricco, A. J. et al. (2020) IEEE Aerosp. Electron. Syst. Mag. 35(3), 6-18. [6] Urgiles, E. et al. (2007) *IEEE Aero. Conference*, 1-15. [7] Seo, S. H. et al. (2019) *Chem. Eng. Journal*, 360, 494-500. [8] Ndiaye, B. et al. (1999) *Rad. Phys. and Chem.*, 55.5, 437-445.