APOLLO LUNAR QUARANTINE

PLANETARY BIOLOGY SUB-COMMITTEE 1-22-70

RECOMMENDATIONS:

A. "THE SUBCOMMITTEE RECOGNIZES THAT THE EVIDENCE FROM APOLLO 11 AND 12 MAKES UNLIKELY THE POSSIBILITY THAT DANGEROUS BIOLOGICAL MATERIAL EXISTS ON THE LUNAR SURFACE. HOWEVER, IF THERE IS ANY ENVIRONMENT OF BIOLOGICAL IMPORTANCE ON THE MOON IT IS AT DEPTH IN THE HIGHLANDS OR POLAR REGIONS. THEREFORE, WE RECOMMEND THAT QUARANTINE BE MAINTAINED AT LEAST FOR APOLLO 13".

B. "WE RECOMMEND ELUCIDATION, AT MSC AND ARC OF THE NATURE OF THE AGENT(S) IN LUNAR MATERIAL WHICH MANIFEST(S) BIO-ACTIVITY IN BACTERIA AND PLANTS, BEFORE THE DISTRIBUTION OF SAMPLES FROM APOLLO 12 AND BEFORE DROPPING OF QUARANTINE PROCEDURES."

SPACE SCIENCE BOARD AD HOC COMMITTEE 2-17-70

SUMMARY OF FINDING AND MAJOR RECOMMENDATIONS

FINDING

"IN APOLLO 13 THE PROPOSED HIGHLAND LANDING SITE AND CORE SAMPLE TO A DEPTH OF 8 FEET CONSITUTE A SUBSTANTIALLY NEW LUNAR ENVIRONMENT IN COMPARISON TO THE LANDING SITES AND SAMPLED AREAS OF APOLLO 11 AND 12 MISSIONS."

SPACE SCIENCE BOARD AD HOC COMMITTEE 2-17-70

RECOMMENDATIONS

"LUNAR QUARANTINE PROGRAM

A MAJORITY RECOMMEND CONTINUANCE OF THE 3-WEEK LUNAR QUARANTINE PERIOD. A MINORITY FAVOR DISCONTINUANCE OF QUARANTINE.

LUNAR SAMPLES

WE RECOMMEND DEVELOPMENT OF PROCEDURAL CHANGES IN THE HANDLING OF LUNAR SAMPLES TO PRECLUDE ALTERATION OF THE SAMPLE PRIOR TO ANALYSIS.

BIOLOGICAL TESTING PROGRAM

WE RECOMMEND THE CONTINUED DEVELOPMENT OF A RESEARCH PROGRAM WITHIN THE LRL TO DEVELOP GREATER CONFIDENCE IN THE ADEQUACY OF THE TEST PROGRAM AND THE VALIDITY OF BOTH NEGATIVE AND POSITIVE FINDINGS."

INTERAGENCY COMMITTEE ON BACK CONTAMINATION
1-15-70

THE COMMITTEE RECOMMENDED:

"1. THAT THE FOLLOWING POSITION AND RECOMMENDATIONS, RELATED TO QUARANTINE, BE TRANSMITTED TO THE ADMINISTRATOR NASA.

THE QUARANTINE ACTIVITIES CONDUCTED FOR THE APOLLO LUNAR PROGRAM WERE ESTABLISHED TO PROTECT TERRESTRIAL LIFE FROM HAZARD DUE TO BACK CONTAMINATION. THE EVIDENCE ACCUMULATED FROM APOLLO 11 AND 12 MISSIONS DEMONSTRATES:

A. THE INCOMPATIBILITY OF GEOCHEMICAL FINDINGS WITH THE EXISTENCE OF CONVENTIONAL LIFE FORMS ON THE MOON,

B. THE COMPLETE ABSENCE OF EVIDENCE OR HINT OF HAZARD TO ANIMAL OR PLANT LIFE IN THE BIOTESTS CONDUCTED ON SAMPLES FROM APOLLO 11 AND 12, AND

C. THAT THE SAMPLES WERE REPRESENTATIVE OF THE VARIOUS TYPES OF TERRAIN, BOTH SURFACE AND SUBSURFACE, MARE AND HIGHLAND.

THE COMMITTEE, THEREFORE, RECOMMENDS TO THE ADMINISTRATOR OF THE NATIONAL AERONAUTICS AND SPACE ADMINISTRATION THAT, IN THE ABSENCE OF NEW INFORMATION, THERE IS NO NEED TO IMPOSE QUARANTINE REQUIREMENTS ON THE CREWS, LUNAR SAMPLES, AND EQUIPMENT ON SUBSEQUENT APOLLO MISSIONS."

SUMMARY

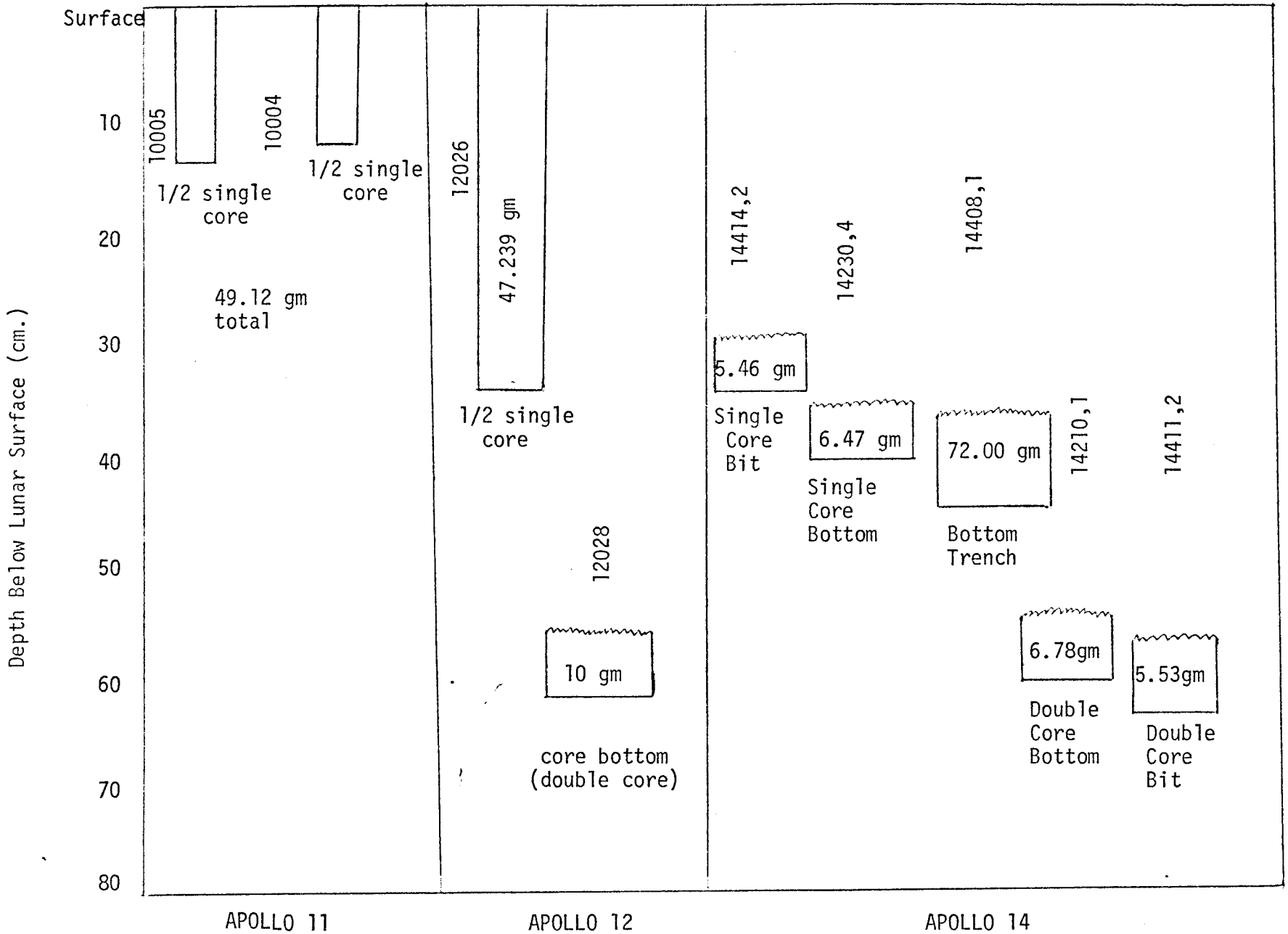
APOLLO 14 QUARANTINE REQUIREMENT

- NEW ENVIRONMENT TO BE VISITED (HIGHLAND)
- SUB-SURFACE SAMPLES TO BE COLLECTED
- LUNAR SAMPLE BIOLOGICAL ACTIVITY NOT DEFINED

STATUS OF QUARANTINE CONTINUATION RECOMMENDATIONS

- NEW ENVIRONMENT TO BE VISITED
  - APOLLO 14 - HIGHLAND
  - APOLLO 15-17 - (GEOLOGICAL DESCRIPTION)
- SUB-SURFACE SAMPLES TO BE COLLECTED
  - APOLLO 11-14 - PROFILE
- LUNAR SAMPLE BIOLOGICAL ACTIVITY NOT DEFINED

SOURCES OF EARLY BIO-SAMPLE



MICROBIAL TOXICITY STATUS

1. EXTRACTS OF APOLLO 11 CORE KILL ALL MICROORGANISMS TESTED.

2. APOLLO 11 SURFACE AND APOLLO 12 SURFACE AND SUBSURFACE SAMPLES DID NOT EXHIBIT TOXICITY.

3. APOLLO 11 CORE HIGHER IN CALCIUM, ALUMINUM, NICKEL AND SCANDIUM THAN APOLLO 11 FINES OR APOLLO 12 SAMPLES.

4. APOLLO 11 CORE HIGHER IN ILMENITE THAN ALL OTHER SAMPLES.

5. AMES REPORTS INHIBITION OF PIGMENT PRODUCTION IN PSEUDOMONAS AERUGINOSA BY APOLLO 11 LUNAR MATERIAL.

6. APOLLO 14 SAMPLE TESTING HAS BEEN INITIATED.

7. NEW APOLLO 11 CORE HAS BEEN REQUESTED ON TWO OCCASIONS

   - SEPTEMBER 24, 1970 - 6 GRAMS REQUESTED FROM EACH CORE
   - JANUARY 18, 1971 - 1 GRAM REQUESTED FROM EACH CORE

BOTANICAL STIMULATION STUDIES

- TWO EXPERIMENTAL DESIGNS, TOBACCO TISSUE CULTURE TESTS, AND SEED GERMINATION TESTS IN CONTACT WITH RADIOACTIVE LUNAR MATERIAL, WERE AUTHORIZED JANUARY 1971.

- AN ALIQUOT OF APOLLO 11 BIO-POOL SAMPLE IS BEING RADIATED IN THE CORE-REACTOR AT TEXAS A & M. THIS MATERIAL WILL BE USED TO PERFORM THE LETTUCE SEED GERMINATION TEST.

- EXTENSIVE STUDIES ARE UNDERWAY WITH TOBACCO TISSUE CULTURES. THE PRELIMINARY FINDINGS OF THESE ARE:

  - CHLOROPHYLL A IS SIGNIFICANTLY INCREASED BY TREATMENT OF TOBACCO TISSUE CULTURES WITH LUNAR MATERIAL. TREATMENT OF SIMILAR CULTURES WITH A SIMULATED LUNAR MATERIAL CAUSED A LESS SIGNIFICANT INCREASE IN CHLOROPHYLL A CONTENT.

  - THE NUMBER OF LIVING CELLS IS GREATER IN TOBACCO TISSUE CULTURE TREATED WITH LUNAR MATERIAL THAN IN UNTREATED CULTURES. THIS IS REFLECTED AT THE CELLULAR LEVEL BY AN INCREASED DENSITY OF THE CYTOPLASM, AN INCREASE IN THE DEVELOPMENT OF THE CHLOROPLASTS, AND A REDUCTION IN THE FORMATION OF THE LARGE VACUOLES WHICH PRECEDE DEATH OF THE CELLS.

  - LUNAR MATERIAL ACCUMULATES ADJACENT TO CELLS UNDERGOING NORMAL NUCLEAR AND CELL DIVISION. THERE IS NO EVIDENCE THAT THE LUNAR MATERIAL EXERTS A TOXICITY TO THE CELLS EVEN WHEN THEY ARE SURROUNDED BY PARTICLES.

  - THERE IS PRELIMINARY EVIDENCE TO INDICATE THAT LUNAR-TREATED TOBACCO TISSUE CULTURES CONTAIN HIGHER AMOUNTS OF CERTAIN CELLULAR CONSTITUENTS SUCH AS AMINO ACIDS, AND SELECTED LIPIDS.

APOLLO 14 QUARANTINE RESULTS: BOTANICAL LABORATORY

ALGAE

1. A BLUE-GREEN, A GREEN, A DIATOM, AND A RED ALGA WERE TREATED WITH APOLLO 14 MATERIAL.

2. THE SHOCK REACTION OBSERVED IN APOLLO 11 AND 12 WAS NOT OBSERVED IN APOLLO 14.

3. ALL ALGAE GREW WELL IN THE PRESENCE OF LUNAR MATERIAL. AS REPORTED FOR APOLLO 11 AND 12, THE BLUE-GREEN, ANACYSTIS NIDULANS APPEARED TO GROW BETTER IN CONTACT WITH THE LUNAR MATERIAL.

APOLLO 14 QUARANTINE RESULTS: BOTANICAL LABORATORY

SEED GERMINATION

1. BROCCOLI, BRUSSEL SPROUTS, CABBAGE, CARROT, CELERY, LETTUCE, RADISH, AND TOBACCO WERE TESTED IN APOLLO 14.

2. AS IN THE CASE OF APOLLO 11 AND 12, LETTUCE SEEDS GERMINATING IN CONTACT WITH THE LUNAR MATERIAL WERE MORE VIGOROUS. THOSE GERMINATING IN CONTACT WITH SIMULATED LUNAR MATERIAL SHOWED LESS STIMULATION.

3. OTHER SPECIES MAY BE SIGNIFICANTLY DIFFERENT IN SUBSEQUENT ANALYSIS, BUT NOT AS MUCH AS THE LETTUCE.

4. THE ABOVE SPECIES WERE MAINTAINED GERM-FREE, AND NO EVIDENCE OF ANY SEEDLING DISEASES WERE NOTED THROUGHOUT THE PERIOD OF THE TEST.

APOLLO 14 QUARANTINE RESULTS: BOTANICAL LABORATORY

LOWER PLANTS

1. THREE FERN SPECIES, ONE SPECIES OF CLUB MOSS, AND ONE LIVERWORT SPECIES WERE CHALLENGED.

2. ALL WERE GROWN IN A NEW ENRICHED MEDIUM WHICH SUPPLIES ALL THE ELEMENTS NEEDED FOR THEIR GROWTH. THIS ENRICHED MEDIUM WAS DEVELOPED AT MSC FOLLOWING THE RESULTS OF APOLLO 11 AND 12.

3. USING THE NEW MEDIUM, ALL CULTURES APPEAR EXCELLENT. THERE IS STILL AN INCREASED GREENING IN THE PRESENCE OF LUNAR MATERIALS AND THE SIMULATED LUNAR MATERIAL.

4. ACCORDING TO RESULTS FROM APOLLO 11 AND 12, THIS DIFFERENCE SHOULD INCREASE IN INTENSITY AS THE CULTURES CONTINUE TO GROW. THESE CULTURES ARE STILL VERY YOUNG AT THE END OF THE MISSION.

APOLLO 14 QUARANTINE RESULTS: BOTANICAL LABORATORY

SEEDLINGS

1. DUE TO A NUMBER OF PROBLEMS, MATURE GERM-FREE SEEDLINGS WERE NOT UTILIZED FOR APOLLO 14.

2. NORMAL SEEDLINGS OF ARTICHOKE, BEAN, CABBAGE, CITRUS, COTTON, CUCUMBER, EGGPLANT, LETTUCE, PARSLEY, PEANUT, PEPPER, RADISH, STRAWBERRY, AND TOMATO WERE CHALLENGED BY RUBBING THEIR LEAVES WITH A SUSPENSION OF LUNAR MATERIAL. SOME OF THESE SEEDLINGS APPROACH MATURITY AT THE TIME OF TREATMENT.

3. NO ADVERSE EFFECT WAS NOTED BY CHALLENGE. DUE TO THE LARGE SIZE OF THE PLANTS AND THEIR RAPID GROWTH RATE, NO DIFFERENCES WERE FOUND IN COMPARING THE CONTROLS TO THE LUNAR-TREATED PLANTS.

APOLLO 14 QUARANTINE RESULTS: BOTANICAL LABORATORY

TISSUE CULTURES

1. CARROT (WILD AND CULTIVATED), CORN, EUPHORBIA, LETTUCE, PINE, RICE, SOYBEAN, SUNFLOWER, AND TOBACCO TISSUE CULTURES WERE CHALLENGED.

2. AS IN THE CASE OF APOLLO 11 AND 12, TOBACCO TISSUE CULTURES APPEAR TO BE DOING BETTER WHEN TREATED WITH LUNAR MATERIAL. PINE CULTURES APPEAR TO BE STORING MORE TANNINS AND EXHIBITING A GREATER CELLULAR MEMBRANE ACTIVITY WHEN CHALLENGED WITH EITHER LUNAR OR SIMULATED LUNAR MATERIALS.

3. SOYBEAN APPEARS TO BE EXHIBITING A SHOCK REACTION SIMILAR TO PINE IN APOLLO 11. THIS REACTION IS RESTRICTED TO AREAS WHERE THE DUST SETTLED AS A THICK LAYER ON THE CULTURES.

APOLLO 14 QUARANTINE RESULTS: BOTANICAL LABORATORY

OVER-ALL FINDINGS

1. 560 GERM-FREE PLANT CULTURES HAVE BEEN EXPOSED TO APOLLO 14 SAMPLE AND HAVE NOT REVEALED A HAZARD ASSOCIATED WITH THE SAMPLES.

2. ESSENTIALLY ALL OF THE RESULTS FOUND, AND REPORTED IN BIOSCIENCE, FOR APOLLO 11 AND 12 SAMPLES WERE FOUND AGAIN IN APOLLO 14. IT IS STILL TOO EARLY TO GIVE QUANTITATION OF DIFFERENCES BUT ALL SYSTEMS APPEARED IN EXCELLENT CONDITION.

SOURCE OF LUNAR SAMPLES FOR MICROBIOLOGICAL ASSAY

Mission	Sample	Sample Type	Parent Sample Numbers and Description
Apollo 11	A	Sub-surface	10005 (1/2 of core #1) 10004 (1/2 of core #2)
	B	Surface	10050 + 10051 + 10052 (all chips and fines)
Apollo 12	C	Sub-surface	12026 (1/2 of core #1) 12028 (bottom of core #2)
	D	Surface	12057 + 12001 + 12003 (all chips and fines) 12052 + 12032 + 12033 (all fines)
Apollo 14	E	Sub-surface	14408 (Trench)
	F	Sub-surface	14411 (Double core bit)
	G	Sub-surface	14414 (Single core bit)
	H	Surface	14422 (unseived fines) 14259 (fines less than 1 mm diameter) 14041 (chip and residue) 14047 + 14049 + 14051 + 14055 + 14063 + 14064 + 14066 + 14068 + 14069 + 14070 + 14071 + 14072 (all residue)

MICROBIAL ASSAY OF LUNAR SAMPLES, APOLLO 11 - 14

Media Used	Sample Employed
Reduced protocol	
a. Agar	A
b. Blood Agar	C
c. Trypticase glucose yeast-extract agar	E
d. Thioglycollate broth	
Complete protocol	
a., b., c., and d.	
e. Czapek Dox Agar	B
f. Sabouraud Dextrose Agar	D
g. Pooled Terrestrial Soil Agar	H
h. Six Aquatic Fluids	
Autotrophic Media	
j. Burk's Nitrogen free medium	C
k. 9K + Ferrous sulfate medium	D
l. 9K + Sulfur medium	E
m. 9K medium	H
n. Distilled water	
Special protocol	
a. Agar	F
b. Trypticase glucose yeast-extract agar	G

Results:

No evidence of replicating organisms detected

APOLLO 14

LUNAR MATERIAL VIROLOGY

SAMPLES: 1. Trench 2. Double Tube Core Bit
3. Single Tube Core Bit 4. Pool 5. Igneous Rock
6. White Rock

Host Systems Challenged	Sample Used for Challenge
Primary African Green Monkey Kidney	1, 4, 2, 3, 5, 6
Primary Human Embryonic Kidney	1, 4, 2, 3, 5, 6
Diploid Human Embryonic Kidney	1, 4, 2, 3, 5, 6
Hetereploid Bovine Kidney	1, 4
Hetereploid Porcine Kidney	1, 4
Primary Duck Embryonic Fibroblast	1, 4
Rainbow Trout Gonadal Tissue	1, 4
Fathead Minnow	1, 4
Grunt Fin	1, 4
*Embryonated Eggs	1, 4, 2, 3, 5, 6
Suckling Mice	1, 4, 2, 3, 5, 6
Mycoplasma Medium	1, 4

No evidence of viruses, bacteria, fungi or other replicating organisms in the systems challenged.

*Random deaths of yolk sac inoculated embryonated eggs not transmissible or due to replicating agent

APOLLO 11

LUNAR MATERIAL VIROLOGY

SAMPLES: 1. Core 2. Fines 3. Pool

Host Systems Challenged	Sample Used for Challenge
Primary African Green Monkey Kidney	1, 2, 3
Primary Human Embryonic Kidney	1, 2, 3
Human Embryonic Lung	1, 2, 3
Primary Bovine Embryonic Kidney	1, 2, 3
Primary Porcine Embryonic Kidney	1, 2, 3
Primary Duck Embryonic Fibroblast	1, 2, 3
Rainbow Trout Gonadal Tissue	3
Fathead Minnow	3
Grunt Fin	3
Embryonated Eggs	1, 2, 3
Mycoplasma Medium	1, 2, 3

No evidence of viruses, bacteria, fungi, or other replicating organisms in the systems challenged.

APOLLO 12

LUNAR MATERIAL VIROLOGY

SAMPLES: 1. Core 2. Pool

Host Systems Challenged	Sample Used for Challenge
African Green Monkey Kidney	1, 2
Human Embryonic Kidney	1, 2
Human Embryonic Lung	1, 2
Hetereploid Bovine Kidney	2
Hetereploid Porcine Kidney	2
Primary Duck Embryonic Fibroblast	2
Rainbow Trout Gonadal Tissue	2
Fathead Minnow	2
Grunt Fin	2
Embryonated Chicken Eggs	2
Mycoplasma Medium	2

No evidence of viruses, bacteria, fungi, or other replicating organisms in the systems challenged.

MAMMALIAN ANIMALS

APOLLO 11 AND 12

GERM-FREE MICE - NO EVIDENCE OF TRANSMISSIBLE DELETERIOUS EFFECT ORIGINATING FROM EXPOSURE TO LUNAR SAMPLES

APOLLO 14

TEST ANIMALS:

SAMPLE	NATURAL	STERILE	CONTROL
TRENCH MATERIAL	34 MICE	12 MICE	20 MICE
BIOPOOLED MATERIAL	46 MICE	12 MICE	24 MICE
BIOPOOLED INSULFLATION	9 GUINEA PIGS		3 GUINEA PIGS

RESULTS:

ALL ANIMALS REMAINED HEALTHY AND NORMAL
DETAILED EXAMINATION OF HISTOLOGICAL SPECIMENS IN PROGRESS.

EFFECTS OF LUNAR MATERIAL ON APOLLO MISSIONS 11, 12, & 14
FISH AND INVERTEBRATES
(PART 1)

Species	Results
Paramecium *Paramecium aurelia*	Initial reduction in fission rates following exposure, rapidly increasing to normal after 4-5 days. All groups normal in morphology.
*Euglena *Euglena gracilis*	Slight reduction in locomotion following exposure with return to normal activity by the fourth day. All groups normal in morphology.
Planaria *Dugesia dorotocephala*	No significant gross or histopathological changes.
**Brown Shrimp *Penaeus aztecus*	No abnormal behavior or significant gross or histopathological changes.
***Pink Shrimp *Penaeus duorarum*	Considerable fighting in all groups early in test. No significant gross or histopathological changes.
Commercial Oyster *Crassostrea virginica*	During missions 11 and 14 severe mortalities were encountered in all groups and no correlation could be shown between the deaths and exposure to lunar material. During mission 12, all oysters remained in excellent health.

*Used on Apollo 11 only
**Used on Apollo 11 and 14
***Used on Apollo 12 only

EFFECTS OF LUNAR MATERIAL ON FISH AND INVERTEBRATES
APOLLO MISSIONS 11, 12, & 14
(PART 2)

Species	Results
German Cockroach *Blattella germanica* (adults & nymphs)	No gross or histopathological changes
House Fly *Musca domestica* (adults & larvae)	No gross or histopathological changes
Greater Wax Moth *Galleria mellonella* (larvae only)	No gross or histopathological changes
Mummichog Minnow *Fundulus heteroclitus*	With the exception of a few fish in each group during mission 12 (lost due to gill congestion from exposure to hypochlorite), all mummichogs remained in excellent health and no unaccountable gross or histopathological changes were found.
+Fathead Minnow *Pimephales promelas*	Chronic mortalities in all groups due to sodium hypochlorite spill. No unaccountable gross or histopathological changes.
++Guppy *Lebistes reticulatus*	No unaccountable gross or histopathological changes found.

+Used on Apollo 11 only
++Used on Apollo 12 and 14

MICROBIOLOGICAL SUPPORT

MISSION: PROVIDE MICROBIOLOGICAL ANALYSES OF BIOTEST SYSTEMS WHICH ARE EXPOSED TO LUNAR MATERIAL AND TO SEGREGATE THESE ANALYSES FROM THOSE ON SAMPLES PREVIOUSLY EXPOSED TO LUNAR MATERIAL, SUCH AS CREW OR SPACECRAFT SAMPLES.

EFFORTS: PROVIDED ANALYSES ON 370 SAMPLES IN SUPPORT OF THE "GERM-FREE" MURINE PROTOCOL, VIROLOGY, BOTANY AND THE INVERTEBRATE AND LOWER VERTEBRATE PROTOCOL.

- STERILITY CHECK OF ALSRC
- STERILITY CHECK OF LUNAR SAMPLE CONTAINERS

RESULTS: NO VIABLE LIFE FORMS IN STERILITY CHECKS

ALL VIABLE LIFE FORMS DETECTED IN THE ANALYSES ARE BIOCHEMICALLY AND MORPHOLOGICALLY COMPATIBLE WITH RECOGNIZED TERRESTRIAL ORGANISMS.

SUMMARY

BASED ON: 3 missions with .

1. NO EVIDENCE OF REPLICATING ORGANISMS

2. NO DELETERIOUS EFFECTS ON LIVING HOST SYSTEMS

3. SUFFICIENT EVIDENCE TO INDICATE THAT "BIOLOGICAL ACTIVITY" NOT DUE TO SELF REPLICATING ORGANISM

4. GEOLOGICAL EVIDENCE THAT A REPRESENTATIVE SAMPLING OF THE MOON HAS BEEN BIOMEDICALLY EVALUATED.

5. NO SPECIFIC EVIDENCE OF PAST OR PRESENT WATER OR ORGANIC CARBON

AND -

6. RUSSIAN REPORT OF NO LIFE DETECTED IN RETURNED SAMPLES

IT IS RECOMMENDED:

1. ELIMINATE ALL QUARANTINE REQUIREMENTS FOR APOLLO 15 - 17

2. CONTINUE TO BIOMEDICALLY CHARACTERIZE SAMPLES RETURNED BY APOLLO 15 - 17.

Fertilizer - does better than human material.

A. Antioxidation sample -
1. activity in seedling.
2. Plate by
3. Extract 20% ste
   buffer.
4. Biochem.

B. Tissue culture cells. K, P, N. difference
1. Biochem.
2. Histology

Walkinshaw reports weekly — ? where?