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OCULAR TOXICITY TESTING OF LUNAR DUST

OECD Recommendations

- OECD 405 – Acute Eye Irritation/Corrosion (adopted April 2002)
  - "In the interest of both sound science and animal welfare, *in vivo* testing should not be considered until all available data relevant to the potential eye corrosivity/irritation of the substance has been evaluated in a weight-of-the-evidence analysis."

http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/OECD/OECDtg405.pdf

OECD Recommendations

- Recommended Steps
  - Evaluate existing human and animal data
    - None available
  - Analyze structure activity relationships
    - Lunar dust has unique morphological characteristics
  - Evaluate physicochemical properties and chemical reactivity
    - No pH extremes or buffering capacity

OECD Recommendations

- OECD Recommended Steps (cont.)
  - Consider other existing information (systemic toxicity)
    - Instillation data (Lam-unpublished data) suggest inflammatory reaction but no corrosivity
  - Consider results from *in vitro* or *ex vivo* tests
  - Assess *in vivo* dermal irritancy or corrosivity

Stillmeadow, Inc.

- AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care) accredited
- Approved Animal Welfare Assurance on file with NIH
- Located in Sugarland, TX

EpiOcular

- Manufactured by MatTek
- Tissues derived from normal, human epidermal keratinocytes
- Cells are differentiated on cell culture inserts to form a multi-layered structure which closely parallels the corneal epithelium

Method

- Tissue dosed with 100 mg dust
  - Lunar dust
  - Amorphous silica
  - SDS
  - Sodium hydroxide
- Positive control = 0.3% Triton X 100
- Negative control = DI water

Method (cont.)

- 3 exposure times
  - 3 minutes
  - 30 minutes
  - 60 minutes
- PBS wash
- 10 minute incubation in culture media
- 3 hour incubation in MTT
- Overnight immersion in extractant solution

Method (cont.)

- Optical density of 200 μL of each replicate recorded at 570 and 630 nm
- ET-50 calculated based on the manufacturer's spreadsheet
- Each dust assigned an irritancy classification based on ET-50

Irritancy Classifications

Non-irritating/minimal	>60 minutes
Mild	30-60 minutes
Moderate	3-29.9 minutes
Severe/Extreme	<3 minutes

Results

	Lunar Dust	Neg. Control	Pos. Control	NaOH	SDS	Am. silica
3 min	1.117	N/A	0.931	0.014	0.275	1.465
30 min	1.023	N/A	0.548	0.011	0.029	1.288
60 min	1.013	1.077	0.187	0.010	0.012	1.449
ET-50	>60 min	N/A	30.6 min	<3 min	<3 min	>60 min
Irritancy	Minimal	N/A	Mild	Severe	Severe	Minimal

Conclusions

- *In vitro* study provides evidence that lunar dust is not severely corrosive or irritating

HOWEVER,

- *In vitro/ex vivo* tests
  - Have a high false negative rates for solids (nearly 50%) – OECD 437
  - Consider only corneal injury
  - Assess only a single endpoint (cell death)

WHEREAS

- *In vivo* tests
  - Provide more realistic response scenario (tearing, inflammatory response, etc.)
  - Allow evaluation of conjunctiva, iris, and cornea as well as the lids and surrounding tissue
  - Provide information about chemical AND mechanical irritation

THEREFORE,

- *In vivo* testing is recommended and approval is pending

Future Plans

- Number of animals
  - 3 rabbits planned
    - Additional animals may be needed if a weak or moderate response is noted
- Particle Size
  - Median diameter = 51 μm
- Amount of Dust
  - 70 mg/rabbit based on bulk density estimate (0.7 g/cm3)
- Washed vs Unwashed
  - Washed at 1 hr
    - recommended by OECD 405
    - Reflects normal procedure
    - Reduces possibility of permanent eye damage