

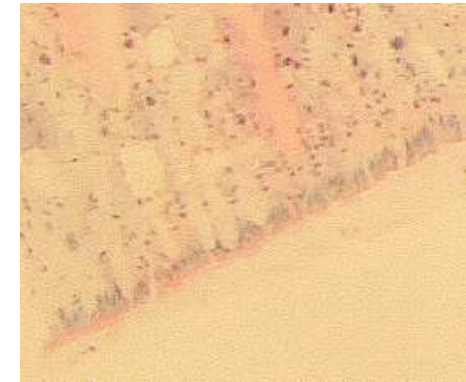
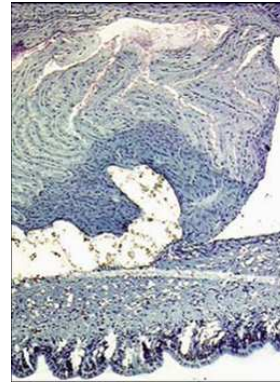
Different applications of the Slug Mucosal Irritation test in pharmaceutical preclinical drug development

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Slugs as test organism

The body wall of slugs is a mucosal surface composed of different layers

- Single-layered columnar epithelium that contains ciliated cells, cells with microvilli and mucus secreting cells (ectodermal)
- Subepithelial connective tissue



- According to the principle of the 3 R's (**R**efinement, **R**eduction and **R**eplacement of laboratory animals) invertebrates are possible alternatives to animals
- *Arion lusitanicus* is a major pest species for the European agriculture
- Easy housing

Principle SMI assay

Exposure to test substance



Irritation



Mucus production (%)

Tissue damage



Release

Protein ($\mu\text{g/ml.g}$)
LDH - ALP (U/l.g)

Applications

- Local tolerance testing: predict the nasal, ocular, oral, GI, vaginal, rectal and parenteral irritation potency of pharmaceuticals, personal care products, house hold products etc... (5-D procedure)
- Screening: predict the (eye) irritation potency of chemicals and active ingredients (1-D procedure)

Advantages of the SMI assay

- Allows single or repeated exposure studies
- Distinguish between *irritation* and *tissue damage*
- Test material can be solid, semi-solid or liquid
- Predicts clinical discomfort: stinging and burning sensations

Validation – Local tolerance test

- More than 200 raw materials and formulations (solutions, semi-solids and solids) with known in vivo irritation data were used to validate the assay for assessing the mucosal tolerance of pharmaceuticals and personal care products
- Based on these data a prediction model was developed that distinguishes between irritation and tissue damage (4 classes)

Overview tested products

Solids

- Powders
- Pellets
- Suppo's
- Films

Semi-solids

- Gels
- Creams
- Pastes
- Foams

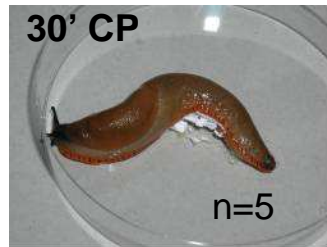
Liquids

- Solutions
- Emulsions
- Suspensions

Local tolerance – 5d test procedure

Test procedure (5-day)

Daily during 5 successive days



30' Contact Period with x amount of the test item

Measure Mucus production

Calculations

Calculate Total Mucus production

Prediction Model

Irritation



Transfer slug to new petri dish. Add 1 ml PBS and remove this after 1 h.

Tissue damage



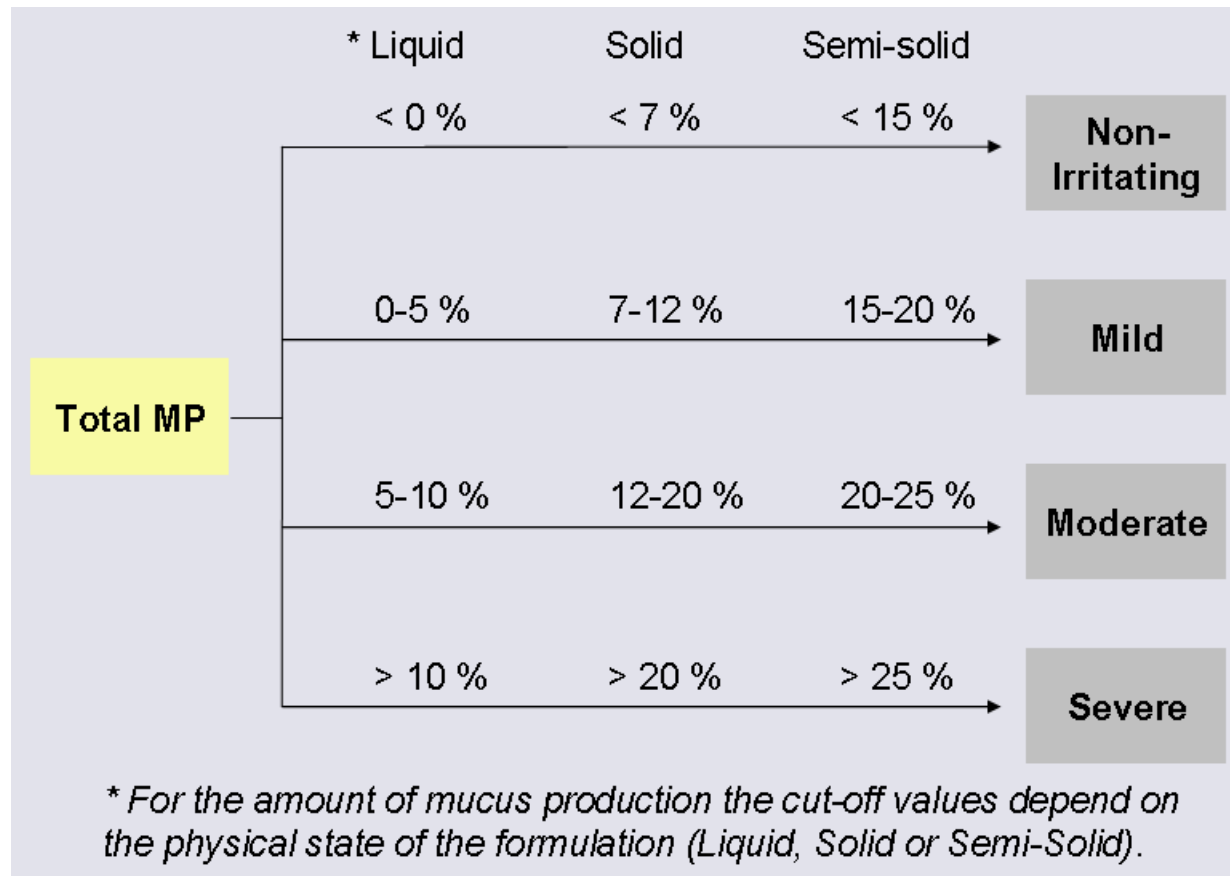
Transfer slug to new petri dish. Add 1 ml PBS and remove this after 1 h.

Measure Protein, LDH & ALP release in samples

Calculate Mean Protein, Mean LDH release and count number of slugs inducing ALP release

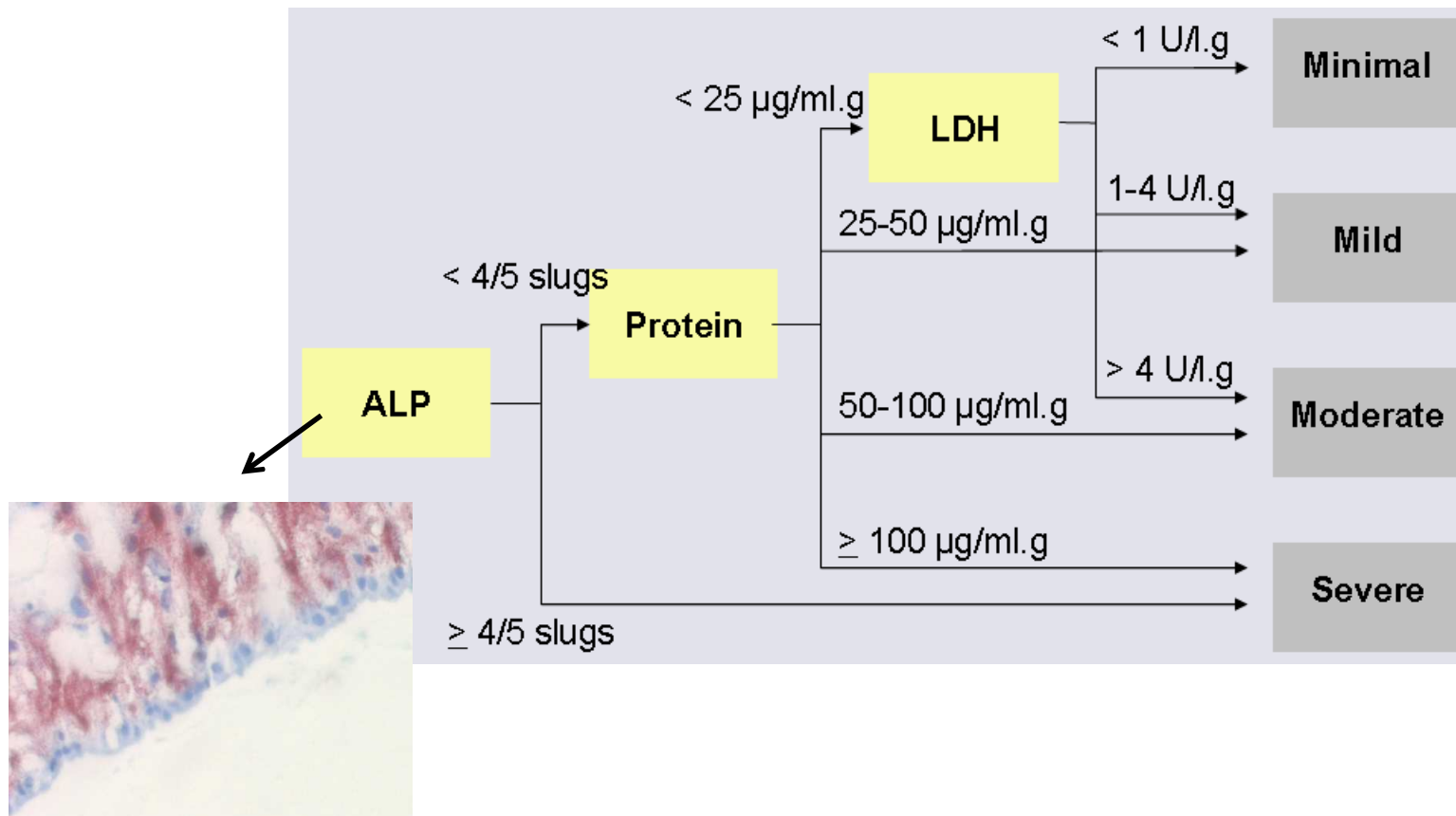
Mucosal tolerance – Prediction Model

Irritation



Mucosal tolerance – 5d test procedure

Tissue damage



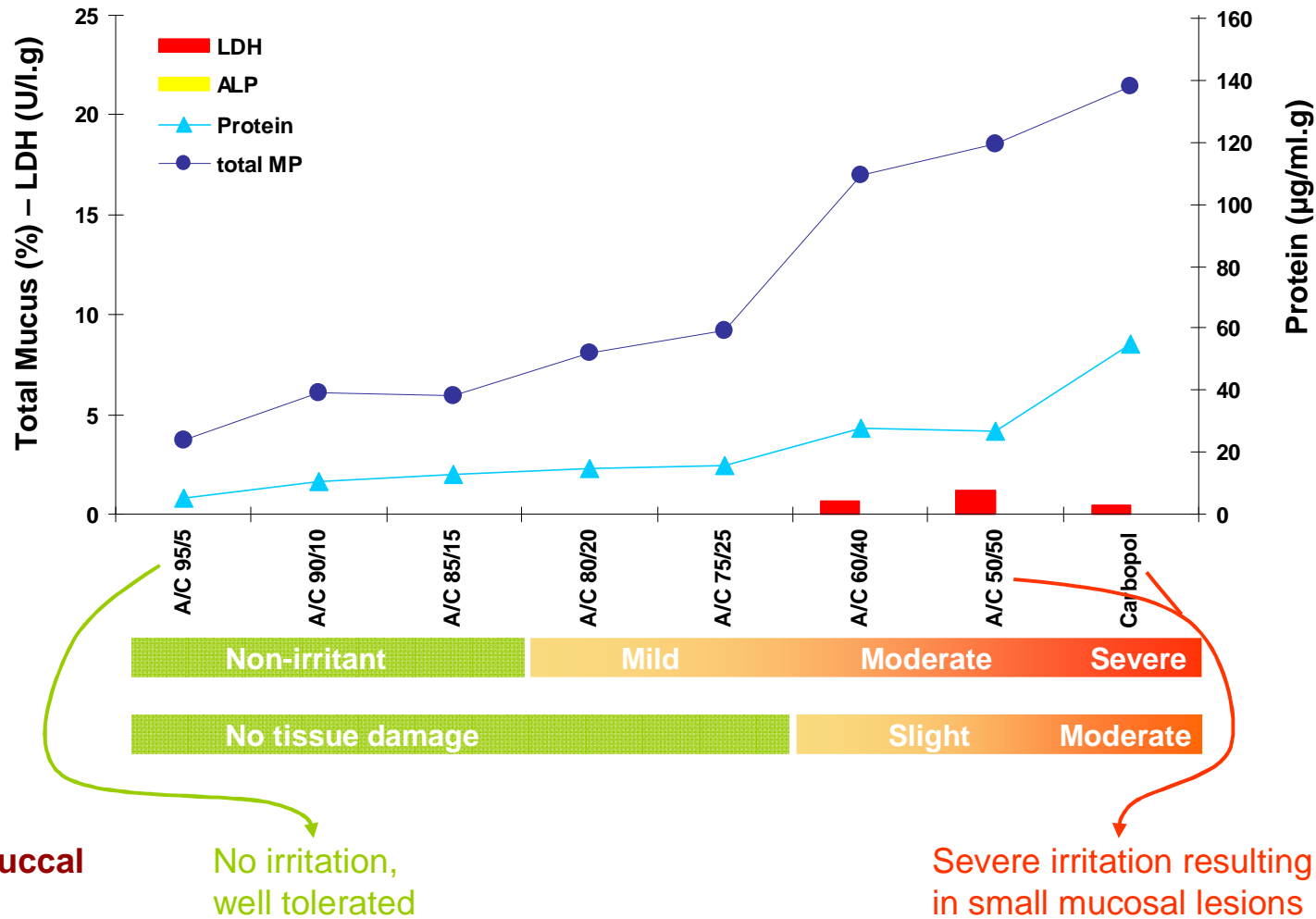
Gels – vaginal application

OTC vaginal lubricants or contraceptives

Formulations	SMI assay						Clinical data	
	Total MP (%)		Protein (µg/ml.g)	LDH (U/l.g)	ALP (n)	Tissue damage	Irritation % (#)	Colposcopic findings
HEC 5%/Glyc 2% <i>(Hydroxyethyl cell.)</i>	1.9 ± 2.9	NI	17 ± 20	-	0/5	No		
HEC 5%/Glyc 10% <i>(Glycerol)</i>	12.3 ± 1.3	NI	8 ± 2	-	0/5	No	5% (20)	
KY® jelly <i>12% glycerol</i>	18.7 ± 3.1	Mild	13 ± 12	-	0/10	No	17% (89)	16% erythema
Gynoll II® <i>Nonoxynol-9 2%</i>	23.4 ± 4.3	Mod	37 ± 26	0.42 ± 0.46	3/10	Slight	45% (20)	45% erythema
Gynol II ES® <i>Nonoxynol-9 3%</i>	27.7 ± 5.5	Sev	67 ± 43	1.05 ± .11	5/10	Mod	87% (15)	47% findings
Conceptrol® (PC) <i>Nonoxynol-9 4%</i>	25.5 ± 2.7	Sev	161 ± 46	3.30 ± 1.16	12/15	Sev	75% (12)	75% findings

Powders – Bioadhesives for buccal application

Concentration - response: Amioca® starch/Carbopol® 974P powder mixtures



Prevalidation – SMI assay EYE irritation

- Multicenter study – 4 partners
- 20 ECETOC reference chemicals for eye irritation (Draize data)
- Study design: training phase – testing phase
 - **Training phase:** demonstration of procedure on first day, repeated testing of the same 5 reference chemicals for 3 days under supervision of the trainer
 - Transferability and within-laboratory variability
 - **Testing phase:** testing of another 15 reference chemicals
 - Between-laboratory variability and performance

Procedure – EYE irritation chemicals

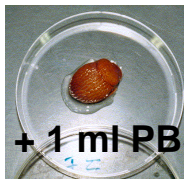
CP 1 60'
1%



Mucus production

60' contact period (CP) - 1% dilution

Sample 1
60'



+ 1 ml PBS

Protein release (P1)

PBS
60'

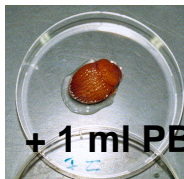
60' rest period

CP 2 60'
3.5%



60' contact period - 3.5% dilution.

Sample 2
60'



+ 1 ml PBS

Protein release (P2)

Prediction model

NC / R36 / R41

Relevance SMI assay – EYE irritation

- All NC compounds (8 chemicals) classified correct by SMI:
3-Methoxy-1,2-propanediol, PEG 400, potassium tetrafluoroborate, glycerol, methylcyclopentane, tween-20, 2,4,5,6-tetraaminopyrimidine, Ethyl acetate

Chemical	EU	Predictions (number)			
		Under	Correct	Over	
Ammonium nitrate*	R36	1	9	-	
1-Octanol	R36	-	4	-	Predictive capacity
2-Ethyl-1-hexanol	R36	-	4	-	NI R36 R41
1-Hexanol*	R36	-	10	-	100% 97% 88%
Acetone	R36	-	4	-	
Imidazole*	R41	1	9	NA	Predictive value
Sodium oxalate	R41	-	4	NA	
Triton X-100 (10%)	R41	1	3	NA	NI R36 R41
Promethazine HCl	R41	-	4	NA	94% 94% 100%
Chlorexidine	R41	-	4	NA	
Cetylpyridinium Br (10%)	R41	2	2	NA	
Benzalkonium Cl (10%)	R41	-	4	NA	

Conclusions

- **Local tolerance testing**

- Accurately predict local tolerance of pharmaceuticals (nasal, buccal, parenteral, vaginal, rectal)
- Test volume needed: liquids (2.5 ml), semi-solids (2.5 g), solids (0.5 g)

- **Eye irritation testing of chemicals**

Successful prevalidation

- High predictive capacity: NC (100%), R36 (97%) and R41 (88%)
- High predictive value: NC (94%), R36 (94%) and R41 (100%)

- **Advantages of the SMI assay**

- Allows single or repeated exposure studies
- Predicts clinical discomfort (stinging and burning sensations)