

Mucosal irritation: SMI Test



- **Objective:** evaluation of the local tolerance (stinging and burning, irritation and tissue damage) of pharmaceuticals, personal care products, house hold products, chemicals etc.

- **Principle:** slugs produce mucus upon contact with in irritating test substance. Tissue damage caused by a test substance, is measured up to 2 hours after each contact period.



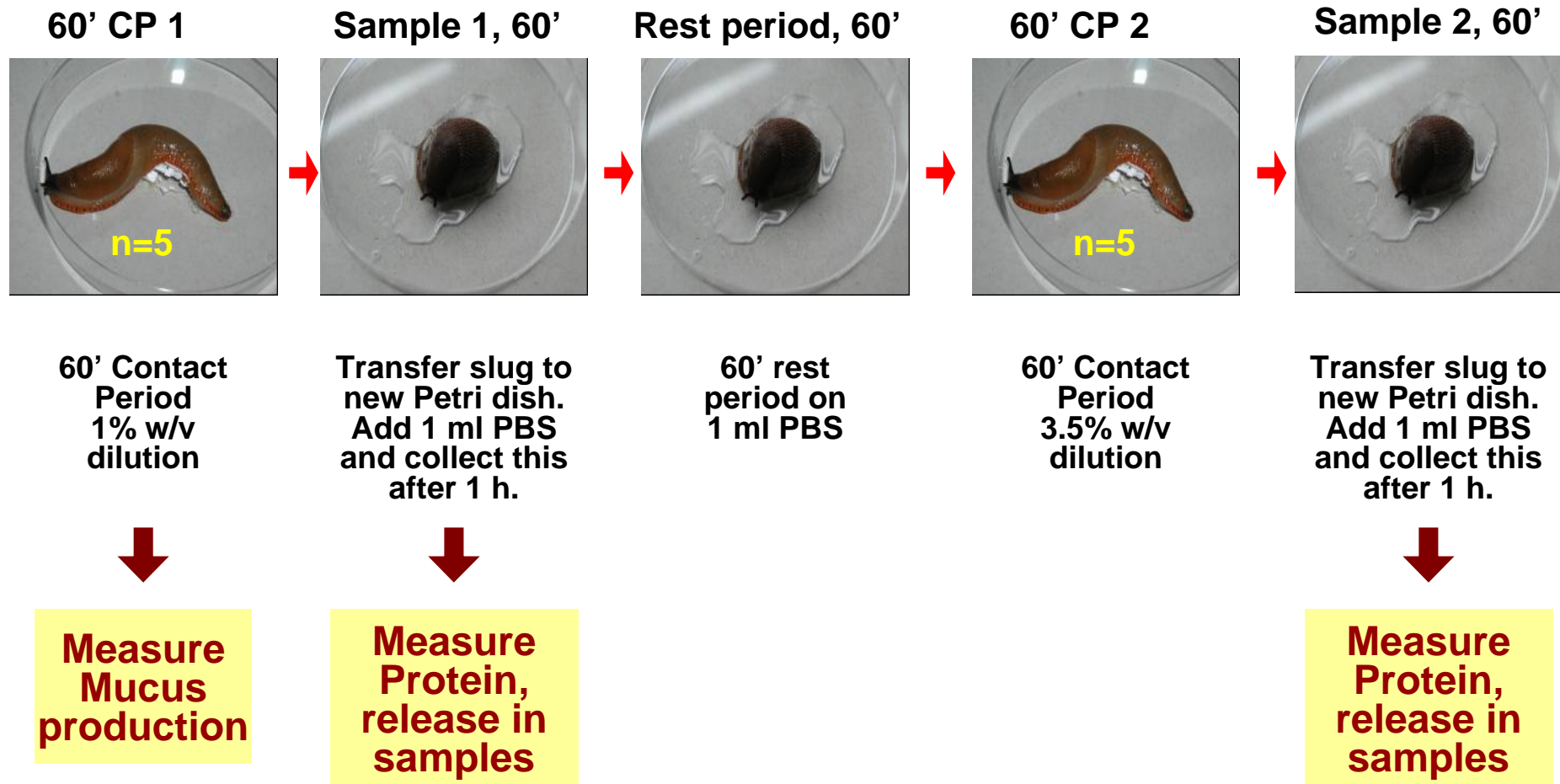
- **Development Stage:** R&D: continuous further development
Validation stage – Prevalidation finished – Validation planned (2009)
Routine: contract services since 2004

- **Results / data:** more than 150 chemicals tested and over 300 formulations tested, 13 peer reviewed papers

- **Applicability Domain:** up to date no restrictions determined

Mucosal irritation: SMI Test

Experimental layout for 1D treatment



Mucosal irritation: SMI Test

Prediction model for 1D treatment

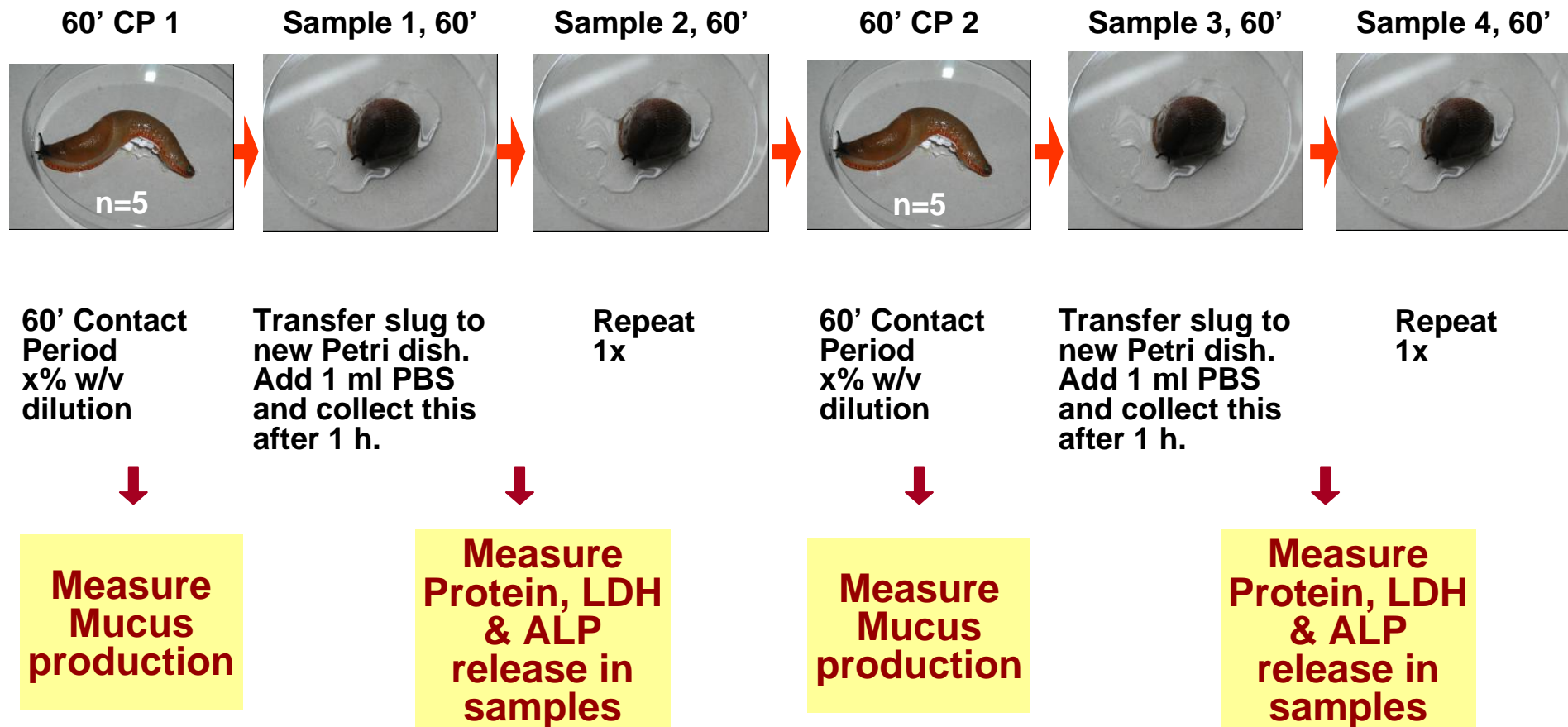
The evaluation of the test results is based upon three endpoints: (1) the mucus produced during the first 60-min contact period with the test item and (2 & 3) the protein release after the first and second 60-min contact period. A prediction model (applying discriminant functions equations) classifies the compounds into one of the 3 eye irritation classes (NI, R36 and R41) (Adriaens et al., 2008).

Prediction model (Fisher's linear classification function coefficients)

EU class	Classification functions
NI	$100.0 \text{ mean}(\text{Log}(\text{MPCP1}+10)) - 3.2 \text{ mean}(\text{Log}(\text{P1}+1)) + 3.1 \text{ mean}(\text{Log}(\text{P2}+1)) - 51.9$
R36	$100.6 \text{ mean}(\text{Log}(\text{MPCP1}+10)) - 4.8 \text{ mean}(\text{Log}(\text{P1}+1)) + 9.7 \text{ mean}(\text{Log}(\text{P2}+1)) - 60.9$
R41	$125.8 \text{ mean}(\text{Log}(\text{MPCP1}+10)) - 2.9 \text{ mean}(\text{Log}(\text{P1}+1)) + 10.1 \text{ mean}(\text{Log}(\text{P2}+1)) - 94.9$

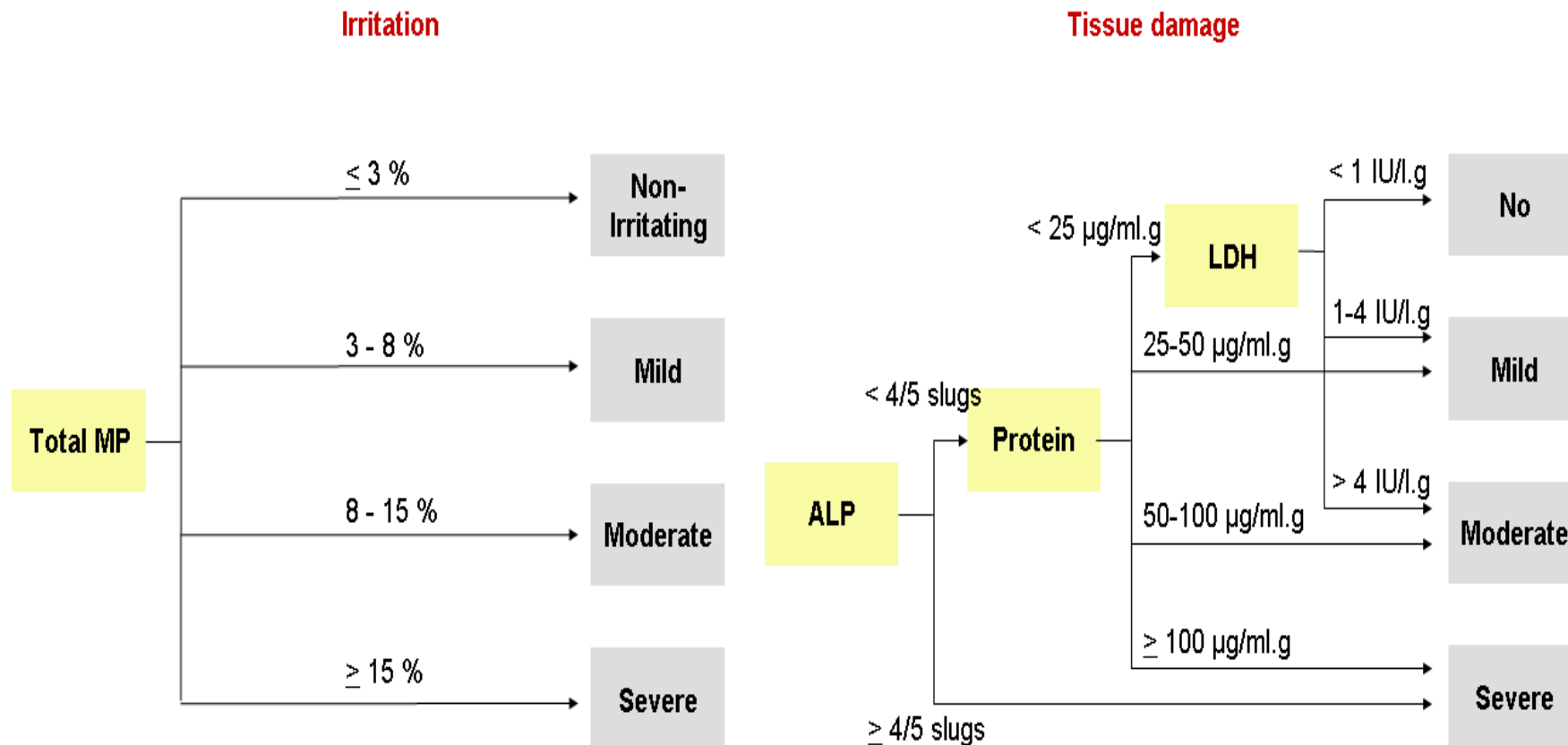
Mucosal irritation: SMI Test

Experimental layout for 1D Screening Concentration Response



Mucosal irritation: SMI Test

Prediction model for 1D Screening Concentration Response

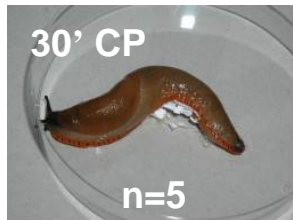


Mucosal irritation: SMI Test

Experimental layout for 5D Local Tolerance Testing (Liquids, semi-liquids and solids)

Test procedure

Daily during 5 successive days



30' Contact Period with 100µl of the test substance

Endpoints

Measure Mucus production

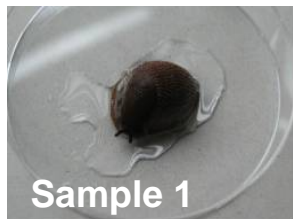
Calculations

Calculate Total Mucus production

Prediction Model

Irritation

Cut-off values for the Total Mucus production are used to classify the compounds into *non-irritants, mild, moderate & severe irritants*.



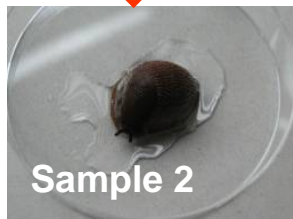
Transfer slug to new petri dish. Add 1 ml PBS and remove this after 1 h.

Measure Protein, LDH & ALP release in samples

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

Tissue damage

A decision tree combining the results of the Mean Protein, Mean LDH and number of slugs showing ALP release is used to convert the results into tissue damage grades: *minimal, slight, moderate and severe tissue damage*



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