2nd PAN-AMERICAN Conference for Alternative Methods
August 23-24, 2018
Rio de Janeiro

UFG UNIVERSIDADE FEDERAL DE GOIÁS

Skin Sensitization: Are we ready for replacement?

Implementation of *in vitro* Tests: Brazilian experience

Dra. Marize Campos Valadares

marizeufg@gmail.com

TOXIN
LABORATÓRIO DE ENSINO E PESQUISA EM TOXICOLOGIA IN VITRO

SKIN SENSITIZATION TESTING

DPRA- OECD 442C
KeratinoSens™, LuSens - OECD 442D
U-SENS™; h-CLAT; IL8-Luc assay - OECD 442E
GARDskin™ .....
...but none *in vitro assay* is recommended as a stand alone...

Draize Test — **1959**

Guinea pig maximization test (GPMT) — **1969**

OECD 429 2002, updated 2018
OECD 442A: LLNA:DA
OECD 442B: LLNA: BrdU-ELISA — **2002/2010**

IN VITRO TESTS RECOMENDED BY **CONCEA** — **2014/2016**

2015/2018

1965

Buehler test (BT)

1981/1992

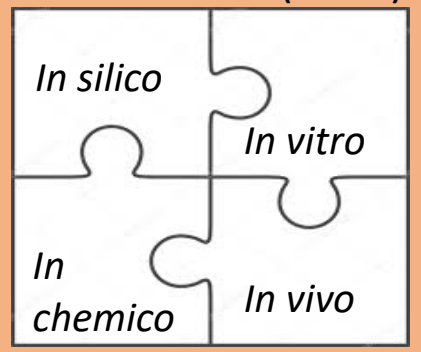
OECD 406
GPMT and BT

2012

Alergenicity: Adverse Outcome Pathway (AOP)

INTEGRATED APPROACHES TO TESTING AND ASSESSMENT- IATA

OECD no. 256 (2016)



SENSITIZERS — NONSENSITIZERS

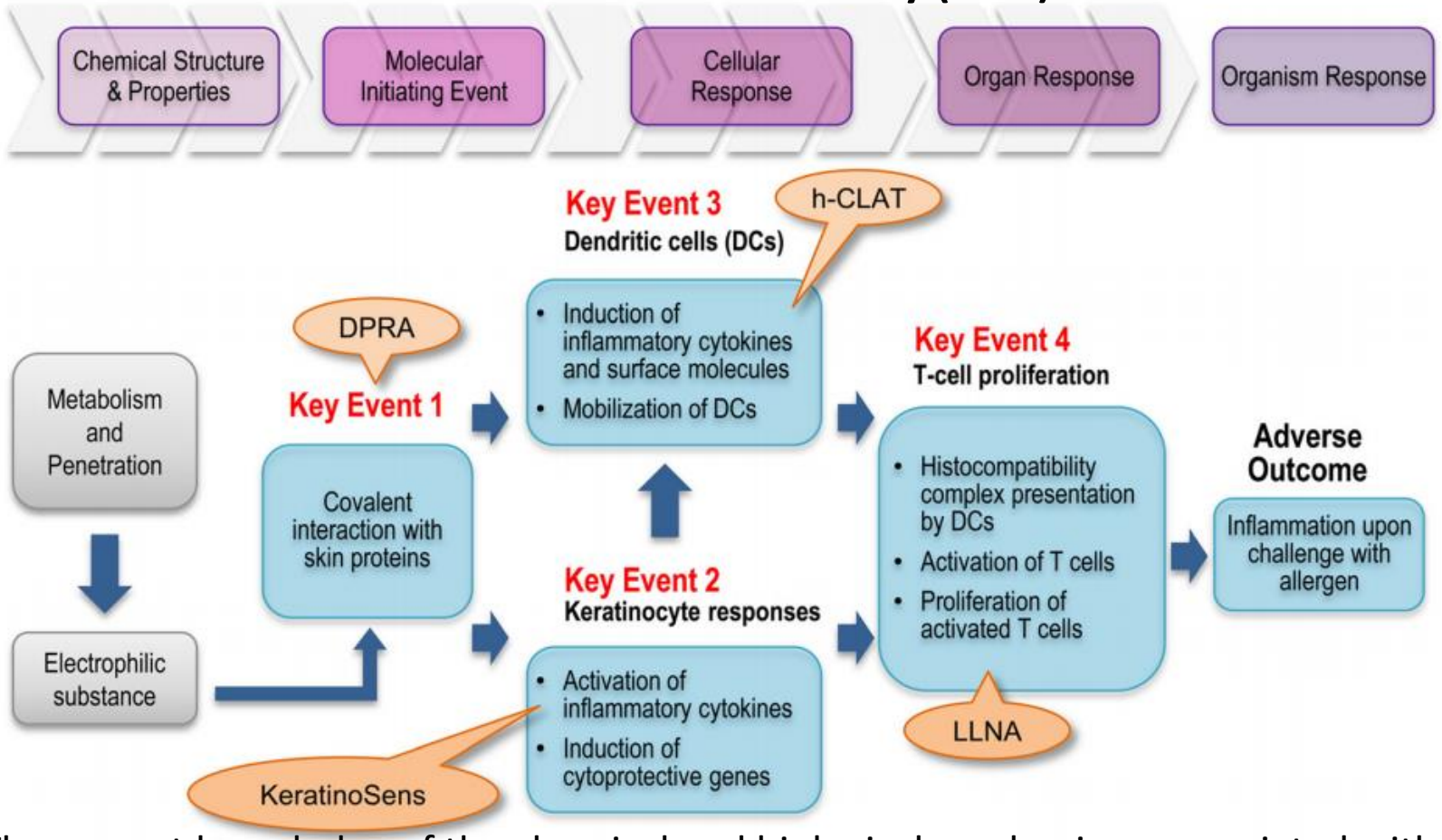
Pre-clinical assays for SS initiated using animals (n=20)

Refinement: initiated in OECD 429 and its derivatives with a validated Test. 2012: the **establishment of the AOP**

Development of *in vitro* tests based on the AOP **2016: OECD** published the IATA to categorize sensitizer or nonsensitizer

IN VITRO TESTS BASED ON THE AOP

Adverse Outcome Pathway (AOP)



The current knowledge of the chemical and biological mechanisms associated with skin sensitization has been summarized as an Adverse Outcome Pathway (AOP), starting with the molecular initiating event (Key 1) through intermediate events (Key 2, 3 and 4) to the adverse effect, namely allergic contact dermatitis.

In vitro Assays for 1R

Key Event 1:
Binding to proteins:
Direct peptide reactivity assay- DPRA

OECD 442C, 2015

Key Event 2:
Keratinocyte cells:
KeratinoSens™
LuSens test method

OECD 442D, 2017/18

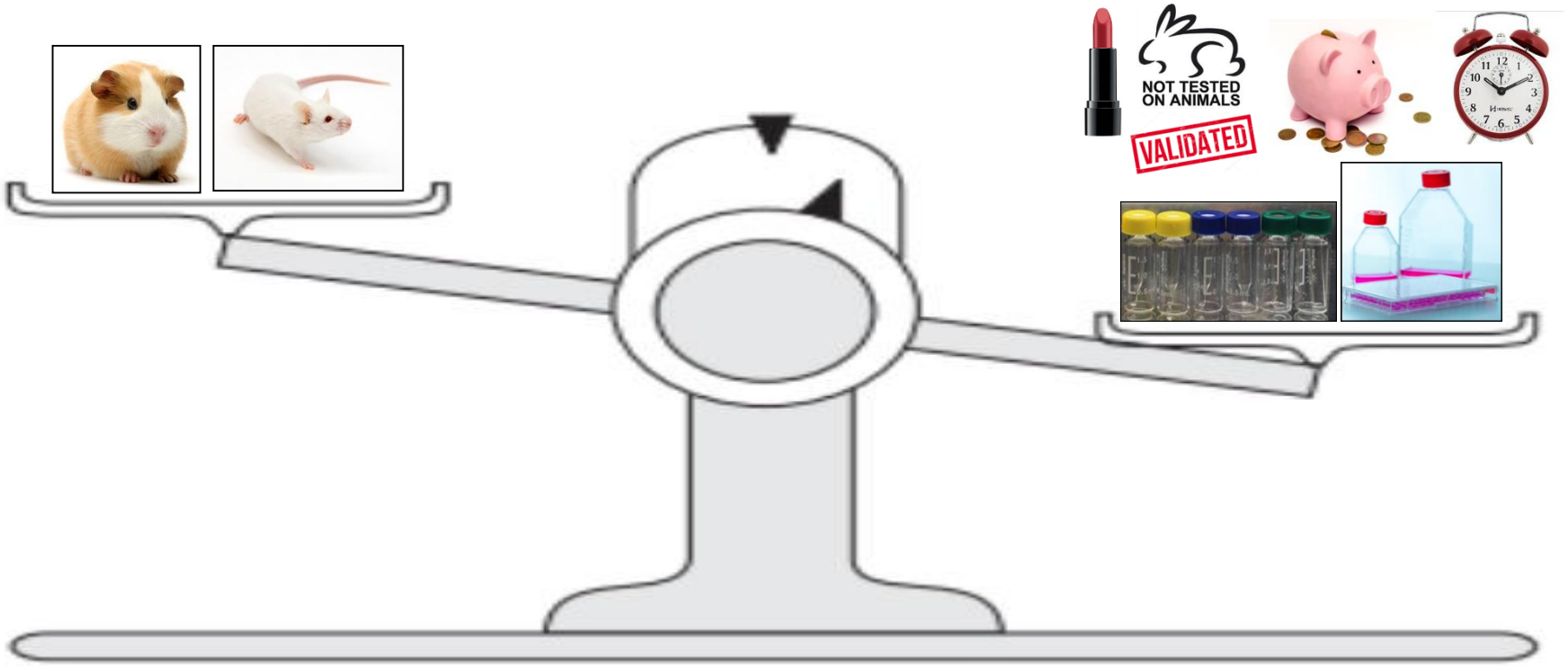
Key Event 3:
Dendritic cells:
h-CLAT
U-SENS
IL-8 Luc assay

OECD 442E, 2017/18

Prediction: *in vivo* x *in vitro*

In vivo assays using animals:
Accuracy of the **72%** in relation to human data

Using In vitro assays:
The accuracy is superior to **72%** in relation to human data or animals data



Assay	Accuracy
DPRA	80%
KeratinoSens™	77%
LuSens test method	74%
h-CLAT	85%
U-SENS™	77%
IL-8 Luc assay	86%

Combination of tests increased the Accuraccy: Ex.: "2-out-3" approach

Test should be considered in combination with other sources of information:
INTEGRATED APPROACHES TO TESTING AND ASSESSMENT-IATA

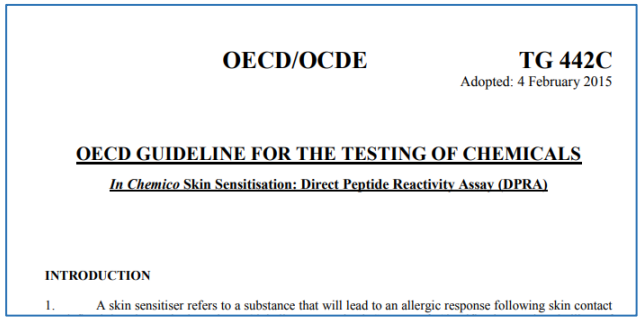
SKIN SENSITIZATION: BRAZIL

National Council for the Control of Animal Experimentation - CONCEA

TOXIN
LABORATÓRIO DE ENSINO E PESQUISA EM TOXICOLOGIA IN VITRO

NORMATIVE RESOLUTION nº18/2014

OECD 429 2002
OECD 442A
OECD 442B



OECD/OCDE TG 442C
Adopted: 4 February 2015

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

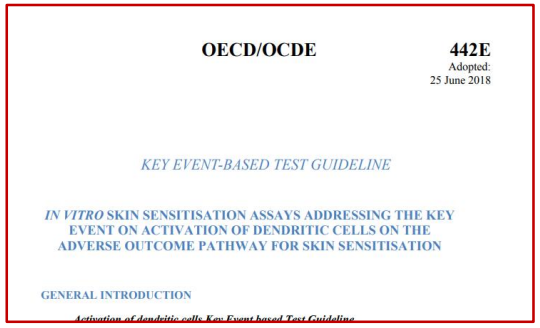
In Chemico Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA)

INTRODUCTION

1. A skin sensitiser refers to a substance that will lead to an allergic response following skin contact

Implementation of *in vitro* tests in the Laboratory of Toxicology *in vitro*:
DPRA; h-CLAT
U-SENS™; KeratinoSens™

NORMATIVE RESOLUTION nº 31/2016
OECD TG 442C
OECD TG 442D

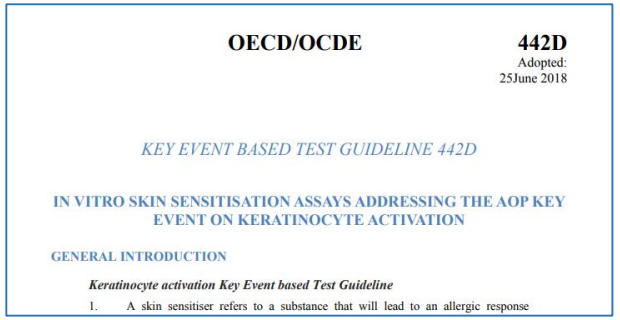


OECD/OCDE 442E
Adopted: 25 June 2018

KEY EVENT-BASED TEST GUIDELINE

IN VITRO SKIN SENSITISATION ASSAYS ADDRESSING THE KEY EVENT ON ACTIVATION OF DENDRITIC CELLS ON THE ADVERSE OUTCOME PATHWAY FOR SKIN SENSITISATION

GENERAL INTRODUCTION



OECD/OCDE 442D
Adopted: 25June 2018

KEY EVENT BASED TEST GUIDELINE 442D

IN VITRO SKIN SENSITISATION ASSAYS ADDRESSING THE AOP KEY EVENT ON KERATINOCYTE ACTIVATION

GENERAL INTRODUCTION

Keratinocyte activation Key Event based Test Guideline

1. A skin sensitiser refers to a substance that will lead to an allergic response

Reagent and Cell lines:
-Suppliers
- Cost
-Donation of cell lines for research only

SKIN SENSITIZATION: BRAZIL

TOXIN
LABORATÓRIO DE ENSINO E PESQUISA EM TOXICOLOGIA IN VITRO

DPRA: What to do with 10 mg of peptide?

Reducing cost of the assay and organic solvent waste

Micro-DPRA (mDPRA)

"minitualization" of the assay

10 to 20X

Volume of reaction: 2 mL

200 µL → 100 µL

SKIN SENSITIZATION: BRAZIL

mDPRA has the same prediction of DPRA

Table 4
In house mDPRA proficiency data.

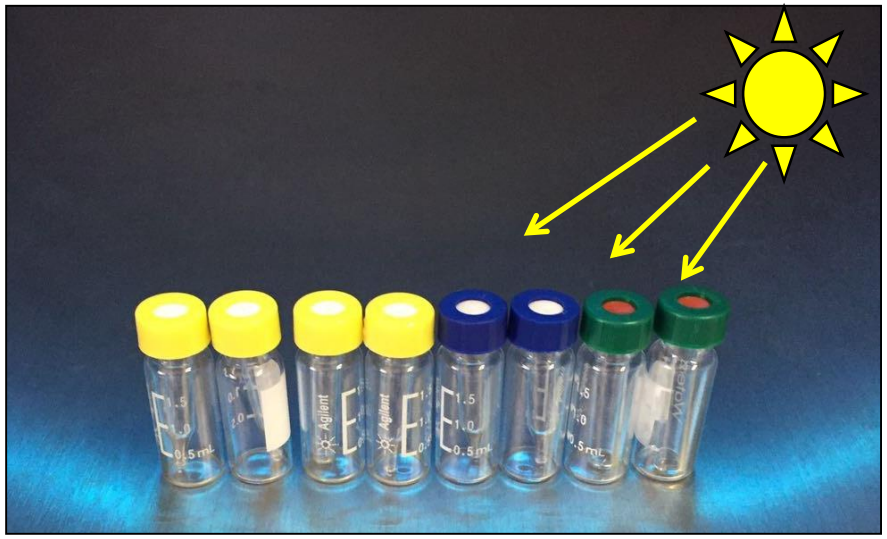
Proficiency substances (CAS number)	Classification		DPRA prediction	*In house* mDPRA proficiency			
	Animal	Human		Cys-peptide depletion	Lys-peptide depletion	Mean depletion	Prediction
Hexane (110-54-3)	NS[a]	NS[e]	−[h,i]	11.49 ± 1.22	0	5.74	−
Propylene glycol (57-55-6)	NS[a]	NS[e,*]	−[b,i]	0	0	0	−
N,N-Dimethylformamide (68-12-2)	NS[b]	S[f]	−[b]	0	0	0	−
Pyridin (110-86-1)	S[c]	S[g]	−[h]	0.29 ± 0.51	8.95 ± 5.24	4.62	−
Eugenol (97-53-0)	S[b]	S[e]	+[b,h]	49.95 ± 1.79	25.57 ± 0.71	37.76	+
2,4-Dinitrochlorobenzene (97-00-7)	S[d]	S[e]	+[b,d,h]	100 ± 0	24.08 ± 0.49	62.04	+
Oxazolone (15646-46-5)	S[d]	S[h]	+[b,d,h]	47.54 ± 3.80	42.80 ± 2.12	45.17	+

Based on mDPRA: Photoallergy assay

TOXIN
LABORATÓRIO DE ENSINO E PESQUISA EM TOXICOLOGIA IN VITRO

Substances potential photosensitizer,
Additional step to mDPRA, the UV exposure; thereby changing the reactivity class of each chemical in comparison to mDPR

Photo-mDPRA



Solar irradiantion simulator

Based on mDPRA: Photoallergy assay

Photosensitizer inducer greater peptide depletion using photo-mDPRA, after UV exposure; thereby changing the reactivity class of each chemical in comparison to mDPRA.

Table 5
Evaluation of skin sensitization and photosensitization of non-phototoxic and phototoxic chemicals using mDPRA and photo-mDPRA.

Chemical (CAS number)	Classification		mDPRA results				Photo-mDPRA results			
	Animal	Human	Cys-peptide depletion	Lys-peptide depletion	Mean depletion	Prediction (react. class)	Cys-peptide depletion	Lys-peptide depletion	Mean depletion	Prediction (react. class)
Non-phototoxic										
L-histidine (71-00-1)	NPho	NPho	0	7.88 ± 3.44	3.94	–	0	0	0	–
Hexachlorophene (70-30-4)	NPho[b]	Pho[c]	23.40 ± 3.45	36.54 ± 12.66	29.97	+ (moderate)	100 ± 0	14.11 ± 1.42	57.05	+ (high)
Chlorhexidine (55-56-1)	NA	Pho[d]	37.63 ± 0.54	0	18.82	+ (low)	44.83 ± 3.01	99.50 ± 0.87	72.17	+ (high)
Phototoxic										
Ketoprofen (22071-15-4)	Pho[a]	Pho[a]	60.27 ± 2.73	13.88 ± 3.59	37.08	+ (moderate)	100 ± 0	0	50.0	+ (high)
Protoporphyrin IX (50865-01-5)	Pho[e]	Pho[f]	47.21 ± 2.55	20.50 ± 4.33	33.85	+ (moderate)	96.18 ± 0.86	15.83 ± 0.66	56.01	+ (high)
Amiodarone HCL (19774-82-4)	Pho[g]	Pho[h]	19.37 ± 7.32	0	9.68	+ (low)	100 ± 0	6.50 ± 6.21	53.25	+ (high)

Based on mDPRA: Photoallergy assay

Limited information is currently available on the applicability of the test methods to multi-constituent substances/mixtures

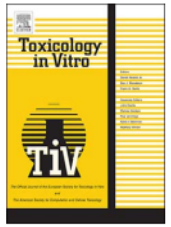
mDPRA + **Photo-mDPRA** ⇨ **photosensitizer**

One assay for two *end-points*: skin sensitization and photosensitization

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit



In vitro assessment of skin sensitization, photosensitization and phototoxicity potential of commercial glyphosate-containing formulations

Renato Ivan de Ávila[a], Gabriel Campos Teixeira[a], Danillo Fabrini Maciel Costa Veloso[b], Larissa Cleres Moreira[a], Eliana Martins Lima[b], Marize Campos Valadares[a,*]

[a] Laboratory of Celullar Toxicology and Pharmacology - FarmaTec, Faculty of Pharmacy, Federal University of Goiás, Goiânia, GO, Brazil
[b] Laboratory of Pharmaceutical Technology - FarmaTec, Faculty of Pharmacy, Federal University of Goiás, Goiânia, GO, Brazil

"Real-life" mixtures ?
Nanomaterials ?

DPRA is useful to categorize “real life” mixtures? formulations of herbicides???

mDPRA and photo-mDPRA glyphosate and formulation “real-life” → Herbicides

Table 6
Evaluation of skin sensitization and photosensitization of glyphosate, POEA and six glyphosate-based herbicides using mDPRA and photo-mDPRA.

Test product	Classification		mDPRA results				Photo-mDPRA results			
	Animal	Human	Cys-peptide depletion	Lys-peptide depletion	Mean depletion	Prediction (react. class)	Cys-peptide depletion	Lys-peptide depletion	Mean depletion	Prediction (react. Class)
Glyphosate	NS[a]/NA	NA/NA	0	3.5 ± 4.94	1.7	–	0	0	0	–
POEA	S[b]/NA	NS[c]/NA	0	0.18 ± 0.31	0.09	–	69.14 ± 3.81	6.19 ± 8.60	37.67	+ (moderate)
Herbicides										
Original Roundup	NS[d]/NA	NS[e]/NPho[e]	0	4.8 ± 8.29	2.4	–	0.21 ± 0.37	0	0.11	–
Glyphosate AKB 80	NA/NA	NA/NA	0	18.9 ± 8.14	9.4	+ (low)	24.96 ± 3.19	3.3 ± 2.85	14.12	+ (low)
Glyphosate Atanor 48	NA/NA	S[f]/Pho[f]	0	8.3 ± 8.26	4.2	–	6.21 ± 3.48	15.53 ± 2.12	10.87	+ (low)
Roundup Transorb R	NS[g]/NA	S[g]/Pho[g]	81.97 ± 0.69	0	41.0	+ (moderate)	81.90 ± 1.63	1.06 ± 1.83	41.48	+ (moderate)
Trop	NS[h]/NA	S[h]/Pho[h]	0	0	0	–	12.58 ± 2.40	16.62 ± 10.24	14.60	+ (low)
Zapp QI 620	S[i]/NA	S[i]/Pho[i]	0	2.2 ± 3.10	1.1	–	45.87 ± 3.08	0	22.94	+ (moderate)

"*in vitro* validated tests are useful to categorize nanomaterials ??? DPRA and U-SENS™

Pallardy, et., all, Frontiers in Immunology, 8, 2017

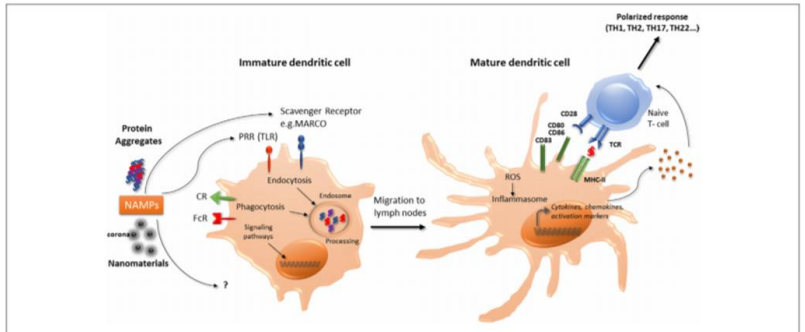


FIGURE 1 | Interaction of nanomaterials and aggregates with DCs. Nanomaterials and aggregates can be internalized by several receptors present at immature DCs membrane, either by endocytic or phagocytic pathways. Protein aggregates will then be processed by DCs, leading to peptide presentation associated with MHC class II molecules to naive T-lymphocytes. Both nanomaterials coated with a corona or protein aggregates may also be seen as NAMPs and interact with PRR. This interaction can act as a danger signal that induces a signaling cascade leading to the transcription of maturation genes. Mature DC will then be able to express co-stimulation molecules and to produce cytokines and chemokines that will trigger naïve T-cells activation and polarization. These products can also increase ROS production and initiate the inflammasome activation. CR, complement receptor; DCs, dendritic cells; FcR, immunoglobulin constant fragment receptor; MHC, major histocompatibility complex; NAMP, nanoparticles-associated molecular patterns; PRR, pattern recognition receptors; ROS, reactive oxygen species; Scavenger R, scavenger receptor; TLR, toll-like receptor.

Key event 1: DPRA

Test-product	% Depletion		%Mean LYS&CYS	Prediction
	LYS	CYS		
Fulerene	7.57 ± 0.33	57.57 ± 0.96	32.57	**+ (moderate)**
Titanium Dioxide	5.54 ± 0.22	50.55 ± 0.75	28.05	**+ (moderate)**
Carbon Nanotube	12.33 ± 0.20	38.94 ± 0.51	25.64	**+ (moderate)**

Key event 2: USENS™

Test product	USENS™ results		
	CV_{70} (µg/mL)	EC_{150} (µg/mL)	Prediction
Reference controls			
(-) Dimethyl sulfoxide	-	-	(-) NS
(-) Glycerol	> 200	ND	(-) NS
(+) Sodium dodecyl sulfate	111.0	53.90	(+)S
(+) 2,4-dinitrochlorobenzene	2.67	0.62	(+)S
(+) Eugenol	56.90	17.90	(+)S
Nanomaterials			
Fulerene	> 200	200.0	(+)S
Carbon Nanotube	> 200	150.0	(+)S

TOXIN
LABORATÓRIO DE ENSINO E PESQUISA EM TOXICOLOGIA IN VITRO

Harmonization of the International regulatory requirement

UFG
UNIVERSIDADE FEDERAL DE GOIÁS

Optimizing vitro Tests Implementation: SERIES OF TRAINING VIDEOS IN SKIN SENSITIZATION ASSAYS: English, Portuguese; Korean; Mandarin

HUMANE SOCIETY INTERNATIONAL

***In Chemico* Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA) – OECD 442C**

HUMAN TOXICOLOGY PROJECT CONSORTIUM

First video training: DPRA

SERIES OF SKIN SENSITIZATION TRAINING VIDEOS

FIRST VIDEO: Direct Peptide Reactivity Assay (DPRA) – OECD 442C

Obrigada!!!!!!!

TOXIN
LABORATÓRIO DE ENSINO E PESQUISA EM
TOXICOLOGIA IN VITRO