



CARDAM: The Centre for Advanced R&D on Alternative Methods

A new *expert platform on alternative toxicology testing* to meet the needs of industry and legislation

Bart De Wever, Ph D.
Director Business Development

Innovation for Sustainable Production - Bruges 22-25 April 2008



What is **CARDAM**:

An initiative funded by VITO, the Flemish institute for Technology and Research

Anticipate to the needs of industry for testing of chemicals and hazardous substances (e.g. REACH)





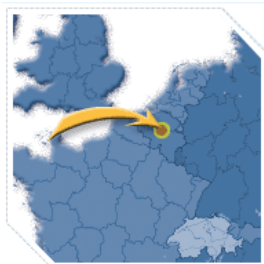
What is **CARDAM**:



: the Flemish institute for Technology and Research



- Autonomous public research company (100% Flemish Government)
- 500 employees, annual budget of 80 M €
- 7 centers of Expertise (animal alternatives since 1995)
- Bridge scientific knowledge between industry/government



Located 45 min. drive from Brussels



Mission of **CARDAM**:

To become a European *industrial* Center of Excellence on alternative methods

Combining unique expertise in

- research and development of new alternative methods
- validation of alternative methods
- *in vitro* contract research and testing services
- consulting and education on alternative approaches



Mission of **CARDAM**:

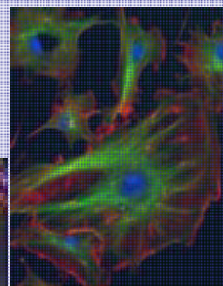
Population



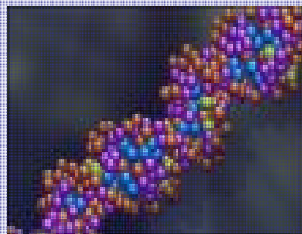
Individual



Cell



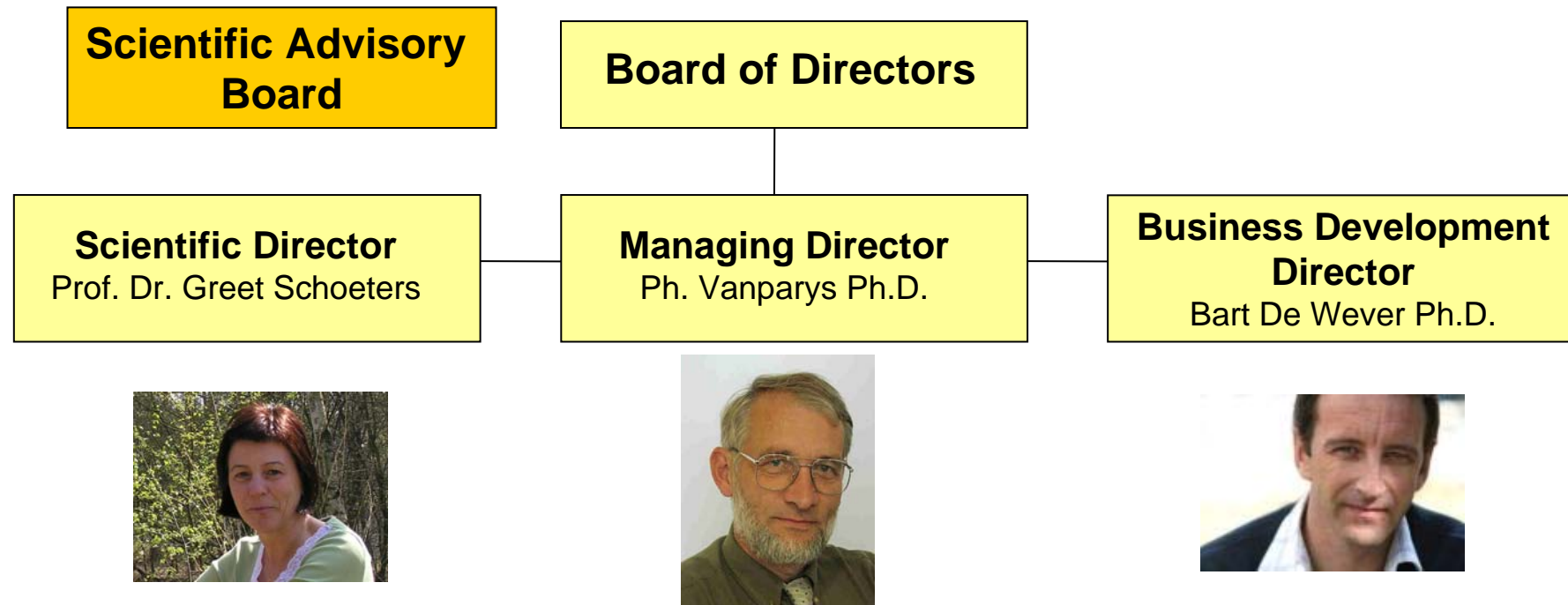
Molecule



Alternative test systems/3R's



Management of **CARDAM** :





Management of **CARDAM** :

Managing Director
Ph. Vanparys Ph.D.



- Biologist
- Senior Research Fellow at Johnson & Johnson Pharmaceutical Research & Development:
 - Head Genetic & In vitro Toxicology (1978-2005)
 - EU Head Mechanistic Toxicology (2005-March 2008)
- April 2008: CARDAM
- >15 years active in the field of alternatives
- Expert for ECVAM
- 27 years experience in GLP testing
- 2005: 3Rs Award “In Recognition Of Advancing The 3Rs” at Johnson & Johnson
- >50 publications in the field of genetic toxicology and alternative testing



Management of **CARDAM** :

Scientific Director
Prof. Greet Schoeters



- Biologist
- Research Unit Manager Environmental Toxicology VITO since 1993 – currently 40 people
- > 18 years experience in alternative test: development, validation and implementation
- Involved in risk assessment and environmental health impact assessment for various international and national organizations
- Member of board of ESTIV
- Professor at University of Antwerp, coordination of study direction Environment and Health- biomedical sciences
- >100 publications in the field of risk assessment and alternative testing



Management of **CARDAM** :

Business Development

B. De Wever Ph.D.



- Biochemical Engineer
- > 15 years experience in in vitro tissue model testing & Business Development in alternative methods
 - 5 years Janssen Research Foundation, Belgium
 - 4 years Advanced Tissue Sciences, USA
 - 7 years SkinEthic Laboratories, France
- ECVAM ESAC External Reviewer
- Business Development Board at Phenion, Germany
- Active member of the EPAA WG1
- COLIPA Eye Irritation Task Force member
- Executive Secretary of IVTIP
- >50 publications in the field of alternative testing



Operational Team of **CARDAM** :

Support by **21 scientific experts** in alternative toxicology testing

coordinate collaboration between authorities and industry to

- develop, validate and implement new alternative methods
- conduct regulatory & pre-clinical studies
- educate and train customers in alternative safety testing



Test facilities of **CARDAM** :

1200 m² of laboratory space:

- 7 cell and tissue culture labs equipped with biohazards, laminar flows, CO₂ incubators and chemical hoods
- Flow cytometer (Calibur), Luminex system-96well platform for protein measurements
- Scanning fluorescence microscope with image analysis and automated slide feeder for micronucleus and comet assay
- Agilent platform and Tecan HS400 hybridisation station for transcriptomics, 2 array scanners - real time PCR (i-Cycler Biorad)
- Inverted fluorescence microscope with time lapse for embryos and Ethovision software for eggs and larvae behavioural studies, in 96- or 24-well set-up and IR-camera
- ICP and HPLC for chemical analysis





Quality Assessment at **CARDAM** :

As part of VITO:

- Environmental Quality assurance – ISO 14001
- Quality assurance ISO 9001

• Specific for **CARDAM** :

- GLP-like for all routine testing and validation studies
- GCCP for all in vitro testing
- ***GLP accreditation expected end 2008***





Expertise of **CARDAM** :

Historical *in vitro* R&D & validation activities at VITO:

- R&D projects: hematotoxicity, bone cell differentiation, immune toxicity, skin and respiratory sensitization, estrogen/androgen aromatase disruption, genotoxicity, acute toxicity, teratogenesis, neuro-behaviour
- 3 ECVAM validation projects: CFU-GM, fish cell lines, estrogen activation assay
- 55 scientific *in vitro* technology papers since 2005



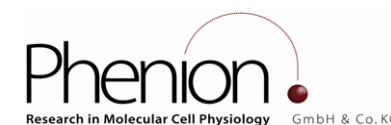


Scientific 'snapshot' at **CARDAM** :

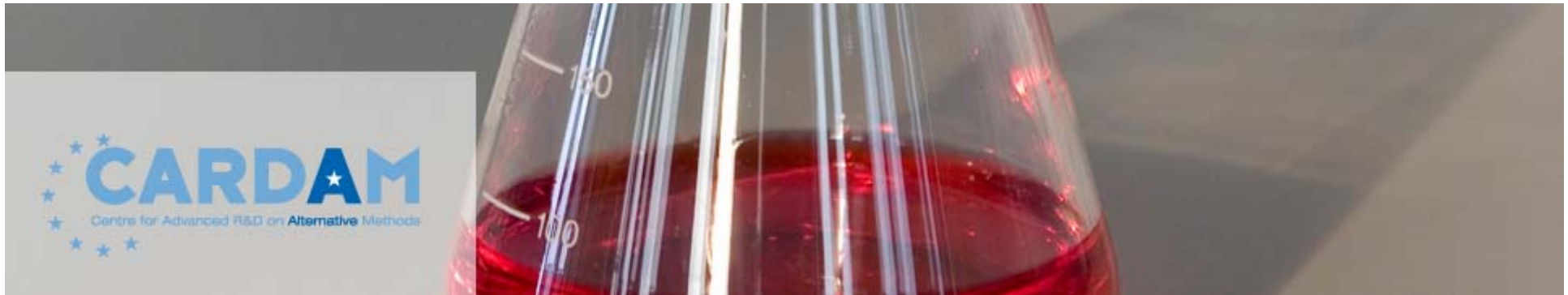
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- Schoeters E, Verheyen GR, Nelissen I, Van Rompay AR, Hooyberghs J, Van Den Heuvel RL, Witters H, Schoeters G, Van Tendeloo VF, Berneman ZN. **Microarray analyses in dendritic cells reveal potential biomarkers for chemical-induced skin sensitization**. Mol Immunol. 2007, 44 (12):3222-33
- E. Schoeters, J.M. Nuijten, R. Van Den Heuvel, I. Nelissen, H. Witters, G. Schoeters, V. Van Tendeloo, Z. Berneman, G. Verheyen, **Gene expression signatures in CD34+progenitor-derived dendritic cells exposed to the chemical contact allergen nickel sulfate**, Toxicology and Applied Pharmacology - 216: 131-149 (2006)
- E. Schoeters, G. Verheyen, R. Van Den Heuvel, I. Nelissen, H. Witters, V.F.I. Van Tendeloo, G. Schoeters, Z.N. Berneman. **Expression analysis of immune-related genes in CD34+ progenitor-derived dendritic cells after exposure to the chemical contact allergen DNCB**, Toxicology in vitro 19(7): 909-913 (2005)
- De Smedt A.C.A., Van Den Heuvel R.L., Van Tendeloo V.F.I., Berneman Z.N., Schoeters G.E.R. **Potency of CD34+progenitor derived dendritic cells to distinguish between sensitisers and irritants**. Toxicology Letters, 156, 3 (2005), 377-389
- G. Verheyen, E. Schoeters, J.M. Nuijten, R. Van Den Heuvel, I Nelissen, H. Witters, V. Van Tendeloo, Z. Berneman, G. Schoeters. **Cytokine transcript profiling in CD34+ progenitor derived dendritic cells exposed to contact allergens and irritants** . Toxicology Letters 155: 187-194 (2005)



Collaborating partners of **CARDAM** :



Innovation for Sustainable Production - Bruges 22-25 April 2008



Current *In vitro* testing services at **CARDAM** :

Genotoxicity

- Vitotox™ test
- Umu-C test (ISO 13829)
- Ames screen test
- Ames test (OECD 471)
- Comet test

Skin sensitization

- Vitosens™ test

Mucosal irritation

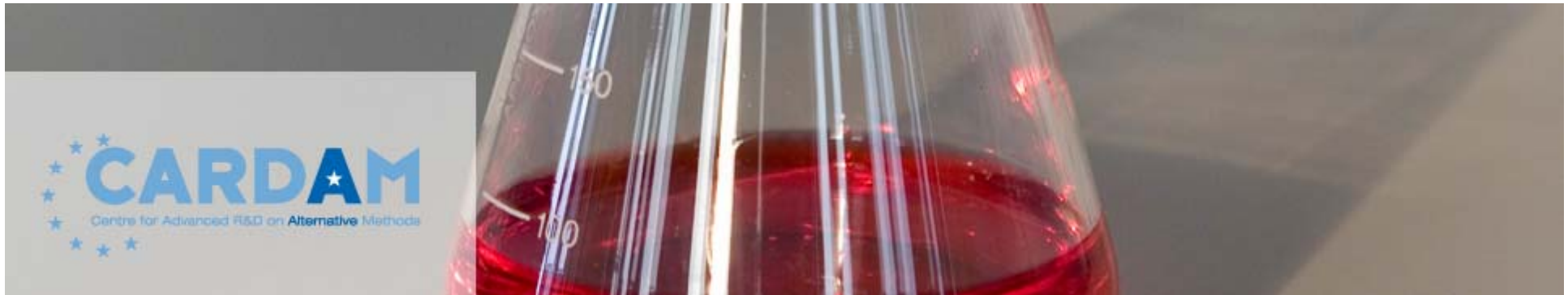
- SMI Slug mucosal irritation test (UGent)

Haematotoxicity

- CFU -GM test
- BFU-E
- CFU-MK

Acute toxicity

- Cell growth inhibition (3T3)
- CFU-GM



Current *In vitro* testing services at **CARDAM** :

Ecotoxicity

Aquatic tests

- Microtox test (OECD 201)
- Daphnia tests (OECD 202, 211)
- Fish tests (OECD 203, 204, 210)

Terrestrial tests

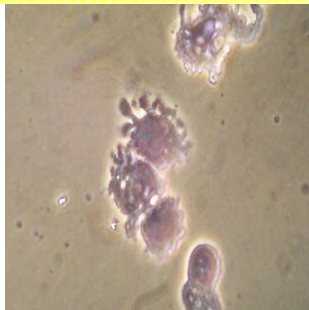
- Microtox-SP test
- Plant tests (OECD 208)
- Worm tests (OECD 207, 222)

Biodegradation tests (OECD 301, 302)



CARDAM VITASENS™

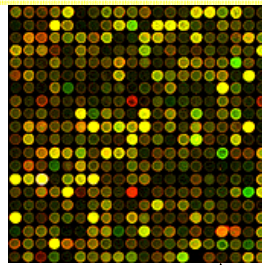
Exposure human dendritic cells



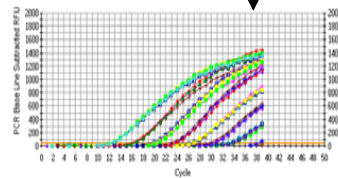
Sensitising chemicals

Non-sensitising chemicals

Gene expression profiles + confirmation

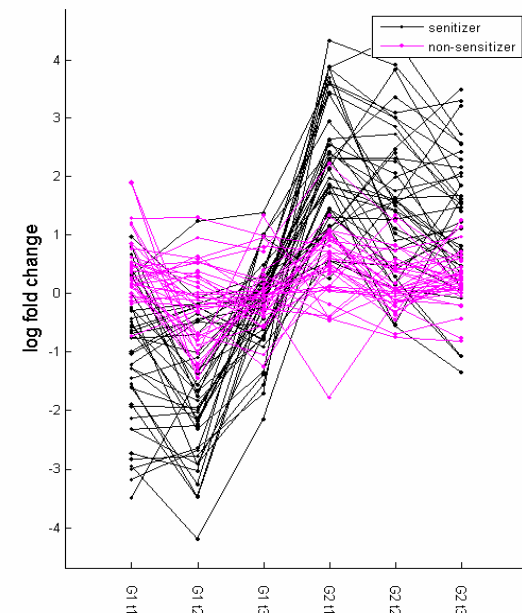


Microarray



Real-time RT-PCR:

**Classification model:
Discriminates for sensitising chemicals
by gene biomarkers**





CARDAM VITOUSENS™

21 compounds tested on 73 donor samples

	Predicted sensitizing	Predicted non-sensitizing	Total		
Sensitizing	32	7	39	Sensitivity = 32/39	82%
Non-sensitizing	1	33	34	Specificity = 33/34	97%
Total	33	40	73	Concordance = (32+33)/73	89%

The predictions are from a cross-validation, leaving out one compound. The contingency is presented on the level of number of donor samples.



Future *In vitro* testing services at **CARDAM** :

* **Skin corrosion**

- Corrositex™ test

* **Skin irritation**

- 3D-models (EPISKIN™, Phenion OS-REP)

* **Eye irritation**

- BCOP test
- PCOP test
- 3D HCE-models (SkinEthic/MatTek)

* **Phototoxicity**

- 3T3 phototox test

Genotoxicity

- In vitro micronucleus test

Developmental and neural toxicity

- Zebrafish test (larvae < 7 days)

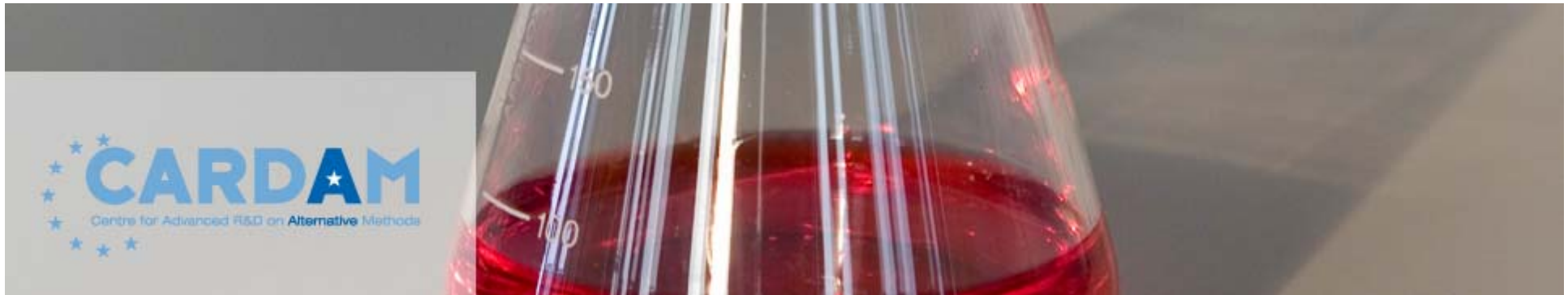
Hepatotoxicity

- Zebrafish test (larvae < 7 days)

Acute toxicity

- CFUM test

Available by fall 2008



Current R&D programs at **CARDAM** :

- Zebrafish model for
 - teratogenesis
 - neurotoxicity
 - liver toxicity
- Respiratory sensitization and toxicity
- Hormone transactivation assays

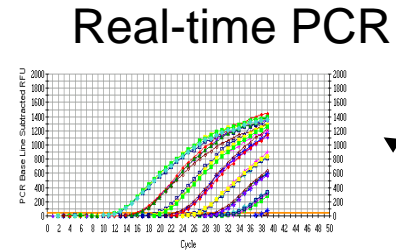




Current R&D programs at **CARDAM** :

Allergy (skin/lung)

Alternative model
(eg. Cell cultures,
zebrafish embryo)

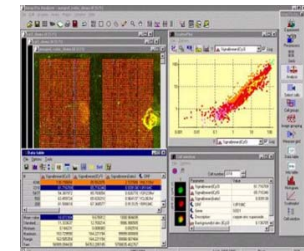


Bioinformatics

Exposure of
chemicals,
nanoparticles,..



Micro-arrays



Neuro-development



Become an active promoter of **CARDAM** :

For the development of alternative methods:

- Sponsor unspecified 3R activities
- Sponsor specified activities in function of your needs
 - regulatory
 - validation
 - screening



Contact **CARDAM** :

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Thank you.