Endocrine active nutrients explored in human bone cell cultures

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Overview

DSM Family - DSM Nutritional Products

Endocrine active substances - in Nutrition

Bone Cell Culture Models

Endocrine Disruptor Consideration

Conclusion
DSMs global presence

2011

World total: 200+ locations, 22,000 employees

North America:
33 locations
4,000 employees

Europe:
64 locations
13,000 employees

China:
31 locations
3,000 employees

India:
8 locations
700 employees

Latin America:
15 locations
1,000 employees

2011 World total: 200+ locations, 22,000 employees
DSM Nutrition at a glance 2011

- Net sales € 3,370m
- EBITDA € 735m
- EBITDA margin 21.8%
- R&D € 200m
- Workforce 8,329
Endocrine active substances in nutrition

In Foods or supplements

- Vitamins
- Carotenoids
- Nutraceuticals
## Nutraceuticals vs Endocrine disruptors

<table>
<thead>
<tr>
<th>Nutraceutical</th>
<th>Endocrine disruptor</th>
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<tbody>
<tr>
<td>• Natural substances from foods that provide physiological health benefits or provide protection against chronic diseases</td>
<td>• Exogenous substances altering function(s) of the endocrine system to cause adverse health effects in an intact organism, or its progeny, or (sub)populations</td>
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<tr>
<td>• Nutritional origin</td>
<td>• Non-nutritional origin</td>
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<tr>
<td>• Molecular effects supportive</td>
<td>• Molecular effects disruptive</td>
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Animal welfare - Ethical commitments

- Reduction of the number of animals tested
- Refinement of the methodology
- Replacement of the animal model
Example: Bone biology
Endocrine active substances (EASs)

• Bone remodeling is a balanced coupling of bone formation and resorption

• Two different cell types play a role: Osteoblasts and Osteoclasts

Source: http://www.bonekey-ibms.org/
Human primary bone cultures
*In vitro* bone models (osteoblasts)

1. **Mesenchymal Stem Cells (BMSC)** isolated from bone marrow, differentiation through Osteostimulation

2. **Primary Osteoblast Cells (pOB)** collagenase-digest of human trabecular bone

3. **Primary Periost Cells** collagenase-digest of periostal layer
Human primary bone culture
Primary osteoblasts

pOB cell culture model

For reproducible results

- Characterize pOB batch using gene expression fingerprint (profiling)

- Perform analysis with 4-6 passages after the harvest of the primary cell culture
pOB Affymetrix GeneChip Array Analysis
Functional expression analysis in primary human osteoblasts

Genistein and the endogenous hormone 17beta-estradiol have identical gene regulation pattern on selected bone marker genes.
DNP Strategy on nutraceutical safety
Toxicology and ADME

- Literature assessment

- *In silico* assessment
  - Toxicity alerts (DEREK Software)
  - Metabolism (METEOR Software)

- Screens
  - Genotoxicity (*in vitro* micronucleus test)
  - Additional investigations as necessary (e.g. nuclear receptor test battery)

- Regulatory safety package
Non-mandatory DNP screening battery for endocrine disruptors

Case-by-case decision depending on:
I. Structural alert
II. Systemic exposure
III. Results of available *in vivo* toxicological information
IV. Regulatory environment
V. Customer expectation

- *In vitro* (with parent and possibly metabolites)
  - ER transactivation assay
  - AR transactivation assay
  - Steroidogenesis assay
  - Aromatase assay
- *In vivo*
  - uterotropic and Hershberger assay (if positive *in vitro*)
Conclusions

Primary bone cell cultures
- Different human primary bone cell culture models available for bone biology (for efficacy testing)
- Certain vitamins, nutraceuticals function through nuclear receptors also in bone tissue (along the endocrine axis)
- Limitation of primary cell culture model for biological questions:
  - No systemic approach
  - Knowledge on pathways important
  - Comparative analysis using controls only: targeted approach

Safety assessment of endocrine disruption (ED) in bone cells
- ED analysis using primary bone cells requests mechanistic knowledge on detrimental homeostasis disruption
  - Whole transcriptome analysis possible (Affymetrix or Illumina), but will require validation
- DNP Safety evaluation approach, OECD recommendation
Thank you for your attention

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