

esnats aims at developing a novel toxicity test platform based on embryonic stem cells (ESCs), in particular human ESCs (hESCs), to streamline the drug development R&D process and evaluation of drug toxicity in clinical studies, reduce related costs and propose a powerful alternative to animal tests. This European collaborative research project gathers 30 leading European researchers in the fields of alternative testing, toxicology, ESC research, genomics, modelling and automation.

Why ESCs?

ESNATS will develop a novel testing system taking advantage of the unique potential of ESCs, including:

- their capacity to self renew
- their pluripotency
- the physiological relevance of ESC-derived somatic cells for toxicity endpoints, at least for murine ESCs
- their easy genetic manipulation.

Research areas

ESNATS is divided into four main research areas, each one representing a sub-project (SP).

- **SP1 Reproductive Toxicity** will focus on the influence of compounds on fertilisation, differentiation into gametes (male fertility), (embryonic) development; teratogenicity (functionality)
- **SP2 Neurotoxicity** will evaluate the effect of compounds on neuronal development and neuronal viability (functionality)
- **SP3 ESC-based toxicogenomics** and toxicoproteomics signatures will focus on the influence of compounds on gene expression and proteomics.

- **SP4 Toxicokinetics, metabolism and modelling** will focus on the usage of *in vitro* physicochemical and ADME data for establishing physiologically based pharmacokinetic (PBPK) models (functionality). Computer-based modelling will be used for *in silico* studies of pharmacokinetics. Special emphasis will be given to the liver metabolism.

These SPs are complemented by three central work packages (cWPs):

- cWP01: Steering Committee
- cWP02: Knowledge management, validation and testing strategies
- cWP03: Methods for automating scale up

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2008-2013

Main Results after year 1

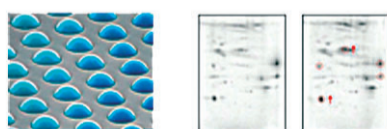
Initiation of the set-up of *in vitro* toxicology systems: Cell culture systems were developed for the use of ESCs in reproductive, developmental and neural toxicity testing, including the differentiation of stem cells into a wide range of cells and tissues, such as neural cell types and engineered neural tissues, cardiomyocytes and spermatocytes (using mouse ES cells). Culture conditions of primary mouse and human hepatocytes were optimised to be able to integrate them as metabolising systems into the testing strategies with ESC derived cells.

Definition of substances to be tested: To develop test systems, it is necessary to use well characterised reference substances with known modes of action. With the help of industrial partners, an initial list of reference substances was therefore established for the development of the ESNATS test systems. Furthermore, industrial and regulatory specifications for assay development were provided.

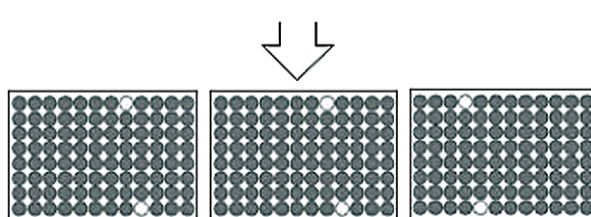
Specification of the requirements for automating the scale-up of stem cell production: In order to ensure later industrial use of the ESNATS test systems, the scale-up of stem cell culture needs to be automated. In the first year, the requirements for automation were specified.

Embryonic Stem cell-based Novel Alternative Testing Strategies

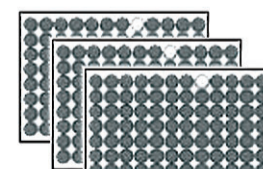
Toxicogenomics, Phosphoproteomics (SP3)



Correlation and identification of markers



- Assay development for Reproductive toxicity (SP1), Neurotoxicity (SP2), ESC-based toxicogenomics/proteomics (SP3), Toxicokinetics, metabolism and modelling (SP4)
- Statistical evaluation of individual assays (cWP02)



Integrated testing strategies and evaluation of strategies (proof of concept study, uncertainty testing) (All SPs and cWPs)



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