

## Project Progress Summary

<b>Section 1. Project Identification</b>		<b>Not Confidential</b>
<b>Title of the Project</b> Protection of the European population from aneugenic chemicals		
<b>Acronym of the Project</b> PEPFAC		
<b>Type of Contract</b> Shared cost research project		<b>Total Project Costs</b> €1.456.693
<b>Contract Number</b> QLK4-CT-2000-00058	<b>Duration</b> 44 months	<b>EU Contribution</b> €1.013.438
<b>Commencement date</b> 1-5-2001		<b>Period covered by the progress report</b> 1-5-2001 to 1-2-2005
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## Objectives of the Project

A key aim of the PEPFAC project was to make use of a complementary methodologies developed by the collaborators to characterise the mechanisms of action of aneugenic chemicals. These methods would be used to produce detailed dose-response data for aneugenic chemicals in both somatic and germ cells suitable for use in regulation and risk assessment. Data would be generated for spindle modifying aneugens which could be predicted to show thresholds of genotoxic activity because of their multiple targets for interaction in contrast to clastogens (chromosome damaging), which could be predicted to have only one or two targets for toxin inactivation or modification. Comparisons would also made with aneugens which modified the fidelity of chromosome segregation by mechanisms other than spindle inhibition. The study would determine the contribution of factors such as apoptosis, cell cycle checkpoint genes, target cell age and culture progression, type of cell division, i.e. mitosis or meiosis, and target tissue to the shapes of the dose response curves and potential thresholds of activity.

Currently, the assessment of the potential genotoxicity of chemicals is based upon the use of *in vitro* screening and detection methods supported by *in vivo* methods to confirm or not whether activity detected in simple test systems such as cultured mammalian cells is reproduced in intact animals. PEPFAC collaborators planned to determine the relative requirements for *in vitro* and *in vivo* methods in the assessment of aneugenic chemicals, and parameters such as the relative sensitivities of somatic versus germ cells and male versus females. The data from the project would be evaluated for its ability to generate a hazard and risk-estimation model for aneugenic chemicals and integrate these models into the regulatory process.

## Results and Milestones

During the project the collaborators have made a number of important developments and discoveries:

- 1) The development and validation of *in vitro* methods to identify the induction of chromosome loss and non-disjunction by aneugenic chemicals. The methodology was used to identify and characterise the aneugenic activity of a wide range of chemicals including both natural and synthetic hormones, xenobiotic metabolites, industrial chemicals and pharmaceuticals.
- 2) The *in vitro* methods were used to characterise the mechanisms of action of individual aneugenic chemicals and produce detailed dose responses data which are suitable for hazard and risk assessments. These included the plastics component bisphenol-A and 17 $\beta$ -oestradiol which were further investigated in the higher order test systems
- 3) The influence of apoptosis upon the formation of micronuclei and the quantification of aneuploidy induction was determined. These studies have demonstrated the role of caspase-3 in the formation of micronuclei and of caspases -8 and -9 in the elimination of micronuclei by apoptosis. These studies indicate that the understanding of the influence of apoptosis will be important in the estimation of the hazard and risk assessment of individual aneugenic chemicals.
- 4) The numerical chromosome changes which occur in oesophageal and gastric cancers were characterised demonstrating that specific tumour types and stages in progression are associated with the aneuploidy of specific chromosomes. These specific aneuploidies have the potential to provide markers of exposure and early indicators of tumour formation. It was shown that the p53 tumour suppressor gene plays a major role in reducing the levels of chromosome instability and subsequent aneuploidy in tumour progression.
- 5) New protocols were developed for studying the induction of toxicity and micronucleus induction in rodent bone marrow and lymphocytes measured *ex vivo* and *in vitro* following aneugen treatment. The studies consistently demonstrated the higher sensitivity of the bone marrow assay. The mouse gut assay for the analysis of induced aneuploidy in

gastrointestinal cells was optimised and shown to be valuable for the assessment of the relative sensitivity of tissues.

- 6) The published data on induced aneuploidy collected in the database provided little evidence of differences in the relative sensitivity of aneugens in different somatic and/or germ cells. However, the more sophisticated methods developed by the collaborators are better able to reveal any differences in aneugen potency. Germ cell studies were combined with bone marrow micronucleus studies to enable comparative analyses to be performed with chemical that generate aneuploidy by a range of different mechanisms. These studies demonstrated that:
  - a. The topoisomerase inhibitor amsacrine induced both hyperploid and diploid sperm but there was no evidence of the induction of somatic changes in bone marrow at equivalent concentrations.
  - b. The tubulin inhibitor nocodazole induced hyperploid sperm but no induction of micronuclei in bone marrow at equivalent concentrations.
  - c. Vanadium salts which inhibit a range of components of the cell division cycle induced hyperhaploid sperm but did not induce micronuclei in rodent bone marrow.

These studies indicate that the relative potency of activity of aneugens in somatic and/or germ cells can only be definitively determined by comparing tissue activities in the same animals and similar exposure conditions.

- 7) Significant progress was made in developing and optimising methods for the analysis of the activity of aneugens female germ cells and its transmission to mouse embryos. These methods were based upon the use of fluorescence *in-situ* hybridisation (FISH} techniques primarily using probes for mouse chromosomes 15 and 16.
- 8) In mouse oocytes there were technical developments which enabled the detection and quantification of the activity of aneugens and provided mechanistic information. which include:

- a. The application of Polscope microscopy as a non-invasive methodology to analyse changes in spindle morphology and size.
- b. A methodology using preantral follicle culture, growth and maturation *in vitro* which allows a major extension in the period over which the consequences of chemical exposure may be analysed.

These methods were used to analyse the mechanisms of action of nocodazole, trichlorofon, diazepam and the hormone metabolite 2-methoxyestradiol.

- 9) During the initial stages of the project a publication appeared which appeared to indicate that the plastic component bisphenol-A was capable of inducing aneuploidy in germ cells specifically mouse oocyte at extremely low exposure concentrations. In view of the potential human exposure to bisphenol-A the collaborators decided to apply their expertise and methods to the comprehensive analysis of the potential aneugenic activity this chemical. The methods used by the collaborators ranged from the study of micronuclei induction in cultured mammalian cell, *in vivo* rodent somatic tissues and male and female germ cells. The studies failed to confirm the reported potency of bisphenol A in oocytes.

## **Benefits and Beneficiaries**

The Project has provided the EU and the international community with a battery of methods capable of detecting and assessing the biological and health significance of aneugenic chemicals. These methods demonstrated that current levels of exposure to bisphenol A did not represent a significant health risk to the European population. The studies also revealed that a range of natural and synthetic hormones were capable of inducing aneuploidy. Such observations may prove of value in future assessments of the potential health impacts of endocrine disrupting chemicals. A range of aneugenic chemicals was shown in the *in vitro* models to have thresholds of activity at low doses. However, current *in vivo* methods are insufficiently sensitive to provide reliable data in the low dose regions. When applied to the analysis of the karyotype of tumour cells the methods provide some evidence that aneuploidy induction may be a useful biomarker of stages of progression.