GENOTYPIC AND PHENOTYPIC VARIABILITY, INDIVIDUAL SUSCEPTIBILITY FACTORS AND INDUSTRIAL GENOTOXICANTS/NEUROTOXICANTS IN OCCUPATIONAL MEDICINE

M. Kirsch-Volders (coördinator) - VUB
H. Veulemans - KUL
H.Thierens - RUG
L. de Ridder - RUG
D. Lison - UCL
P. Vielle - UCL
C. Laurent - ULg

Promotor: Prof. Dr. M. Kirsch-Volders

Scientific collaborators: A. Tremp
M. De Boeck

Free University Brussels
Faculty of Science
Department of Biology
Laboratory of Cellular Genetics

I. INTRODUCTION

I.1. Context and general frame of the research

The *in vivo* effect of a given toxic agent on the cellular machinery and its consequence on human health are dependent on many interactions between the agent and the different levels of physiology. Before a chemical toxicant reaches its final target(s), its systemic concentration can be modulated by varying absorption rates and its chemical structure can be modified by activation or detoxification enzymes. Physical mutagens/carcinogens can be absorbed differentially by the tissues separating the target from the surface of the body. Once the agent reaches its target(s) the lesion induced can be directly fixed, repaired or transformed into a mutation or a functional deficiency. Alternatively the toxicant can induce necrosis or the damaged cells can be blocked at a given stage of the cell cycle and triggered to apoptosis. Most of the genes coding for the proteins responsible for the above-described mechanisms show inter-individual variability.
Variability occurs at the genetic level (GENOTYPIC VARIATIONS): individuals carrying one or more alleles leading to a stronger effect of the toxicant, for instance by better activation, less efficient repair capacity and/or absence of checkpoint control will conceivably be more susceptible for the disease. Genes may be absent, as in individuals homozygous for the non-functional allele of glutathione transferase classes, or enzyme activity can differ across the population by several orders of magnitude, as with cytochrome P4502D6 (Wormhoudt et al., 1999).
In addition to genetic polymorphisms, the expression of many of these genes is also variably regulated according to environmental factors (PHENOTYPIC VARIATIONS): diet, occupational exposure, life style factors, smoking habits, alcohol consumption, drugs, fasting, … stimulate or inhibit the expression of these genes. Induction of CYP2E1 activity upon chronic consumption of ethanol is for instance expected to increase the activation of several toxicants (Girre et al., 1994).

Besides disease susceptibility, variability factors are also important for the interpretation of several biological tests used in occupational medicine to assess the intensity of exposure, and hence the risk of occupational disease. So far, biological monitoring methods and biological limit values applied in occupational medicine have been developed on the assumption that individuals do not differ significantly in their biotransformation capacities, which is not the case. Integration of a measurement of variability in a biological monitoring programme could represent a significant refinement of the method and hence a better assessment of the risk of occupational disease.

The fascinating progress made in genome analysis and the identification of several classes of genes involved in disease susceptibility (e.g. cancer, neurological disorders, pulmonary diseases and reproductive/developmental defects) point now to the possibility of identifying carriers of specific allelic combinations or polymorphisms related to an increased risk for occupationally induced illnesses. For instance the estimation of cancer risk might thereby progress from a probability calculated for a given population exposed to a known agent to a more individualised health counselling. For example, in low dose exposure to ionising radiation individual radiosensitivity might be more important than the dose itself with respect to cancer risk. Potential applications of variability parameters for exposure biomonitoring have so far received much less attention.

This potentially new approach in occupational health practice, called susceptibility analysis, might very fruitfully complement the existing biomonitoring approaches. However, the technical capacity to perform such tests and the reliability of these tests in terms of public health significance has to be examined. The possible implementation of such tests in occupational medicine also poses major societal and ethical questions, which need adequate answers.

I.2. Aims of the research

The project aimed at combining susceptibility biomarkers with biomarkers of exposure and of effect that are sensitive, specific and predictive enough to make reliable measurements.

The specific aims for the VUB were:

1. Validation of the techniques for:

    1.1. The genotyping of DNA repair genes (hOgg1, XRCC1, XRCC3) in collaboration with the UCL.

    1.2. The DNA strand break repair phenotype assay using the comet assay for the assessment of the speed and efficiency of in vitro DNA repair after challenging with a reference mutagen (styrene oxide, ionizing radiation) of cultivated cells derived from several donors. Differences in *in vitro* repair could be an indication for individual susceptibility after chronic exposure.

2. Mechanistic research on the genotoxic effects of hard metal dust (WC-Co) with the aim of choosing adequate biomarkers

3. Predictivity of genotyping (in particular of genes involved in DNA repair) en DNA strand break repair phenotype for genotoxic effects in biomonitoring of occupational exposure to mutagens.

    3.1 Integration of susceptibility parameters, in addition to the classical exposure and effect parameters, for the assessment of the genotoxic risk due to exposure to styrene, by means of a biomonitoring study (in collaboration with the KUL and UCL).
    3.2 Integration of susceptibility parameters, in addition to the classical exposure and effect parameters, for the assessment of the genotoxic risk due to exposure to cobalt, by means of a biomonitoring study (in collaboration with the UCL).
    3.3 Integration of susceptibility parameters, in addition to the classical exposure and effect parameters, for the assessment of the genotoxic risk due to exposure to ionizing radiation, by means of a biomonitoring study (in collaboration with the RUG).

4. Exploratory approach of the micro-array technology for the detection of gene expression of relevant genes after occupational exposure, in collaboration with the UCL.

5. Study of the ethical and legal aspects of the use of susceptibility tests in occupational medicine (in collaboration with the legists of the UCL).

II. THEORETICAL FRAME

Why genotyping and phenotyping?

Genetic polymorphisms provide us with the ability to study interindividual differences in susceptibility to exposure and diseases, to analyse whether the risk of cancer associated with particular environmental exposure differs with respect to functionally different polymorphisms of genes. Biomarkers of susceptibility include polymorphisms in drug/carcinogen metabolism, in DNA repair capacity, and in genes that control cell cycle, cell death and immune response. The identification of susceptibility genes could therefore lead to possible prevention programmes directed to high-risk individuals. In particular from the theoretical point of view it is expected that the variation in individual responses to a same concentration of genotoxicants will be larger at low concentrations than at higher concentrations (saturation effect). Therefore the assessment of genetic susceptibility may be quite important to define exposure limits (safety factor) at concentrations encountered in occupational exposure.
In table 1 an overview of the advantages and disadvantages of genotyping versus phenotyping is given. Genotyping has the advantage to be technically easy and inexpensive, not influenced by the environment, giving definite answers but is more distal to the disease. Phenotyping is more proximal to the disease and integrating effects of several genes, including environmental factors, however the methodologies are not yet fully validated and tend to be expensive. Examples of the latter include the in *vitro* challenging assays and microarrays (**Touil et al., 2002**; Ahsan and Rundle, 2003).
Thus, both genotyping and phenotyping are important, which is why we combined both of them in this project.

Table 1: Some advantages and disadvantages of genotyping versus phenotyping (**Decordier et al., 2004**)

	Genetic polymorphisms	Functional/phenotype measures
- Proximity to disease on causal chain	More distal	More proximal
- Expected relative strength of association	Weaker	Stronger
- Inductive or inhibitory effects of exposures	Not integrated into the measure	Integrated into the measure
- Epigenetic processes	Not integrated into the measure	Integrated into the measure
- Effects of post-transcriptional/translational Alterations	Not integrated into the measure	Integrated into the measure
- Relative degree of measurement error	Lower	Higher
- Measures single gene processes	Yes	Yes
- Measures multi-gene processes	Yes, but with difficulty	Yes
- Temporal stability of measure	Stable	Less stable
- Modifiability and applicability for Prevention trials	Not modifiable and not applicable	Modifiable and applicable
- Person-to-person transmission within family and applicability for family-based analysis	Transmission is determined by Mendel's law and is applicable	Less deterministic transmission and not applicable

- Ethical considerations	Higher	Lower
- Array-based simultaneous measurement	Challenging, because amplification	More feasible, because of many genes/gene products of each gene is involved individual implification is not involved

DNA repair genes

When a cell undergoes genotoxic damage, multiple outcomes are possible: if the damage is severe, the cell may die (by apoptosis or necrosis), the damage can be completely repaired, or the damage repair may be imperfect/absent, leading to the formation of mutations that are fixed during replication and/or the following mitosis. There are several DNA repair mechanisms: base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), and double strand break repair (DSBR).

The human Ogg1 gene is localized on chromosome 3p25, and encodes a DNA glycosylase that excises 8-hydroxyguanine (8-OH-G) from oxidatively damaged DNA through BER. 8-OH-G is a major form of oxidative DNA damage induced by reactive free radicals. It is highly mutagenic *in vitro* and *in vivo*, yielding G:C to T:A transversions, since it directs the incorporation of cytosine and adenine nucleotides opposite the lesion. Polymorphisms in the hOgg1 gene play a possible role in the ability of the cell to repair 8-hydroxyguanine.

XRCC1 plays an important role in BER. It is located on chromosome 19q13.2. The XRCC1 protein complexes with DNA ligase III and DNA polymerase ß to repair gaps left during BER. XRCC1 mutant cells show a heightened sensitivity to ionising radiation, UV, hydrogen peroxide and mytomycin C. Shen et al. identified three coding polymorphisms in the XRCC1 gene at codons 194 (Arg to Trp), 280 (Arg to His) and 399 (Arg to Gln) (Shen et al., 1998).

XRCC3 is located on chromosome 14q32.3, and is a member of the Rad51 DNA-repair gene family. It functions in the homologous recombination repair pathway by repairing double-strand breaks (DSBR). XRCC3 mutant cells show an extreme sensitivity to DNA cross-linking agents like MMC and cisplatin.

Our VUB laboratory was responsible for the validation and application of the genotyping of the hOgg1, XRCC1 and XRCC3 repair genes, and performed the global repair phenotype assay for these genes in the styrene and ionising radiation biomonitoring studies.

Cobalt and its compounds

Exposure to cobalt can result in adverse health effects in different organs or tissues, including the respiratory tract, the skin, the hemapoietic tissues, the myocardium or the thyroid gland. In addition, teratogenic and carcinogenic effects have been observed in experimental systems and/or in humans.
For the general population, the diet is the main source of exposure to cobalt, since cobalt is an essential constituent of vitamin B_{12} (hydroxycobalamin).

The carcinogenic potential of cobalt and its compounds was evaluated by the International Agency for Research on Cancer (IARC) in 1991 (IARC, 1991), the general conclusion was that cobalt and its compounds are possibly carcinogenic to humans (group 2B).

Occupational exposure to cobalt-containing dust has been associated with pulmonary toxicity including asthmatic reactions, fibrosing alveolitis (hard metal disease) and lung cancer. While the asthmatic responses are caused by any cobalt species, the development of cancer and fibrosing alveolitis is mainly ascribed to the simultaneous exposure to cobalt and tungsten carbide particles in workers of the hard metal industry (Lison, 1996; Moulin et al., 1998) and in diamond polishers (Nemery, 1990). Hard metals generally consist of a mixture of tungsten carbide (WC) and metallic cobalt (Co) particles (96:4) and are produced for their extreme hardness and high resistance properties. The exact mechanisms underlying the human carcinogenicity of hard metal dust still needed to be elucidated, but simultaneous exposure to cobalt and tungsten carbide seemed to be required. The demonstration that hard metals produce large quantities of active oxygen species, directly and indirectly through the inflammatory reaction that they induce in the lung and, in parallel, release cobalt ions may provide important mechanistic bases for explaining their human carcinogenicity (Lison et al., 1995). Indeed, oxygen free radicals are highly reactive and able to alter the integrity of lipids, proteins and DNA (Halliwel & Gutteridge, 1999). In addition, cobalt ions may readily bind to proteins involved in essential cellular processes such as DNA repair, cell cycle control and apoptosis (Hartwig, 2001). Considering genotoxicity, it was previously demonstrated that the WC-Co particle mixture induces more DNA damage and chromosome/genome mutations in human lymphocytes *in vitro* than its individual components (Anard et al., 1997; Van Goethem et al., 1997). It was shown that Co(II) can inhibit the incision and polymerisation step of nucleotide excision repair (Kasten et al., 1997), which would indicate a co-mutagenic potential of the element. No animal data on *in vivo* mutagenicity and/or carcinogenicity of hard metals are available.

The objective of part of this study was to investigate the possible underlying (geno)toxic mechanisms of the carcinogenic potential of the WC-Co mixture. The relationship between induction of initial DNA damage, mutations and cell survival after exposure to hard metal in comparison to its individual components was evaluated, both *in vitro* and *in vivo*.

III. METHODS

III.1. Validation of methods

Genotyping of repair genes

hOgg1

The techniques for the determining of the genotypes of DNA repair genes were developed at the UCL. Gina Plas, connected to the VUB, learned this technique at the UCL.

A C-G mutation in exon 7 in 40% of the alleles of hOgg1 modifies the wild type gene product, changing a serine into a cysteine. This Ser/Cys, and especially Cys/Cys genotypes would supposedly be more sensitive for oxidative damage.

For the detection of the polymorphisms, we use the "CAPS" technique (Cleaved Amplified Polymorphic Sequences). The first important step is the choice of restriction enzyme (in the case of hOgg1, the enzyme Fnu4HJ recognizes the GC↓CGC sequence in the Cys variant) and primers (20 to 30 bases long) on both sides of the restriction polymorphism. The concerned DNA fragment is amplified through PCR (polymerase chain reaction, yielding a 234 bp product), then incubated with the restriction enzyme, followed by electrophoresis on an agarose gel. The detection of the obtained fragments is done through staining of the DNA with ethidium bromide. The number and position of bands on the gel allows us to determine the genotype; in the case of hOgg1, the Cys variant is cut by the Fnu4HI enzyme (164, 46 and 21 bp fragments), while the Cys variant is not (213 and 21 bp fragments).

XRCC1 and XRCC3

For the detection of XRCC1 and XRCC3 polymorphisms it was decided, with an eye on a uniform way of working within the EU project (Cancer Risk Biomarkers QLRT-2000-00628), to adopt the protocols of Prof. A. Hirvonen (FIOH, Helsinki). These protocols are a modification of the method of Lunn et al. (1999). 2 multiplex PCR reactions are used, one detecting the polymorphisms of codons 194 and 399 of XRCC1, the other of codon 280 of XRCC1 and codon 241 of XRCC3.

For the first analysis (XRCC1-194 and –399), after a Hot Start PCR, MspI is used for the digestion. This enzyme shows, in addition to an unchanged band of 174 bp, digested bands of 21 and 292 bp; 21, 292 and 313 bp; and 313 bp for the Arg/Arg, Arg/Trp and Trp/Trp polymorphisms of codon 194 respectively. For codon 399, a difference can be made between the non-digested band of 615 bp of the Gln allele, and the 221 and 374 bp digested bands of the Arg allele. For the analysis of the XRCC1-280 and XRCC3-241 polymorphisms, the digestion (after a Hot Star PCR) was performed with RsaI and NlaIII. A 140 bp digestion product is found for the XRCC1 Arg allele, and a 280 bp one for the His allele. Fragments for 335 and 233 bp are found for the XRCC3 Thre an Met alleles respectively. All samples also show an unchanged band of 102 bp NlaIII digestion product.

The global repair phenotype assay

The global repair phenotype assay allows the fast and efficient evaluation of *in vitro* DNA repair. It used the alkaline comet assay, a microgel electrophoresis technique for the detection of DNA breaks and alkali-labile sites on the cellular level. At different times, before and after *in vitro* treatment with a mutagen, cells are taken and processed in the comet assay. This way the induction and repair on DNA damage after *in vitro* treatment can be followed, and the residual DNA damage present at a given time can be calculated. Interindividual differences in the progress of the curve of residual DNA damage reflect variations in susceptibility (phenotypic) or adaptive response after chronic exposure.

Figure 1: scheme for the global repair phenotype with styrene oxide

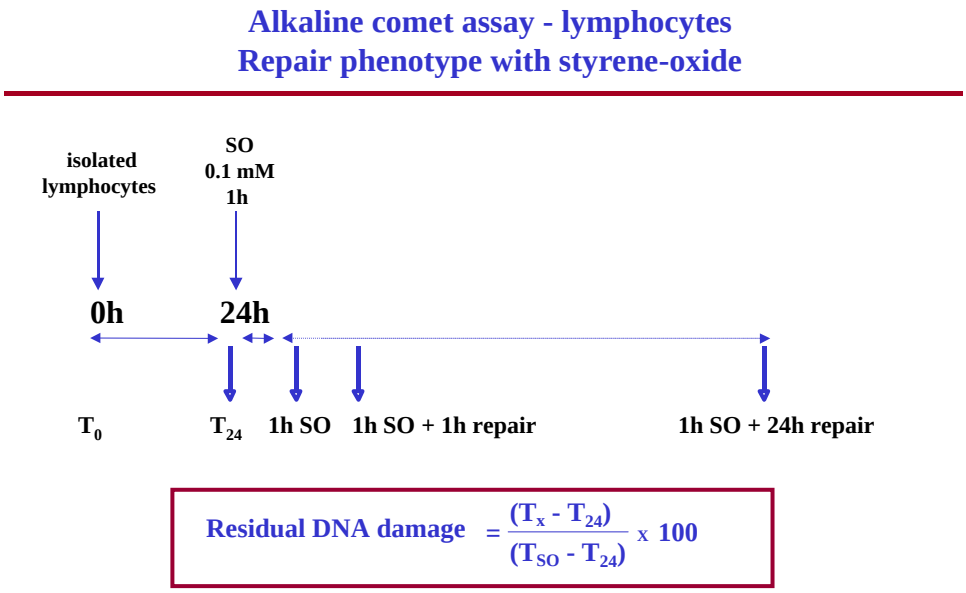
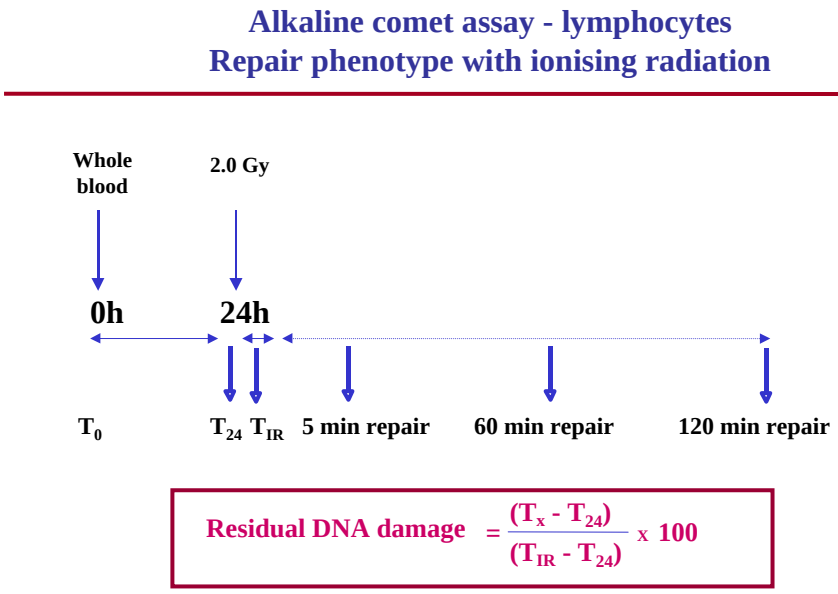


Figure 2: scheme for the global repair phenotype with ionising radiation



The comet assay

This technique is named after the shape of the images (comets) seen under the microscope. It is a microgel electrophoresis technique, which allows to measure DNA damage cell by cell. The alkaline version detects DNA breakage, alkali labile sites, open repair sites and cross links. For this technique cells are mixed with agarose gel, which is spread onto a microscope slide. The cells are lysed with high salt concentrations and detergents. The remaining nuclear DNA is then denaturated in an alkali buffer and electrophoresed in the same buffer. The DNA

fragments migrate out of the nucleus, towards the positive pole. After electrophoresis, the slides are stained with a fluorochrome, like ethidium bromide. An image analysis system is used to measure several damage parameters. The most useful are tail length (TL), measured from the centre of the nucleus towards the end of the tail, the percentage of DNA in the tail (TD) and tail moment (TM = TL x TD).

To allow detection of oxidative DNA damage, the Fpg enzyme (Formamidopyrimidine DNA glycosylase), which detects mainly 8-hydroxydeoxyguanine and so called Fpg-sensitive sites, may be included (Hartwig et al., 1996).

III.2. Mechanistic research on the genotoxic effects of hard metal dust

In vitro experiments

- *In vitro* genotoxicity of hard metal dust and its single components in human PBMC.

The *in vitro* genotoxicity of WC-Co and its component Co and WC, on human peripheral blood mononuclear cells (PBMC) was assessed with the alkaline comet assay, and the cytokinesis-block micronucleus test.

Cell treatment for alkaline comet assay:

Twenty-four hours after the onset of PHA stimulation, 10 µl of particle suspension was added to the PBMC. Treatment time was 15 minutes in the dose-effect experiments and for detection of Fpg-sensitive sites and 15 minutes, 1, 2, 4, 6, 14, 24, 48 and 72 h in the time-dependency experiments. EMS (ethyl methane sulfonate), used as the positive control, was diluted in PBS and exposure was for 2 hours. H_2O_2, used as control to assess Fpg activity, was diluted in PBS and exposure proceeded on ice for 5 minutes. After treatment, the cells were centrifuged for 10 minutes at 400 g. The supernatant was removed and the cell pellet was resuspended in PBS and immediately processed for the comet assay.

Cell treatment for *in vitro* cytokinesis-block micronucleus test:

Thirty-two hours after the onset of PHA-stimulation, the PBMC were treated with 4.0 µg/ml Co-equivalent of $CoCl_2$, Co, WC and WC-Co, 0.15 µg/ml MMC (mitomycin C) and 0.04 µg/ml nocodazole. The test substances were left in the cultures from the 24 or 32 h treatment point until harvest at 72 h. At 44 h, cytochalasin B was added and at 72 h the cells were fixed and spread onto slides. The micronucleus test was combined with fluorescence in situ hybridisation, using an pan-centromeric probe.

- *In vitro* genotoxicity of different combinations of cobalt and metallic carbides in human PBMC.

The tested metal carbides were Cr_3C_2, Mo_2C and NbC, and WC as the reference carbide. The carbides were chosen on the basis of previously published data on cytotoxicity (Lison & Lauwerys, 1995). Their ability to induce DNA single strand breaks and alkali-labile sites was assessed by means of the alkaline comet assay and their potential to induce chromosome/genome mutations by means of the *in vitro* cytokinesis-block micronucleus test

on PBMC from two. PBMC were exposed to 0.6, 3.0 and 6.0 µg/ml Co-equivalent for 15 minutes corresponding to 10.0, 50.0 and 100.0 µg/ml of the carbide or the Co-carbide mixture.
For the alkaline comet assay, the cells were processed immediately. For the micronucleus test, the cells were further incubated at 37°C. As positive controls, EMS (2 mM, 2h) and MMC (0.15 µg/ml, 48h) were used for the comet assay and micronucleus test, respectively.

- Comparative *in vitro* genotoxicity of hard metal and metallic cobalt in primary rat lung cells.

The *in vitro* genotoxicity of Co and WC-Co was assessed in primary rat alveolar macrophages (AM) and type II pneumocytes (AT-II) by means of the alkaline comet assay. The dose-dependency of the DNA damaging capacity of the two particle types was compared. The choice of the concentrations (3.0, 6.0, 12.0 and 24.0 µg/ml Co-equivalent) was based on the results obtained in human PBMC. The treatment time of 4 hours was based on preliminary experiments with AM and AT-II cells. Cells were also exposed to EMS (2µM) for 2h at 37°C, as a positive control.
In parallel, the viability of the lung cells was analysed by using the trypan blue exclusion test.

- Interference of hard metal dust and its single components with DNA repair *in vitro* in human PBMC.

The objective of this part of the work was to determine whether, as was previously shown for $CoCl_2$, that metallic Co, WC and the WC-Co particle mixture are able to interfere with DNA repair *in vitro* in human PBMC. As a model to evaluate the effect on base excision repair, methyl methane sulfonate (MMS) was chosen.

PBMC were treated with MMS (5.5 µg/ml) for 2 h, 24 h after PHA stimulation. Thereafter, the cells were centrifuged at 400 g for 10 min and then, after removal of the supernatant, immediately submitted to the comet assay or resuspended in fresh medium and further incubated in complete medium during 2h in the absence (*post-incubation*) or the presence (*post-treatment*) of 1.2 µg/ml Co-equivalent of Co, WC-Co or WC. Single and simultaneous exposure of cells to MMS and 1.2 µg/ml Co-equivalent of Co, WC-Co and WC were also included. After treatment, the cells were centrifuged at 400 g for 10 minutes, the supernatant was removed and the cell pellet resuspended and processed for the comet assay.

- *In vitro* apoptogenic effects of hard metal dust and its single components in human PBMC.

PBMC were treated with Co, WC, WC-Co and $CoCl_2$ at a concentration where *in vitro* genotoxicity was previously observed (Anard et al., 1997; Van Goethem et al., 1997). Treatment with metal compounds was started together with PHA stimulation or 24 hours after. Apoptosis was determined by Annexin-V staining, Cell Death ELISA, and flow cytometric identification of cells with a low DNA content. The involvement of specific apoptosis pathways was studied by the use of general and specific inhibitors.

In vivo genotoxicity of hard metal dust in the rat

Rats were treated with 16.6 mg WC-Co/kg body weight through intra-tracheal instillation. This dose induced significant but relatively limited pulmonary toxicity.

The comet assay was performed on PBMC and AT-II pneumocytes 12, 48, 72, 96 and 120h after intra-tracheal instillation. The micronucleus test was performed on PBMC and AT-II pneumocytes 72h after intra-tracheal instillation.

The micronucleus test

A micronucleus (MN) is formed during the metaphase/anaphase transition of mitosis (cell division). It may arise from a whole lagging chromosome (aneugenic event leading to chromosome loss) or an acentric chromosome fragment detaching from a chromosome after breakage (clastogenic event), which do not integrate in the daughter nuclei.
Scoring of micronuclei can be performed relatively easily and on different cell types relevant for human biomonitoring: lymphocytes, fibroblasts and exfoliated epithelial cells, without an extra *in vitro* cultivation step.
An *ex vivo* / *in vitro* analysis of lymphocytes in the presence of cytochalasin-B, an inhibitor of actins (added 44 hours after the start of cultivation), allows to distinguish easily between mononucleated cells which did not divide and binucleated cells which completed nuclear division during in vitro culture. Indeed, in these conditions the frequencies of mononucleated cells provide an indication of the background level of chromosome/genome mutations accumulated *in vivo* and the frequencies of binucleated cells with MN a measure of the damage accumulated before cultivation plus mutations expressed during the first *in vitro* mitosis.
The combination of the micronucleus assay with fluorescence in situ hybridisation (FISH) with a probe labelling the (peri-)centromeric region of the chromosomes (FISH assay) allows discrimination between micronuclei containing a whole chromosome (centromere positive micronucleus) and an acentric chromosome fragment (centromere negative micronucleus).
In the absence of cytochalasin B, mononucleated cells are analysed for the presence of micronuclei.
In the presence of cytochalasin B, mononucleated cells are recommended to be harvested at 24 hours post-PHA stimulation as there can be no doubt at this time-point that MN within such a cell are a result of *in vivo* rather than *ex vivo* division. Binucleated cells are recommended to be harvested at 72 hours post-PHA stimulation.

MNMC = the frequency of micronuclei per 1000 mononucleates.
MNCB = the frequency of micronucleated binucleated cells per 1000 binucleated cells.

Detection of apoptosis

- Annexin-V staining

After treatment, the cells are incubated with a mixture of annexin and propidium iodide. The cells are dropped onto microscope slides and analysed under a fluorescence microscope. This was distinction between early apoptotic cells (annexin-V positive/propidium iodide negative), late apoptotic/secondary necrotic cells (annexin-V positive/promidium iodide positive) and necrotic cells (annexin-V negative/propidium iodide positive) can be made.

- Flow cytometry

After treatment, the celles are washed in PBS and stained with propidium iodide. Samples are analysed with the CellQuest Pro software on a FACSCalibur flow cytometer (BECTON Dickinson). Detection of apoptotic cells happens on the basis of cell granularity and DNA content. With this approach, apoptotic cells can be identified as very condensed, granular cells with a sub-G1 DNA content.

- Cell Death ELISA

The measurement op apoptotic DNA degradation was quantified with the "cell death detection plus assay" (Roche Molecular Biochemicals), which measures the amount of histone-bound DNA fragments in the cytosol.

III.3. Biomonitoring studies

Styrene

The aim of the biomonitoring program of occupational exposure to styrene, a collaboration between KUL, UCL and VUB, was the assessment of external en internal exposure, biological effective dose, early biological effects and metabolic susceptibility, using a wide range of biomarkers:

- External dose: styrene in the air **(KUL)**
- Internal dose: mandelic acid in urine **(KUL)**
- Biological effective dose: haemoglobin adducts **(KUL)**
- Early biological effect: comet assay (DNA breaks and alkali labile sites)
  (VUB) sister chromatid exchange (chromatid recombination)
  micronucleus test (chromosome and genome mutations)
- Metabolic susceptibility: interindividual variability parameters: genotyping of cytochrome P450 mono-oxygenase (CYP2E1), microsomal epoxide hydrolase (EPHX) and glutathion-S-transferase (GSTT1 and GSTM1) **(UCL)**

Smoking is a possible confounding factor. The amount of cotinine in urine was measured as a marker for smoking behaviour. The workers were also exposed to lead chromate, so the concentrations chrome in urine and lead in blood were also determined. All the analyses were performed on workers exposed to styrene, as well as on the control group.

- Repair susceptibility: determining of repair genotypes : hOGG1, XRCC1 and XRCC3 **(VUB)**
- global repair phenotype **(VUB)**

The studied population is a cohort of 44 workers from the glass fibre reinforced polyester industry, and 44 matched controls. The control and exposed groups were matched for age, sex, smoking habits, ethnical origin, socio-economic status, … The controls were not exposed to any carcinogen.
The VUB was responsible for the implementation of the comet assay, the global repair phenotype, micronucleus test on nasal cells, de *ex vivo/in vitro* micronucleus test on lymphocytes, and the genotyping of all repair genes.

Cobalt

The main aim of the Co biomonitoring study was to assess the importance of susceptibility biomarkers for the improvement of the biomonitoring of workers exposed to mutagens/carcinogens. For this we determined the genotype for hOGG1, XRCC1 and XRCC3 for all workers analysed in the previous study on biomarkers for exposure and genotoxic effects (**De Boeck et al., 2000a**). The studied workers were classified according to homozygosity or heterozygosity for the wild type or mutated alleles, and smoking behaviour. The studied group is a cohort of 60 exposed workers from the hard metal industry, and 35 matched controls. This project was executed in collaboration with D. Lison (UCL).

Ionising radiation

The aim of this study was to combine genotyping and *in vitro* challenge phenotype to assess individual susceptibility of workers exposed to low dose ionising radiation, which is known to induce oxidative damage and DNA strand break.
32 external workers involved in the revision of the reactors of the nuclear plant of Doel were sampled. This type of workers is known for its high exposure during a relatively short time. Of course these workers are the most critical exposed population of a nuclear plant, which is why they were chosen for this biomonitoring study. Blood was also taken from 31 controls. in collaboration with the RUG, several cytogenetic endpoints were examined to assess the biological effects of the work conditions of these workers. The VUB was responsible for the *ex vivo/in vitro* micronucleus test and the comet assay for the detection of background DNA damage and global repair phenotype.

For further information on all of the methods, see annex I.

IV. RESULTS

IV.1. Validation of methods

Genotyping of DNA repair genes

In the hOgg1 protocol, we reduced the concentration of $MgCl_2$ from 2.5 tot 2 mM to avoid contaminating bands. We also lowered the concentration of Taq which was unnecessarily high (2.5 U/50µl) to 1U/50µl.

In the XRCC1 194-399 protocol we increased the $MgCl_2$ concentration to 3 mM and changed the 194:399 primer ratio to 1:5 in order to get multiplex bands of equal intensity.

In the XRCC1 280-XRCC3 241 protocol, we made the following modifications: we added three bases in the 3' end of the 241R primer in order to obtain uniform annealing temperatures in the PCR reaction; we increased the dNTP concentration from 200 to 400 µM of each dNTP to improve the yield of the PCR reaction; and we increased the concentration of $MgCl_2$ from 1.67 to 2 mM, since any increase in dNTP concentration requires an increase in the concentration of magnesium in order to make the reaction work. We performed single locus PCR instead of multiplex PCR on some old and diluted DNA samples. Single locus PCR

reaction required adjustment in cycling conditions, and the presence of BSA in a concentration of 0.5 µg/µl.

The global repair phenotype assay

Details concerning on work on the validation of the global repair phenotype assay can be found in the following publications/Ph.D.'s from our laboratory:

- De Boeck at al, 2000b
- Touil N., 2001
- De Boeck M., 2002

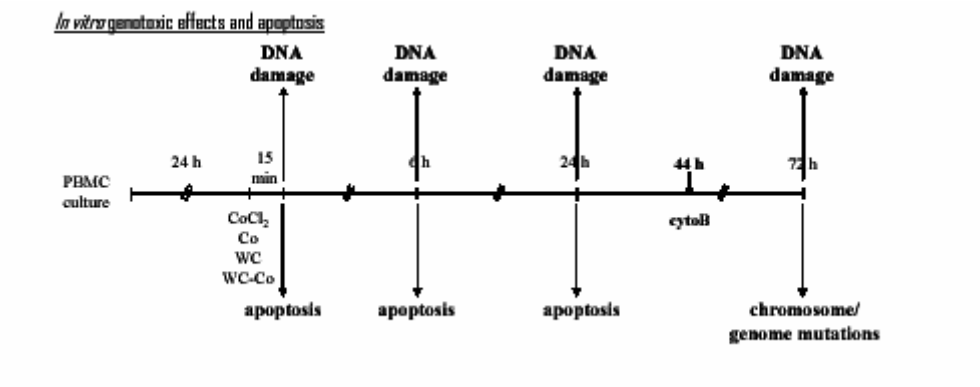
IV.2. Mechanistic research on the genotoxic effects of hard metal dust

Previously, it was demonstrated, using the alkaline comet and elution assays and the cytokinesis-block micronucleus test, that the WC-Co mixture is more genotoxic *in vitro* in PBMC than its individual components (Anard et al., 1997; Van Goethem et al., 1997). The main aim of this part of the project was to investigate whether induction of initial DNA damage, occurrence of mutations and alterations of cell survival could help to explain the human carcinogenic potential of the WC-Co mixture in comparison to its individual components. *In vitro* and *in vivo* experimental approaches were combined. In Table 2 and Figure 3, an overview is given of the different (geno)toxicity endpoints measured, comparing Co, $CoCl_2$, WC and WC-Co in most of the studies.

Table 2: (Geno)toxicity endpoints determined in *in vitro* and *in vivo* experimental tests. +: positive effect; -: no effect; ±: small (non-significant) effect; nd: not determined; FB1: sensitive to fumonisin B1 inhibition; casp: caspase; AM: alveolar macrophages; AT-II: type II pneumocytes; BAL: broncho-alveolar lavage cells; PBMC: peripheral blood mononucleated cells; Fpg: formamido pyrimidine DNA glycosylase.

	Co	$CoCl_2$	WC	WC-Co
In vitro in human PBMC				
DNA breaks and alkali labile sites	±	±	-	++
Fpg sensitive sites	-	nd	-	-
Micronuclei	+	nd	±	++
Chromosome loss	+	+	-	+
	+	+	+	±
Apoptosis	FB1 casp 8 & 9		FB1 casp 9	not FB1 casp 9
DNA repair inhibition	+	nd	±	+
In vitro in rat lung cells				
DNA breaks and alkali labile sites	++ (AT-II) + (AM)	nd nd	nd nd	+ (AT-II) ++ (AM)
In vivo in rat lung cells and PBMC				
DNA breaks and alkali labile sites	nd	nd	nd	- (AT-II, BAL, PBMC)
Micronuclei	nd	nd	nd	+ (AT-II) - (PBMC)

Figure 3: Overview of the different (geno)toxicity endpoints measured.



In vitro, WC-Co induced a dose and time-dependent change in DNA migration in human PBMC. $CoCl_2$ induced approximately the same extent of DNA damage as Co but no statistically significant increase was observed. WC alone had only a marginal effect on DNA migration. The induction of micronuclei by Co and WC and their mixture paralleled their DNA damaging capacity. Co and, in a more pronounced way, WC-Co, are clastogenic and/or aneugenic.
In addition to $CoCl_2$ (Resende de Souza Nazareth, 1976; Farah, 1983), the present study provided the first proof of the potential of other forms of cobalt (Co and WC-Co) to induce chromosome loss in human PBMC, while WC alone did not.

It was clear that the elevated genotoxicity in terms of DNA migration and induction of chromosome/genome mutations that was observed for the WC-Co mixture was not limited to this particle combination. Other carbides (at least Nbc and Cr_3C_2) were able to interact with metallic Co particles, leading to increased genotoxicity, as most clearly evidenced by the *in vitro* cytokinesis-block micronucleus test. In the case of Mo_2C, no enhanced mutagenic effect was observed when combined with Co. The physico-chemical interaction with Co leading to this enhanced genotoxicity was not specific for WC and is at least partially determined by the particle surface area.

When extending the *in vitro* genotoxicity testing to more relevant target cells, *i.e.* primary rat alveolar macrophages (AM) and type II pneumocytes (AT-II), it became apparent that specific cell type characteristics play an important role in their sensitivity toward hard metal dust and its single components. The previously demonstrated higher genotoxic effect of the WC-Co mixture was confirmed in AM but not in AT-II cells, possibly related to the strong antioxidant activity of the latter cell type.

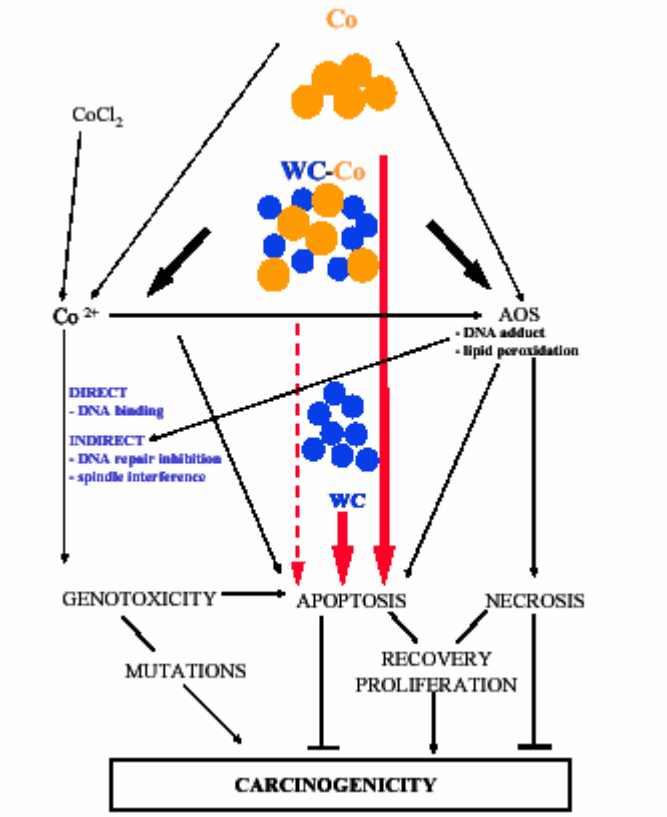
As was previously demonstrated for $CoCl_2$ (Hartwig et al., 1991; Kasten et al., 1997), other cobalt species (Co and WC-Co) or probably the Co ions released there from were able to interfere with DNA repair. The cobalt compounds caused persistence of MMS-induced DNA damage as detected with the alkaline comet assay. Inhibition of DNA repair is considered as a co-mutagenic effect. Simultaneous treatment of PBMC with MMS and metal compounds led to results suggestive for the presence of DNA-protein crosslinks and/or inhibition of DNA attack of MMS.

Apoptosis was assessed by Annexin-V staining, flow cytometry and cell death ELISA assay. Metallic Co particles were able to induce apoptosis *in vitro* in human PBMC; the effect was, at least in part, reproducible by treatment with $CoCl_2$, indicating a possible role for Co ions in the apoptogenic effect. Although so far considered as biologically inert, WC particles also induced apoptosis but with different kinetics. After 24 hours exposure, as compared to its individual components, WC-Co had an additive apoptotic effect in the ELISA assay. WC-induced apoptosis was largely dependent on caspase-9 activation while Co-induced apoptosis involved both caspase-9 and -8 activation. Apoptosis induced by the tested WC-Co mixture was mainly abolished by caspase-9 inhibitor and probably resulted from the combination of WC specific apoptosis in monocytes and Co specific apoptosis in both monocytes and lymphocytes. The carcinogenic potential of WC-Co may therefore be related to the combination of its higher mutagenic and apoptogenic capacities.

Concerning the *in vivo* approach in the rat, WC-Co particles induced micronuclei in possible target cells of the alveoli, type II pneumocytes, after a single intra-tracheal instillation. This is the first proof of the *in vivo* mutagenic potential of hard metal dust. The highest increase in micronucleated type II pneumocytes occurred at the time required for these cells to complete one division. In PBMC of WC-Co treated animals, no increases in DNA migration and micronuclei frequency were observed.

Taken together, the different events underlying the human carcinogenic potential of the WC-Co mixture identified here are the elevated *in vitro* genotoxic damage (higher frequency of DNA lesions and chromosome/genome mutations), the lower rate of *in vitro* apoptosis, the capacity to interfere with DNA repair and the *in vivo* mutagenic potential in possible target type II pneumocytes. In other words, the combination of direct genotoxic effects (direct binding of Co to DNA, as reported in the literature), indirect genotoxic effects (production of DNA and/or lipid damaging AOS, inhibition of DNA repair, interaction with tubulins leading to chromosome loss) and disturbance of apoptotic cell death are, in addition to the increase in necrosis (cytotoxicity) reported in the literature, all mechanisms that may contribute to the elevated risk for lung cancer development after exposure to hard metal dust. The relationship between the different endpoints measured and carcinogenesis is well established and allows viewing the obtained results within the frame of the different mechanisms of disturbance of cell homeostasis (Figure 4).

Figure 4: Possible mechanisms involved in carcinogenesis induced by hard metal dust.



DNA breaks/alkali labile sites, chromosome/genome mutations and apoptosis were all detected after a 15 minutes *in vitro* treatment with WC-Co (6.0 µg/ml Co-equivalent). This period is long enough to liberate the majority of the AOS (Lison et al., 1995) and to solubilise most of the cobalt (own data). Hence, it may be inferred that the produced AOS and cobalt ions are responsible for the observed effects. The difference in effect seen between Co and WC-Co should then especially be ascribed to a lower peak intensity of the AOS burst (in the case of Co) since at 15 minutes, approximately the same amount of ions was then available to the cells.

Tungsten carbide has long been considered to be biologically inert. In the present study, a marginal, but not dose-dependent, effect on DNA migration was observed and although an increase in micronucleated binucleates was observed, this was not dose-dependent. No increase in centromere positive micronuclei (aneugenic event) was demonstrated. In contrast, WC was shown to be an effective inducer of apoptosis mainly through activation of caspase 8. Its effect on DNA repair inhibition was equivocal. Although WC does not cause cytotoxicity (necrosis), as demonstrated in the literature (Lison & Lauwerys, 1990), its apoptogenic capacity may still affect normal tissue homeostasis and could therefore potentially be harmful. It may be useful to look for an alternative composition of hard metal dust, with similar technical characteristics but with no or very limited hazardous effects.

IV.3. Biomonitoring studies

Ionizing radiation (**Aka et al., Radiation Research, submitted**)

The aim of this study was to assess the link between hOgg1, XRCC1, XRCC3 genotypes and the *in vitro* DNA strand break repair phenotype in populations and individuals exposed to low doses of gamma rays, and to identify the main determinants of genotoxicity following lose dose exposure to ionizing radiation.

The genotype frequencies we found for hOgg1, XRCC1 and XRCC3 (see table 3) are in agreement with literature values for the Caucasian population, and the allele frequencies do not deviate significantly from the Hardy-Weinberg equilibrium.

Table 3: Distribution of hOGG1, XRCC1 and XRCC3 genotypes in the study populations

Gene	Genotype	Controls (%)	Exposed (%)	Total (%)
hOGG1^{326}	Ser/Ser	22 (71)	17 (53)	39 (62)
	Ser/Cys	09 (29)	13 (41)	22 (35)
	Cys/Cys	00 (00)	02 (06)	02 (03)
XRCC1^{194}	Arg/Arg	30 (97)	26 (84)	56 (90)
	Arg/Trp	01 (03)	05 (16)	06 (10)
XRCC1^{280}	Arg**/**Arg	30 (97)	28 (90)	58 (93)
	Arg**/**His	01 (03)	03 (10)	04 (07)
XRCC1^{399}	Arg**/**Arg	10 (32)	13 (43)	23 (38)
	Arg**/**Gln	17 (55)	10 (33)	27 (44)
	Gln/Gln	04 (13)	07 (24)	11 (18)
XRCC3^{241}	Thr/Thr	13 (42)	15 (48)	28 (45)
	Thr/Met	16 (52)	10 (32)	26 (42)
	Met/Met	02 (06)	06 (20)	08 (13)

The link between genotype and genotoxic endpoints as a result of exposure to IR is relatively clear.

1. hOGG1^{326} and XRCC1^{280} seem to influence the repair of oxidative base and single strand breaks detected by the alkaline comet assay and the *in vitro* repair phenotype assessed by the same technology.
2. XRCC1^{280} influences the induction of double strand breaks expressed as MNMC in mononucleated peripheral blood lymphocytes (PBMC) in the cytochalasin –B assay.
3. XRCC3^{241} influences the repair of double strand breaks expressed as MNCB in binucleated PMMC in the cytochalasin-B assay.

These data suggest that besides double strand breaks, the single strand breaks induced by IR which were not repaired by base excision (deficiency of hOGG1 and or XRCC1), are replicated into double strand breaks during the next S-phase and if not repaired (deficiency of XRCC3), giving rise to micronuclei in the following mitosis. On the basis of these results, it is not possible to select a single genotype for prediction of the individual susceptibility to ionising radiation. A combination of genotyping of hOGG1, XRCC1 and XRCC3 polymorphisms is advised. As an alternative or complement, the *in vitro* DNA strand break phenotype, which integrates several repair pathways, is recommended.

Considering the population studied, their exposure to a mean cumulative dose of 15.7±8.0 mSv did not induce significant genotoxic effects. Although a slightly higher frequency of TD, MNCB and MNMC was observed in the exposed workers compared to the control workers, it was not statistically significant. Identification of MN arising from chromosome breakage by FISH (Fluorescence In Situ Hybridisation) with pericentromeric probes might have provided a more sensitive assay. On the other hand, the study of repair efficiency with the *in vitro* challenge with IR indicates that the *in vivo* exposed workers repaired their DNA damage more efficiently than the control workers. This might be due to the induction of repair enzymes (adaptive response) by sub-chronic exposure to low dose IR.

Finally one should underline that workers with variant hOGG1^{326} or XRCC3^{241} genotypes who also smoke are at higher risk for induction of DNA damage or MN, respectively. These workers who smoke and who are exposed to IR represent a specific population requiring a closer medical surveillance because of their increased mutagenic/carcinogenic risk.

Cobalt (**Mateuca et al., in preparation**)

The potential genotoxic effects of Co-containing dust in workers currently exposed to the TLV-TWA (20µg/m3) for cobalt particles alone or associated to carbide particles in hard metal (WC-Co) were studied by determination of 8-OHdG in urine, and the comet assay and the ex vivo/in vitro MN test in PBMC (**De Boeck et al., 2000a**). No significant increase of genotoxic effects was observed in workers exposed to cobalt-containing dust as compared to controls. However multiple regression analysis indicated that workers who smoked and were exposed to hard metal dusts have elevated 8-OHdG and MN values. It was concluded that this particular group of workers needs closer medical surveillance.

This study aimed at assessing whether at this level of exposure to Co compounds, genetic polymorphisms may contribute to explain particularly high or low genotoxic effects, providing an improved way to do prevention at individual level. Since our mechanistic studies demonstrated the role of oxidative damage and DNA strand breakage in the induction of genotoxic effects by Co compounds, we selected several DNA repair polymorphisms involved in the repair of the Co-induced DNA lesions. The predictivity of DNA repair genotypes on genotoxic effects were analysed by comparison of the genotoxic effects of each genotype in exposed versus corresponding controls and by multiple regression analysis taking into account, age, exposure type, smoking, and interaction between genotypes, smoking and exposure.

1) Genotype frequencies in exposed and control workers

The frequencies of the observed genotypes for the hOGG1, XRCC1 and XRCC3 genes are summarized in table 4.

Table 4 a. Distribution of hOGG1, XRCC1 and XRCC3 genotypes in workers exposed to Co and matched controls

Gene	Genotype	Control (%)	Exposed (%)	Total (%)
hOGG1	*Ser/Ser*	10 (66)	15 (68)	25 (68)
	Ser/Cys	4 (27)	6 (27)	10 (27)
	Cys/Cys	1 (7)	1 (5)	2 (5)
XRCC1 194	*Arg/Arg*	14 (93)	18 (82)	32 (86)
	Arg/Trp	1 (7)	4 (18)	5 (14)
	Trp/Trp	0 (0)	0 (0)	0 (0)
XRCC1 280	*Arg/Arg*	14 (93)	20 (91)	34 (92)
	Arg/His	1 (7)	1 (5)	2 (5)
	His/His	0 (0)	1 (4)	1 (3)
XRCC1 399	*Arg/Arg*	6 (40)	10 (46)	16 (43)
	Arg/Gln	6 (40)	6 (27)	12 (33)
	Gln/Gln	3 (20)	6 (27)	9 (24)
XRCC3 241	*Thr/Thr*	3 (20)	7 (32)	10 (27)
	Thr/Met	8 (53)	9 (41)	17 (46)
	Met/Met	4 (27)	6 (27)	10 (27)

Table 4 b. Distribution of hOGG1, XRCC1 and XRCC3 genotypes in workers exposed to WC-Co and matched controls

Gene	Genotype	Control (%)	Exposed (%)	Total (%)
hOGG1	*Ser/Ser*	5 (42)	15 (56)	20 (51)
	Ser/Cys	6 (50)	10 (37)	16 (41)
	Cys/Cys	1 (8)	2 (7)	3 (8)
XRCC1 194	*Arg/Arg*	12 (100)	26 (96)	38 (97)
	Arg/Trp	0 (0)	1 (4)	1 (3)
	Trp/Trp	0 (0)	0 (0)	0 (0)
XRCC1 280	*Arg/Arg*	11 (92)	25 (93)	36 (92)
	Arg/His	1 (8)	2 (7)	3 (8)
	His/His	0 (0)	0 (0)	0 (0)
XRCC1 399	*Arg/Arg*	1 (8)	10 (37)	11 (28)
	Arg/Gln	8 (67)	13 (48)	21 (54)
	Gln/Gln	3 (25)	4 (15)	7 (18)
XRCC3 241	*Thr/Thr*	6 (50)	16 (59)	22(56)
	Thr/Met	5 (42)	9 (33)	14 (36)
	Met/Met	1 (8)	2 (8)	3 (8)

2) Comparison of the genotoxic effects of each genotype in exposed workers versus corresponding controls

In the cobalt study no significant difference between control and exposed workers was found for any genotype. In the WC-Co study micronuclei frequencies (MNCB) were significantly higher in the exposed workers with Arg/Arg genotypes for $XRCC1^{194}$ or $XRCC1^{280}$ than in corresponding controls with the same genotype ($p = 0,021$ and $p = 0,035$, respectively). Significant differences between genotypes of the same genes were found for $XRCC1^{399}$ and TD of Cobalt controls (the Gln/Gln genotype being more sensitive), for hOGG1 and MNMC of the WC-Co exposed population (the Ser/Cys genotype being more sensitive), and XRCC3 and 8-OHdG of the WC-Co exposed population (the Met/Met genotype being more sensitive).

3) Multiple regression analysis

When the exposed populations were compared to their corresponding control population, taking into account only the significant independent variables ($p<0,05$), the multiple regression analysis indicates that:

- in workers exposed to Co alone , TD is determined by the interaction between XRCC1 (280 and 194) and hOGG1, the interaction between smoking and exposure and the interaction between $XRCC1^{194}$ and smoking. MNCB are determined by smoking status and MNMC by hOGG1. The level of 8-OHdG is determined by the interaction between $XRCC1^{399}$ and hOGG1, and age.

- in workers exposed to WC-Co, TD is determined by the interaction between $XRCC1^{280}$ and hOGG1. MNCB are determined by exposure status. MNMC are determined by the interaction between $XRCC3^{241}$ and hOGG1, and smoking status. The level of 8-OHdG is determined by the interaction between exposure and smoking, the interaction between $XRCC1^{399}$ and smoking, and $XRCC3^{241}$.

When the respective exposed groups are compared to the combined control population, the multiple regression analysis indicates that:

- in workers exposed to Co alone, TD is determined by the interaction between $XRCC1^{280}$ and hOGG1. MNCB are determined by the interaction between $XRCC1^{194}$ and smoking, and the interaction between $XRCC3^{241}$ and smoking. MNMC are determined by age, urinary Co, the interaction between exposure and smoking status, the interaction between smoking and hOGG1, and hOGG1. The level of 8-OHdG is determined by the interaction between $XRCC1^{399}$ and hOGG1, the interaction between smoking and hOGG1, the interaction between $XRCC1^{399}$ and exposure, and $XRCC1^{399}$.

- in workers exposed to WC-Co, TD is determined by the interaction between $XRCC1^{280}$ and hOGG1. MNMC are determined by the interaction between $XRCC3^{241}$ and hOGG1 and the interaction between exposure and smoking. The level of 8-OHdG is determined by the interaction between $XRCC3^{241}$ and exposure, and $XRCC1^{399}$.

Styrene (**Godderis et al., Environmental and Molecular Mutagenesis, submitted**)

In this study, a set of biomarkers was applied on workers to evaluate whether low occupational exposure to styrene can cause genotoxic effects and to assess the influence of individual susceptibility on the relationship between styrene exposure and genotoxic effect. The average styrene exposure was low (9.5 ppm ± 9.6) compared with the current ACGIH TLV-TWA for styrene of 20 ppm.

Genotyping was performed for DNA repair genes and for several genes coding for enzymes involved in styrene metabolisation (see Table 5).

Correlation and regression analysis revealed a positive association between TD and chromium concentration (all under current BEI-TLV of 30 μg/g cr.). The chromium exposure of the referent group is probably originating from a combination of cigarette smoking and diet, since no significant chromium exposure could be documented during the production process. Since the referent group had a higher chromium concentration compared with the exposed group and since the TD in comet assay in this study depends on both styrene-adduct formation and chromium exposure, these findings could possibly contribute to explain the absence of a significant difference in TD between the exposed and non-exposed population.

Frequencies of MNMC, MNCB and MN in nasal cells differed significantly between the exposed and referent group. We could also confirm the effect of age on MN frequencies (MNCB). The frequencies of MNMC and MNCB were higher among the smokers compared with non-smokers in the exposed population. MNMC and MNCB also seemed to be both induced by a long styrene exposure. The positive association between duration of employment and frequencies of MNMC and MNCB in our study confirms the fact that MN frequencies are good biomarkers for accumulated chromosomal damage (Kirsch-Volders and Fenech, 2001).

Lymphocytes of non-smoking styrene workers showed a higher level of residual DNA damage after 24 hours in vitro repair after SO induced DNA damage than the non-smoking referents. Moreover, the longer the duration of employment in a styrene environment, the more residual DNA damage was found after 24 hours. In contrast, in-vitro DNA repair after 1 hour seemed to be enhanced by recent styrene exposure and alcohol. The differences in early and late DNA strand break repair phenotype could be due to different lesions and different mechanisms repairing them. Early repair mainly involves the excision of small lesions such as oxidised bases and non-bulky adducts by hOGG1 (Lu et al. 2001). In our study we found an influence of the hOGG1 polymorphisms on the early DNA strand break repair phenotype in the referent population. Individuals with two hOGG1 Ser^{326} alleles showed less residual DNA damage than individuals possessing one hOGG1 Cys^{326} allele. After excision in a later phase, the abasic site is restored by endonuclease action, removal of the sugar residue, DNA synthesis using the other strand as a template and ligation (Goode et al., 2002). XRCC1 is a group of enzymes involved in this phase. It has been stated that polymorphisms in base excision repair enzymes are important for the removal of styrene induced alkyl-DNA adducts and γ-radiation induced DNA damage (Dypbukt et al., 1992;Wood et al., 2001). The early in vitro repair capacity in our study was decreased in individuals with the XRCC1 His^{280} allele. Tuimala et al reported increased frequencies of in vitro bleomycin-induced chromatid breaks in lymphocytes of individuals with XRCC1 His^{280} allele (Tuimala et al., 2002). However, this finding needs to be interpreted carefully and needs further clarification since individuals with this XRCC1 codon 280 variant are rare (only 5% in our study population). In our study,

individuals, with the XRCC1 Arg^{194} allele revealed a decreased late in vitro DNA strand break repair capacity. On the other hand, polymorphisms on XRCC1 codon 194 did not seem to influence the results of genotoxicity assays.

Table 5: Distribution of GSTT1, GSTM1, EPHX1, CYP2E1, NAT2, hOGG1, XRCC1 XRCC3 genotypes

		Referents			**Exposed**			**All**		
GST	***GSTT1***	+	-		+	-		+	-	
		36(84%)	7 (16%)		37(84%)	7 (16%)		73(84%)	14(16%)	
	GSTM1	+	-		+	-		+	-	
		25(58%)	18(42%)		16(36%)	28(64%)		41(47%)	46(53%)	
EPHX	***EPHX1 113***	Tyr/Tyr	Tyr/His	His/His	Tyr/Tyr	Tyr/His	His/His	Tyr/Tyr	Tyr/His	His/His
		17(40%)	24(56%)	2 (4.7%)	23(52%)	15(34%)	6 (14%)	40(46%)	39(45%)	8 (9%)
	EPHX1 139	Arg/Arg	His/Arg	His/His	Arg/Arg	His/Arg	His/His	Arg/Arg	His/Arg	His/His
		1 (2%)	13(30%)	29(67%)	1 (2%)	13(30%)	30(68%)	2 (2%)	26(30%)	59(68%)
CYP2E 1	***Intron 6 DraI***	D/D	D/C	C/C	D/D	D/C	C/C	D/D	D/C	C/C
		33(80%)	8 (20%)	0 (0%)	33(75%)	10(23%)	1 (2%)	66(78%)	18(21%)	1 (1%)
Nat2	***Allele 4***	Present	Absent		Present	Absent		Present	Absent	
		22(52%)	20(48%)		20(48%)	22(52%)		42(50%)	42(50%)	
hOGG1	***hOGG1 326***	Ser/Ser	Ser/Cys	Cys/Cys	Ser/Ser	Ser/Cys	Cys/Cys	Ser/Ser	Ser/Cys	Cys/Cys
		28(65%)	15(35%)	0 (0%)	24(57%)	18(43%)	0 (0%)	52(61%)	33(39%)	0 (0%)
XRCC1	***XRCC1 194***	Arg/Arg	Arg/Trp	Trp/Trp	Arg/Arg	Arg/Trp	Trp/Trp	Arg/Arg	Arg/Trp	Trp/Trp
		34(77%)	10(23%)	0 (0%)	35(83%)	7 (17%)	0 (0%)	69(80%)	17(20%)	0 (0%)
	XRCC1 399	Arg/Arg	Arg/Gln	Gln/Gln	Arg/Arg	Arg/Gln	Gln/Gln	Arg/Arg	Arg/Gln	Gln/Gln
		22(50%)	16(36%)	6 (14%)	13(31%)	20(48%)	9 (21%)	35(41%)	36(42%)	15(17%)
	XRCC1 280	Arg/Arg	Arg/His	His/His	Arg/Arg	Arg/His	His/His	Arg/Arg	Arg/His	His/His
		41(95%)	2 (5%)	0 (0%)	42(95%)	2 (5%)	0 (0%)	83(95%)	4 (5%)	0 (0%)
XRCC3	***XRCC3 241***	Thr/Thr	Thr/Met	Met/Met	Thr/Thr	Thr/Met	Met/Met	Thr/Thr	Thr/Met	Met/Met
		16(37%)	20(47%)	7 (16%)	17(40%)	18(43%)	7 (17%)	33(39%)	38(45%)	14(16%)

Frequencies of MNMC and MNBC were lower in individuals with one or two XRCC1 Gln^{399} alleles. The influence of the XRCC1 polymorphisms on genotoxic effects after occupational exposure to ionising radiation (see IV.3.a.) seems to confirm our findings.

Subjects, who are GSTT1 negative, showed higher residual DNA damage after 1 hour repair of SO induced DNA damage. This means that SO has been less efficiently detoxified causing more DNA damage, which requires more and longer repair. GSTT1 activity plays a role in the metabolism of styrene. Although, glutathione conjugation is considered as a minor route in SO detoxification in humans, it still seems to influence the results of genotoxic assays (Haufroid et al., 2002; Shield and Sanderson, 2001). In our study non-exposed GSTM1 negative referents also showed higher frequencies of MNMC than GSTM1 positive referents.

We found an influence of the EPHX1 polymorphisms in exon 3 and in exon 4 on the genotoxic effects in the exposed population. The presence of a His^{139} allele in styrene-exposed subject was associated with increased frequencies of MNMC. Higher frequencies of MN in nasal cells were detected in individuals possessing one or more His^{113} alleles. Our data could not reveal an association between EPHX1 activity and TD.

IV.4. Exploratory approach of the DNA array microchips technology for genotyping and phenotyping in occupational medicine

See the UCL (promotor D. Lison) part of this report.

IV.5. Study of the ethical and legal aspects of the use of susceptibility tests in occupational medicine

During this programme, we were in constant contact with our legist partners of the UCL (promotor P. Vielle), and exchanges of information and opinions took place during several meetings.

V. DISCUSSION

The aim of the research performed by the laboratory of Cell Genetics was to assess the predictivity of genotyping for DNA repair genes and phenotyping for single strand breakage DNA repair (in association with genotyping for relevant metabolising genes) for genotoxic effects in workers exposed to mutagens/carcinogens.

This objective was applied to three occupational exposures: low chronic exposure to ionising radiation (nuclear power plant workers), styrene, and cobalt-containing dust. These studies were performed in collaboration with our colleagues from the RUG, KUL and UCL respectively.

In a first approach, validation of the genotoxicity biomarkers (comet assay, micronucleus assay) was completed, methodologies for genotyping of Base Excision Repair genes (hOGG1 and XRCC1) and double strand DNA break repair (XRCC3) were developed, and the

applicability of the comet assay for the *in vitro* phenotyping of strand break repair was assessed.

The choice of the adequate biomarkers for the styrene and the ionising radiation population were well known from literature, but not well established for cobalt and tungsten carbide in association with cobalt. Therefore, mechanistic studies were undertaken to determine which cytogenetic effects were induced *in vitro* in human lymphocytes and *in vivo* in rat lung cells by cobalt alone, or cobalt in association with tungsten carbide. These studies, summarized in the Ph.D. of Marlies De Boeck, demonstrated that tail DNA assessed by the comet assay and micronuclei assessed by the cytochalasin-B assay are sensitive biomarkers for exposure to cobalt-containing dust.

The predictivity of the genotyping and phenotyping for genotoxic effects in occupationally exposed individuals can be summarized as follows:

1. Nuclear plant workers

The link between genotype and genotoxic endpoints as a result of exposure to IR is relatively clear.

- hOGG1^{326} and XRCC1^{280} seem to influence the repair of oxidative base and single strand breaks detected by the alkaline comet assay and the *in vitro* repair phenotype assessed by the same technology.
- XRCC1^{280} influences the induction of double strand breaks expressed as MNMC in mononucleated peripheral blood lymphocytes (PBMC) in the cytochalasin –B assay.
- XRCC3^{241} influences the repair of double strand breaks expressed as MNCB in binucleated PMMC in the cytochalasin-B assay.

These data suggest that besides double strand breaks, the single strand breaks induced by IR which were not repaired by base excision (deficiency of hOGG1 and or XRCC1), are replicated into double strand breaks during the next S-phase and if not repaired (deficiency of XRCC3), giving rise to micronuclei in the following mitosis.

On the basis of these results, it is not possible to select a single genotype for prediction of the individual susceptibility to ionising radiation. A combination of genotyping of hOGG1, XRCC1 and XRCC3 polymorphisms is advised. As an alternative or complement, the in vitro DNA strand break phenotype, which integrates several repair pathways, is recommended.

Considering the population studied, their exposure to a mean cumulative dose of 15.7±8.0 mSv did not induce significant genotoxic effects. Although a slightly higher frequency of TD, MNCB and MNMC was observed in the exposed workers compared to the control workers, it was not statistically significant. Identification of MN arising from chromosome breakage by FISH (Fluorescence In Situ Hybridisation) with pericentromeric probes might have provided a more sensitive assay. On the other hand, the study of repair efficiency with the *in vitro* challenge with IR indicates that the *in vivo* exposed workers repaired their DNA damage more efficiently than the control workers. This might be due to the induction of repair enzymes (adaptive response) by sub-chronic exposure to low dose IR.

Finally, one should underline that workers with variant hOGG1^{326} or XRCC3^{241} genotypes who also smoke are at higher risk for induction of DNA damage or MN, respectively. These

workers who smoke and who are exposed to IR represent a specific population requiring a closer medical surveillance because of their increased mutagenic/carcinogenic risk.

2. Cobalt/hard metal workers

Confirmation on larger populations is needed before drawing definitive conclusion. As major findings, one may underline that:

- Workers who are of Arg/Arg genotypes for XRCC1^{194} or XRCC1^{280} and who are exposed to WC-Co might be at higher risk for induction of micronuclei, and therefore of cancer.
- TD is significantly determined by the interaction between hOGG1 and XRCC1^{280}, in both the Co and WC-Co exposed workers, confirming the fact that the Base Excision Repair pathway of oxidative damage is triggered by exposure to cobalt-containing dusts.
- MNMC are determined by hOGG1 in cobalt-exposed workers but not in WC-Co exposed workers; on the contrary the interaction between hOGG1 and XRCC3 is significant only in the WC-Co exposed workers. This might suggest that double strand breakage is more frequent in WC-Co exposed workers than in Co-exposed workers.
- MNCB are significantly related to the interaction between smoking with XRCC1^{194} and XRCC3 in Cobalt exposed workers.
- The level of 8-OHdG in urine is positively correlated to the interaction between hOGG1 and XRCCI^{399} and negatively correlated with XRCC1^{399} in Co-exposed workers. This could be expected since the more efficient the repair of oxidative DNA damage, the more oxidised bases are found in the urine. This however means that 8-OHdG in urine is only an indirect measure of exposure.

These results indicate that polymorphisms for genes which code for repair enzymes capable to repair oxidized bases (hOGG1), single stranded DNA breaks (XRCC1) and double stranded DNA breaks (XRCC3) are involved in the variation of the extent of genotoxic effects induced in workers exposed to Co-containing dust. Interactions between these polymorphisms seem critical and therefore no single genotyping can be advised in particular. Moreover, the interaction with smoking is a key factor, which is not surprising since smoking itself can induce oxidative damage.

3. Styrene workers

The study on workers exposed to styrene and matched referent workers was performed in order to examine the importance of genetic polymorphisms in biotransformation and DNA repair enzymes on N-terminal Hb adducts levels and genotoxic effects.

Frequencies of micronuclei (MNMC and MNCB) in lymphocytes, and in nasal cells differed significantly between the exposed and referent group. Individuals with a high level of N-terminal valine adducts revealed higher TD as evaluated by Comet assay. The *in vitro* strand break repair capacity after 24 hr was less efficient in lymphocytes of non-smoking styrene workers. Duration of employment in a styrene environment was positively associated with most of the results of the genotoxicity assays (MNMC, MNCB, RD 24hr). The influence of genetic polymorphisms of metabolising and repair enzymes on the Hb-adduct and results of the genotoxicity tests was also studied. Hand laminators possessing one or more EPHX1 His^{113} allele showed higher frequencies of micronuclei in nasal cells than homozygote

individuals EPHX1 Tyr^{113}. Higher frequencies of micronuclei in mononucleated cells were associated with the presence of EPHX1 His^{139} allele. Individuals possessing one or more XRCC1 Gln^{399} allele showed higher frequencies of MNMC and MNCB. Analysis of *the in vitro* DNA strand break repair phenotype data showed a faster onset of DNA repair after 1 hour in individuals heterozygous for XRCC1 Arg^{280} and in individuals possessing GSTT1. Higher residual DNA damage 24 hours after *in vitro* styrene oxide exposure was found in individuals homozygous for XRCC1 Arg^{194} than heterozygous individuals.

In conclusion, our data suggest that chromosome/genome mutations are formed in workers exposed to low concentration of styrene. Duration of exposure, age and smoking habits are important variables to consider in studies evaluating genotoxic effects on workers. Genotyping of metabolising and DNA repair enzymes are useful for the assessment of individual genotoxic risk to styrene. The in vitro DNA strand break phenotype might be a valuable method to estimate the repair capacity of workers.

Promotor: Prof. Dr. H. Veulemans

Scientific collaborators: L. Godderis, MD
N. Van Nimmen

Catholic University Leuven
Faculty of Medicine
Laboratory of Occupational Hygiene and Toxicology

1 Influence of genetic polymorphisms on biomarkers of exposure and genotoxic effects in styrene-exposed workers.

1.1. Introduction

1.1.1. Context and frame of the research

To date, cancer risk is assessed mainly through epidemiological follow-up and investigation of exposure-effect relationships (e.g. genotoxicity endpoints) at population levels. However, individual characteristics may influence this relationship and appear to have a significant influence on the development of cancer. In order to improve cancer risk assessment in workers exposed to low levels of carcinogens it has become more and more clear that it is necessary to include the identification of genotypes and phenotypes of enzymes involved in metabolism and DNA repair (Mohrenweiser et al. 2003; Mutti 1999).

Styrene causes its genotoxic effects predominantly through the formation of styrene oxide (SO). Styrene has been suspected to be a causal agent for lympho-hematopoietic malignancies (Kogevinas et al. 1994; Kolstad et al. 1994). The fate of styrene in humans with respect to uptake and disposition is well understood (Sumner and Fennell 1994). The first step in the major metabolic pathway is the formation of styrene 7,8-oxide (SO) by the cytochrome P450-mediated monooxygenase system (CYP). SO is hydrated to styrene glycol by microsomal epoxide hydrolase (EPHX1) or conjugated with glutathion in enzyme-catalyzed reactions (glutathion S-transferases, GST). Different studies indicated the importance of inter-individual variability in styrene biotransformation enzymes activities (Vodicka et al. 2001; Vodicka et al. 2002). DNA repair enzyme polymorphisms, in particular those capable to repair oxidative damage and/or alkyl adducts, may be important for the determination of an individual cancer risk (Maki-Paakkanen et al. 1991; Marczynski et al. 2000). Different genetic polymorphisms in DNA repair have been described.(Hu et al. 2002) hOGG1 is involved in base excision repair of 8-oxoguanine oxidative damage. The XRCC1 repair system plays an important role in base excision repair and in rejoining DNA strand breaks. Three polymorphisms in *XRCC1* (*Arg194Trp, Arg280His, and Arg399Gln*) have been described (Tuimala et al. 2002). XRCC3 is involved in the homologous recombinational repair of cross-links and chromosomal double-strand breaks (Brenneman et al. 2000).

1.1.2. Aims of the research

The aims of the present study were therefore: 1) to evaluate whether low level exposure to styrene can induce genotoxic effects as assessed with well validated methods for measuring exposure and genotoxic effects; 2) to examine whether individual differences in genotoxic responses can be explained by genetic polymorphisms in genes coding for enzymes involved in styrene metabolism and/or DNA repair.

1.2. Methods

1.2.1. Sampling

The survey was carried out in 2000-2001 and was a co-operation between the KUL, VUB and UCL. Styrene-exposed male workers (n=44) from a fibreglass-reinforced plastic factory volunteered to participate in this study. A referent group of 44 male production workers was

selected from two plants in the same region. An attempt was made to match the exposed and referent groups for age (41.6 ± 9.6, 40.9 ± 9.0), smoking habits (48% smokers in both groups), socio-economic status and ethnical origin (all Caucasian). Every participant filled in a questionnaire recording items such as age, life-style factors (alcohol consumption, smoking habits), duration of employment in styrene-using industry and medical history. The main characteristics of the study population are presented in Table I. Recent exposure to tobacco smoke was evaluated by the determination of cotinine in urine. Packyears, calculated as the number of cigarettes smoked a day divided by 25 and multiplied by the number of years smoking, was used as a measure for chronic cigarette smoke exposure. Lead in blood and chromium in urine were determined because some colour additives, containing lead chromate, were sometimes used during the production process of plastic containers.

During 3 months urine samples were collected every Thursday at the end of the shift for all exposed participants. Mandelic acid (MA) concentration was determined in the collected urine as described elsewhere (Severi et al. 1994a) and styrene airborne exposure (ppm) was then calculated as follows: styrene exposure(ppm)=0.065*MA(mg/gr cr.) - 3.6 (Ikeda et al. 1982). Weekly urine sampling of participants continued until blood and nasal cells were collected for N-terminal valine adducts determination, genotyping and genotoxicity assays. The average styrene exposure per participant over the 3 months was calculated and was used as a parameter of exposure during the previous months. The recent styrene exposure is the calculated exposure based on MA on the day of blood sampling. Blood samples were taken by venipuncture in heparinized Vacutainer and EDTA tubes. Nasal mucosa cells were sampled using a cell brush (Accellon Multi, Medesign, Germany) after gently rinsing the nose with physiological saline (Sterimar, Rhone-Poulenc Rorer, Belgium). Micronucleus scoring in nasal cells and the DNA strand break repair assay were only performed in a subgroup consisting solely of non-smokers.

1.2.2. Measurements and analysis procedure

Haemoglobin-adduct determination

N-terminal valine adducts were analysed by Gas Chromatograph-Mass spectrometry (Hewlett-Packard 6890 series gas chromatograph equipped with an autosampler and a 5973 series mass selective detector) according to the protocol described by Severi et al. (Severi et al. 1994b). The calibration curve for the analytical method was linear with a limit of detection of 1 pmol/g globin. A value of 0.5 pmol/g Hb was used in statistical analysis for N-terminal valine adducts levels under detection limit.

Genotoxicity assays and in vitro repair phenotype

Ficoll-Paque isolated PBMC from exposed and referent workers were analysed for levels of DNA breaks/alkali-labile sites using the alkaline version of the comet assay with modifications as described by De Boeck et al. (De Boeck et al. 1998) including internal standards of untreated and EMS-treated K562 cells (De Boeck et al. 2000b). Percentage of DNA in the tail (TD) was recorded as DNA migration parameter. For details on the methodology we refer to the report of the VUB. Whole-blood cultures and nasals slides were prepared and processed for scoring of micronuclei as described in De Boeck et al. (De Boeck et al. 2000a). For details we refer to the report of the VUB. Data are presented as frequencies of micronucleated binucleates per 1000 binucleates (‰ MNCB) and of micronucleated mononucleates per 1000 mononucleates (‰ MNMC) and number of micronucleated cells per

1000 cells (MN). All slides were analysed with an Olympus light microscope (1250x magnification).

For the determination of the in vitro strand break repair phenotype, cultures of isolated PBMC were prepared and incubated at 37°C for 24h. To determine the in vitro DNA repair capacity, cells were treated with SO (0.1mM) during 1 hour. After removal of the SO, cells were either immediately processed for the comet assay or further incubated to allow DNA repair during 1 or 24h. Thus, at different time points before and after SO treatment, cells were harvested and comet slides were prepared (4 sets of 2 slides per person). For details we refer to the report of the VUB.

Genotyping analysis

Blood samples were obtained in EDTA tubes from all participants in order to perform genotyping analyses. *CYP2E1 intron 6 DraI* genotypes (alleles *D* and *C*) were assayed with a PCR-RFLP-based method described in the report of the UCL (Haufroid et al. 1998). *GSTM1* and *GSTT1* polymorphisms were analysed by the method described by Arand et al. (1996) which allows detection of individuals with an homozygously deleted gene (*GSTM1null* or *GSTT1null*).(Arand et al. 1996) Two common *EPHX1* polymorphisms were detected in *exon 4* (His^{139}/Arg^{139}) and *exon 3* (Tyr^{113}/His^{113}) (see report UCL). The following gene polymorphisms of DNA repair enzymes were investigated: *hOGG1* (Ser^{326}/Cys^{326}), *XRCC1* (Arg^{194}/Trp^{194}, Arg^{280}/His^{280}, and Arg^{399}/Gln^{399}), and *XRCC3* (Trp^{241}/Met^{241}). All genotype analyses were performed using PCR-RFLP. For details we refer to the report of the VUB.

1.3. Results

1.3.1. Styrene exposure

The data on styrene exposure are presented in Table I. As shown in Figure 1 the exposure per sample week was not constant over time (ANOVA, $p<0.0001$). There were some weeks with high exposure moments (MA of 393.8 ± 355.2 mg/g cr corresponds with a styrene concentration of 29.2 ± 26.7 ppm) followed by weeks of low styrene exposure (MA of 102.9 ± 120.7 mg/g cr corresponds with a styrene concentration of 3.09 ± 4.2 ppm). Figure 2 shows the average styrene exposure in the exposed population. The calculated average styrene exposure of the exposed population was 9.5 ppm ± 9.6. All referents had a mandelic acid concentration below the detection limit. The mean recent styrene exposure was 12.4 ± 16.6 ppm. There was a statistically significant positive correlation between average styrene exposure and recent styrene exposure ($r=0.802$, $p<0.001$). Although there was a minor job rotation, workers with longer seniority were exposed to higher styrene levels since both exposure parameters also correlated positively with the duration of employment ($r=0.830$, $p<0.001$; and $r=0.701$, $p<0.001$ respectively).

The results of lead concentration in blood were all under the reference value (15 µg/100ml) (Lauwerys and Hoet 2001). In the exposed and referent group, 3 and 7 individuals respectively showed chromium in the urine above reference value (1 µg/g cr.) (Lauwerys and Hoet 2001), but still were all under the current biological exposure limit defined by the ACGIH (30 µg/g cr) (ACGIH 2003). In the selected subgroup of non-smokers, there were 4 referents with a chromium concentration in urine above the reference value.

1.3.2. Genotyping results

The genotype frequencies were not significantly different from that predicted by the Hardy-Weinberg equation. There was no statistically significant difference between exposed and referent groups in the frequency of polymorphisms (Chi-square and Fisher exact tests).

1.3.3. Differences between referent and exposed population: N-terminal valine adducts concentration and genotoxicity assays

N-terminal valine adducts concentration

There was no significant difference for N-terminal valine adducts between the styrene-exposed workers and referents (Table II). Smokers and non-smokers did not exhibit different levels of N-terminal adducts (M-W *U*, p=0.425). Considering data of the exposed population only, no significant effect of smoking or exposure could be detected. Constructing a multiple linear regression model with Hb adduct levels of all participants revealed a significant positive influence of recent styrene exposure (R^2=0.064, slope=0.095, p=0.017).

Genotoxicity assays and in vitro DNA strand break repair phenotype

For the detailed results of the genotoxicity assays and the in vitro DNA strand break repair phenotype, we refer to the report of the VUB. We found a significant positive correlation (r=0.29, p=0.008) between N-terminal valine adducts and DNA damage as evaluated by alkaline comet assay by measuring % of Tail DNA (TD). Although none of the participants showed a urinary Chromium concentration above exposure limit, correlation analysis showed a positive association between TD and urinary Chromium concentration (r=0.34, p=0.002). In the multiple linear regression analysis, both predictors (N-terminal adduct, slope=0.017; and urinary Chromium concentration, slope=0.042) revealed a significant positive influence on TD (R^2=0.18, p<0.001). Similar results (R^2=0.35, p<0.001) were found when considering only the data of the exposed population (N-terminal adduct, slope=0.022; and urinary Chromium concentration, slope=0.134)).
There were no significant associations between N-terminal valine adducts and any of the measure endpoints: micronucleus (MN) frequencies in binucleated (CB), mononucleated (MC) lymphocytes and in nasal cells (only determined in a subgroup of non-smoking subjects); and the in vitro repair phenotype.

1.3.4. Influence of genotypes on levels of N-terminal valine adducts, results of genotoxicity assays and in vitro DNA strand break repair phenotype.

The influence of genotypes on N-terminal valine adducts, results of genotoxicity assays and DNA strand break repair phenotype was evaluated by M-W *U*. None of the considered polymorphisms of biotransformation enzymes did influence N-terminal valine adduct levels. Multiple linear regression using the whole data set and using the data of referent and exposed group separately could not reveal any significant influence of the considered genotypes of metabolizing and repair enzymes.

1.4. Discussion

In this study, a set of biomarkers was applied on workers to evaluate whether low occupational exposure to styrene can cause genotoxic effects and to assess the influence of individual variability on the genotoxic effects. The average styrene exposure was low (9.5

ppm ± 9.6) compared with the current ACGIH TLV-TWA for styrene of 20 ppm. We were not able to detect a significant difference in N-terminal Hb adduct levels between the exposed and the referent populations. However, multiple regression analysis showed that the N-terminal Hb-adduct level was positively associated with recent styrene exposure (in ppm). Increased N-terminal adduct levels have been mainly found in studies with occupational exposures above or around the current TLV. Christakopoulos et al. (1993) found that Hb adduct levels correlated with the concentration of mandelic acid in urine (Christakopoulos et al. 1993b). The mean MA concentration in that study was 9.5 mmol/L, corresponding to a styrene exposure of 75 ppm. Fustinoni et al. found a significant difference in Hb adduct levels between workers and controls when the data for the workers were stratified for high exposure (average estimated was 23.5 ppm) (Christakopoulos et al. 1993a; Fustinoni et al. 1998). Our results indicate that a significant level of N-terminal valine adducts above background cannot be detected when occupational styrene exposure is low (± 10 ppm).

We found that TD correlated with N-terminal adduct level. A 3 year follow-up study in hand lamination workers (exposed to 10-50 ppm) could not reveal an association between N-terminal Hb adducts and SSB (Vodicka et al. 1999). In an earlier study a significant correlation was reported between O^6-dG-styrene adduct levels and amounts of DNA strand breaks.(Vodicka et al. 1995). N-terminal Hb adduct levels can be used as surrogates for DNA adducts (Farmer 1995). The correlation found between TD and N-terminal Hb adduct levels could indicate that the DNA damage in the form of single strand breaks was a result of covalent binding of styrene to DNA.

Correlation and regression analysis revealed a positive association between TD and chromium concentration (all under current BEI-TLV of 30 µg/g cr.). The chromium exposure of the referent group is probably originating from a combination of cigarette smoking and diet, since no significant chromium exposure could be documented during the production process. Recent research showed an increase in Tail Moment in chrome-plating workers (urinary Chromium: 7.31 µg/g cr. ± 4.33) compared with non-exposed individuals (Gambelunghe et al. 2003). It has been stated that SSB are formed during the reductive conversion of chromium(VI) in the cells (Blasiak and Kowalik 2000). Since the referent group had a higher chromium concentration than the exposed group and since the TD in comet assay in this study depends on both styrene-adduct formation and chromium exposure, these findings could possibly also contribute to explain the absence of a significant difference in TD between the exposed and non-exposed population.

Neither univariate analyses nor multivariate analyses could reveal an influence of the genotypes of biotranformation enzymes on the results of the N-terminal valine adducts. The relation between low ambient styrene exposure and the results of N-terminal adduct levels, genotoxicity assays and in vitro repair capacity respectively in workers does not seem to be influenced by the genotype. This could be due to a lack of statistical power in a study of this size.

In conclusion, we can state that low exposure to styrene (under current TLV) induces genotoxic effects in workers as assessed by well validated methodologies: N-terminal valine adducts, Comet assay, MNMC, MNCB and nasal MN. Individuals with a higher biologically effective dose (evaluated by haemoglobin adducts) showed a higher percentage of single-strand breaks. It is clear that the influence of polymorphisms in metabolising and DNA repair enzymes on genotoxicity needs further clarification. Therefore, biomonitoring studies on a high number of genotyped individuals are necessary to explore these relationships.

1.5. Tables and Figures

Table I: Characteristics of the study population

	Referent group (n=44)			Exposed group (n=44)		
	Mean ± SD	Range	n (%)	Mean ± SD	Range	n (%)
Age (years)	40.9 ± 9.0	22.0-58.0	,	41.6 ± 9.6	21.0-59.0	,
Smoking	,			,		
Smokers	,	,	21 (48%)	,	,	21 (48%)
Pack years[a]	16.8 ± 9.7	1.3-33.6	,	13.7 ± 10.5	0.24-35.0	,
Cotinine in urine (mg/l)	0.9 ± 1.1	ND-3.0	,	1.9 ± 1.0	ND-4.8	,
Exposure data	,			,		
Average mandelic acid (mg/g cr.)	ND	ND		201.6 ± 148.3	ND-618.2	
Calculated styrene exposure (ppm)	ND	ND	,	9.5 ± 9.6	ND-36.6	,
Calculated recent styrene exposure (ppm)	ND	ND	,	12.4 ± 16.6	ND-73.0	,
Calculated maximum styrene exposure (ppm)	ND	ND	,	31.0 ± 28.8	1.5 ± 116.6	
Duration of employment in styrene environment (months)	0.0	0.0	,	170.7 ± 123.8	7.0-455.0	,
Lead in blood (µg/dl)	5.4 ± 3.1	1.7-15.0	0 (0%)[b]	4.6 ± 1.7	2.1-10	0 (0%)[b]
Chromium in urine (µg/g cr.)	1.2 ± 3.1	0.05-18.6	7(16%)[c]	0.5 ± 0.9	0.1-5.1	3 (7%)[c]

[a]packyears only calculated for the current smokers: ((number of cigarettes/day)*years smoking)/25
[b]number (%) above reference value (15 µg/100ml)
[c]number (%)above reference value (1 µg/g cr.)
ND= not detected

Table II: N-terminal adduct levels and biomarkers of genotoxicity

	Referents			Exposed			M-W *U*
	n	Mean ± SD	Range	*n*	Mean ± SD	Range	p-value
N-terminal adduct level (pmol/g hb)	44	3.08 ± 3.30	ND-13.06	44	5.23 ± 3.49	ND-25.52	0.131
Comet assay: TD (DNA % in tail)	44	0.80 ± 0.34	0.20-1.62	37	0.80 ± 0.31	0.15-1.60	0.878[b]
MNMC (MN/1000 mononucleated lymphocytes)	41	0.11 ± 0.20	0.00-0.60	38	0.71 ± 0.88	0.00-4.00	**<0.001**
MNCB (MN/1000 binucleated lymphocytes)	41	2.65 ± 1.94	0.00-10.0	38	3.93 ± 2.75	0.00-13.50	**0.02**
MN Nasal (MN/1000 nasal cells)[a]	17	0.23 ± 0.31	0.00-1.00	23	0.52 ± 0.49	0.00-1.50	**0.04**[b]
RD 1h (% residual DNA damage 1 hour after in vitro SO)[a]	17	100.53 ± 9.43	86.53-124	17	98.41 ± 16.70	38.53-115.25	0.71
RD 24h (% residual DNA damage 24 hours after in vitro SO)[a]	17	13.35 ± 9.96	-0.54-33.0	17	40.55 ± 38.82	1.82-108.92	**0.04**

[a] only determined in a subgroup of non-smoking individuals
[b] p-value of unpaired T-test

Figure 1: Evaluation of MA concentrations (mg/g cr.) per week in exposed workers

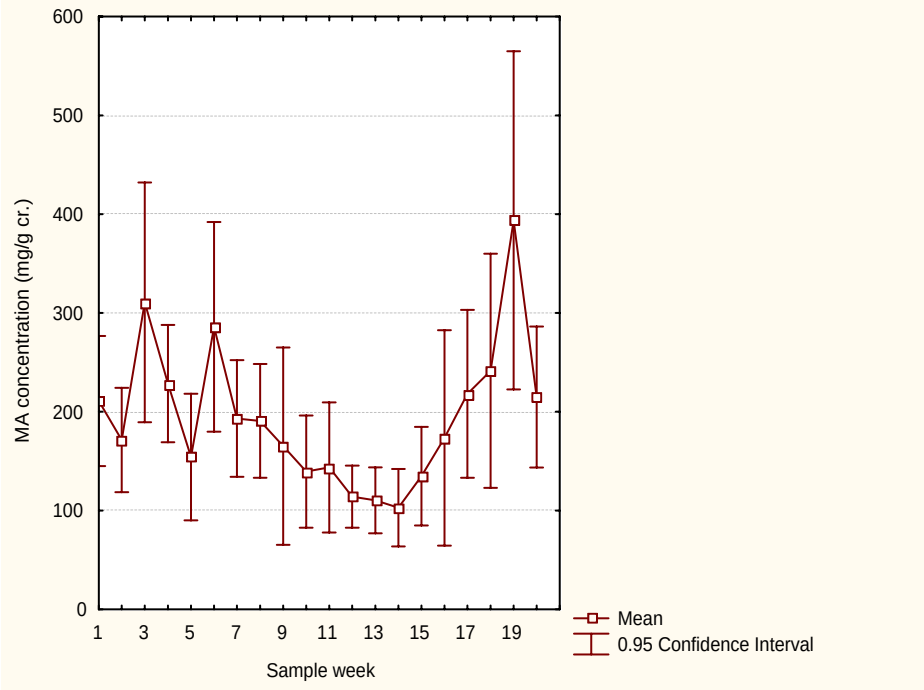
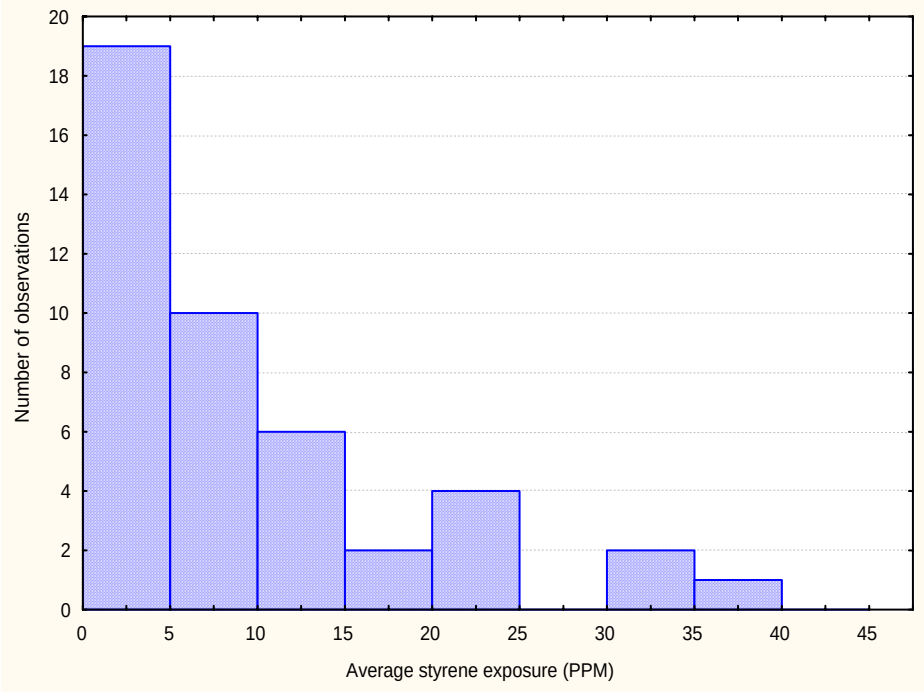


Figure 2: Distribution of average styrene exposure (ppm) in exposed workers (n=44)



2. Opioid analgesics.

2.1. Introduction

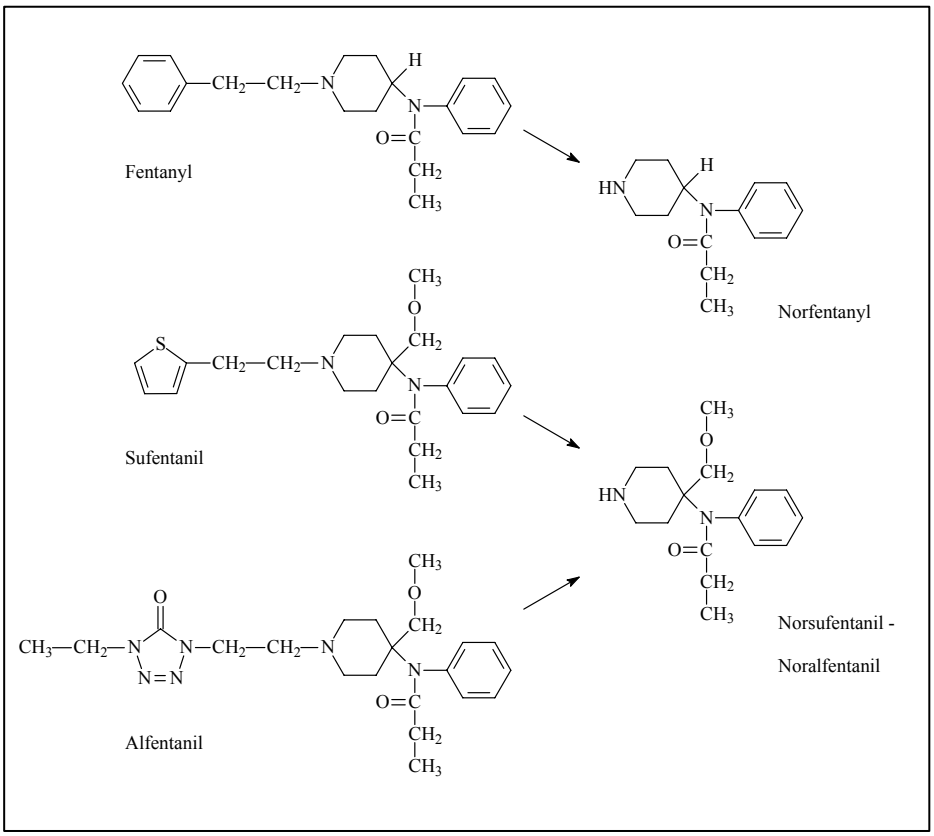
2.1.1. Context and general frame of the research

Fentanyl, sufentanil and alfentanil are members of the 4-propananilido-piperidine class of synthetic narcotic analgesics. These opioid compounds are more potent than the prototype analgesic morphine, and are less likely to produce complicating adverse effects upon acute administration. As a result of these properties, these drugs are among the most widely used agents to produce surgical anesthesia (Valaer et al., 1997). Pharmacological properties of the opioid analgesics include the induction of analgesia, sedation, respiratory depression, bradycardia, miosis and truncal rigidity.
In humans fentanyl, sufentanil and alfentanil are extensively metabolized and only a few per cents of the original doses are excreted unchanged in urine. The main metabolic pathway of the opioid analgesics is the CYP3A4 mediated oxidative N-dealkylation at the piperidine nitrogen, resulting in the formation of nor-metabolites (Fig. 1) (Goromaru et al., 1984, Meuldermans et al., 1988). Research has shown that a wide range of activities of this CYP3A4 enzyme exists in human populations potentially inducing inter-individual variability in drug metabolism and clearance.

Prior to formulation in various dosage devices, fentanyl, sufentanil and alfentanil are synthesized as neat chemicals. As with other pharmaceutical ingredients that are specifically designed to modify biological function, production workers can be placed at risk of experiencing pharmacological effects if exposures are not adequately controlled. Following exposure, primary adverse effects of the opioid analgesics may include dose-related sedation, associated with a risk of acute or delayed respiratory depression, bradycardia and hypotension (Willens et al., 1993). To limit and control the potential exposure and the health risk associated of workers engaged in the synthesis and formulation of these potent narcotics, monitoring programs are required. In addition to industrial hygiene measurements, primarily focussing on the assessment of respiratory exposure, biological monitoring aims at assessing the individual workers' uptake of the compound and the related risk. In view of the highly lipophilic nature of especially sufentanil and fentanyl and to a less extent of alfentanil, absorption through the skin could present an important concomitant route of exposure. Moreover, fentanyl and alfentanil metabolism and clearance may be subject to inter-individual variability (Kharasch et al., 1977, Labroo et al., 1977), and selecting the appropriate biomarker of exposure could potentially provide additional information on the individual susceptibility of exposed workers.

Several assays for measuring fentanyl-like compounds in biological fluids of patients have been reported. Among these assays, techniques based on immunological principles and instrumental techniques, including both liquid and gas chromatographic separations with diverse detection systems, are most widely used (Fryirsa et al., 1997, Dotsikas et al., 2002, Dufresne et al., 2001, Choi et al., 2001, Mautz et al., 1994, Shou et al., 2001). Monitoring methods for the detection of occupational exposure to opioid analgesics are not reported in the literature. In order to measure ultra low levels of the fentanyls and their major metabolites in urine, which might be expected after occupational exposure, new and sensitive analytical methods had to be developed and validated.

Fig. 1: Structure of fentanyl, sufentanil and alfentanil and their major metabolites formed through CYP3A4 mediated oxidative dealkylation.



2.1.2. Aims of the research

The aims of the present study therefore were:

(1) To develop and comprehensively validate highly sensitive gas chromatographic-mass spectrometric (GC-MS) analytical methods to detect ultra low levels of fentanyl, alfentanil and sufentanil in environmental samples and to determine these opioid analgesics and their metabolites in urine of potentially exposed opioid production workers.

(2) To conduct a field study in which the above developed methods were applied to measure external and internal exposure levels of pharmaceutical production workers to fentanyl.

(3) To identify and validate a relevant biomarker for inter-individual variability in workers' opioid metabolism and clearance and to test the hypotheses indicated in the theoretical frame.

(4) To screen pharmaceutical production workers for acute or chronic neurological effects.

2.2. Theoretical frame

The main metabolic pathway of fentanyl, alfentanil and sufentanil is the CYP3A4 mediated oxidative N-dealkylation at the piperidine nitrogen, resulting in the formation of nor-metabolites. Research has shown that a wide range of activities of this CYP3A4 enzyme exists in human populations. The enzyme activity can be investigated in vivo in patients treated with the opioid analgesics during surgery or for chronic pain disorders. The clearance of the drugs will be used as metabolic probes for CYP3A4 activity. In workers this method would not be applicable. Therefore, an alternative methodology will be adopted, using

endogenous steroid substrates and metabolites (e.g. 6-β-hydroxycortisol/cortisol) as metabolic probes (Joellenbeck et al., 1992). This method has to be validated in a pilot study in patients treated with the drugs by comparing the results obtained with both substrates.
In this framework the following hypotheses will be tested:

- "The possible individual susceptibility to the opioids caused by CYP3A4 deficiency, can be evaluated directly by measuring the metabolising rate of these opioids themselves and indirectly by measuring the metabolising rate of endogenous hormone substrates of the same enzyme".
- "Workers with reduced activity in the opioid metabolizing enzyme CYP3A4 are more at risk of experiencing neurological effects as measured by the neurotoxicological end-points in this study".

2.3. Methods

2.3.1. Sampling

Pilot study in patients

In order to evaluate intra- and inter-individual differences in drug metabolizing capacity, a pilot study was undertaken involving 50 patients treated with fentanyl for the relieve of chronic cancer related pain. In this group the clearance of the drug will be used as a probe for the phenotypic polymorphism in CYP3A4.
Urine samples were collected from each patient at three consecutive days on which a constant fentanyl nominal dose was administered through the application of Durogesic® Patches. Urine samples consisted of a spot sample or a 24h-collection, depending on the mental and physical status of the patient. After collection, the urine samples were stored at –30°C until fentanyl and norfentanyl analysis was performed. For each patient the actual fentanyl dose administered was measured through the analysis of residual fentanyl content in the removed Durogesic® patches.

Field study in workers

A detailed survey was conducted in a pharmaceutical production department in December 2001. During a three weeks fentanyl production campaign, four workers were monitored, of whom two operators were directly involved in fentanyl production, one worker was charged with surveillance and one operator was involved primarily in sufentanil synthesis. Despite significant measures that have been taken by the pharmaceutical industry to control the exposure of production workers, low-level background exposures to these substances with incidental peak exposures were demonstrated to occur in previous research.
During fentanyl production cycles at which tasks were performed involving the neat finely divided fentanyl powder, the production workers were equipped with a Mururoa® personal protective suite and independent air supply. At these occasions external exposure monitoring was performed inside the protective suite. All other tasks were executed using routine protective measures as coveralls, special shoes, gloves and occasionally safety masks and at all these occasions, sampling was performed outside the protective equipment.

Air sampling

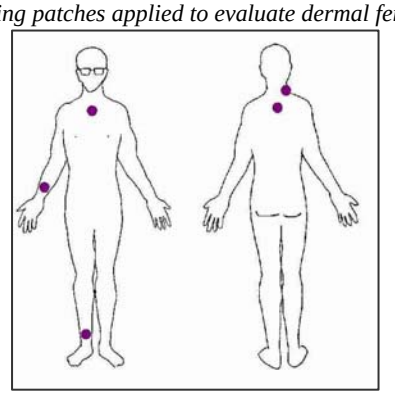
Personal inhalable exposure was measured by means of a IOM sampling head mounted near the breathing zone of the worker. For each worker 2 personal air samples were taken daily during each half shift and supplementary short duration samples were taken at special high risk related tasks. Total airborne particulate matter was collected on glass fibre filters (Pall

Corporation) with air samplers (Gilair 5 ®, Sensidyne Inc.) at a flow of 2,0 ml/min. Filters were stored at –30°C until fentanyl analysis.

Dermal sampling

Dermal exposure to fentanyl was measured in two surveys. The first survey focussed on the quantification of fentanyl exposure distribution across the body and served to select the most appropriate body part to be sampled in the estimation of total dermal body exposure. In this survey five sampling patches were placed on different locations of the body: arm, back, chest, neck and lower leg (fig. 2). The sample patches consisted of a transparent dressing with an 4 x 2,5 cm absorbent pad (OpSite, Smith & Nephew Medical Limited) and were worn during a half shift at occasions on which high risk related tasks were performed. At the end of this half shift also hand wipes were taken as described hereafter. The second survey involved the estimation of total dermal body exposure through a simple hand wiping protocol. In this survey the operators involved were asked to wipe their finger tops with a swab previously immersed in isotone saline and subsequently repeat this action with an alcohol-wetted wipe. All patches and hand wipes were transferred to individual extraction vials and stored at –30°C until fentanyl analysis.

Fig. 2: Body location of the five sampling patches applied to evaluate dermal fentanyl exposure.



Urine sampling

Urine samples were collected daily at three time intervals: (i) in the morning at arrival on the job, (ii) at the end of the first or at the beginning of the second half shift and (iii) at the end of the working day. The weight of the collected samples and the excretion periods were registered. As soon as possible after collection, the urine samples were stored at –30°C until sample preparation and fentanyl and norfentanyl analysis.

2.3.2. Measurement

<u>Determination of airborne and dermal fentanyl</u>

Sampled and QC control glass fibre filters, hand wipe samples and dermal sampling patches were transferred to individual extraction vials and 10 ml or 20 ml of a citrate/sodium hydroxide buffer (pH 6) was added. The samples were shaken automatically during 30 minutes. For each sample three replicates of 1 ml of the extract were transferred to new sample tubes. The samples were basified with 10 N NaOH and 50 µl of an internal standard solution containing deuterated analogues was added. The samples were applied to a 1 ml EXtrelut® NT1 SPE column. Elution was carried out using 6 ml of a mixture of *n*-heptane/*iso*-amylalcohol (98,5/1,5 v/v). The extracts were evaporated at 50°C using a gentle stream of nitrogen, reconstituted in 30 µl of methanol and analyzed via GC-MS.

Determination of urinary fentanyl and norfentanyl

Aliquots of 1 ml of each urine standard and sample were basified with 10 N NaOH and 50 µl of an internal standard solution containing deuterated analogues was added. The analytes of interest were extracted using the SPE protocol described above. For the determination of fentanyl, cooled residues were reconstituted in 30 µl of methanol. For the determination of the opioid nor-metabolites, the cooled residues were derivatized with 100 µl of a 0,1 M pentafluorobenzoyl chloride solution in chloroform. The residues were allowed to react overnight (16 hours) at 4°C. After that period, the samples were dried under a gentle stream of nitrogen and the residues were reconstituted in 30 µl of methanol and analyzed via GC-MS.

GC-MS analysis

The analyses were carried out on a Hewlett-Packard 6890 series gas chromatograph equipped with an autosampler and a 5973 series mass selective detector (MSD) in electron impact (EI) mode (70 eV). For the determination of fentanyl, a 5 µl aliquot of the sample was introduced in a splitless way onto a 30-m DB35-MS (J&W). For the analysis of the nor-metabolites, a 30-m DB5-MS (J&W) column was used. The GC separation was obtained using a program with an initial oven temperature of 70 °C that was increased at a rate of 60 °C/min to a final temperature of 280 °C. The mass selective detection system was operated in the selected ion monitoring (SIM) mode. Base ion fragments occurring at *m/z* 245 for fentanyl, and *m/z* 250 for d_5-fentanyl were monitored and used for subsequent quantification. The norfentanyl-metabolite derivatives were monitored using their specific molecular ion fragments at *m/z* 426 for norfentanyl and *m/z* 431 for the internal standard analogue d_5-norfentanyl.

Determination of urinary cortisol/6-β-hydroxycortisol ratio

Urinary cortisol and 6-β-hydroxycortisol will be determined by the GC-MS analytical procedure recently developed and published by Luceri et al. (2001). Briefly, 1 ml urine samples were fortified with d_2-cortisol as internal standard and were applied to a 1 ml Extrelut ® SPE column and eluted with 6 ml of dichloromethane. Both analytes of interest were subsequently derivatized to dimethoxime tri-(trimethyl-silyl)-ethers (MOX-TMS) and analyzed via GC-MS using specific ion fragments (SIM).

Evaluation of neurological end-points

The following parameters will be examined markers of neurotoxicity from exposure to opioid analgesics:

- Test for simple reaction times (visual/auditive) with the computerized Neurobehavioral Examination System (NES).
- Complex visuomotor reaction times.
- Increased pain thresholds through EMG.
- Respiratory depression as parameters for acute neurological effects.
- A questionnaire for symptoms of drug dependence/craving as instrument for evaluating chronic neurological effects.

2.3.3. Analysis procedure

Urinary fentanyl and norfentanyl concentrations were adjusted for urinary creatinine content (patients, workers) or urinary flow (workers). It should be noted that samples with a urinary creatinine content of less than 0,3 g/L were not included in the analysis.

Time weighted average (TWA) respiratory exposure was calculated for each worker for each half shift period (4h) and for each working day (8h).
Dermal exposure surface concentrations (µg/cm²) (as determined through the analysis of skin patches and hand wipes) were transformed to an 8h time weighted average (TWA) exposure concentration per body part (µg/body part) by multiplying the fentanyl concentration of the individual sampler with the anatomical dimensions described by EPA (1989). Total dermal body contamination (µg/8h) was calculated by adding the fentanyl TWA surface concentration of the individual body parts. In the calculation it is assumed that each of the samplers represent a skin region with a certain surface area at which the fentanyl contamination is uniformly present.
Mixed ANCOVA and Variance Components Method of Analysis was used to estimate variance components based on maximum likelihood methods (REML).
Pearson correlation coefficients were used to investigate the relationship between workers' dermal exposure at individual skin regions and total dermal body exposure and to explore the relationship between various markers of exposure. In these analyses logarithms of the exposure values were used.
Multiple regression analysis was performed to analyse the relationship between several independent or predictor variables and a selected outcome.

2.4. Results

2.4.1. Pilot study in patients

Intra- and inter patient variations in urinary concentrations of fentanyl (blue line) and norfentanyl (green line) corrected for administered dose are shown in figure 3. A similar graph representing intra- and inter-patient variability in urinary norfentanyl/fentanyl ratios is shown in figure 4.

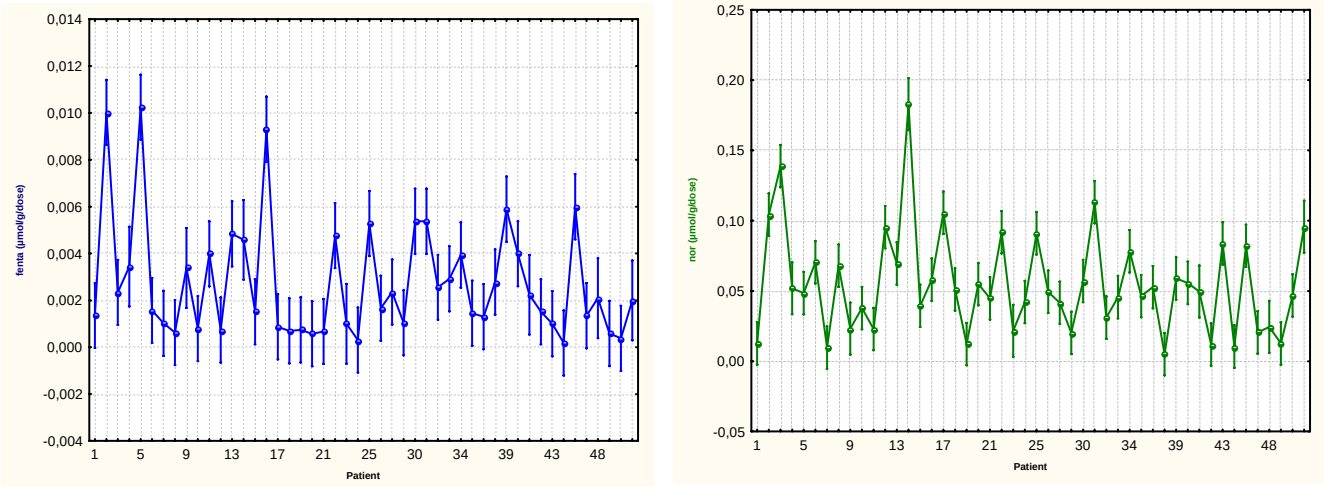


Fig. 3: Intra- and inter-patient variations in urinary fentanyl and norfentanyl concentrations (µmol/g) corrected for administered dose. Vertical bars denote 0,95 confidence intervals.

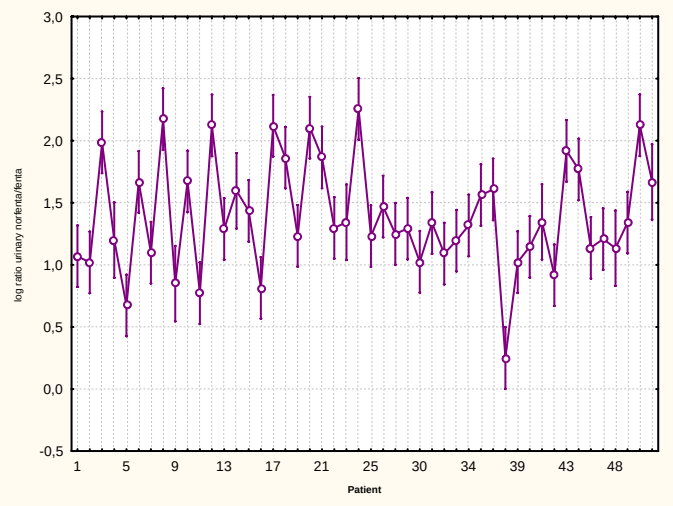


Fig. 4: Intra- and inter-patient variations in urinary norfentanyl/fentanyl ratios. Vertical bars denote 0,95 confidence intervals.

The above graphs indicate that a potentially large variability exists in fentanyl clearance and durg metabolism -as measured through the urinary norfentanyl/fentanyl ratio- between patients. Moreover, for the three parameters examined, the estimation of variance components (REML) indicated that the majority of the observed variance was patient related, accounting for respectively 77% (urinary fentanyl), 96% (urinary norfentanyl) and 80% (ratio norfentanyl/fentanyl) of the total variance (table 1).

Table 1: Mixed ANCOVA and Variance Components method of analysis to estimate variance components based on maximum likelihood methods (REML).

Parameter examined	Variance effect	Variance compone	Variance %	Asymptotic p
Urinary fentanyl	Between patients Within patients	0,17 0,051	77% 23%	0,000016 < 0,00001
Urinary norfentanyl	Between patients Within patients	0,13 0,0051	96% 4%	0,000011 < 0,00001
Urinary norfentanyl/ fentanyl	Between patients Within patients	0,18 0,047	80% 20%	0,000010 < 0,00001

Finally, when exploring the relationship between urinary fentanyl or norfentanyl and the administered dose, multiple linear regression revealed that both parameters are significantly ($p<0,0001$) but rather weakly correlated with the administered dose ($r = 0,56$ and $r = 0,63$ respectively) indicating that inter-patient variability exists in drug clearance and metabolism. However, in the multiple regression analysis, non of the parameters indicated in the literature (age, sex, BMI) were found to have significant influence on the examined variable, again indicating that other parameters, like CYP3A4 activity might be an important determinant.

2.4.2. Field study in workers

Dermal sampling

Figure 5 shows the results of the assessment of fentanyl exposure distribution across selected parts of the body in an elective operator, involved in the fentanyl production. All patches applied clearly show detectable amounts of fentanyl. In figure 6 the same data are shown

complemented with the measured fentanyl exposure of the arms and hands. It is clear that in this worker, the arms and hands are highly contaminated body parts. Figure 7 shows the same graph for two other operators. Operator C was charged with surveillance tasks and clearly shows lower fentanyl dermal exposure than operator A, as was expected. In this operator hands and arms were also manifestly contaminated but not consistently more than the other body parts. Operator D was primarily involved in the sufentanil synthesis and clearly shows the lowest fentanyl dermal exposure across the body, again being not consistently most elevated on arms or hands. These results might indicate that different operators have various, possibly task related sources of dermal contamination.

In order to select the most appropriate body part to be sampled in the estimation of total dermal fentanyl body exposure, the correlation between the calculated total dermal exposure and the exposure of the different body parts was explored. Pearson correlation coefficients (r) are shown in table 2. Fentanyl contamination at the arms showed a highly significant correlation with total dermal exposure, when all four operators were included, as well as when the operators were catogerized in two groups (fentanyl production workers and other operators). For the total group of 4 operators high correlations were found between fentanyl contamination on all skin regions examined and total dermal exposure. Correlation between fentanyl contamination in the neck region and on the chest is no longer significant at the α = 0,05 level in the fentanyl production group. For the other operators only the relationship between fentanyl contamination on the arms and total body dermal exposure is significant at the indicated significance level. Again, these results might indicate that in these two groups workers' dermal exposure originates from different sources. Nevertheless, the assessment of fentanyl skin contamination on the arms appears to be an appropriate parameter in estimating total body exposure in all workers included.

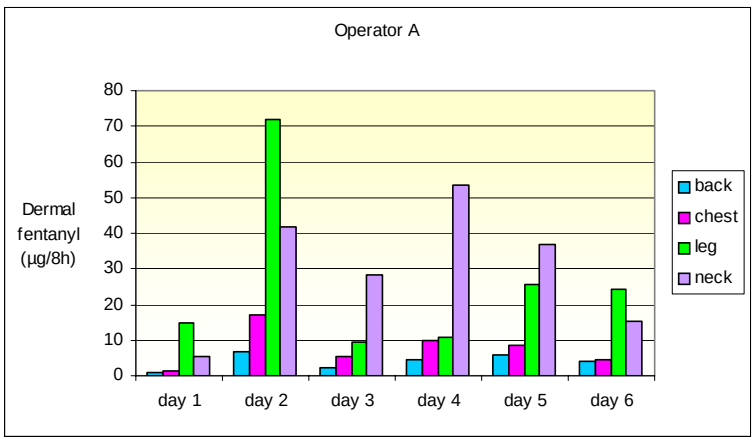


Fig. 5: Operator A Fentanyl dermal exposure measured through the analysis of patch samplers.

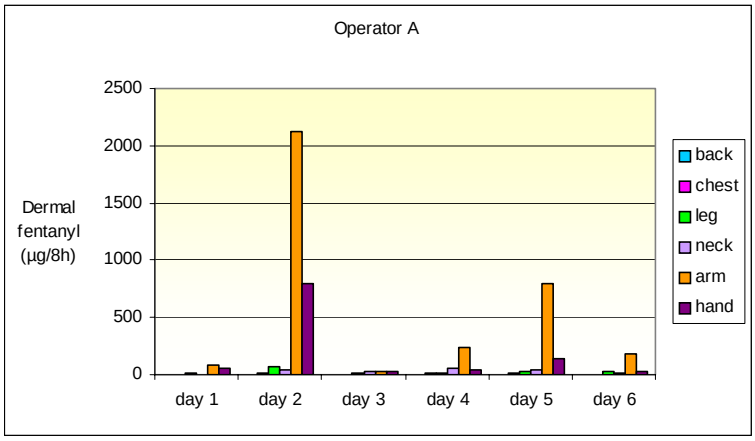


Fig. 6: Operator A Fentanyl dermal exposure measured through the analysis of patch samplers and hand wipes.

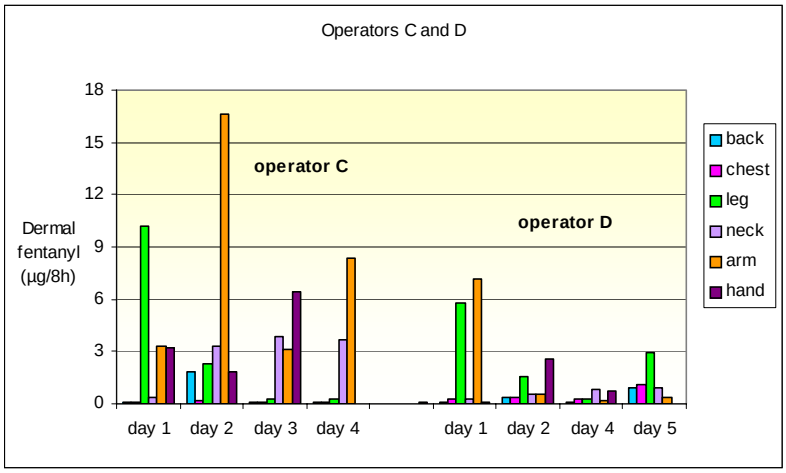


Fig. 7: Operator C and D Fentanyl dermal exposure measured through the analysis of patch samplers and hand wipes.

Table 1. Pearson correlation coeffcients (r) between fentanyl level (µg/8h/body part) at individual skin regions and calculated total body dermal exposure (µg/8h)

Skin region	All (N=4)	Fentanyl production workers (N=2)		Other operators (N=2)	
Neck	0,87	0,56	(NS)	0,71	
Back	0,78	0,79		0,28	(NS)
Chest	0,81	0,61	(NS)	0,07	(NS)
Legs	0,84	0,85		0,46	(NS)
Arms	0,97	0,97		0,92	
Hands	0,90	0,87		0,52	(NS)

(NS) not significant at the α =0,05 level

Figure 8 shows the results of the analysis of the hand wipes taken in the second survey. The fentanyl production workers (N=2) show a highly significant ($p < 0{,}0001$) higher contamination of the hands than the other operators (N=2), as was expected.

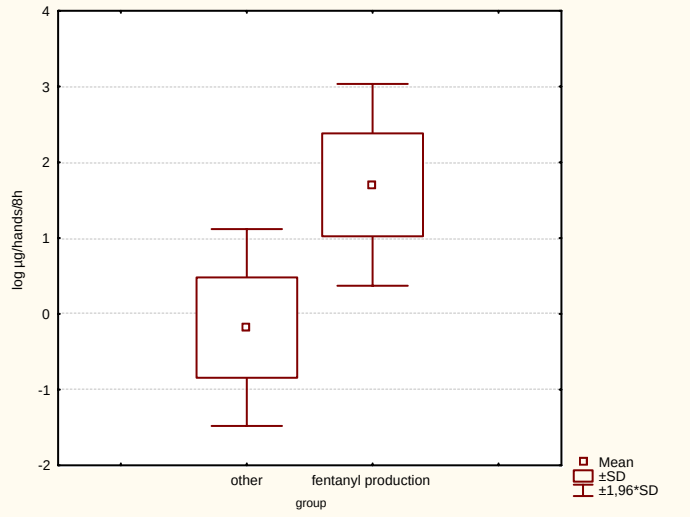


Fig. 8: Fentanyl exposure of the hands, measured through the analysis of hand wipes.

Air sampling

Figure 9 shows the results of the determination of the time-weighted respiratory exposure integrated over each have shift as compared to the current Occupational Exposure Limit (OEL) (100 ng/m³) for operator A. It is clear that on a number of occasions potential respiratory exposure exceeds the current OEL. It should be noted however that personal inhalable dust samples were taken outside any respiratory protection used, except for the datapoints indicated as white bullets.

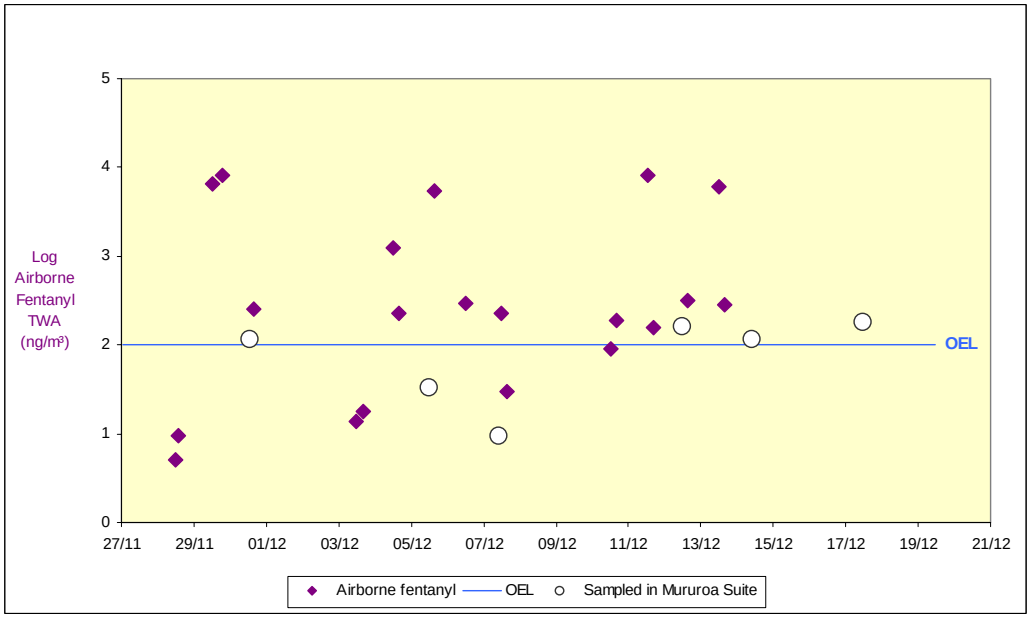


Fig. 9: Operator A Fentanyl potential respiratory exposure as compared with the Occupational Exposure Limit (OEL). Samples taken in the Mururoa protective suite reflect actual respiratory exposure levels and are indicated in white bullets.

These datapoints are derived from personal air samples taken inside the Mururoa® protective suite and hence reflect actual respiratory exposure, more or less fluctuating around the current OEL.

Urine sampling

Figure 10 shows the urinary fentanyl clearance profile in operator A. The first urine sample, collected before fentanyl production was started, showed no detectable amount of fentanyl. All subsequently collected urine samples had detectable amounts of fentanyl, ranging from 15 pg/ml to 1560 pg/ml urine, corresponding with a urinary clearance of 18 to 2750 pg fentanyl/min.

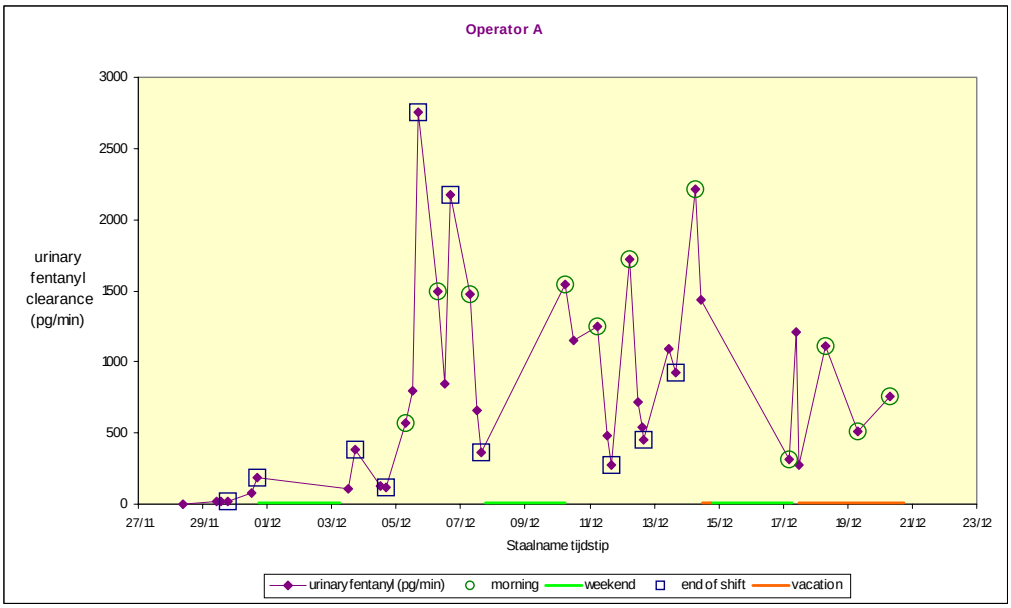


Fig. 10: Urinary fentanyl clearance (pg/min) in operator A.

As was observed from the concentration versus time plots in figure 10, urine samples collected during the weekends and on holidays, unexpectedly also showed detectable fentanyl and norfentanyl levels. It was hypothesized that a delayed fentanyl clearance appeared to occur, possibly as a result of unintentional and prolonged exposure through contamination of the worker's skin. This intriguing hypothesis was subject to further exploration during a follow-up study involving -amongst other parameters- the evaluation of dermal fentanyl exposure (data not shown).

Correlations between markers of exposure

Simple regression analysis revealed a highly significant correlation (r=0,79) between urinary fentanyl clearance at the end of each half shift and dermal fentanyl contamination on the hands, the latter serving as an estimate for total dermal fentanyl exposure averaged over the same period of time (fig. 11). Also a significant (p=0,001) but less pronounced correlation (r=0,36) appears between urinary fentanyl clearance at the end of each half shift and time weighted average respiratory exposure for the same time period. However, using multiple stepwise regression analysis, it was shown that although the correlation between fentanyl clearance and hand contamination levels remains highly significant (p<0,0001), the according correlation with respiratory exposure is no longer significant (p=0,28). These data probably

indicate that the observed respiratory fentanyl levels and fentanyl dermal exposure of the hand are interrelated and urinary fentanyl levels and clearance is dominantly determined by fentanyl dermal exposure. Hence, it was concluded that fentanyl absorption through the skin could present a major route of exposure as compared to respiratory exposure.

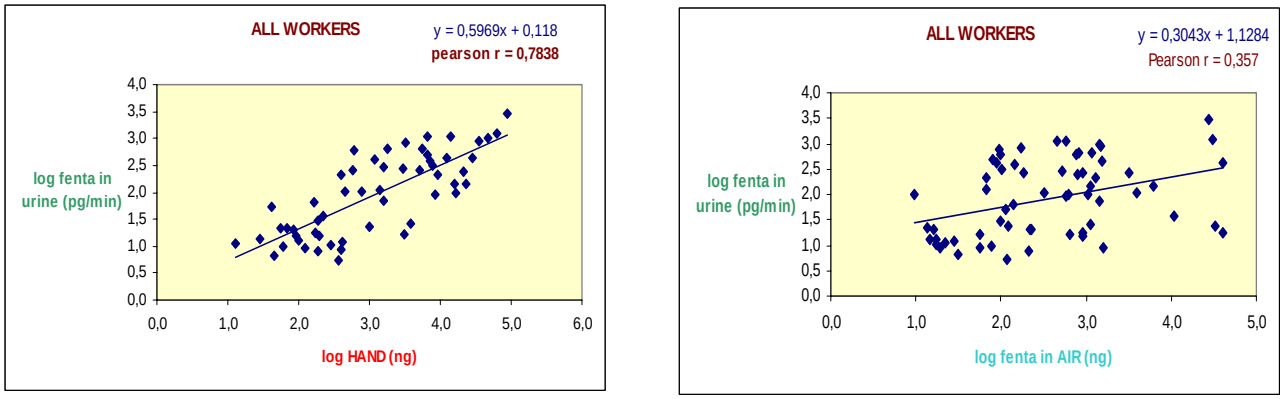


Fig. 11: Relationship between urinary fentanyl clearance (log fenta in urine (pg/min) and fentanyl contamination levels of the hand (log hand (ng)) and between urinary fentanyl clearance (log fenta in urine (pg/min) and fentanyl respiratory exposure (log fenta in air (ng)). All values were log-transformed and were averaged over a half shift period (4h time weighted average value).

Inter-individual susceptibility

Up till now, the limited sample size (n = 4) in the conducted field study in workers, does not allow for the exploration of inter-individual susceptibility. However, the experience and knowledge gained through the research work described above including both the pilot study in patients and the assessment of internal and external opioid exposure in workers will be adopted in future surveys in the exploration of workers' susceptibility in the pharmaceutical industry.

2.5. Discussion

Highly specific and sensitive analytical methods were developed to measure fentanyl in personal air samples and dermal patch samplers and to determine this drug and its major metabolite in urine of exposed workers. The same methodology was applied for the determination or urinary fentanyl and norfentanyl profiles in patients.

In order to evaluate inter-individual differences in drug metabolizing capacity, a pilot study was undertaken involving 50 patients treated with fentanyl for the relieve of chronic cancer related pain. In this group the clearance of the drugs was used as a probe for the phenotypic polymorphism in CYP3A4. It was shown that considerable variability exists between patients' metabolising capacity as expressed by the ratio of urinary norfentanyl/fentanyl. The use of drug clearance as a probe for CYP3A4 activity will be further validated using endogenous steroid substrates and metabolites (e.g. 6-β-hydroxycortisol/cortisol) as an alternative metabolic probe.

In a first survey involving a group of four fentanyl-exposed workers, a systematic evaluation was performed regarding potential and actual respiratory exposure and a collection of urine samples was organised in the frame of a biological monitoring strategy. Spatial distribution of dermal fentanyl contamination was investigated by means of dermal exposure patch samplers placed on five anatomical regions of the body, complemented by the use of hand wipe samples. An underlying assumption in this assessment is that the exposure is uniformly distributed within the discreet anatomical region. Fentanyl production workers showed high dermal contamination levels, especially for the hands and the arms, being significantly lower in the other workers. Dermal contamination of the chest, neck region, back and legs had only a minor contribution to total dermal body exposure in the first group, while contamination of these body regions significantly contributed to total dermal exposure in the second. Therefore, it is assumed that dermal exposure in both groups of workers takes place in a different way, being task related in the fentanyl production workers and being probably caused by direct contact with contaminated surfaces in the other workers.

For the four workers included in the field study, total body dermal exposure showed statistically significant correlation coefficients with exposure measured at the individual skin regions, especially the arms. However, in the subsequent survey, total body dermal exposure was estimated through the much more simple hand wiping protocol described.

Pearson correlation coefficients were used to investigate the relationship between various markers of exposure and to explore the importance of different exposure pathways. Total body fentanyl dermal exposure was found to be highly correlated with urinary biomarkers of exposure, while this correlation was less pronounced in the case of respiratory exposure. Multiple stepwise regression analysis revealed that fentanyl absorption through the skin could present a major route of exposure. In these settings, the assessment of the workers' integrated individual opioid uptake through a biological monitoring strategy is essential. Moreover, fentanyl metabolism and clearance may be subject to inter-individual variability and selecting the appropriate biomarker of exposure could potentially provide additional information on the individual susceptibility of exposed workers.

It is expected that this study would later serve as a model for the assessment of occupational exposure and individual workers' susceptibility for other pharmaceutical ingredients having similar effects.

Promotor: Prof. Dr. H. Thierens

Gent University
Faculty of Medicine
Dept. of Anatomy, Embriology, Histology and Medical Physics
Biomedical Physics & Radiation Protection

I. Introduction

I.1. Context and general framework of the research

Ionising radiations are known as a strong carcinogen. Exposure of populations of workers at the workplace takes place especially in the medical and the nuclear sectors. In Belgium more than 40.000 workers are occupationally exposed to radiation, and the dose received in the execution of certain jobs amounts to 10 mSv/year, which means a risk for cancer mortality of 1 over 50 in a 40 year professional career according to currently applied risk estimation methods.

Studies of radiotherapy patients have shown that 1 to 2 % of these patients overreact to the therapy, resulting in early or late complications due to a high sensitivity with respect to radiation. This together with the enhanced radiosensitivity and cancer susceptibility observed in patients suffering from certain genetic syndromes points to the genetic base for radiosensitivity. Workers with this genetic burden will have a significantly enhanced risk for a radio-induced malignancy when exposed at the workplace compared to the average risk evaluation. Knowledge of the individual radiation sensitivity of the worker can play a very important role in the framework of the preventive policy of the occupational medicine. In contradiction to the population approximation, resulting in an average risk estimation assuming that all individuals are reacting identically to the exposure, an individually tailored preventive approach taking into account the individual radiosensitivity becomes desirable.

During the last decennium, a lot of scientific progress has been made in the analysis of the human genome. The "Human Genome project" has shown a relatively large genetic variation at the individual level. Investigation of the correlation between the individual genotype and the *in vivo* radiosensitivity in radiotherapy patients can lead to genetic profiles associated with hypersensitivity to radiation. The enhanced chromosomal radiosensitivity observed in a number of populations of cancer patients and in clinically hyperradiosensitive radiotherapy patients points to the possibility to use chromosomal radiosensitivity assays for the assessment of the individual radiosensitivity at the phenotype level. In the framework of the project we investigated the possibility and usefulness to apply "State of the Art" radiosensitivity assays at the genotypic and phenotypic level in occupational medicine in the framework of the protection of the worker against the hazards of ionising radiation. The essential question to be solved here is if the considered tests are sufficiently sensitive, specific and predictive to be used for a reliable biomonitoring for individual radiosensitivity. Application of individual genotypic and phenotypic information in an occupational setting in the framework of prevention and health at the workplace automatically involves a lot of ethical and juridical questions. This kind of implications linked to the project was treated by a team of jurists and ethicists participating to the network.

I.2. Aims of the study

The main aim of our contribution to the project is the investigation of the value, the usefulness and the applicability of candidate-biomarkers for the determination of the individual susceptibility to ionising radiation at the genotypic and phenotypic level in the occupational medicine of radiation workers. The candidate-biomarkers have to be sufficiently sensitive, specific and predictive to be used for a reliable biomonitoring for individual radiosensitivity.

In the first place cytogenetic techniques were optimised and standardised for the determination of the chromosomal radiosensitivity as phenotypic biomarker for radiosensitivity. Also, molecular techniques for analysis of single nucleotide polymorphisms (SNP's) were elaborated for identification of SNP-profiles as a genotypic biomarker associated with an enhanced radiosensitivity. Indeed, SNP's represent about 90 % of the naturally occurring DNA sequence variations.

For the validation of the applied techniques, a population of radiotherapy patients with clinical evaluation of the radiosensitivity was used. The value of genotoxicity assays for the individual assessment of the radiosensitivity in occupational medicine was evaluated by a study of a population of external radiation workers from the nuclear industry involved in the overhaul of nuclear reactors. In the elaboration of the study, a discussion of the socio-ethical aspects of the project due to the use of genetic information for assessment of the individual radiosensitivity at the workplace became spontaneously prominent.

II. Theoretical framework

The *in vivo* effects of exposure to ionising radiation of cells and tissues and the consequences for the human health are dependent on the interactions between radiation, cells and tissues. After internal and external exposure as is the case in an occupational setting, this depends in the first place on the absorption of radiation by the exposed tissues: the absorbed dose. The absorption of radiation in the tissues induces DNA strand breaks in the cells of these tissues. These DNA strand breaks can be repaired, fixed, or transformed in a mutation. The latter effect of exposure to ionising radiation can lead to carcinogenesis.

Variability in these mutagenic and carcinogenic effects exists at the genetic level: individuals carrying one or several alleles with polymorphisms leading to less efficient DNA repair capacity and/or deregulation of the checkpoint control of the cell cycle will have a higher susceptibility for radiation induced cancer. More than 100 genes are involved in the detection of DNA damage and DNA repair. These genes are responsible for base excision repair (BER), nucleotide excision repair (NER), non-homologous-end-joining (NHEJ), homologous recombination (HR), cell cycle control and mechanisms of oxidative damage repair. Literature data show that several SNP's in genes involved in these processes are associated with radiosensitivity and cancer predisposition.

A reduced DNA capacity results also in an enhanced chromosomal damage in peripheral blood lymphocytes after *in vitro* irradiation of a blood sample. Individuals with an enhanced *in vitro* chromosomal radiosensitivity also have an enhanced risk for radiation-induced malignancies. Studies of patients with genetic syndromes associated with an enhanced radiosensitivity and cancer predisposition such as ataxia-telangiectasia have shown that the scoring of the number of chromatid breaks in dividing lymphocytes after *in vitro* irradiation in the G2 phase of the cell cycle (G2 assay) is a sensitive cytogenetic technique for assessment of the radiosensitivity. The same holds for the number of micronuclei in lymphocytes after division following an *in vitro* irradiation in the G0 phase (the micronucleus assay).

In the framework of the project we investigated the sensitivity, specificity and the predictive value of the cytogenetic techniques mentioned above for assessment of the *in vitro* chromosomal radiosensitivity in view of a possible application in occupational medicine. These techniques for radiosensitivity assessment were applied to a population of external workers occupationally exposed to radiation in the nuclear industry. On the other hand, we also elaborated molecular techniques for the analysis of polymorphisms, to gather information on genotypes associated with an enhanced radiosensitivity and enhanced risk for radiation induced cancer.

III Methods

III.1. Populations under study

Populations for the validation studies

For the validation of the chromosomal radiosensitivity assays and the studies at the genotype level a population was considered of 48 patients, treated in the past with radiotherapy for cervix uteri and endometrium carcinoma, in the Radiotherapy Department of the University Hospital Gent. For these patients the clinical radiosensitivity during and after the radiotherapy treatment was evaluated according to the RTOG (Radiation Therapy Oncology Group) scale. Grade 1 and 2 are mild and moderate reactions while grade 3 and 4 are severe to life-threatening complications. All these patients received a nearly identical treatment and the data with respect to the physical dosimetry were recorded. A blood sample was taken of these patients at least 6 months after the end of the radiotherapy treatment. The age of the patients at time of venepuncture ranged from 35 to 81 years with an average value of 64 years. The chromosomal radiosensitivity assays were validated by study of the correlation between *in vivo* and *in vitro* radiosensitivity. Genotypic information on radiosensitivity resulted from a comparison of the SNP analysis data between the patient group with high clinical radiosensitivity and the group without and with mild reactions. This study was executed in collaboration with Prof. M. van Eijkeren of the Radiotherapy Department.

For the validation of the chromosomal radiosensitivity assays, a second population was considered consisting of breast cancer patients with a putative genetic predisposition. Patients fulfilling one of the following selection criteria were included in the study: (1) patients diagnosed with breast cancer before the age of 35 years, (2) patients with bilateral breast cancer before the age of 50, (3) patients with three first degree relatives affected with breast/ovarian cancer, (4) patients with two relatives first/second degree with breast/ovarian cancer detected before the age of 50. Sixty-two breast cancer patients were included in the study with age ranging from 29 to 69 years and average value 45 years. A blood sample was taken at least 9 months after the radio/chemotherapy. This study was performed in collaboration with the Department of Medical Genetics, University Hospital Gent, Director Prof. A. De Paepe.

60 female workers of the University Hospital Gent, who were not exposed to radiation or other mutagenic agents, served as a control population. The study of this control population was executed in collaboration with Dr. R. Morthier of the occupational medical service of this hospital (IDEWE). The age of these women ranged from 23 to 60 years with average value of 37 years.

For all these studies the approval of the Ethical Committee of the University Hospital was obtained. All participants to the studies were informed on the content and signed an informed consent before venepuncture.

Population of workers of the nuclear industry

The application of radiosensitivity assays in occupational medicine is most relevant for the workers with the highest radiation burden. With respect to the nuclear industry, the external

workers involved in the overhaul of reactors ("jumpers") are the most critical population. In the execution of these activities they receive in some cases relatively high doses in a relatively short period (up to 10 mSv per month). The activities are jobs related to the cleaning, the maintenance and the repair of components in the "hot" zone of the reactors. The number of external workers in the nuclear power plant Doel is important: this group comprises about 63% of all workers (1636). The group of external workers is a difficult population for biomonitoring as this group consists mainly of temporary workers. Physical dosimetry is obligatory for these workers, and the occupational medicine service is responsible for this. Consequently, biomonitoring of this population was only possible in strong collaboration with the local occupational service, Department head Dr. M. Barbé (CBMT).

In the framework of the project a biomonitoring programme was elaborated for the populations of external workers involved in the overhaul of the four nuclear reactors of the nuclear power plant Electrabel Doel. These revisions took place in the period from April to September 2000. The study was executed in collaboration with the Medical Service NPP-Doel. The revision activities were performed by ten companies. A study of the doses received by external workers in the nuclear power plant Doel in the period 1990-2000 allowed a selection of three companies with workers receiving the highest radiation burden and this indeed due to the revision activities of the nuclear reactors.

A first company is responsible for the mechanical and electrical repair and maintenance activities of the steam generators, and workers of this company can be considered as "jumpers". A second company is executing cleaning and mechanical maintenance activities in the "hot" zone, while a third company is performing isolation works around cooling-water circuits. The average doses of these workers amount to 2-5 mSv per year with maximal doses of 10 mSv per year.

The population under study exposed to radiation consisted of 41 male workers of the three companies selected. Before participation to the study the workers were informed about the aims of the study. Written informed consent was obtained from all donors before blood sampling. The participants to the study were asked to fill in a questionnaire dealing with data important for the study: age, smoking habits, and medical exposures. The age of the participants to the study varied from 18 to 55 years with mean value of 30.6 years. Thirteen workers were non-smokers. For the smoking population, the average number of cigarette-years, defined as the number of cigarettes consumed per day multiplied by the number of years of smoking, was 208 with a variation from 40 to 750 cigarette-years. The average number of cigarettes consumed per day within the population of smokers amounted to 16.9 with a variation from 6 to 25 cigarettes. The accumulated dose received by the workers up until before the overhaul of the reactors, according to the official dosimetry records, ranged from 0 to 68.7 mSv with average value of 13.9 mSv.

The dose received by the workers during the revision activities was continuously monitored by physical methods (electronic dosimetry). A first blood sample, 10 ml blood in a heparinized tube, was taken from each participant to the study immediately before start of the activities. This sample was the material used for the determination of the individual chromosomal radiosensitivity of the worker pre-exposure. On the other hand a fraction of the blood sample was used for the micronucleus assay as effect-biomarker.

A second blood sample, 10 ml blood in a heparinized tube and 5 ml in an EDTA tube, was taken directly after the revision activities in the "hot" zone according to the following criteria:

(1) for workers involved in the overhaul of one reactor directly after these activities, (2) for workers involved in the overhaul of more reactors after the revision with the highest estimate for the dose involved. This second blood sample allowed a study of the effect of the exposure on the individual chromosomal radiosensitivity and a study of the use of the micronucleus assay as effect-biomarker. The average exposure time amounted to 5.2 weeks with a variation between 1 to 8 weeks. The average dose received during this period was 3.0 mSv with a maximal dose of 9.5 mSv.

For the studies performed by the Laboratory for Cellular Genetics of the Free University Brussels (VUB), a control population of 31 male administrative workers of the nuclear power plant Doel not exposed to radiation was considered in addition to the population of external radiation workers. Age of this population ranged from 18 to 57 years with average value of 37 years.

III.2. Procedures of analysis

G2 assay

The first assay for assessment of the chromosomal radiosensitivity, applied systematically within the project, is the G2 assay on peripheral blood lymphocytes. In this assay the number of chromatid aberrations (breaks and gaps) is scored in lymphocytes in metaphase after an *in vitro* irradiation of a blood sample in the G2 phase of the cell cycle. The high chromosomal radiosensitivity of ataxia telangiectasia patients is most striking after irradiation in the G2 phase of the cell cycle. Very critical for the G2 assay is the temperature of 37 °C in the short period of 30 min between the irradiation and the addition of colcemid to block cell division. This is not a problem in the research group as the irradiation equipment is in the same building as the cell culture laboratory.
After optimisation of the *in vitro* dose and the cell culture times the following protocol was elaborated: starting from an heparinized blood sample, cell cultures are started and stimulated with 10 µl of a 1 % solution of phytohaemagglutinin (PHA). After 71h incubation at 37°C, cell cultures are irradiated at 37°C with a 0.4 Gy dose of ^{60}Co-γ rays. Thirty minutes after irradiation, colcemid (0.15µg/ml) is added. After 60 min, cell cultures are arrested by putting them on ice. After an hypotonic shock with 0.075 M KCl during 15 min, the cells are fixed in a methanol-acetic acid mixture (3:1), dropped on clean slides and stained with 6 % Romanowsky Giemsa.

For the scoring of the chromatid breaks and gaps in the metaphases, a metaphase-finder is used. This is a fully automated system for the search and localisation of metaphases on microscope slides using a scanning platform. The automated scanning with autofocus at a speed of 5 camera fields per sec results in a total scanning time of 10 min per slide. During the scan all metaphases selected are shown on a high resolution screen allowing an evaluation of the quality of the metaphase spreadings. After the scan the system allows automated relocation of the selected objects so that they can be scored for aberrations under the microscope. The system is also equipped with a motorized slide feeder, which can contain eight slides. A comparative study was performed between the results of the G2 assay obtained with manual scoring of the metaphases and those obtained with automated localisation with the metaphase finder system. This study showed no statistically significant differences. For the G2 assay, chromatid aberrations are scored systematically in 50 metaphases by two scorers on coded slides.

Micronucleus assay

A second assay applied in the assessment of the chromosomal radiosensitivity is the micronucleus (MN) assay for peripheral blood lymphocytes after *in vitro* irradiation in the G0 phase of the cell cycle. For the G0 MN radiosensitivity assay, two *in vitro* irradiation protocols were applied: an irradiation with 3.5 Gy ^{60}Co γ-radiation given at a high dose rate (1 Gy/min) (HDR-MN) and an irradiation with the same dose given at a low dose rate (4 mGy/min) (LDR-MN). The latter procedure was applied because literature data indicate a higher sensitivity of the MN assay for chromosomal radiosensitivity when an *in vitro* dose is given at low dose rate. Phytohaemagglutinin (PHA) was added to the blood culture immediately after the irradiation. Cytochalasin B (6 µg/ml) was added 24 h after stimulation to block cytokinesis. After incubation at 37 °C during 70 h, the cells were harvested, treated with a cold hypotonic solution of 0.075 M KCl and fixed with methanol-acetic acid (10:1). Finally the cells were dropped on clean slides and stained with Romanowsky-Giemsa.
Micronuclei were scored in 1000 binucleated cells according to the criteria defined by Fenech.

The dose-rate sparing (DRS) effect of the LDR irradiation compared to the HDR irradiation was calculated using the equation:

$$DRS = (1-Y_{LDR}/Y_{HDR})\times 100$$

with Y_{LDR} the micronucleus yield at low dose rate and Y_{HDR} the micronucleus yield at high dose rate.

SNP analysis

The SNP analysis started from genomic DNA extracted from peripheral blood lymphocytes. For the detection two techniques were applied: the RFLP (Restriction Fragment Length Polymorphism) technique and the SnaPshot technique, which is commercially available. Both techniques require a first polymerase chain reaction (PCR) with external primers for amplification of the DNA fragment with the known polymorphism. When the RFLP technique is applied, specific restriction enzymes are used, which are cutting or not, according to the presence or absence of the polymorphism. The DNA fragments are separated and visualised with gel electrophoresis. In the application of the SnaPshot technology a second PCR with a primer, just binding before the polymorphic DNA locus, is performed. This primer is elongated with a fluorescent dideoxynucleotide (ddNTP) during the reaction. After denaturation, the fluorescent-labelled PCR fragments are analysed by capillary electrophoresis (ABI 310 Genetic Analyser).

IV. Results

IV.1. Study of the intra- and interindividual variability of chromosomal radiosensitivity assays

Crucial characteristics of a radiosensitivity assay are sensitivity and reliability. To investigate these aspects for the HDR-LDR MN assays and the G2 assay a study was performed starting from blood samples of a number of donors. The intra-individual variation was studied for two donors by collecting 10 blood samples over a period of one year. On the other hand the interindividual variation was assessed by blood samples of twelve donors. Also, the reproducibility of the assays was studied based on the results of six cultures of the same blood sample. In the table below the values for the coefficient of variation CV (defined as the ratio of the standard deviation and the mean value expressed in percent) for the inter-culture, the intra-individual and the interindividual data are compared.

TABLE 1. CV (%) values of the inter-culture, the intra-individual and the interindividual data for the different chromosomal radiosensitivity assays applied

	G2 assay	**HDR-MN assay**	**LDR-MN assay**
Inter-culture	6	5	10
Intra-individual	15	10	17
Interindividual	17	9	18

Conclusion of this study is that the intra-individual variability is only slightly lower than the inter-individual variability for the different assays in a population of healthy donors. The low CV values for the inter-culture data show that that the intra-individual variations cannot be due to culture effects or scoring.

IV.2. Study of the radiosensitivity of a population of radiotherapy patients: validation of the G2 assay and SNP-analysis of the XRCC1 gene

For the validation of the techniques applied within the project a retrospective study of a population of radiotherapy patients treated for gynaecological tumors was performed. In this study a group of 18 patients with moderate and severe reactions on the therapy (grade 2 and more on the RTOG scale) was considered as radiosensitive population, while a group of 30 patients without or with mild reactions (grade 0-1 on the RTOG scale) was adopted as control population. For seven patients mitogenic stimulation of the lymphocytes was impossible, very probably due to the radio/chemotherapy from the past.

For the G2 assay as radiosensitivity assay, the following mean values and standard deviations for the G2 index (being the number of chromatid breaks and gaps per metaphase) were obtained: 1.34±0.21(SD) for the radiosensitive population versus 1.15±0.20(SD) for the control population. Application of the Student's t-test shows that the difference in G2 results between both groups is statistically significant ($p = 0.01$). Splitting of the radiosensitive population in a group with moderate complications (RTOG scale grade 2) and a group with severe complications (RTOG scale grade 3 and 4) gives the following values for the G2 index: 1.32±0.21(SD) and 1.39±0.24(SD) respectively. The difference between these two groups is not statistically significant and this predominantly because of the relatively low number of patients with severe complications (7).

TABLE 2: Frequencies of the different genotypes considered in different polymorphisms of the XRCC1 gene

194 Arg/Trp C>T

	Graad 0-1	Graad 2-4	Controlegroep
CC	24/32 (75%)	16/16	20/25 (80%)
CT	7/32 (22%)	0/16	5/25 (20%)
TT	1/32 (3%)	0/16	0/25
q	**14%**	**0%**	**10%**

280 Arg/His G>A

	Graad 0-1	Graad 2-4	Controlegroep
GG	30/32 (94%)	14/16 (88%)	25/25
GA	2/32 (6%)	2/16 (12%)	0/25
AA	0/32	0/16	0/25
q	**3%**	**6%**	**0%**

399 Arg/Gln G>A

	Graad 0-1	Graad 2-4	Controlegroep
GG	11/32 (34%)	4/16 (25%)	7/25 (28%)
GA	16/32 (50%)	10/16 (63%)	12/25 (48%)
AA	5/32 (16%)	2/16 (12%)	6/25 (24%)
q	**40%**	**44%**	**48%**

632 Gln/Gln G>A

	Graad 0-1	Graad 2-4	Controlegroep
GG	13/32 (41%)	4/16 (25%)	10/25 (40%)
GA	17/32 (53%)	8/16 (50%)	14/25 (56%)
AA	2/32 (6%)	4/16 (25%)	1/25 (4%)
q	**33%**	**50%**	**32%**

In spite of the clear and statistically significant difference in G2 index between controls and radiosensitive patients, and that the radiosensitive patients have a high G2 score on the average, the latter is not systematically the case at the individual level. Using the 90^{th} percentile of the normal control population as "cut off" value for the G2 index above which a patient is considered to have an enhanced chromosomal radiosensitivity, 4 out of 18 patients

of the clinically radiosensitive population have a normal value for the G2 index. On the other hand 8 out of 30 patients without/with mild reactions have a G2 index exceeding the 90th percentile limit. In the application of the G2 assay as individual biomarker for clinical radiosensitivity with the 90th percentile of the control population as cut off value, the sensitivity (78 %) and the specificity (73%) of the assay is thus restricted and not satisfactory.

For the group of radiotherapy patients a SNP analysis was performed for polymorphisms at 4 loci of the XRCC1 gene which are involved in cancer predisposition according to literature data: 194 Arg/Trp C>T, 280 Arg/His G>A, 399 Arg/Gln G>A, 632 Gln/Gln G>A. In Table 2 on the preceding page the results of this SNP analysis are summarized. In addition to the frequencies of the different genotypes the allele frequency q is also given for the population of radiotherapy patients with normal response and with mild reactions to the therapy (RTOG 0-1), the population with radiosensitive patients (RTOG 2-4) and a population of 25 healthy volunteers. This table shows that the polymorphisms 280 Arg/His G>A and 399 Arg/Gln G>A have no influence on the radiosensitivity status. The genotype associated with polymorphism 632 Gln/Gln G>A would result in a higher radiosensitivity while the genotype associated with polymorphism 194 Arg/Trp C>T seems to have a radioprotective effect.

IV.3. Study of the radiosensitivity of a population of breast cancer patients: validation of the chromosomal radiosensitivity assays.

The application of the micronucleus and G2 assays as biomonitor for radiosensitivity was also validated based on a study of breast cancer patients versus a control population of healthy female workers of the University Hospital Gent.

With respect to the micronucleus assay, both the HDR-MN assay with irradiation of a blood sample at high dose rate (1 Gy/min) and the LDR-MN assay with irradiation at low dose rate (4 mGy/min) were applied.
For the control population the HDR-MN assay resulted in an average number of MN of 741±105(SD) per 1000 binucleated cells while for the breast cancer population an average value of 841±143(SD) was obtained. Application of the Student's t-test indicated a statistically significant increase ($p < 0.001$). Using the 90th percentile of the normal population as cut off value for increased radiosensitivity, 45% of the patients are radiosensitive compared to 11% of the control population.

Application of the LDR-MN assay for the control group resulted in an average number of MN of 346±59(SD) per 1000 binucleated cells while for the breast cancer patients an average value of 442±89(SD) was obtained: a statistically significant increase ($p < 0.001$; Student's t-test). Using the 90th percentile of the control population as underlimit for increased radiosensitivity,61 % of the breast cancer patients has an enhanced radiosensitivity, compared to 12 % of the controls.

Also the G2 assay showed a statistically significant increase in radiosensitivity in breast cancer patients compared to the control group ($p < 0.001$). The average value of the G2 index (number of chromatid aberrations per metaphase) after a 0.4 Gy irradiation of a lymphocyte culture in the G2 phase of the cell cycle was 1.03±21(SD) for the control population, while the mean value of the G2 index in the population of breast cancer patients was 1.24±26(SD). Using the 90th percentile of the control population as cut off value for increased chromosomal

radiosensitivity, 43 % of the breast cancer patients are radiosensitive, while this is only 10 % for the controls.

The genetic base for the observed enhanced *in vitro* chromosomal radiosensitivity of breast cancer patients is not clear at all. An analysis of the data in combination with the results of mutation analysis showed that mutations in the BRCA1 or BRCA2 genes with a very strong cancer predisposition are not responsible for the enhanced *in vitro* radiosensitivity.

For the control population the interindividual variation was compared to the intra-individual variation for the different chromosomal radiosensitivity assays. The results are summarized in Table 3 below.

TABLE 3. The CV value for the intra-individual and interindividual variation within the control population for the different chromosomal radiosensitivity assays applied in the study.

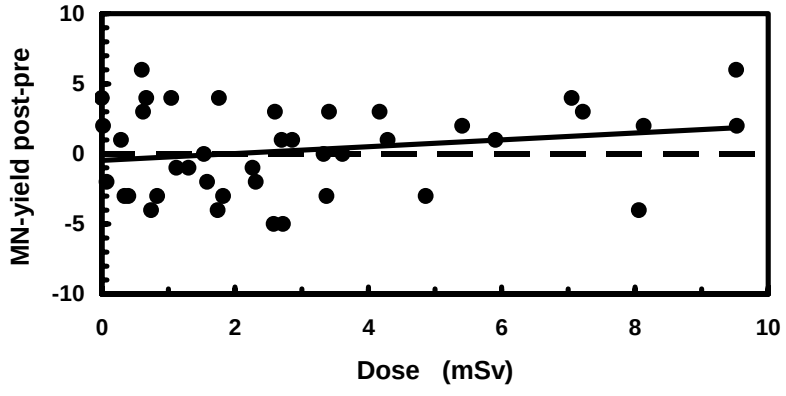
	G2 assay	**HDR-MN assay**	**LDR-MN assay**
Intra-individual	15	9	10
Interindividual	20	14	17

This study indicates that both the HDR-MN and LDR-MN assays and the G2 assay are able to assess an increased radiosensitivity at population level. This study was performed in collaboration with Prof. A. Vral, Laboratory of Histology, University Gent.

IV.4. Investigation of the radiosensitivity of a population of radiation workers of the nuclear industry.

Results of the MN assay as exposure monitor for workers exposed to ionising radiation

In this study the micronucleus frequency of radiation workers was determined before and after exposure, while the received radiation dose was continuously monitored by detailed physical dosimetry. This is the ideal experimental set up to investigate the value of the micronucleus assay as individual bio-effect monitor in the nuclear industry for the maximal dose values currently received in an occupational setting. The individual data of the workers expressed as the difference between the number of micronuclei per 1000 binucleated cells post- and pre-exposure are represented in this figure as a function of the dose received.



This figure shows that the individual increase in the micronucleus yield after the exposure is not strongly correlated with the dose received. A linear regression of the increase in the number of micronuclei, $MN_{post-pre}$, versus the dose received H, gives the following relationship:

$$MN_{post-pre} = 0.244(\pm 0.180)\ H - 0.46(\pm 0.72)$$

The Spearman's correlation coefficient r has the value 0.10 ($p = 0.53$), indicating a weak correlation between $MN_{post-pre}$ and H. An increase of the number of MN is observed for only about half of the workers (21 of the 41 workers). Statistics with the Wilcoxon test do not show a significant increase post-exposure ($p = 0.69$).

This leads to the conclusion that dose values below 10 mSv as received nowadays in the nuclear industry cannot be detected with the micronucleus assay without centromere-detection, even with knowledge of the number of MN before the exposure.

Analysis of the baseline micronucleus frequencies before the exposure during the reactor overhaul

A statistical correlation analysis was performed between the baseline micronucleus frequencies before the exposure, the dose of the workers received during the past years, age and smoking habits. As could be expected a very good correlation (Spearman's correlation coefficient $r = 0.45$; $p = 0.004$) was found between the number of MN before the exposure and the age of the workers. A linear regression analysis shows an increase of 0.228 MN/year per 1000 binucleated cells. The observed age dependence can be considered as an internal quality control to the data.

After correction of the individual MN frequencies for the age dependence and adjustment to the mean age of 30.6 years, the studied population was sorted in three classes according to smoking habits (non-smokers; 1-12 cigarettes a day; 13-25 cigarettes a day). Application of the Mann-Whitney test to these subpopulations does not show significant changes in MN yield (non-smokers mean MN 8.04; 1-12 cigarettes a day mean MN 7.89; 13-25 cigarettes a day mean MN 8.27; $p > 0.10$). A similar analysis with respect to the number of cigarette-years also showed no correlation with the MN yield.

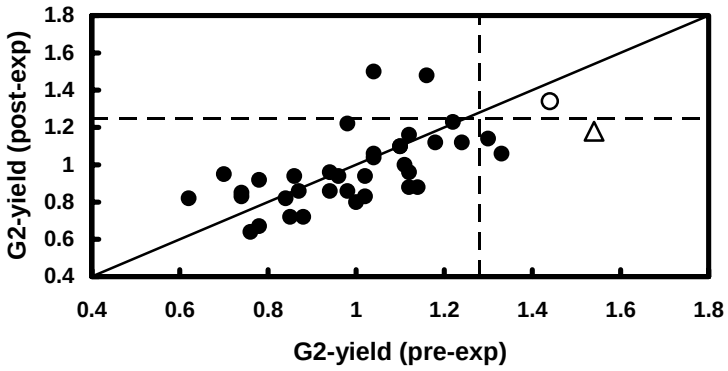
An analysis of the individual age-corrected MN yields versus the accumulated dose received in the past, before the exposure during the reactor overhaul, showed almost no correlation. A linear fit obtained by linear regression analysis to the data gave the following relationship:

$$MN = 0.0067\ (\pm 0.024)\ H_{cum} + 7.98\ (\pm 0.59)$$

with H_{cum} the accumulated dose in mSv. The Spearman correlation coefficient r is 0.04 indicating a very weak correlation between the number of MN and the accumulated dose.

Results of the G2 assay as biomonitor for radiosensitivity of workers exposed to radiation

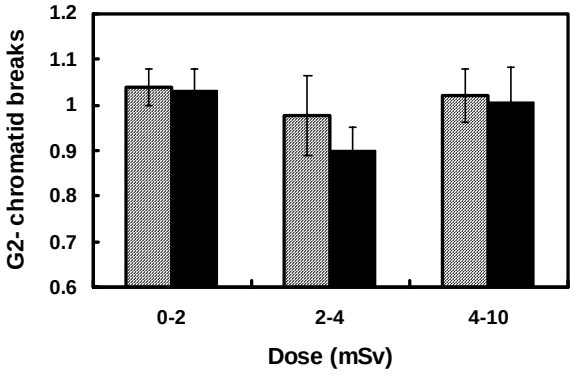
An analysis of the G2 index (number of chromatid breaks and gaps per metaphase) before the exposure does not show any effect of age and smoking habits. The individual values of the G2 index of the workers before and after the exposure are presented in this figure. The diagonal



represents the behaviour expected if the G2 index has the same value pre- and post-exposure.

Based on the cut off value determined by the 90th percentile, indicated in the figure by the dashed lines, four workers are radiosensitive pre-exposure while three are post-exposure. One worker (indicated by the open circle) is radiosensitive before as well as after exposure. The figure shows an acceptable correlation of the results of the G2 assay pre- and post-exposure ($r = 0.74$; $p = 0.001$). The difference between the average G2 index before (1.02 ± 0.21(SD)) and after exposure (0.99 ± 0.20 (SD) is not statistically significant (Wilcoxon test, $p = 0.16$). Further statistical analysis of the data results in an average intra-individual variation of 9 % while the interindividual variation amounts to 20 %.

To find out if the results of the G2 assay are affected by the exposure during the revision, the mean values for the G2 index for the three dose groups (0-2 mSv; 2-4 mSv; 4-10 mSv) pre- and post-exposure are compared in the figure below. The error bars represent the standard deviations of the mean. The hatched bars represent the values pre-exposure, the black bars the values post-exposure. This figure also shows no significant differences before and after the exposure.

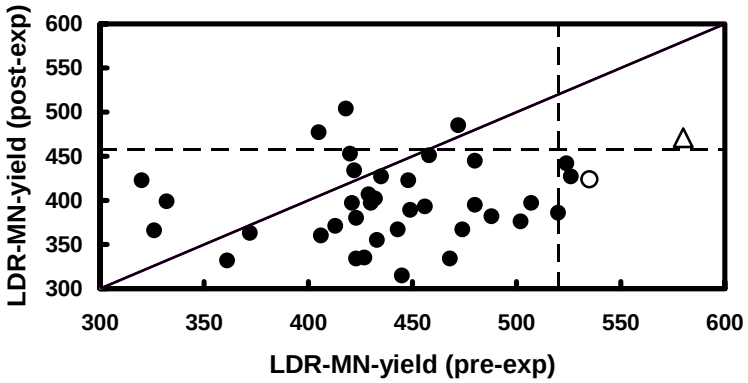


This is confirmed by the analysis of the individual increase of the G2 index post-exposure compared to pre-exposure versus the dose received by the workers H. All the data consistently

show that the result of the G2 assay as a radiosensitivity assay is not influenced by the occupational exposure.

Results of the micronucleus assay as biomonitor for radiosensitivity of workers exposed to radiation

A comparison of the individual results of the LDR-MN assay pre- and post-exposure is presented in the figure below. The diagonal line represents the behaviour expected if the micronucleus yield has the same value pre- and post-exposure. The figure shows that the correlation between the LDR-MN data pre- and post-exposure is poor (r = 0.20) in contrast to the results of the G2 assay.

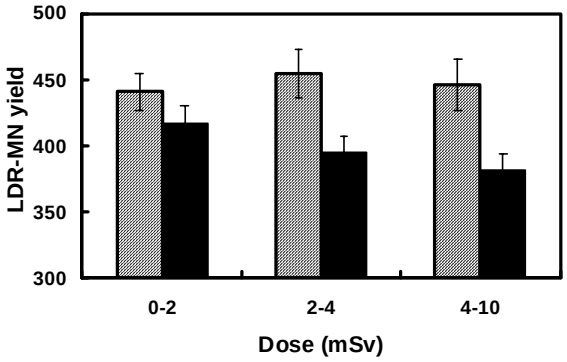


For the LDR-MN data, an almost systematic shift to lower MN yields is observed after the exposure, leading to a significant (p = 0.0002) decrease of the average LDR-MN value after the exposure (400±45(SD)) compared to before the exposure (445±58(SD)). This tendency is also present in the HDR-MN data, but it is less pronounced: 788±77(SD) post-exposure versus 826±80(SD) pre-exposure.

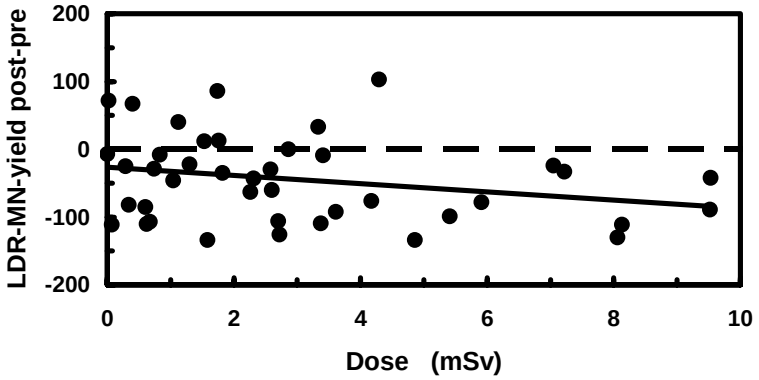
The dashed lines in the figure represent the 90th percentiles. Based on these values as cut off for enhanced radiosensitivity, one worker (indicated in the figure by the triangle) is a candidate for further investigation, as his micronucleus yield value exceeds the 90th percentile before as well as after the exposure for the LDR-MN assay.

With respect to the interindividual variations of the results of the HDR-MN and LDR-MN assays, similar values were obtained pre- and post-exposure: HDR-MN assay CV = 9 %; LDR-MN assay CV=12 %. As the results of the LDR-MN en HDR-MN assays are affected by the dose received during the reactor overhaul, a study of the intra-individual variation has no use.

In order to assess the effect of the dose received during the revision on the results of the HDR-MN and LDR-MN assays, the average micronucleus yields for the three dose groups of workers (0-2 mSv; 2-4 mSv; 4-10 mSv) pre- and post-exposure were compared. This comparison is presented below for the LDR-MN assay. The error bars represent standard deviations on the mean.

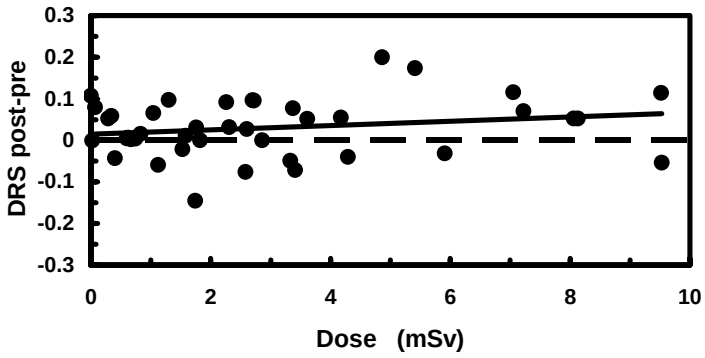


The hatched bars represent the values pre-exposure, the black bars the values post-exposure. This figure shows clearly that the exposure causes a dose-dependent reduction in chromosomal radiosensitivity.
An analysis of the individual data confirms this conclusion. In this figure the individual change in the result of the LDR-MN assay post-exposure compared to pre-exposure is plotted versus the dose received during the overhaul activities.



This figure shows undeniably that a dose in the range 5-10 mSv, received in a short period of one to a few weeks, induces a reduction of the chromosomal radiosensitivity. The data obtained with the HDR-MN assay lead to a similar conclusion but somewhat less pronounced.

Combination of the data obtained in the HDR-MN and LDR-MN assays after *in vitro* irradiation of blood samples at high and low dose rate allows us to calculate the "Dose rate sparing" (DRS) factor, representative for the DNA repair capacity. In this figure the individual data of the change of the DRS factor post-exposure versus pre-exposure are plotted versus the dose received in the revision.
This figure shows that for all workers with a dose exceeding 5 mSv the occupational exposure induces an enhanced DNA repair, which can be explained by the adaptive response phenomenon. In our study the occupational *in vivo* exposure is the "conditioning" dose while the 3.5 Gy *in vitro* dose of the blood sample acts as the "challenging" dose. The fact that the adaptive response effect is not observed for all workers is in agreement with literature data regarding the adaptive response induced by *in vitro* irradiation. These data also show a large interindividual variability.



The fact that the adaptive response does not show up in the G2 assay results is possibly due to the time between blood sampling and start of the culture and the *in vitro* irradiation three days later. For the HDR-MN and LDR-MN assays the irradiation takes place at the start of the culture a few hours post-sampling. Indeed, literature data indicate that the adaptive response effect is only a few days active after the time of the conditioning dose. An analysis of the results of the chromosomal radiosensitivity assays of the blood samples received before the exposure during the reactor revision versus the accumulated dose received in the past does not show any effect of the dose in agreement with the relatively short lifetime of the effect.

V. Discussion

The extensive studies with chromosomal radiosensitivity assays performed in the framework of present project have shown their usefulness in the assessment of radiosensitivity of populations. On the other hand the specificity and sensitivity of the results based on one blood sampling is too low to apply these assays for an individual radiosensitivity assessment in the framework of occupational medicine. In the population of radiation workers two individuals emerged with a systematic enhanced chromosomal radiosensitivity based on two blood samplings in a period of one to a few weeks. A validation study of a population of radiotherapy patients as performed in the framework of the project but taking into account the result of several blood samplings for assessment of the sensitivity and specificity of the chromosomal radiosensitivity assays would give a definite answer to the value of these assays at the individual level. Anyway, multiple sampling at different points in time in an occupational medicine setting is difficult in daily practice.

The study of the radiation workers has shown also that recent exposures (up to a few days before the venepuncture) have an influence on the individual chromosomal radiosensitivity status by the mechanism of the adaptive response. The study performed in the framework of the project is the first study which shows directly by assessment of the chromosomal radiosensitivity that an occupational exposure of 5-10 mSv in a short period has a positive effect on the DNA repair and reduces the radiosensitivity. The effect of exposure to radiation and other mutagenic factors, and also of physiological factors in the blood, on the result of chromosomal radiosensitivity assays is a serious drawback for the phenotypic approach of individual radiosensitivity assessment.

At the occasion of the studies of the chromosomal radiosensitivity of workers from the nuclear industry and in control populations, a debate started with different inter-occupational medicine services on the social-ethical aspects of biomonitoring of susceptibility. The following themes were treated:

- What is the aim of genetic biomonitoring to follow up workers in addition to the techniques applied today?
- How strong is the difference between conventional and the genetic techniques with respect to accuracy, relevance, necessity and consequences?
- What kind of social problems can be introduced by genetic methods in the daily occupational medicine practice?
- How can the results of genetic susceptibility studies be reported to the participants?

A lot of attention was paid to the communication duty by which an individual has to be informed on the results of assays of studies as performed within the present project. The problem is that at the moment, the chromosomal radiosensitivity assays are sufficiently reliable for a population but not for an individual susceptibility assessment. Therefore we decided to communicate the population results anonymously and not to communicate the individual results to the participants. Individual reporting would lead to conclusions concerning themselves and their family members that go much too far taking into account the restricted specificity of the assays.

Studies of patients treated with radiotherapy have shown that the patient-related variability in complications is caused by genetic factors. There is a general agreement that with the exclusion of patients with genetic syndromes associated with a high radiosensitivity this variability is not the result of mutations in one gene but that the individual radiosensitivity is

the result of several interindividual variations in the genome. Individuals with alleles with polymorphisms associated with a less efficient DNA repair capacity and/or deregulation of the checkpoint-control of the cell cycle will have a higher susceptibility for radiation-induced cancer. More than 100 genes are involved in the detection of DNA damage and DNA repair. Combination of polymorphisms in these genes will at least to a large extent determine the individual radiosensitivity in a population of healthy individuals. In the framework of present project we elaborated molecular techniques for individual analysis of "Single Nucleotide Polymorphisms". SNP's represent about 90% of the naturally occurring DNA sequence variations. The effect on the radiosensitivity of four SNP's in the XRCC1 gene, playing a crucial role in the base excision repair process, and for which it is known that they are associated with cancer predisposition was investigated within the framework of the project. This kind of studies has to be extended to the large number of genes involved in DNA repair, cell cycle control and mechanisms of repair of oxidative damage. The results of these studies have to be the base for "radiogenomics", leading to an individual radiosensitivity assessment starting from the individual genomic profile of the genes just mentioned. Recently it was shown that summing up the risk alleles is a first simple method for individual radiosensitivity assessment. An important advantage of the genotypic approach for assessment of the individual susceptibility is that the result is not affected by exposures and physiological factors in the blood. Genomic DNA can be extracted from peripheral blood lymphocytes present in a blood sample. The molecular techniques elaborated within the project can be applied at a large scale.

In conclusion, we can state that chromosomal radiosensitivity assays are useful and sensitive for studies of the radiosensitivity of populations but cannot be applied at the moment at an individual level based on one blood sample, partly because of the observed effect of mutagenic exposures and physiological factors in the blood on the result of the assays. For an individual radiosensitivity assessment, a genotypic approach by "radiogenomics" based on an extensive SNP-analysis seems necessary.

Promotor: Prof. Dr. L. de Ridder

Scientific collaborator: Prof. Dr. A. Vral

Gent University
Faculty of Medicine
Dept. of Anatomy, Embriology, Histology and Medical Physics
Laboratory of Histology

I. INTRODUCTION

I.1. Context and general frame of the research.

The in vivo effect of exposure to ionising radiation at the cellular level and the effect on human health is dependent on the interactions between ionising radiation and tissues. In case of external irradiation this effect depends primarily on the absorption of radiation by the involved tissue. When radiation is absorbed by the target organs it will induce DNA damage such as single strand breaks (ssb), double strand breaks (dsb), base damage, … in the cells of the target tissues. Dependent on the complexity and the amount of the damage the cell will repair, not repair or missrepaired the damage. Missrepaired damage resulting in mutations and chromosome aberrations can lead by a multistep process to carcinogenesis.
The most lethal lesion induced by ionizing radiation is the DNA dsb, which when not repaired or missrepaired by the cell will lead to the formation of chromosome aberrations. Literature data have shown that the number of chromosome aberrations induced by ionising radiation can vary among different individuals and are correlated with individual differences in 1) the repair capacity of the radiation induced lesions and 2) the cell cycle checkpoint mechanisms. Mutations in DNA repair genes, cell cycle checkpoint genes, … would by this lead to an enhanced chromosomal radiosensitivity and increased cancer risk.
In the framework of this DWTC programme we will develop techniques that will allow us to detect individual differences in radiosensitivity and this as well at the chromosomal (cytogenetic analysis) as genetic (RNA expression, gene profile) level.

I. 2. Aims of the research

The major aim of our research team (RUG1, promotor Prof. Dr. L. De Ridder) and RUG2 (promotor Prof. Dr. H. Thierens) is to investigate the value and the application of genotoxicity assays as candidate biomarkers of susceptibility to ionising radiation in the field of nuclear medicine of radiation workers. These assays should be characterised by a high reproducibility and sensitivity to allow reliable monitoring of the radiosensitivity of radiation workers.

To achieve this goal we will develop, optimise and standardise genotoxicity assays that can be used as radiosensitivity biomarkers. As candidate assays to standardise we choose the G2 and G0-MN assay as elevated sensitivity to ionising radiation with these assays has already been demonstrated in cancer prone genetic disorders (such as ataxia telangiectasia and Nijmegen breakage syndrome) and in significant proportions of patients with common cancers (Scott et al.,1998 & 1999, Terzoudi et al., 2000). In this programme the optimised and standardised G2 and G0-MN assay will then be applied and validated by means of 1) a population of breast cancer patients this as literature data have shown that about 30-40% of breast cancer patients show an enhanced chromosomal sensitivity with these techniques and 2) a population of radiotherapy patients of which the in vivo clinical radiotoxicity is known. The value of these cytogenetic assays for the identification of radiosensitive individuals in nuclear medicine will be investigated in a population of radiation workers from nuclear industry.

As enhanced chromosomal radiosensitivity is probably correlated with defects in DNA repair genes or cell cycle checkpoint genes we will also investigate genetic variations (RNA expression, DNA profile) in those individuals (workers and patients) that were characterised in our study by an enhanced G2 or G0-MN response.

Activities carried out to study these goals:
- development and optimisation of G2 assay and G0-MN assay (different irradiation procedures).
- analysis of the reproducibility and sensitivity of the G2 and G0-MN assays.
- application of the G2 and G0-MN assay in a population of radiation workers (see report RUG2, Prof. Dr. H. Thierens).
- validation of the G2 and G0-MN assay in a population of breast cancer patients.
- validation of the G2 and G0-MN assay in a population of radiotherapy patients (see report RUG2, Prof. Dr. H. Thierens).
- investigation of the mechanism underlying the formation of G2 chromatid breaks
- correlation analysis between enhanced chromosomal radiosensitivity and the expression of genes involved in the processing of radiation induced DNA damage.

II. THEORETICAL FRAME

Defects in DNA damage processing genes (DNA repair genes, cell cycle checkpoint genes) are likely to affect chromosomal radiosensitivity. In a large number of patients with inherited cancer-prone disorders such as ataxia-telangiectasia, Nijmegen breakage syndrome and hereditary retinoblastoma an enhanced chromosomal radiosensitivity has been demonstrated (reviewed in Scott et al.,1999). More recently elevated chromosomal radiosensitivity has also been observed in significant proportions of patients with sporadic cancers with no obvious family history (Scott et al., 1998 & 1999; Terzoudi et al., 2000). In breast cancer patients the elevated chromosomal radiosensitivity is confirmed in several independent studies (Parshad et al, 1996; Patel et al., 1997; Scott et al., 1998 & 1999; Terzoudi et al., 2000; Baria et al., 2001; Riches et al., 2001). In these studies the G2 assay and G0-MN assay were used to evaluate chromosomal radiosensitivity. The fact that enhanced chromosomal radiosensitivity is also observed amongst blood relatives of radiosensitive cancer patients points to the heritability of chromosomal radiosensitivity (Roberts et al., 1999; Burrill et al. 2000). These findings support the view that enhanced chromosomal radiosensitivity may be a marker for cancer predisposing genes of low penetrance. The suitability and application of genotoxicity assays, such as G2 and G0-MN assay, as candidate biomarkers for susceptibility to ionising radiation is by this also of major importance in the industrial medicine of radiation workers.
Furthermore recent studies investigating the underlying mechanisms of this enhanced sensitivity in response to radiation place DNA damage processing as a central theme of radiosensitivity. The experimental evidence on cellular radiosensitivity suggests the involvement of genes operating in cell cycle control and apoptosis, and DNA repair genes. Identification of genetic variables in the processing of radiation induced DNA damage may help to re-evaluate and re-define existing radioprotective measures by incorporating a factor of individual radiosensitivity, enriched in both qualitative and quantitative terms.

III. METHODS

III.1. Analysis procedure

1.Optimized G2 assay: the G2 assay procedure of the Paterson Institute, Manchester (Scott et al. 1999) was followed with some minor changes:
0.5 ml of heparinized blood is added to 4.5 ml of complete culture medium (37°C) consisting of RPMI-1640 medium supplemented with 10 % FCS, 0.05 % L-glutamine + peniciline / streptomycine (50U/ml peniciline en 50µg/ml streptomycine; Gibco). 10 µl phytohaemagglutinine (PHA- P) (1% PHA-P oplossing; Difco, Biotrading) is added as

mitogen. Two cultures per donor are set up: one to be irradiated while one serves as control. After 70h incubation in a CO_2 incubator at 37°C the cultures are irradiated with a dose of 0.4 Gy gamma rays at 37°C. At 30 min post-irradiation colcemid is added (0.15µg/ml; Sigma-Aldrich) and 60 min later the cultures are arrested by putting them on ice for 5 min. After a cold hypotonic shock with 0.075M KCl, for 15 min on ice, the cells are fixed once in cold methanol:acetic acid (3:1) and placed at 4°C overnight. The next day the cells are fixed for another 3 times with cold methanol:acetic acid (3:1). Suspensions of cells are dropped on clean dry slides, stained with 6% Romanowsky-Giemsa for 20 min and coded. Fifty well spread metaphases are analysed for the appearance of chromatid breaks and gaps. The radiation induced numbers of chromatid breaks and gaps are obtained by subtracting the spontaneous yields from the yields obtained in the irradiated samples. Per sample 2 slides are made and the analyses are done by 2 scorers (per sample each analyst scores 25 metaphases on one of the duplicate slides).

2. Optimised micronucleus (MN) assay:
For the MN assay 0.5 ml of blood is added to 1.5 ml of complete culture medium (see G2 assay).
Per sample, depending on the study, different cultures are set up: culture 1 is irradiated at 37°C, with a dose of 3.5 Gy Co-gamma rays given at a high dose rate (HDR) (1Gy/min); culture 2 is irradiated at 37°C, with a dose of 3.5 Gy Co-gamma rays given at a low dose rate (LDR) (4mGy/min); culture 3 is irradiated at 37°C, with a dose of 3.5 Gy Co-gamma rays given at HDR (1Gy/min) but PHA is added with a delay of 6 h after irradiation (HDR-DS (delayed stimulation)); culture 4 is sham irradiated. The sparing effect of LDR irradiation compared to HDR irradiation (DRS) = $1 - Y_{LDR}/Y_{HDR}$ is calculated and used as a measure for the repair capacity of the cell.
24 h after PHA stimulation cytochalasin B (6µg/ml; Sigma-Aldrich) is added to block cytokinesis. After a 70h culture period at 37 °C in a CO_2 incubator the cells are harvested by a cold hypotonic shock followed by fixation in a methanol : acetic acid : ringer (0.9% NaCl) solution (10:1:11) and placed at 4°C overnight. The next day the cells are fixed for another 3 times with cold methanol:acetic acid (10:1). Suspensions of cells are dropped on clean dry slides, stained with 6% Romanowsky-Giemsa for 20 min and coded. MN are scored in 1000 binucleate cells (light microscopy, 10x40) according to the criteria of Fenech (1993). The radiation-induced numbers of MN are obtained by subtracting the spontaneous yields from the yields obtained in the irradiated samples. Per sample 2 slides are made and the analyses are done by 2 scorers (per sample each analyst scores 500 binucleate cells on one of the duplicate slides).

The detailed protocols and results of the optimisation and standardisation experiments are published in Radiation Research (**paper 3** in annex II).
In the framework of the optimisation and standardisation of the G2 assay procedure our research group participated in "The technical workshop on the G2 assay" which was held at the University of St.-Andrews in Scotland and which resulted in a paper published in Int. J. Rad. Biol. (**paper 4** in annex II).

III.2. Materials

1. Study related to the optimisation , reproducibility, reliability and sensitivity of G2 and G0-MN assay as biomarkers of radiosensitivity..
- fourteen healthy volunteers, 4 males and 10 females aged between 23 and 55 years old, were tested for their sensitivity towards ionising radiation by means of the G2- and G0-MN assay.

For 12 of these volunteers blood samples were taken once while the two other volunteers gave blood on nine occasions over a time period of one year. From one blood sample multiple cultures per assay were set up to check the reproducibility of both assays in a situation where the variations due to individual or experimental parameters could be excluded. For one donor identified as radiosensitive with the G2 assay the assay was repeated twice to confirm the results. This study was approved by the Ethical Commission of our University.

2. Application of the G0-MN assay and G2 assay on a population of radiation workers from the nuclear industry.

The application of chromosomal radiosensitivity assays in nuclear medicine is most relevant for workers that are exposed to the highest doses. In nuclear medicine this are the external workers involved in the revision of the reactors ("jumpers"). During the revision they receive relative high doses during a short period (up to 15 mSv/month). The work involves cleaning, maintenance and repair of the components in the hot zone of the reactor.

In the framework of the project a biomonitoring programme has been worked out for populations of external workers involved in the revision of the 4 nuclear reactors in Doel. This study has been performed in close collaboration with the nuclear medicine dept. of Doel (Dr. M. Barbé). A detailed procedure can be found in the report of RUG2, promotor Prof. Dr. H. Thierens.

3. Validation of the G0-MN assay and G2 assay in a population of breast cancer patients.

- in this study chromosomal radiosensitivity has been investigated by means of the G2-assay and the G0-MN assay in an extensive group (n=62) of breast cancer patients with a family history or early onset of the disease. A small number of these patients are carrier of a BRCA 1 or 2 mutation.
- heparinized blood samples were obtained by venepuncture from 60 normal healthy women, aged between 23 and 60 years (mean 37 ± 12) and from 62 breast cancer patients, aged between 29 and 66 years (mean 44 ± 10), during a period of 2 years. The study of the breast cancer patients was performed in collaboration with the Department of Medical Genetics, University Hospital Gent, Belgium. Patients are referred to genetic consultation because of familial or early onset breast cancer. Women fulfilling one of the following selection criteria were analysed for presence of a mutation in BRCA1 and BRCA2 as described.

1. 3 first degree relatives affected with breast and/or ovarian cancer (n=27)
2. breast cancer patients from a family where at least in 2 first and/or second degree relatives breast and/or ovarian cancer is detected before the average age of 50 years (n=24)
3. patients with bilateral breast cancer and both tumors diagnosed before an average age of 50 (n=8; 5 of them also fulfil criteria 1 and 3 of them fulfil criteria 2)
4. all patients diagnosed with breast cancer before the age of 35 years without a family history (n=11)

This group of breast cancer patients was selected for mutation analysis of the BRCA1 and BRCA2 genes, because a positive family history and/or diagnosis at young age is a significant risk factor for the development of hereditary breast cancer. The blood samples were collected at varying times after breast surgery and radio/chemotherapy (range 9 months to 21 years). However the majority of the samples were received 2-3 years after therapy. Data on the tumour stage, tumour type, chemotherapy, radiotherapy, oestrogen-receptor and progesterone-receptor status were also collected. In this study we performed the G2- and the MN-assay on blood samples of the patients and on concurrent samples of healthy women.

All patients were given genetic counselling and signed an informed consent.

4.Validation of the G0-MN assay and G2 assay in a population of radiotherapy patients.
A detailed description of this population can be found in the report of RUG2, promotor Prof. Dr. H. Thierens.

5. Study of the mechanism underlying the formation of G2 chromatid breaks.
- for this study the G2 assay was performed on blood samples of 4 healthy donors that were irradiated with low LET γ-rays and high LET neutrons.

6. Correlation analysis between enhanced chromosomal radiosensitivity and the expression of genes involved in the processing of radiation induced DNA damage.
- from all the blood samples obtained in the breast cancer study (patients and healthy controls) half of the quantity was used to isolate lymphocytes that were frozen down in liquid nitrogen. These lymphocytes are and will be further used as starting material for gene expression analysis in samples from healthy controls and cancer patients for which the chromosomal radiation response is known.

IV. RESULS AND DISCUSSION.

1. Study related to the optimisation , reproducibility, reliability and sensitivity of G2 and G0-MN assay as biomarkers of radiosensitivity..
From the start of the project the G2 and G0-MN assays have been optimised (protocols see III.1. analysis procedures) and the reproducibility, reliability and sensitivity of these assays as biomarkers to identify radiosensitive individuals has been analysed.
The results of this study have been published in Radiation Research (**paper 3** in annex II) and in Int. J. Low Radiation (**paper 11** in annex II). For a complete overview of all the results and discussion we refer to the papers of which the reference is given in appendix.

Summary of the results and discussion:
Although it has been shown in literature that cancer patients have a higher mean G2 and G0-MN value compared to healthy individuals the use of these assays for the assessment of individual radiosensitivity and susceptibility is still questionable. In order to allow the quantification of an increased risk for a given individual the inter-individual variation should clearly exceed the inter-experimental / intra-individual variation found for a given endpoint in the normal population. Because of this, an accurate assessment of the inter- and intra-individual variation is essential. However, with regard to the G2 assay and the G0-MN assay these variations have not been investigated systematically and the results are not always straightforward. Because of this lack of clarity we wanted, as part of the DWTC programme, to investigate the reproducibility and sensitivity of the G2- and G0-MN assay and optimise the assays prior to their application as biomarkers of individual radiosensitivity and susceptibility to cancer. For this, the G2 assay and the G0-MN assay (HDR, LDR, HDR-DS) were performed on repeated blood samples of 14 healthy volunteers taken over a time period of 1 year.
The repeat experiments revealed that the intra-individual variability (Table 2) was not significantly different from the inter-individual variability (Table 1) for both G2 and G0-MN assay. As the intra-individual variability determines the reproducibility of the assay, our results highlight the limitations of these endpoints in detecting reproducible differences in radiation sensitivity between individuals within a normal population.

Table 1. Summary of the data used for the study of the interindividual variation among donors for different chromosomal radiosensitivity assays.

Donor/ Gender	**Date G2 (M/D/Y)**	**G2 index**	**Date MN (M/D/Y)**	**HDR-MN**	**LDR-MN**	**HDR-DS-MN**	**DRS**
1/M	7/2/99	1.14	10/16/00	0.752	0.315	0.603	0.58
2/F	7/2/99	1.14	10/16/00	0.652	0.303	0.519	0.54
3/F	10/22/99	0.92	10/16/00	**0.925**	0.332	0.671	0.64
4/M	10/22/99	0.98	12/11/00	0.778	0.358	0.650	0.54
5/M	10/7/99	0.80	-	-	-	-	-
6/F	12/9/99	1.04	-	-	-	-	-
7/M	1/27/00	1.14	10/27/00	0.826	0.432	**0.780**	0.48
8/F	1/27/00	1.28	10/27/00	0.769	0.366	0.688	0.52
9/F	3/20/00	0.92	3/20/00	0.845	0.309	0.599	0.63
10/F	3/20/00	1.02	3/20/00	0.808	0.305	0.620	0.62
11/F	3/20/00	1.18	3/20/00	0.808	0.337	0.637	0.58
12/F	3/20/00	1.10	3/20/00	**0.935**	0.311	0.856	0.67
13/F	12/11/00	**1.58**	3/13/00	0.791	**0.454**	0.603	**0.43**
14/F	12/11/00	1.20	3/13/00	0.829	**0.502**	0.664	**0.40**
15/F	-	-	3/13/00	0.763	0.405	0.683	0.47
Mean		1.10		0.806	0.364	0.659	0.55
SD		0.19		0.073	0.065	0.085	0.09
CV(%)		17		9	18	16	13
90% P		1.35		0.900	0.447	0.768	0.44

*G2 index : number of breaks and gaps/metaphase
HDR-MN, LDR-MN, HDR-DS-MN : number of MN/binucleate cell
DRS : $1-Y_{LDR}/Y_{HDR}$ (Y=yield)

Another important problem associated with the sensitivity of these chromosomal assays is that although the mean radiation induced yield of aberrations is significantly higher in patient samples than in samples of normal individuals, considerable overlap exists between both groups for the G2 and MN scores. Because of this, for individual assessment of sensitivity, a cut-off point has to be determined to define whether an individual is radiosensitive or not. In our study the 90^{th} percentile, derived from adopting a normal distribution for the inter-individual radiosensitivity (Table 1), was taken as cut-off point for radiosensitivity (this in accordance with the studies of Scott et al. 1998 &1999).

Table 2. Summary of the data used for the study of the intraindividual variation for two donors for different chromosomal radiosensitivity assays.

Donor 7

Date G2 (M/D/Y)	**G2 index**	**Date MN (M/D/Y)**	**HDR-MN**	**LDR-MN**	**HDR-DS-MN**	**DRS**
10/7/99	0.80	-	-	-	-	-
1/27/00	1.14	1/27/00	0.826	0.432	**0.780**	0.48
1/31/00	1.18	1/31/00	**0.907**	**0.451**	0.700	0.50
2/8/00	0.92	2/8/00	**0.922**	0.410	0.686	0.56
2/17/00	1.28	2/17/00	0.849	0.326	0.718	0.62
3/20/00	1.20	3/20/00	**0.980**	0.358	0.686	0.64
9/10/00	1.14	9/10/00	0.786	0.290	**0.778**	0.63
10/10/00	1.08	10/10/0	0.706	0.361	0.675	0.49
10/16/00	1.22	-	-	-	-	-
Mean	1.11		0.854	0.375	0.718	0.56
SD	0.15		0.092	0.058	0.044	0.07
CV (%)	14		11	16	6	12

Donor 8

Date G2 (M/D/Y)	**G2 index**	**Date MN (M/D/Y)**	**HDR-MN**	**LDR-MN**	**HDR-DS-MN**	**DRS**
10/22/99	1.24	-	-	-	-	-
1/27/00	1.28	1/27/00	769	0.366	0.688	0.52
1/31/00	0.94	1/31/00	815	**0.458**	0.626	0.44
2/8/00	**1.58**	2/8/00	845	**0.469**	0.654	0.45
2/17/00	1.20	2/17/00	764	**0.468**	**0.797**	**0.39**
2/25/00	1.16	2/25/00	724	0.394	0.634	0.46
9/10/00	1.06	9/10/00	647	0.269	0.638	0.58
10/10/00	1.02	16/10/00	700	0.371	0.678	0.47
10/16/00	**1.38**	-	-	-	-	-
Mean	1.21		752	0.399	0.673	0.47
SD	0.20		68	0.073	0.059	0.06
CV (%)	16		9	18	9	14

Although we accept that a population of e.g. breast cancer patients has a mean G2 and G0-MN value that is significant higher than the mean value obtained in a normal population, the results of our repeat experiments show that care has to be taken when evaluating individual sensitivity. Indeed, the results of donor 7 and 8 (Table 2) show that in most occasions the G2 and G0-MN values were within the normal range (90^{th} percentile as cut-off) but in a few occasions also high values were obtained (data in bold). Based on these high values the individuals would have been regarded as sensitive. Thus, the value of chromosomal aberration assays for assessment of the individual radiosensitivity based on one blood sample seems very questionable. The reason why occasionally high values are obtained within the population under study and more especially within the same individual is not yet clear.

Table 3. Summary of the data used for the study of the variation between different cultures for donor 13 for different chromosomal radiosensitivity assays.

Culture	G2-index	HDR-MN	LDR-MN	DRS
1	1.12	0.770	0.535	0.31
2	0.98	0.831	0.428	0.49
3	1.10	0.750	0.413	0.45
4	1.00	0.825	0.457	0.45
5	1.02	0.747	0.429	0.43
6	1.10	0.821	0.462	0.44
Mean	1.05	0.791	0.454	0.43
SD	0.06	0.039	0.044	0.06
CV (%)	6	5	10	15

Setting up multiple cultures from the same blood sample (Table 3) showed that the inter-culture variability is clearly smaller compared to the intra- and inter-individual variabilities (Table 2 and 3). This points to a good assay reproducibility when the influence of external parameters is excluded. The experimental set up of our experiments, in which at least 2 volunteers were tested simultaneously, allowed us to conclude that the high and variable G2 values found in repeated samples of the same donor taken over a time period of 1 year are not due to experimental parameters such as medium, serum batch, PHA, irradiation conditions, etc., but are really an intrinsic characteristic of the individual (Table 1-2).

These findings raise the question whether the high intra-individual variations found in present study may be due to the fact that "a blood sample" as starting material is not exactly reproducible as the composition of blood (cells and serum) varies according to changes in hormone levels, diet, immune status,…. These parameters may have an influence on the individual's behaviour towards ionising radiation. Furthermore, as the ratio of lymphocyte subsets in the blood may vary over time within the same individual this may influence the number of lymphocytes going into apoptosis and by this also influence the level of radiation induced MN.

Due to the high intra-individual variability and the absence of a significant difference with the inter-individual variability we consider that results obtained with the G0-MN and G2 assay which are based on one single measurement are insufficient to determine the radiosensitivity of an individual. To allow reliable conclusions the results should be based on multiple measurements from multiple blood samples. For the determination of the sensitivity of a group of individuals the assays pose no problems and can be regarded as sensitive and reliable.

The fact that we found no correlation between G2 and G0-MN sensitivity in the same individuals, which was also reported by Scott et al. (1998, 1999), may point to the fact that these assays are reflecting different mechanisms of chromosomal radiosensitivity. As until now little is known about the mechanism underlying the formation of chromatid breaks observed in the G2 assay we further studied, in the framework of the DWTC programme on susceptibility, the formation of G2 chromatid breaks (results 5).

Concerning the methodology of the G2 assay a workshop has been organised in which a standardized protocol for the G2 assay, which was approved by several research groups, has been put forward. The results of this workshop have been published in Int. J. Rad. Biol.. For a complete overview of all the results and discussion we refer to the paper (**paper 4** in annex II).

.

Summary of the results and discussion:
Although requiring stringent experimental conditions to achieve good reproducibility, the G2 assay has potential as a sensitive marker for cancer susceptibility, and is particularly useful in population studies. Immediate culture of blood is preferable, but overnight storage of blood either at ambient temperature or at 4 degrees C does not appear significantly to affect G2 scores. Transport of blood may lead to additional variability in assay results and should be well controlled. Although reproducibility is generally good, G2 scores on blood from certain individuals appear to show significant variability in repeat samples. Thus, determination of an individual's radiosensitivity may require multiple assays on different occasions. While it is recognized that the distinction between aligned and mis-aligned discontinuities has no scientific basis, some laboratories have decided for the purpose of record-keeping to score all aligned discontinuities as gaps, and mis-aligned discontinuities as breaks. In all cases the final G2 score should comprise the sum of all gaps and breaks.

Our group also cooperated in the international HUMN (human micronucleus project) project which aims to standardise the MN assay. By the HUMN project a large international study has been set up to compare the intra- and interlaboratory variations in MN scoring in binucleate cells. The results of these studies, in which a large number of participants were involved, have been published in Environmental and Molecular Mutagenesis (**paper 1** in annex II) and in Mutation Research (**paper 5** in annex II). For a complete overview of all the results and discussion we refer to the papers of which the reference is given in appendix.

2. Application of the G0-MN assay and G2 assay on a population of radiation workers from nuclear industry.
A population of radiation workers from nuclear industry has been investigated to evaluate the value of the above mentioned cytogenetic assays to assess individual radiosensitivity of workers in nuclear medicine. The results of these studies are discussed in detail in the report of RUG2, promotor Prof. Dr. H. Thierens, and are published in Int. J. Rad. Biol. (**paper 6** in annex II) and in Int. J. Low Radiation (**paper 10** in annex II). An important conclusion from the study published in IJRB is that in vivo exposure to low doses of ionising radiation results in a reduction of the radiation response in G0 lymphocytes of workers and this probably due to the "adaptive response phenomenon". With the G2 assay no adaptive repons could be demonstrated.

3. Validation of the G0-MN assay and G2 assay in a population of breast cancer patients.
The results of this study have been published in British Journal of Cancer (**paper 7** in annex II). For a complete overview of the results and discussion we refer to the paper.

Summary of the results and discussion:
The chromosomal radiosensitivity of breast cancer patients with a known or putative genetic predisposition was investigated and compared to a group of healthy women (details of patient population see III.2. Material) . The chromosomal radiosensitivity was assessed with the G2 and the G0-MN assay. For the G2 assay lymphocytes were irradiated in vitro with a dose of 0.4 Gy ^{60}Co γ-rays after 71h incubation and chromatid breaks were scored in 50 metaphases. For the G0-MN assay lymphocytes were exposed in vitro to 3.5 Gy ^{60}Co γ-rays at a high dose rate (HDR) or low dose rate (LDR). 70h post-irradiation cultures were arrested and micronuclei were scored in 1000 binucleate cells (detailed description see III.1. Analysis procedure).

Table 1. Mean values, standard deviations, ranges and
Percentage of radiosensitive breast cancer patients and controls

Assay	Parameter	normals	Patients
Age	Range	23-60	29-69
	Mean ± SD	37 ± 12	45 ± 10 *
G2	Population size [a]	51	54
	Mean ± SD [b]	1.03 ± 0.21	1.24 ± 0.25 *
	Range [b]	0.6-1.55	0.76-1.82
	Cut off value [b,d]	1.29	
	% sensitive [d]	10	43 **
MN-HDR	Population size [a]	53	49
	Mean ± SD [c]	741 ± 105	841 ± 143 *
	Range [c]	495-969	546-1166
	Cut off value [c,d]	876	
	% sensitive [d]	11	45 **
MN-LDR	Population size [a]	49	51
	Mean ± SD [c]	346 ± 59	442 ± 89 *
	Range [c]	247-499	263-635
	Cut off value [c,d]	422	
	% sensitive [d]	12	61 **

[a] number of successful blood samples; [b] number of chromatid breaks per cell
[c] number of micronuclei (MN) per 1000 BN; [d] 90th percentile of the controls was selected as cut off
* Significantly different from controls ($p<0.05$) (unpaired t-test)
** Significantly different from controls ($p<0.05$) (Chi-square test)

The results demonstrated that the group of breast cancer patients with a known or putative genetic predisposition was on the average more radiosensitive than a population of healthy women and this as well with the G2 as with the HDR and LDR MN assay (Table 1). With the G2 assay 43% of the patients were found to be radiosensitive. A higher proportion of the patients were radiosensitive with the G0-MN assay (45% with HDR and 61% with LDR) (Table 1). No correlation was found between the G2 and the G0-MN chromosomal radiosensitivity. This again points to the fact that different DNA repair mechanisms or cell cyclus checkpoint mechanisms are operating when cells are irradiated in G0 or G2 phase of the cell cycle. Of the different subgroups considered, the group of the young breast cancer patients without family history showed the highest percentage of radiosensitive cases and this as well in the G2 (50%) as in the G0-MN assay (75-78%) (Table 2).

Table 2. Mean values, standard deviations, ranges and percentage radiosensitivity of 1) breast cancer patients with a family history and a BRCA 1 or 2 mutation, 2) non-BRCA patients with a family history and 3) young breast cancer patients without a family history

assay	parameter	BRCA1	BRCA2	BRCA1+BRCA2	non-BRCA	Young
G2	Population size [a]	4	7	11	43	10
	Mean ± SD [b]	1.20 ± 0.34	1.32 ± 0.18 *	1.28 ± 0.24 *	1.23 ± 0.26 *	1.24 ± 0.26 *
	Range [b]	0.78-1.6	1.18-1.68	0.78-1.68	0.76-1.82	0.8-1.65
	% sensitive [c]	25	29	27	47 **	50 **
MN-HDR	Population size [a]	2	7	9	40	8
	Mean ± SD [d]	787 ± 140	827 ± 144	818 ± 136	846 ± 146 *	940 ± 165 *
	Range [d]	688-886	644-1050	644-1050	546-1166	621-1166
	% sensitive [c]	50	29	33	48 **	75 **
MN-LDR	Population size [a]	2	7	9	42	9
	Mean ± SD [d]	457 ± 42	470 ± 130 *	467 ± 114 *	437 ± 83 *	472 ± 95 *
	Range [d]	427-487	263-635	263-635	267-579	305-576
	% sensitive [c]	100 **	71 **	78 **	57 **	78 **

[a] number of successful blood samples; [b] number of chromatid breaks per cell;
[c] 90th percentile of the controls was selected as cut off; [d] number of micronuclei (MN) per 1000 BN;
* Significantly different from controls ($p<0.05$) (unpaired t-test)
** Significantly different from controls ($p<0.05$) (Chi-square test)
No significant differences were found between the different subgroups(unpaired t-test and Chi-square test)

4.Intermediate results of the validation study in a population of radiotherapy patients.

A second validation study has been started in a population of radiotherapy patients. This study is done in close collaboration with RUG 2 (promotor Prof. Dr. H. Thierens). An important endpoint of this study is to investigate if the in vitro radiosensitivity observed with the G2 and G0-MN assay correlates with the in vivo clinical radiosensitivity observed in about 1% of radiotherapy patients. The suitability of cytogenetic assays to predict in vivo radiosensitivity is still controversial or not known. Data collection on in vitro and in vivo radiation response are essential for a good understanding of the genetic factors responsible for radiation susceptibility. Genetic variations will be studied by means of SNP (single nucleotide poymorphisms) analysis in DNA repair genes and cell cycle checkpoint genes that are associated with in vivo and in vitro radiosensitivity. Some studies have shown that altered responses for some of these genes are associated with cancer predisposition and clinical radiation-hypersensitivity. The characterization of genetic variations associated with higher risks of radiation toxicity should provide a solid scientific basis for the understanding of health effects of low doses and low dose rates and how information on individual radiosensitivity should be incorporated in risk assessment management. This knowledge can also be used to

develop new high throughput techniques for the identification of individuals characterised by an increased risk to develop adverse health effect due to ionising irradiation.

In the framework of this study blood samples were already collected from about 30 patients. Preliminary results obtained in this study on chromosomal radiosensitivity in radiotherapy patients and the analysis of polymorphisms in DNA damage processing genes are discussed in more detail in the report of RUG2 (promotor Prof. Dr. H. Thierens). Data will be further collected and worked out in future projects.

5. Study of the mechanism underlying the formation of G2 chromatid breaks.
The research team of RUG1 (promotor Prof. L. De Ridder) and RUG2 (promotor Prof. H. Thierens) also studied in more detail 'G2 radiosensitivity' this as previous studies have shown that different DNA repair mechanisms or cell cyclus checkpoint mechanisms are operating when cells are irradiated in G0 or G2 phase of the cell cycle. The results of this study have been published in Int. J. Rad. Biol. (**paper 8** in annex II).
For a complete overview of all the results and discussion we refer to the paper.

Short summary of the results and discussion:
The aim of this study was to determine, by means of the G2 assay, the number of chromatid breaks induced by low LET γ-rays and high LET neutrons, and to compare the kinetics of chromatid break rejoining for radiations of different quality.
The G2 assay was performed on blood samples of 4 healthy donors that were irradiated with low LET γ-rays and high LET neutrons. In a first set of experiments a dose-response curve for the formation of chromatid breaks was carried out for γ-rays and neutrons with doses ranging between 0.1 and 0.5 Gy. In a second set of experiments the kinetics of chromatid break formation and disappearance was investigated after a dose of 0.5 Gy using post-irradiation times ranging between 0.5h and 3.5h. For the highest dose of 0.5 Gy the number of isochromatid breaks were also scored.

Table I. *Dose response for the formation of chromatid breaks following irradiation with Co-60 gamma rays and fast neutrons.*

	Donor 1		Donor 2		Donor 3		Donor 4		Mean + S.D.	
	chromatid breaks*		chromatid breaks*		chromatid breaks*		chromatid breaks*		chromatid breaks*	
Dose (Gy)	Co-60	neutron	Co-60	neutron	Co-60	Neutron	Co-60	neutron	Co-60	neutron
0,1	0,3	0,32	0,48	0,28	0,52	0,36	0,34	0,48	0,41 ± 0,11	0,36 ± 0,09
0,2	0,74	0,7	0,72	0,52	0,9	0,68	0,72	0,88	0,77 ± 0,09	0,70 ± 0,15
0,5	1,55	1,61	1,65	1,61	1,88	2,1	1,63	1,67	1,68 ± 0,14	1,75 ± 0,24

* number of chromatid breaks are given per metaphase ; per donor 50 metaphases were scored.

Table II. *Chromatid break rejoining in function of the time (h) after irradiation with a dose of 0,5 Gy Co-60 gamma rays or fast neutrons.*

Time (h) after IR	Donor 1 chromatid breaks* Co-60	Donor 1 chromatid breaks* Neutron	Donor2 chromatid breaks* Co-60	Donor2 chromatid breaks* neutron	Donor3 chromatid breaks* Co-60	Donor3 chromatid breaks* neutron	Donor 4 Chromatid breaks* Co-60	Donor 4 Chromatid breaks* neutron	Mean ± S.D. chromatid breaks* Co-60	Mean ± S.D. chromatid breaks* neutron
0,5	1,06	1,58	1,02	1,36	1,62	1,48	1,74	0,86	1,36 ± 0,37	1,32 ± 0,31
1	1,76	1,54	1,52	1,48	2,6	2,16	1,78	1,76	1,92 ± 0,47	1,74 ± 0,31
1,5	1,55 (0)	1,61(0)	1,65 (0)	1,61(0)	1,88 (0)	2,1(0)	1,63 (0)	1,67 (0)	1,68 ± 0,14	1,75 ± 0,24
2,5	0,66 (4)	0,38 (0)	0,62 (0)	0,6 (0)	0,74 (3)	0,46 (2)	0,7 (10)	0,56 (2)	0,68 ± 0,05	0,5 ± 0,1
3,5	0,2 (2)	0,2 (12)	0,34 (8)	0,42 (22)	0,6 (10)	0,3 (10)	0,36 (23)	0,42 (4)	0,38 ± 0,17	0,34 ± 0,11

* number of chromatid breaks are given per metaphase ; per donor 50 metaphases were scored.
( ) : % of labelled metaphases (per donor 50 metaphases were scored).

No significant differences in the number of chromatid breaks were observed between low LET γ-rays and high LET neutrons for the 4 donors at any of the doses given (Table 1). The dose response curves for the formation of chromatid breaks are linear for both radiation qualities and RBE values equal to one were obtained. Scoring of isochromatid breaks at the highest dose of 0.5 Gy revealed that high LET neutrons are however more effective at inducing isochromatid breaks (RBE of 6.2). The rejoining experiments further showed that the kinetics of disappearance of chromatid breaks following irradiation with low LET γ-rays or high-LET neutrons are not significantly different (Table 2). T ½ = 0.92h for γ-rays and t ½ = 0.84h for neutrons were obtained.
To conclude our results demonstrate that, applying the G2 assay, at low doses of irradiation the induction as well as the disappearance of chromatid breaks is independent of the LET of the radiation qualities used (0.24 keV/µm ^{60}Co-γ-rays and 20 keV/µm fast neutrons). As these radiation qualities produce the same initial number of dsb, our results are in support of the signal model (P.E. Bryant 1998) which proposes that chromatid breaks are the result of an exchange process which is triggered by a single dsb.

6. Preliminary results concerning the correlation analysis between enhanced chromosomal radiosensitivity and the expression of genes involved in the processing of radiation induced DNA damage.
As chromosomal radiosensitivity is probably the result of defects in genes involved in the processing of DNA damage such as DNA repair genes, cell cycle checkpoint genes, stress genes, … a very important aim of the recent programme on susceptibility was to investigate the correlation between both endpoints.

In a first study we investigated the expression of DNA repair proteins in different subpopulations of lymphocytes (T and B lymphocytes). In a previous study (Vral et al. IJRB, 73, 549-555, 1998) we demonstated that the MN response in B lymphocytes irradiated with low doses of gamma rays was higher compared to T lymphocytes. Repair proteins involved in

the non homologous endjoining (NHEJ) repair mechanism were chosen as according to literature NHEJ is the main mechanism to repair radiation induced dsb in mammalian cells. Protein expression of the 3 most important genes involved in NHEJ: Ku 70, Ku86 and DNA-PKcs were analysed by Western Blot. Activity measurements of the catalytic subunit of DNA-PK were also performed. From our results we could conclude that enhanced chromosomal radiosensitivity observed in B cells is not due to defects in the DNA repair enzymes DNA-PKcs, Ku70 & 86. The results of this paper have been published in Int. J. Rad. Biol. (**paper 2** in annex II).

In a second study, which is not yet completed, we wanted to investigate if the enhanced chromosomal radiosensitivity observed with G2 assay and G0 micronucleus assay in breast cancer patients and radiation workers can be correlated with an altered expression (mRNA) of DNA damage processing genes. We started to study differential mRNA expression by real-time quantitative PCR (Q-PCR) on interleukine 2 (IL2) cell cultures of lymphocytes of 1) a group of healthy controls that showed a normal response and 2) a group of individuals (patients, workers) which showed a radiosensitive response with G2 and G0-MN assay. We opted for IL2 cell cultures because this allows us to dispose of a large number of lymphocytes what will allow repetitive experiments. IL2 cell cultures have the advantage, compared to EBV-transformed cell lines that they represent primary cells. To amplify differences in gene expression between the group of normal responders and radiosensitive cases the IL2 cell cultures will be in vitro irradiated.

Below follows an overview of the preliminary results:
1) PCR primers have been developed for the following genes: 1) genes involved in homologous recombination (RAD51, XRCC2, XRCC3, RAD52), 2) genes involved in non-homologous endjoining (NHEJ) (XRCC4-XRCC7, Ligase 4), 3) cellcycle checkpoint genes (G2/M: Chk1, Chk2, cdk1(cdc2/cyclin B) and 4) other genes involved in the processing of DNA damage such as: ATM, CDKN1A (=p21/waf1), GADD45, PARP, XRCC1, DDB2.
2) Preliminary real time quantitative PCR experiments have been performed. Our first results showed that the RNA was not optimal. By Agilent technology we saw that the RNA was strongly degraded. At the moment the cause of this problem is still under investigation.
3) As we want to investigate if the enhance chromosomal response is correlated with a differential gene expression we have to be sure that IL-2 cell cultures show the same radiation response as blood cultures. To investigate this: A) the G0 MN assay and G2 assay for PBLs were optimised for IL2 lymphoblastoid cells and B) the chromosomal radiation response was compared between PBLs in a fresh blood sample and the IL2 lymphoblastoid cells derived from them.

3A) Optimisation of MN assay and G2 assay for IL2 lymfoblastoid cells.
Optimised protocol: lymphocytes are isolated from the fresh blood samples and frozen down in liquid nitrogen. To set up IL2 cell lines frozen lymphocytes are brought in culture and stimulated with PHA and IL2. PHA is only added at the start of the culture while IL2 is added each time the cultures are passaged. De stimulated IL2 cultures are grown for 17-20 days before they are irradiated and MN assay and G2 assay were performed. For MN assay cytochalasin is added immediately after irradiation with 2 Gy gamma rays and cultures are harvested 48 h after irradiation. This protocol yielded the best results. For G2 assay, different protocols were tried out but the obtained yield of good metaphases was to low in all procedures. For this reason the comparative study was only performed with the MN assay.

3B) comparative study between peripheral blood lymphocytes and IL2 lymfoblastoid cells.
For this study blood samples were selected from 20 normal responding control donors and 11 breast cancer patients that showed an enhanced response with the G0-MN assay. The results showed that the significantly enhanced radiation response obtained in fresh blood samples of breast cancer patients (408MN/1000) versus healthy controls (292MN/1000) after an in vitro dose of 2 Gy was not observed in the IL2 lymphoblastoid cells derived from the same individuals. For IL2 lymphoblastoid cells the MN response was comparable in the patients (382MN/1000 BN cells) and healthy controls (400MN/1000 BN cells). The reason why the enhanced chromosomal radiosensitivity observed in blood samples disappeared in IL2 lymphoblastoid cells is not solved yet and will be further investigated.
Other alternatives such as the use of EBV transformed cell lines are under investigation at the moment. If also here the chromosomal response would disappear we will have to perform the gene expression studies on the frozen stock of PBLs . This however has the disadvantage that the amount of material is restricted. The results of these studies are in preparation for publication.

7. Future perspectives:
In the current DWTC programme studies were started to investigate the correlation between chromosomal radiosensitivity observed in a certain number of individuals (patients, workers) and the **expression of DNA damage processing genes**. As these investigations are only in a first stage they will be further worked out in future programmes. The detection of mutations, polymorphisms in DNA damage processing genes correlated with in vitro chromosomal radiosensitivity is a very important issue in the framework of susceptibility of workers and patients.

Another important issue that still needs further investigation in future Radiation Protection and Health Programmes is related to the suitability of the chromosomal aberration assays for **individual risk assessment**. Although the present studies have shown that the G2 and MN assays are suitable and reliable techniques for radiosensitivity assessment in population studies, their value for individual risk assessment is still unclear.

Promotor: Prof. Dr. D. Lison

Catholic University of Louvain
Industrial Toxicology and Occupational Medicine Unit

I. INTRODUCTION

I.1. Context and general frame of the research

The general objective of the programme was to develop new tools for the assessment of inter-individual variability/susceptibility in occupational medicine.
As indicated in the general introduction, variability is important to consider when assessing exposure (variability in exposure biomonitoring) or the consequences of exposure (variability of effects or susceptibility).

Among the different sources of variability, genetic factors (genetic polymorphisms) contribute, for some genes, significantly. The most obvious examples are the polymorphisms for glutathione-S tranferases *GSTM1* and *GSTT1* which are absent in respectively 50 and 20 % of the Caucasian populations. The introduction of molecular biology methods allows to detect these polymorphisms easily (genotyping). The UCL team was responsible for the development of most of the genotyping methods necessary for the present programme.

Beside genetic polymorphisms, other factors may influence the functional activity of an enzyme involved in xenobiotic biotransformations or DNA repair. In particular, the expression or the activity of the enzyme *in vivo* can be measured (phenotyping methods). The UCL team devoted a large part of their efforts during this programme to develop a tool that would allow to phenotype CYP2E1, one of the most important enzyme involved in the biotransformation of industrial compounds and the expression of which is regulated both at the gene level and post-transcriptionally.

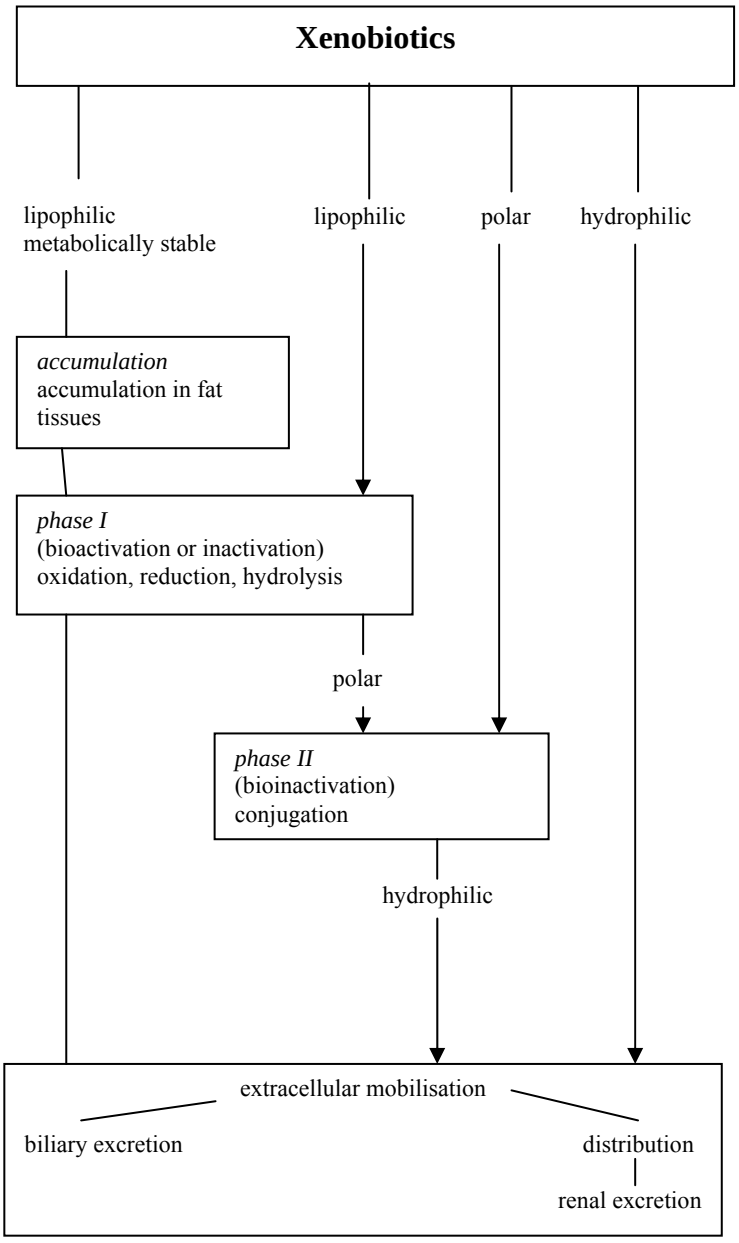
The tools developed at UCL were implemented in several experimental and field studies in collaboration with other partners. The UCL group was in charge of two specific studies exploring the role of inter-individual variability: the first one dealt with biomarkers of exposure to styrene in human volunteers, the second examined the respiratory impairment in workers exposed to cobalt dusts.

I.2. Theoretical considerations.

For the interpretation of biological monitoring data, measured values are to be compared with reference limit values, established on the allowance of the uptake of a certain amount of a chemical agent which is considered to be acceptable for the preservation of the health of the subject. Such reference limit values are proposed by several national or international organisations such as the American Conference of Governmental Industrial Hygienist (ACGIH), which proposes Biological exposures indices (BEI), or the "Deutsche Forschungsgemeinschaft" (DFG) which proposes "Biologischer Arbeitsstoff-Toleranz-Wert" (BAT).

The most useful biomarkers of exposure should be "health-based", i.e. derived from long term follow-up studies of workers allowing the definition of an exposure level without adverse effects in the majority of the workers. Under those conditions limit values may be set up on the basis of dose-effect/response relationships and such values are generally called "***biological action levels***" (BAL). Examples of biomarkers for which a quantitative relation between internal dose and adverse health effects have been identified are relatively scarce: lead in blood, cadmium in urine or carboxyhaemoglobin.
For most substances, however, the dose-effect/response relationship is insufficiently assessed and biological limit values are therefore derived from occupational exposure limits (OEL) in air such as threshold limit values (TLVs) proposed by the ACGIH. These limit values are then called "***biological exposure indexes***" (BEI) as they represent the concentration of the agent that will occur in the body fluids after an eight hour time weighted average inhalation exposure at the OEL. Under these conditions, biological monitoring is more an assessment of exposure intensity than of the potential risk to health.
So far, BEIs have been developed on the assumption that individuals do not differ significantly in their biotransformation capacities. It is clear, however, that this is not the case because wide inter-individual differences exist in the metabolism of xenobiotics. Among the sources of variability, inter-individual differences in uptake (lung, gut or skin) and nutritional heterogeneity may be cited. A fraction of the variability observed in the relationship between the concentration of a biomarker in a body fluid and the ambient concentration of the corresponding toxicant could also be explained by differences in biotransformation capacities. When dealing with those inter-individual variability factors, two situations must be clearly distinguished: (a) the measured biomarker is directly implicated in the toxicity mechanism of the chemical. In this case, the knowledge of its concentration is sufficient for a health assessment without a need to take into account any inter-individual variability factor , since the biomarker itself integrates such variability factors; for this reason this kind of biomarker offers a clear advantage compared to ambient monitoring. The specific carboxylic acids produced *in vivo* from ethylene glycol derived ethers (e.g. methoxyacetic acid in urine (MAA) to assess exposure to methoxyethanol, …) or 2,5-hexanedione as a specific urinary metabolite for n-hexane exposure illustrate this situation; (b) when the link between the biomarker of exposure and the toxic mechanism is not clearly established or even absent, there is a need to identify the sources of the inter-individual variability in order this time to better assess individual exposure. This is of importance and still remains a better approach than ambient monitoring especially to assess residual exposure when individual protective devices are worn (which is a more and more frequent practice in many countries). For this purpose, integration of data on individual metabolic capacity could represent a significant refinement for the interpretation of currently used BEI by integrating, for example, data on genotype and/or phenotype of metabolic enzymes relevant for the biotransformation pathway of the chemical of interest.

In order to select adequate genotyping and/or phenotyping methods, a detailed characterisation of the biotransformation pathways followed by the chemical of interest and the identification of particular enzyme isoforms implicated in each step of the metabolism are, of course, of primordial importance. Most of these preliminary studies are performed *in vitro* using human liver microsomes. The reactions catalyzed by xenobiotic-biotransforming enzymes are generally divided into two groups, called phase I and II. Phase I reactions involve hydrolysis, reduction and oxidation. These reactions expose or introduce a functional group (-OH, -NH_2, -SH or –COOH), and usually result in only a small increase in hydrophilicity. Phase II biotransformation reactions include glucuronidation, sulfation, acetylation, methylation, conjugation with glutathione (mercapturic acid synthesis) and conjugation with amino acids (such as glycine, taurine and glutamic acid). The cofactor for these phase II reactions reacts with functional groups that are either present in the xenobiotic or are introduced/exposed by phase I biotransformation. Most phase II biotransformation reactions result in large increase in xenobiotic hydrophilicity, hence they greatly promote the excretion of foreign chemicals.



Among all the enzymes implicated in the biotransformation of xenobiotics, several are of primordial importance for the biotransformation of industrial and/or environmental toxicants (generally smaller molecules than drugs). For phase I reactions, cytochrome P450 (CYP), in particular isoforms CYP1A1 and CYP2E1, have been involved in the metabolic activation of many precarcinogens such as polycyclic aromatic hydrocarbons (PAHs) for CYP1A1 and benzene, dimethylnitrosamine and vinyl chloride for CYP2E1. Another phase I enzyme largely implicated in the biotransformation of industrial and/or environmental toxicants is microsomal epoxide hydrolase (mEH or EPHX1). This enzyme catalyses the hydrolysis of reactive aliphatic and arene epoxides generated by CYP enzymes to more water soluble dihydrodiol derivatives (detoxification pathway). In certain instances however, chemical products of EPHX1 metabolism (*trans*-dihydrodiols) may be further derivatized to secondary epoxide species which are poor substrates for EPHX1. These diols-epoxides are often highly reactive and have been involved in processes such as teratogenesis and initiation of cancer. Finally, as phase II enzymes, gluthathione S-transferases (GSTs) are a family of dimeric enzymes which play an important role in the detoxification of numerous industrial and/or environmental toxicants. These enzymes catalyze the conjugation of electrophiles with glutathione thereby inactivating potential cytotoxic and/or genotoxic substances. Others enzymes of importance for the biotransformation of industrial chemicals are N-acetyl transferases, UDP-glucuronosyl transferases, sulfotransferases,…

For all previously cited enzymes, differences in the gene nucleotide sequence, called genetic polymorphisms, have been described in the general population, including Caucasians. Each gene can be found in a wild and most frequent form, called “wild allele”, or in one (for bi-allelic polymorphisms) or more (for multi-allelic polymorphisms) variant forms, called “variant or rare alleles”. As each gene is present in double exemplar, a particular genotype is defined as a combination of two alleles (hetero- or homozygotes). Such polymorphisms can have an effect on the enzymatic function and ideally, the relationship between a particular genotype and phenotypic catalytic activity of the enzyme should be established at least *in vitro*. For *CYP1A1* (located on chromosome 15), two polymorphic loci have been largely studied. The polymorphism of greatest interest is located in the catalytic region of CYP1A1 (exon 7) and leads to the substitution of isoleucine by valine (Ile/Val). The presence of valine is thought to increase the catalytic activity of the enzyme. This mutation is closely linked with another polymorphism which can be detected by a MspI restriction fragment length analysis and that is characterised by two alleles: m1 (absence of MspI site) and m2 (presence of MspI site). The m2 allele is believed to be associated with a higher enzyme inductibility. For *CYP2E1* (located on chromosome 10), three different polymorphisms detectable with TaqI (A2/A1), DraI (D/C), RsaI and PstI (c1/c2) restriction enzymes as well as a 96 bp-insertion polymorphism have been described. While reduced CYP2E1 activity in the presence of the rare C allele (*CYP2E1*6*) has been suggested, the *in vivo* significance of most of these polymorphisms is, as yet, far from clear. For EPHX1 (located on chromosome 1), two polymorphic sites have been observed in exon 3 (Tyr113/His113) and exon 4 (His139/Arg139). Based on *in vitro* studies, the variant allele of one of these sites (exon 3) correlated with reduced EPHX1 activity whereas the variant in the other site (exon 4) resulted in increased EPHX1 activity. Finally, for the cytosolic GSTs which include five subfamilies (A, alpha; M, mu; P, pi; T, theta; Z, zeta), two polymorphic genes have been largely investigated, i.e. *GSTM1* and *GSTT1*, and it has been estimated that about 50 and 15% of Caucasians lack the gene, respectively.

II. AIMS OF THE RESEARCH

The *general objective* of the programme was to explore the possibility to develop new methods to assess inter-individual susceptibility/variability factors and to assess their usefulness in occupational medicine.
Based on the previous experience of the partners, two test-toxicants, styrene and cobalt, were selected for these investigations.

The *specific objectives* for the UCL group were:

1. development of phenotyping (CYP2E1) and genotyping methods (CYP2E1, CYP2B6, GSTs and EPHX1, DNA repair enzymes and HLA polymorphisms).

2. exploration of the "DNA array microchips" technology for genotyping and phenotyping in occupational medicine (in collaboration with VUB).

3. experimental study (*in vitro* and *in vivo*) of the impact of the considered polymorphisms (CYP2E1, GSTs and EPHX1) on the biotransformation of styrene (in collaboration with the other partners).

4. experimental study of the impact of polymorphisms on the repair of oxidative damage induced by cobalt (in collaboration with VUB).

5. biomonitoring study exploring the susceptibility in a population of workers exposed to styrene (in collaboration with the other partners).

6. biomonitoring study exploring the susceptibility in a population of workers exposed to cobalt.

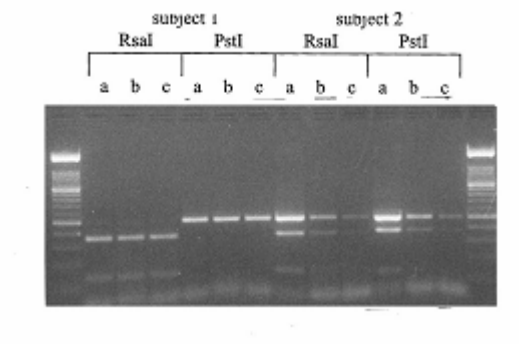
7. study of the ethical and legal implications of the introduction of susceptibility tests in occupational medicine (in collaboration with the other partners of the network, lawyers in particular).

III. ACHIEVED RESULTS

1. Development of phenotyping and genotyping methods.

1.1.Genotyping

Genotyping was performed after extraction of genomic DNA from an EDTA-anticoagulated blood sample. Methods based on classical PCR restriction fragment polymorphism (RFLP) were developed for a large array of biotransformation enzymes (*CYP2E1* c1/c2, D/C, A1/A2 and insertion polymorphisms or CYP2E1*5B, *6, *1B and *1D, respectively), *GSTM1*, *GSTT1*, *EPHX1* exon 3 Tyr113/His113 and exon 4 His139/Arg139) and for one enzyme involved in the repair of oxidative DNA damage (hOGG1 Ser326/Cys326).



Example of a RFLP analysis for CYP2E1 polymorphisms

A Real Time PCR allelic discrimination method was also developed to confirm the PCR RFLP results for the exon 4 polymorphism in the EPHX1 gene that could be influenced by another polymorphism in codon 119. These tools were implemented in several of the experimental and field studies discussed below.
CYP2B6 genotyping was developed elsewhere in the frame of the specific KUL-study on the biotransformation of fentanyl.

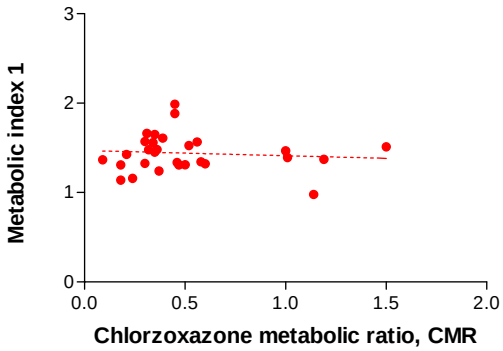
The presence of the polymorphisms leading to the expression of Glu69β was determined by a reverse-sequence specific oligonucleotide (SSO) test using a probe specific for the GAG sequence in exon 2 of the HLA-DPB1 gene (Innolipa, Innogenetics). The results were expressed as presence or absence of the polymorphisms without consideration of the allelic combinations (see point 6 below).

1.2. Phenotyping

Although the most accurate assessment of CYP2E1 activity is based on direct hepatic 2E1 measurement and therefore requires a liver biopsy, alternative approaches have been developed, to be used on a large scale with non-invasive methods. Until now, the "gold standard" method requires the use of a probe drug (chlorzoxazone, CZX) which has to be administered to the individual for which a phenotyping test is needed. CZX is a drug formerly used as a myorelaxant and which is extensively metabolised in the liver to 6-hydroxychlorzoxazone (HCZX), a reaction mediated by CYP2E1. The chlorzoxazone test is generally based on the administration of a single oral 500 mg (or 250 mg) dose to fasting individuals. Various CZX pharmacokinetic indexes have been proposed to reflect CYP2E1

activity : plasma CZX $t_{1/2\beta}$, HCZX renal excretion, CZX oral clearance, fractional clearance to HCZX and HCZX area under the concentration curve (AUC). Among these, CZX fractional clearance to HCZX is the most direct measure of CYP2E1 activity and often serves as a standard method to which other proposed approaches are compared. However this procedure is time consuming, inconvenient (urine collection,…), and requires subject compliance and catheterization for up to 12 hours. The ratio of plasma CZX/HCZX concentrations (chlorzoxazone metabolic ratio, CMR) measured 2 hours after the oral CZX dose has been largely used as an alternative to CZX fractional clearance but the correlation between both methods is quite low (r^2 = 0.16-0.28) suggesting that single-point ratios do not accurately measure CYP2E1 activity. Furthermore, recent *in vitro* studies suggest that CYP1A1, CYP1A2 and possibly CYP3A are involved in CZX 6-hydroxylation limiting the specificity of CZX for CYP2E1 phenotyping.

We conducted a field study to assess the value of CMR in refining biomonitoring of exposure. Exposure to styrene was selected as a paradigmatic situation (Haufroid et al. 2002a). 31 workers from a fibreglass-reinforced plastics factory took part in the study. Ambient styrene concentration was determined during the whole workshift by passive sampling. Each worker received a 500-mg chlorzoxazone (CZX) tablet at the beginning of the workday, and blood was taken after 2 h for CMR and CYP2E1 genotypes determination. Urine was collected at the end of the shift for the determination of styrene-specific metabolites. The integration of CMR value as an independent variable to explain inter-individual variability in urinary metabolite excretion was not conclusive.

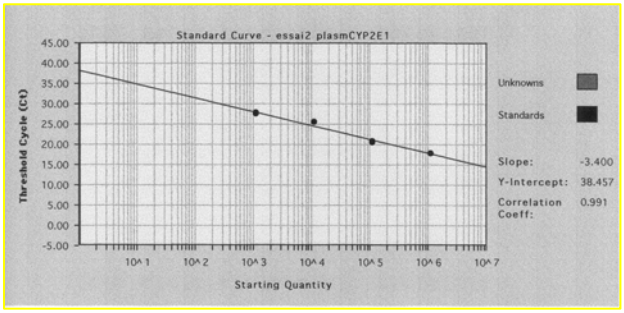


The metabolisation of styrene (metabolic index 1 : mandelic and phenylglyoxylic acid/styrene in air) does not correlate with the chlorzoxazone metabolic ratio (CMR)

An additional objective was to analyse the relationship between CYP2E1 activity as assessed by the chlorzoxazone metabolic ratio (CMR) and frequent CYP2E1 genotypes (CYP2E1*5B, *6, *1B and *1D). While the only worker heterozygous for CYP2E1*1D allele presented the highest value of CMR, a trend to lower CMR value for individuals possessing at least one mutant CYP2E1*6 allele compared with homozygous wild type was observed.

It was concluded that in this population of workers, the CMR test was able to detect a slight influence of some genotypes on the activity of the CYP2E1 enzyme, but it must be recognised that this method is not appropriate for refining the biological monitoring of industrial compounds that are metabolised by CYP2E1.

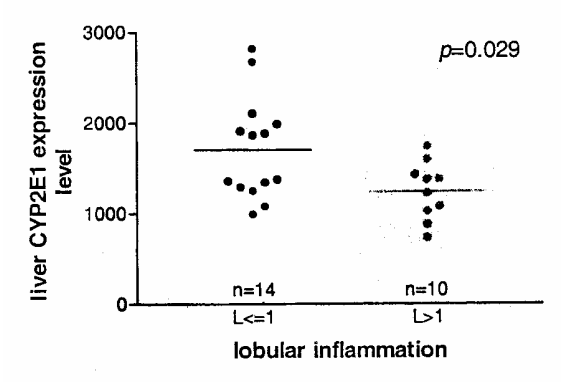
CYP2E1 phenotyping on a large scale, such as for the screening of large numbers of samples, for epidemiological studies or even as an adjunct to improve a biological monitoring programme, requires the implementation of more practical methods. A CYP2E1 phenotyping method by reverse transcription and real-time PCR was developed and extensively validated analytically and biologically. This method allows the quantification of the number of mRNA copies coding for CYP2E1 in human circulating lymphocytes and was published in the international literature (Haufroid et al. 2001).



Example of a calibration curve for CYP2E1 mRNA quantification by RT-real time PCR

The biological validity of this method was examined in several clinical situations known or suspected to involve alterations in CYP2E1 expression :

- diabetics : we have shown in a group of 30 diabetics with unstable disease (glycated Hb >6%) that the RT-PCR test on a lymphocyte sample was able to detect a systemic induction of CYP2E1 expression that could be expected in this disease,
- hepatitis : the same methodology was applied in 30 paired liver biopsy and lymphocytes samples from patients with viral hepatitis or controls. In the group of patients, a reduction of CYP2E1 expression was noted, especially in association with centrolobular lesions, which is consistent with our knowledge of the tissular distribution of this enzyme in the liver. In contrast, as expected, no modification was found in peripheral lymphocytes that were not affected by this type of pathology,



CY2E1 expression levels according to Ludwig's histological score of lobular inflammation

- phenotype-genotype relationship : we have also shown that the expression of CYP2E1 in lymphocytes of individuals bearers of a C allele (D/C polymorphism of the *CYP2E1*gene) is significantly reduced (Haufroid et al. 2002a), which is consistent with the published data,
- N-methylpyrrolidone (NMP) : the quantification of messenger RNA for CYP2E1 was also implemented in a study carried out in volunteers from our laboratory, dealing with the metabolism of NMP after dermal application and the role of CYP2E1 in the

biotransformation of this industrial solvent (Ligocka et al. 2003). Three experimental approaches were used : in the rat, *in vitro* in human microsomes, and in human volunteers. NMP was administered dermally (40 mg/kg) to OFA rats to examine the influence of CYP2E1 inhibition (5 mg/kg diethyldithiocarbamate, DETC, 30 min before) and CYP2E1 induction (after 4 days of fasting). The main NMP metabolite 5-hydroxy- N-methylpyrrolidone (5HNMP) in the urine fractions collected during the following 48 h was analysed by gas chromatography-mass spectrometry. CYP2E1 inhibition led to a statistically significant retardation of 5HNMP excretion in urinary fractions collected during the first 12 h. In the group of fasted rats, a two-fold increase of CYP2E1 activity was observed in comparison with the control group. During the first 6 h after dermal administration of NMP to fasted rats, about 33% of the dose was excreted in urine versus 22% in controls. In vitro, NMP (15 mM) was incubated (up to120 min) with human liver microsomes and the formation of 5HNMP followed Michaelis-Menten kinetics with V(max) of 1.1 nmol/min per mg protein and K(m) of 2.4 mM. The formation of 5HNMP was inhibited by 35% in the presence of a monoclonal antibody against CYP2E1, but not by CYP1A2 antibody. In a dermal application experiment, 12 humans volunteers were exposed by means of a dermal patch to 300 mg NMP; five urine fractions were collected during the 48 h following the onset of application in order to measure the major metabolites 5HNMP and 2-hydroxymethylsuccinimide (2HMSI). Before NMP application, a blood sample was collected for the quantification of CYP2E1 mRNA in peripheral blood lymphocytes (PBLs). The mean dermal absorption of NMP was 67.9%. The highest amount of 5HNMP was excreted in urine in the fraction collected between 6-12 h (12.6% of dose), while 2HMSI peaked in fractions 12-24 h and 36-48 h (3.3 and 3.2% of dose, respectively). A significant relationship was found between CYP2E1 mRNA content in PBLs and the amount of both the metabolites excreted in urine within 24 h ($r(2)=0.54$, $P<0.01$). It was concluded that CYP2E1 is involved in the first steps of NMP metabolism in the rat and, to a lesser extent, in humans. Since large variations in CYP2E1 activity exist in the human population (at least 5-fold range), it seems justified to take into account the activity of this enzyme in an individual for an accurate interpretation of biological monitoring of exposure to NMP when relying on 5HNMP and/or 2HMSI determination in urine.

Overall, these data tend to support the validity of the method developed that allows to detect responses consistent with the biology of the investigated situations. These observations were summarised in two publications (Ligocka et al. 2003, Haufroid et al. 2003).

These data were also presented on the occasion of the international symposium on biomonitoring organised in Banff in September 2001.
In addition, this methodology was applied in the framework of an European research programme in which volunteers exposed to a controlled concentration of styrene and workers were monitored for a range of biomarkers of exposure (Haufroid et al. 2002b).

The genotyping methods developed for this programme were also valorised in a study conducted in collaboration with a Japanese team that was interested in the biotransmformation of dimethylformamide (DMF) in workers (Nomyiama et al.2001). This study examined whether consideration of the *1C/*1D CYP2E1 insertion polymorphism is important for interpreting the biological monitoring of exposure to N,N-dimethylformamide (DMF) in Japanese workers. The insertion genotype, airborne DMF exposure on the last day of a work week, and NMF in urine sampled just after the last workshift of the week were determined in 44 male and female Japanese workers. The allelic frequency of this CYP2E1 polymorphism

was 0.261 in this Japanese population of workers. The CYP2E1 insertion polymorphism did not contribute to NMF levels even after consideration of BMI or alcohol intake.

The results indicated that CYP2E1 insertion polymorphism does not appear to be an important determinant for the interpretation of biological exposure to DMF by the measurement of urinary NMF.

2. exploration of the "DNA array microchips" technology for genotyping and phenotyping in occupational medicine.

In collaboration with Prof Kirsch-Volders (VUB), several contacts were taken to explore the possibilities to transpose our results, obtained with artisanal genotyping and phenotyping methods, to a more sophisticated technology.
Two types of technologies were explored :

- DNA microchips allowing the quantification of gene expression
- Genotyping macroarrays designed to detect single nucleotide polymorphisms (SNPs).

Contacts were taken with 3 partners : the research group of Prof J. Remacle (Facultés ND de la Paix in Namur), the group of Dr P. Van Hummelen (Centre for Microarray Technology, VIB) in Leuven, together with an industrial partner.

So far, the contacts are still exploratory because the numbers of genes or polymorphisms examined in the frame of our projects remain limited and did not appear to justify the use of such sophisticated and expensive technologies.

3. experimental study of the impact of the considered polymorphisms (CYP2E1, GSTs and EPHX1) on the biotransformation of styrene.

A study was conducted in human volunteers recruited and exposed to styrene vapors in the frame of a European project (Suscepstyrene) (Haufroid et al. 2002). Styrene is one of the chemicals for which biological monitoring programs have been validated and implemented in environmental studies and occupational medicine. However, inter-individual differences in the urinary excretion exist both for the main end-products (mandelic acid and phenylglyoxylic acid) and for its specific mercapturic acids (phenylhydroxyethylmercapturic acids, PHEMA). This limits to a certain extent the use of these metabolites for an accurate assessment of styrene exposure. In a group of 26 volunteers selected with relevant genotypes, and exposed to styrene vapours (50 mg/m3, 8 h) in an inhalation chamber, we evaluated whether genotyping or phenotyping relevant drug-metabolizing enzymes (CYP2E1, EPHX1, GSTM1, GSTT1 and GSTP1) may help to explain the observed inter-individual variability in the urinary metabolite excretion. Peripheral blood lymphocytes were used for genotyping and as reporter cells for the phenotyping of CYP2E1 and EPHX1.

The GSTM1 genotype was clearly the most significant parameter explaining the variance in urinary PHEMA excretion (6-fold lower in GSTM1 null subjects; $P < 0.0001$) so that systematic GSTM1 genotyping should be recommended routinely for a correct interpretation of PHEMA urinary levels.

Variant alleles CYP2E1*6 (7632T>A) and His113EPHX1 were associated with a significant reduction of, respectively, the expression (P = 0.047) and activity (P = 0.022) of the enzyme in peripheral blood lymphocytes.

In combination with GSTM1 genotyping, the phenotyping approach also contributed to improve the interpretation of urinary results, as illustrated by the combined effect of CYP2E1 expression and GSTM1 allelic status that explained 77% of the variance in PHEMA excretion and allows the recommendation of mercapturates as specific and reliable biomarkers of exposure to styrene.

Determinants of the urinary excretion of styrene metabolites (including CYP2E1 phenotyping).

Dependent variables	*Independent variables*	Partial r^2	*Slope*	*p* value*
MA excretion	CYP2E1 mRNA	0.22 Model r^2: 0.22	positive	0.016
Total VP excretion	BMI	0.13	negative	0.066[a]
Total mercapturic acids	**GSTM1** Tobacco consumption	0.71 0.07 Model r^2: 0.78	positive negative	0.0001 0.014
Total mercapturic acids**	*GSTM1**CYP2E1 mRNA	0.77 Model r^2: 0.77	positive	0.0001

* partial r^2 *p* value.
Tested independent variables were five unrelated polymorphisms (*GSTM1*, *GSTT1*, *GSTP1*, *EPHX1*(exon3 and 4)), CYP2E1 mRNA expression in PBLs, BMI, alcohol consumption, tobacco consumption and age.
** The same independent variables were selected with three first order interaction term *GSTM1**CYP2E1 mRNA, *GSTM1**GSTT1 and *GSTM1***GSTP1* (after exclusion of one "outlier").
[a] first independent variable selected by the model but excluded in the first step because p>0.05.

This work was published in the international literature and the ECETOC science award 2003 was presented to V. Haufroid for this innovative publication in the field of toxicology.

4. experimental study of the impact of polymorphisms on the repair of oxidative damage induced by cobalt (in collaboration with VUB).

see VUB report

5. biomonitoring study exploring the susceptibility in a population of workers exposed to styrene (in collaboration with KUL, ULg et VUB).

The UCL-team was responsible for the genotyping of the biotransformation enzymes in another study conducted in workers exposed to styrene. This study comprised 44 workers exposed to styrene and 44 matched referents and examined the influence of genetic polymorphisms in biotransformation and DNA repair enzymes on N-terminal Hb adducts levels and genotoxic effects (Godderis et al., submitted). MA concentration in urine was 201.57 mg/g cr ± 148.32, corresponding to a calculated average airborne styrene exposure of 9.5 ppm ± 9.6. Individuals with a high level of N-terminal valine adducts revealed higher DNA damage as evaluated by Comet assay (r=0.29, p=0.008). Frequencies of micronuclei

(MN/1000 cells) in mononucleated lymphocytes (MNMC) (0.71 ± 0.88 vs 0.11 ± 0.20, p<0.0001), binucleated lymphocytes (MNCB) (3.93 ± 2.75 vs 2.65 ± 1.94, p=0.02) and MN in nasal cells (0.52 ± 0.49 vs 0.23 ± 0.31, p=0.04) differed significantly between the exposed and referent group. Early and late in vitro repair capacity was also influenced by styrene exposure. The influence of genetic polymorphisms of metabolising and repair enzymes was studied. Higher frequencies of MNMC were found in individuals possessing *XRCC3 Met*^{241} allele, in individuals with *XRCC1* Gln^{399} allele and in styrene-exposed workers homozygous for *EPHX1 His*^{139} (p=0.044). Analysis of the vitro DNA strand break repair phenotype data showed a faster onset of DNA repair after 1 hour in individuals heterozygous for *XRCC1 Arg*^{280} (p=0.043) and in individuals possessing GSTT1 (p=0.043). Higher residual DNA damage 24 hours after in vitro SO exposure was found in individuals homozygous for *XRCC1 Arg*^{194} (p=0.013). It has to be stated that multivariate analysis could not reveal any significant influence of the genotype on the results of the assays, probably due to a low number of participants in this study.

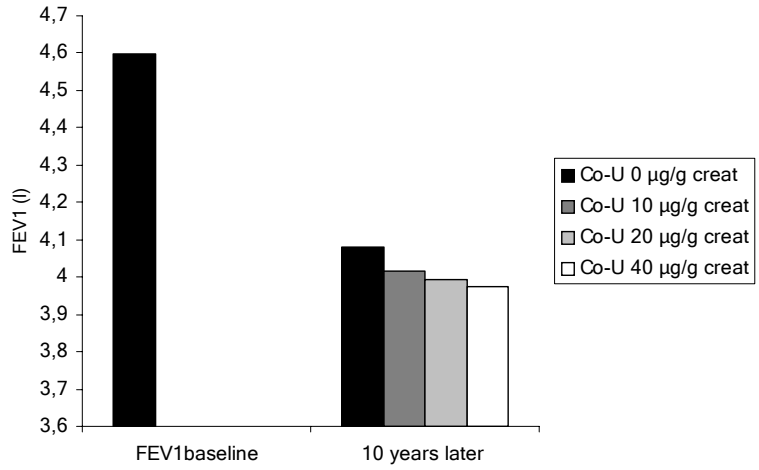
In conclusion, the data suggest that chromosome/genome mutations are formed in workers exposed to low concentrations of styrene. Duration of exposure, age and smoking habits are important variables to consider in studies evaluating genotoxic effects on workers. Genotyping of metabolising and DNA repair enzymes are important for the assessment of individual genotoxic risk to styrene. The in vitro DNA strand break phenotype might be a valuable method to estimate the repair capacity of workers.

6. biomonitoring study exploring the susceptibility in a population of workers exposed to cobalt.

The types of respiratory disorders that have been related to occupational exposure to cobalt include bronchial asthma, chronic bronchitis and the so-called hard metal disease (HMD), a fibrosing alveolitis that has mainly been described in workers exposed to a mixture of cobalt metal and carbides in the hard metal industry. Because of the characteristics of the disease, such as its low prevalence in exposed workers or the lack of correlation with the intensity or duration of exposure, authors have suggested the existence of a possible genetic susceptibility to HMD. Notably the substitution of a lysine residue by a glutamate in position 69 in the HLA-DP beta chain (Glu69β) has been reported to be weakly associated with HMD. An increased risk of lung cancer has also been reported in the hard metal industry.
A cross-sectional study carried out at the end of the eighties in a plant producing metallic cobalt, cobalt oxides and salts did not detect signs of fibrotic disease in the 82 investigated Co workers, based on chest radiographs and measurements of lung volumes and diffusing capacity. However, exposed workers complained more often of respiratory symptoms than controls and a negative relationship between lung function parameters (mainly FEV1) and the intensity or duration of exposure was found. Since these findings suggested a risk of respiratory impairment, technical and hygienic improvements were enforced in the plant to reduce exposure and a periodic monitoring of the lung function of the workers was implemented from 1988 on. Workers were thus examined at intervals for spirometric parameters and exposure to cobalt was quantitatively assessed at least annually by the measurement of cobalturia. Based on this 13-year surveillance programme (1988-2001), enough information was available to investigate in a longitudinal design a possible effect of cobalt exposure on the lung function parameters.

We therefore analysed these data with the objective of identifying the determinants of lung function changes in workers from this cobalt producing plant and included in the respiratory monitoring programme between 1988 and 2001. 122 male workers with at least three lung function tests (FEV1, FVC) during the follow-up period were assessed. The possible association of spirometric changes with cobalt exposure was examined cross-sectionally and longitudinally by using a random coefficient model, taking into account other potential influential variables such as smoking, age, previous respiratory illness, exposure to other lung toxicants and the presence of Glu69β, a HLA-polymorphism possibly associated with hard metal-induced lung diseases. Cobalt exposure significantly decreased over the follow-up period, as reflected by the measurements in air and in urine. The cross-sectional analysis in 2001 did not reveal any association.

The main finding was that cobalt exposure contributed to FEV1, not FVC, decline over time, only in smokers. No influence of Glu69β polymorphism was detected. Although the amplitude of the additional decrement associated with exposure was relatively small (<20%) compared to the expected decline in a smoker, the results indicated that further efforts to reduce exposure and to encourage workers to quit smoking are still warranted.



Expected FEV1 decrements (ml) after 10 years of exposure to cobalt calculated for a 30-y.old smoking worker, based on the estimates provided by the random coefficient model

7. **study of the ethical and legal implications of the introduction of susceptibility tests in occupational medicine.**

The UCL-researchers involved in this programme were in constant contact with the partners examining the ethical and legal aspects of susceptibility and genotoxicity testing in occupational medicine. Exchanges of information and opinions took place on the occasion of the regular meetings organised by the coordinator and through more frequent and informal meetings between the researchers. D. Lison was a member of the accompanying doctoral committee of Mrs N. Hautenne (Prof P. Vielle, UCL).

D. Lison was also invited by the Commission of the social affairs of the Belgian Senate to advise senators on the law projects dealing with genetic testing in occupational medicine introduced by senators Mahoux et Van Lerberghe. This proposal has now been published in the form of a Royal Decree.
D. Lison gave also a presentation on the use of genetic tests in occupational medicine during the workshop organised by OSTC in October 2003 (see annex II).

8. **valorisation**

PhD theses

Most of the data on genotyping, phenotyping and styrene metabolism were included in the doctoral thesis of V. Haufroid (D. Lison promoter, l'UCL) presented on 27 November 2002.

D. Lison was also co-promoter of the doctoral thesis of M. De Boeck (M. Kirsch-Volders promoter, VUB) presented on 29 October 2002.

The data on lung function in workers exposed to cobalt compounds will be integrated in the PhD thesis of Dr V. Verougstraete (D. Lison promoter, UCL).

Publications

The data generated during this research programme were valorised by a total of 21 scientific publications (see Annex II).

Promotor: Prof. Dr. P. Vielle
Scientific collaborator: N. Hautenne

Catholic University of Louvain
Department of Economical and Social Law

CHANGES IN THE CONCEPT OF OCCUPATIONAL HAZARD
IN RESPONSE TO THE USE OF GENETICS, AND LEGAL IMPLICATIONS
FOR THE PREVENTION OF AND COMPENSATION FOR OCCUPATIONAL DISEASES

INTRODUCTION

1- Context and motivation of the study

This research relates to the use of genetic tests in employment relations. This research is carried out in the framework of an interdisciplinary (biology, genetics, occupational medicine, toxicology…) and inter academic network.

The scientific component of the project considers the capacity and the utility of having genetic tests in the context of workplace health prevention. The legal component of the project is intended to accompany this scientific reflection with a study of the conditions for application of these tests. This question gives rise today to a number of legislative interventions at the Belgian and international levels. For example, UNESCO's recent draft international declaration, the opinion of the European Group on Ethics, the Belgian law of January 28, 2003 relating to medical examinations in the framework of employment relations, … (UNESCO, 2003; groupe européen d'éthique, 2003) These interventions translate the fears caused by unregulated use of genetic tests, and show the urgent need to consider the principles and values which could form the basis of a clear and securing legal framework.

2- Objectives of the research

The legal component of the project is intended to evaluate the various parameters that make it possible to answer the question of the "applicability" of genetic tests in the workplace. What are the values concerned? What are the regulatory devices allowing it to be answered? Are these devices sufficient?

More particularly, it aims at highlighting the potential consequences of the introduction of genetics into employment relations, from the point of view of the way in which employment and social security law constructs the concept of "occupational hazard," and the way in which this hazard is apprehended in the legal system of prevention and reparation for occupational diseases.

Theoretical framework

1. In the first phase, the theoretical framework of fundamental rights and, more specifically, the right to respect for private life, was selected in order to analyze the issues arising from the use of genetic tests in the workplace. Medical tests and *a fortiori* genetic tests constitute an attack on the physical integrity and the private life of the person. As such, they are in theory prohibited. Certain tests are, however, legitimate if they meet conditions set by law. This work enabled us to answer certain questions and to formulate certain conditions for the application of genetic tests.

2. In the second phase, the perspective adopted is that of employment and social security law. The theoretical framework is based on the concept of occupational hazard that emerges from

Ewald's analysis in his work on the Welfare State.

The Welfare State emerges, according to Ewald, following the historical conjunction of two events: the scientific discovery of probability and statistics on the one hand and, on the other, an awareness of the insufficiency of the liberal model, and of the need to find another model in response to the problems arising from the development of industrialization and to the phenomenon of industrial accidents.

The discovery of statistics and probability represents the technical means which made it possible to rethink the rule of justice in terms of mutualisation, and hence to modify ways of envisaging the sharing of responsibility between individuals, the market and the State. The liberal model invite done to find the cause of the harm and to hold its author responsible, on the basis of the principle that from "natural" distribution of good and bad emerged a principle of justice. With probability and statistics, we have the means of rethinking the relationship between the individual and society (the whole and the part).

In addition, the development of industrialization with its accidents and catastrophes highlights the insufficiencies of the liberal model, and sets in motion the divorce between the rule of justice and the most obvious requirements of equity.

This conjunction of factors allows the emergence of the concept of occupational hazard based on the idea that social questions are not solved by the principle of freedom. It would henceforth be necessary to resort to obligation and thus to the law. The concept of occupational hazard undergoes a scission between causality and attribution of responsibility for risk, that is to say by the intuition that a principle of responsibility no longe relates to the objective cause of the damage (new relation between man and nature).

This system engenders a new rule of equality : no longer the formal equality necessary to the birth of the contract, that is to say equality of right; but a factual equality based on the prior factual identification of inequalities. Equality is indexed on an idea of average and balance, by a regular appraisal of the relationship of society to itself. Equality is determined concretely by an average.

J. Rawls explains why our notion of justice, based on equality, can only be understood starting from a principle of radical ignorance as to our individual destinies (Rawls, 1997). It is here that the question arises of genetic tests, and their consequences for the principle of solidarity and the social contract (Ewald, 1993).

Our principal hypothesis is as follows: the conjunction between the use of genetic information in the workplace and the current socio-economic context, helping to transform the concept of risk (by allowing/accentuating anticipation and individualization of risk), will bring about a transformation of the legal system of prevention and reparation for occupational hazards.

METHODS

1- Historical and descriptive approach

Analysis of the evolution of the concept of occupational hazard, through successive legislation as well as parliamentary work, as regards both prevention and compensation for the risk of

occupational disease.

2 - Multidisciplinary and prospective approach

Multidisciplinary
- Study of the roles and functions of genes in pathological processes, thanks to discussions with the biologists, geneticists and doctors involved in the SSTC project, and starting from readings of various works of popular science.

- Integration of a sociological dimension for the comprehension of the models under which the concepts of risk and genetics are constructed in employment relations.

Prospective
- Taking stock (in the current socio-economic context) of the changes in the concept of "occupational hazard" in response to the use of genetic tests in the context of employment relations, in order to extract models of evolution of the systems of prevention and compensation.

3 –Contextualized approach

- Putting the results in perspective in the current economic and social context (productivity, competitiveness, flexibility, globalisation, …) which determines working conditions.

RESULTS AND DISCUSSION

1- Scientific approach to genetics

The first part of this research was devoted to the comprehension of genetics from a scientific point of view. In this respect, participation in an interdisciplinary network allowed regular contacts and discussions which facilitated a good comprehension of the concepts.

- Comprehension of certain scientific concepts: distinction between monogenic and multifactorial disease, genetic polymorphism, tests of susceptibility and monitoring tests, early biomarkers of effects, adducts, processes of metabolisation, sensitivity of tests, specificity, reliability, predictive value, prevalence, false positives, false negatives, mutagenic or genotoxic substances, …

- Comprehension of certain mechanisms: metabolisation of substances, measurements of exposure, the role and limits of genetics in the pathological processes of occupational diseases.

- Distinction between genetic susceptibility tests and genetic monitoring tests. The purpose of susceptibility tests I to identify, at the genome level, certain genes known to be involved in pathological processes. Genetic monitoring tests relate to the analysis of the early effects on the genetic material of certain substances known to be genotoxic or mutagenic. These are the early biomarkers of effects. These early genetic "lesions" could reveal the existence of an increased risk of a disease (*e.g. certain occupational cancers*).

2- Analysis of the international, European and Belgian legal framework

An analysis of all of the provisions applicable to the problem was carried out in order to determine the outline of an existing legal framework as well as the conditions imposed by various legal orders. This study is a necessary precondition to the comprehension of the issues, in view of adopting a regulatory framework. These provisions were then scrutinized starting from three important questions pertaining to the application of genetics to workplace health prevention. These are: the question of discrimination ; of workplace health monitoring ; and of consent. This analysis enabled us to examine coherence between regulatory systems.

- on the subject of discrimination, we note that there is unanimity on the need to prohibit any discrimination based on genetic characteristics. However, there are deep divergences on the means to be implemented for the enforcement of this prohibition (prohibition of any access to genetic information; prohibition of carrying out a test and/or requesting information obtained elsewhere; limitation of access to the test for certain purposes(problem of control);free and informed consent; …).
- on the subject of workplace health monitoring, the coexistence of multiple sources does not always encourage coherence and unity of concepts. Many questions remain outstanding as to precise limits of workplace health monitoring : What purposes could justify carrying out a genetic/HIV test prior to recruitment, and thus a selection on this basis? What does the concept of "immediate protection " of the person cover? Can protection of the health of the worker colleagues, and/or other third parties (suppliers, customers, …) also be called upon? Do these tests have to be expressly permitted or expressly prohibited by law? Is workplace health monitoring a matter of "public order". In this case, will the genetic test necessarily be obligatory or optional? Does the Occupational Health doctor have exclusive competence?
- Lastly, on the question of consent, provisions diverge appreciably. Some make consent an exception to the prohibition of genetic tests. Other provisions make it a condition of validity of the test. Lastly, some estimate that consent is not enough, given the inequality between employer and worker, to justify carrying out a genetic test.

3- Genetic tests and the right to respect for private life

This part consisted of studying and understanding, from a theoretical point of view, the concept of private life and its application to the employment relationship. It related to the following question: in the absence of specific legislation as regards genetic tests in the framework of recruitment or the execution of the contract of employment, do fundamental rights, and particularly the right to respect for private life, offer a satisfactory answer to the questions raised by the application of genetic tests by the employer?

We thus confronted the use of genetic tests with the conditions imposed by article 8 of the European Convention on Human Rights sanctioning the right to respect for private life. This study enabled us to check whether the conditions required by this provision are satisfied concerning the application of genetic tests in a professional context (Convention de sauvegarde des droits de l'homme et des libertés fondamentales, 1950). These conditions are as follows: legality, finality and proportionality. They allow us, at an early stage, to answer certain questions and to exclude the use of certain tests (e.g. tests of genetic susceptibility to non occupational diseases before recruitment, see below). Furthermore, this first part of our research made it possible to determine the limits of the theoretical framework of "fundamental rights" in the particular context of industrial relations between an employer and workers/candidates. In fact, we think that this framework does not make it possible to

approach all of the questions raised by the application of genetics in the occupational context.

4-Sociological approach to genetics

This part enabled us to re-insert the use of genetics into the context of the employment relationship, to study its consequences and implications, and in particular:

- To understand the mechanisms and the issues involved in the social representations of genetics in the workplace (social aspects of genetics) as an instrument of workplace health. The employment relationship is marked by a certain distribution of power and control. Sociological studies (Lebeer et al., 1997; Draper, 1986) have shown that the social representations of genetics (susceptibility and monitoring tests) held by the workers and the employers diverge appreciably. Thus, in a schematic way, these studies reveal that trade unions are more opposed to tests of genetic susceptibility for reasons of discrimination, and rather in favour of genetic monitoring tests for reasons of health protection. Conversely, employers are rather in favour of tests of susceptibility in particular for reasons related to the cost of prevention, and unfavourable to monitoring which they consider likely to "demotivate" the workers. Rather than scientific "objectivity", these are the social representations which generally underpin legislative work.

- To analyze the various arguments advanced in the debate on genetic exceptionnalism, i.e. the question of knowing if genetic information is different from other medical information to the point of necessitating the adoption of specific legislation, as the Belgian legislature did recently (law of January 28, 2003). The main arguments and counter-arguments studied within the framework of this debate can be briefly summarized as follows:

  - the first argument is based on the predictive capacity of genetics, that is to say on the capacity of genetic information to "predict" the future health of individuals. However, genetic information is not the only information concerning the future health of the person. This prediction can in fact also result from information relating to family history or other medical or non-medical criteria.
  - the second reason advanced to justify specific treatment of genetic information concerns the implications of genetic information for family members. But again, genetic information is not unique in having consequences for the family. For example, information relating to HIV (risk of contamination) or a cancer developed following exposure to toxins present in the home environment.
  - the potentially discriminatory character of genetic information is also called upon. But there too, there is no question of any specificity of genetics. Discrimination exists and will exist as long as there remain differences between individuals, whether these are of genetic origin or not.
  - It has also been advanced that the specificity of genetic information rests on its individual character. But other information has an even more identifying character than genetic information (which can be identical in the case of twins): for example the analysis of the human iris, which is unique even in the case of identical twins.

Thus it seems that genetic information does not differ fundamentally from other medical information, which implies that it is neither necessary, nor even desirable, to treat it differently from other medical information (Murray, 2001; Lemmens & Austin, 2001; Gostin & Hodge, 1999).

However, some maintain that genetics radically modifies our conception of health and disease. Indeed, genetics has created a "population of healthy, but at-risk individuals" (Ross, 1992; Nelkin, 1992). This modification highlights the need to shift the focus of medicine from *post facto* medical intervention to a medicine of prevention and risk reduction. Lazzarini maintains that the specificity of genetic information lies inforcing us to examine in one context all the threats to our autonomy and privacy which have accrued over the last fifty years (Lazzarini, 2001).
We think that if genetics does not differ "objectively" from other medical techniques, the distinction is marked in the social representation of genetics which modifies/will modify our epistemological categories. Thus, the transformation of the concept of risk will oblige us to rethink the systems of prevention and reparation. In particular, the representation of genetics is impregnated with the idea that tomorrow we will have simple and inexpensive tools allowing an unquestionable prediction of diseases but also possibly of other non-pathological characteristics (homosexuality, scientific curiosity, …). Genetics is perceived as a means of forecasting all our future ills, a technique to make bodies transparent, with positive aspects related to this "transparency" in terms of eradication of diseases, and negative aspects such as the feeling of a future entirely written in our genes. This representation of genetics is/will be necessarily at work in the acceptance by society of genetic diagnostic techniques, but will also underlie any possible legislative intervention. In addition, in the framework of employment relations, these representations translate the relations and distribution of power.

5- Genetic tests and occupational hazard

This part had as an aim the analysis and comprehension, in employment and social security law, of the construction of the concept of occupational hazard in the mechanisms of workplace health protection (*ex ante*risk) and of occupational diseases (*ex post*risk), as well as of the bonds which link them.

1°- Occupational hazard in the system of workplace health protection

The study of Belgian (laws of 1899, R.G.P.T., law of 1952, law of 1996, Code on wellbeing, …) and European (Framework Directive, …) regulation as regards workplace health protection, as well as reference texts on the subject, allowed us to define the concept of risk and its evolution.

Thus, the representation of risk is no longer founded on a technical model of risk from a single cause, but on an ensemble of different, interdependent risk factors. Consequently, the prevention of occupational hazards is necessarily subsumed within the framework of asystemic, multi-causal and multidisciplinary approach whose centre of concern becomes "the man at work".

The question of the insertion of "genetic risk" in the study, analysis and prevention of occupational diseases forms part of the problem of weighting between collective risks and individual risks.

A study of the respective competences of the agents involved in protection was first carried out, in order to better determine the roles and responsibilities of each. We can briefly mention that the employer retains final responsibility for workplace health protection. The employer, however, has a largely symbolic scope since the adoption of the occupational disease scheme. One can summarize the mission of the prevention adviser-Occupational Health doctor as

follows: epidemiological research, disease prevention, and monitoring of health. The workers also collaborate in the policy of health and safety at work.

Within the framework of the monitoring of workers' health (AR May 28, 2003, *M. B* ,June 16, 2003), no provision expressly prohibits recourse to the use of genetic tests by the Occupational Health doctor, who has, subject to the principle of proportionality, a certain freedom in the choice of examinations to be carried out.

The question then arose of knowing if, taking into account the current economic and social context, an over estimation of individual risk, including genetics, within the framework of prevention, was not likely to transform the principles of prevention into practices of selection.

Thus, what to make of individual genetic information in the framework of a model of prevention which is supposed to "privilege collective measures of prevention over individual measures" and "to adapt the work to the man and not the reverse" (article 6 2 d) and h) of the European Framework Directive and article 5 e) and f) of the law of August 4, 1996 relating to wellbeing?

And how to avoid, given the costs of prevention, the danger of slippage towards practices of selection or exclusion, instead of practices of collective prevention such as the lowering of exposure levels to a threshold acceptable to all the workers?

2°- occupational hazard in the system of compensation for occupational diseases

A. The concept of occupational hazard in the legal system for occupational diseases

Systematic analysis of the legislation (1927,1963,1970,1990) on occupational diseases as well as parliamentary work and the reading of various texts relating to this matter, as well as many articles, allow us to describe the evolution of the concept of occupational hazard such as it is apprehended by the legal system for occupational diseases (extension, standardisation, evolution of causality, collectivisation v. socialisation of risk, …)

Following this analysis, we can make the following observations:

- It is the concept of occupational hazard built into the legal system for industrial accidents (the only one which existed at the time) that was used as the "model" for the legal system for occupational diseases. For several aspects, the legislator reasons by analogy.
- The system enshrines and leads to a standardization of risk. Prevention then becomes a simple cost to be integrated into business management. The values related to workplace health protection become secondary.
- The concept of risk is built on successive legislation relating to occupational diseases, on a collective, past and environmental model.
- the extension of the concept of risk depends fundamentally on the context, and on political and social choices (extension of the legal system, multiplication of the number of diseases covered) (Vogel, 1994).
- causality remains, paradoxically, a central problem within the framework of the legal system of reparation for occupational diseases, even though the choice of the model of occupational hazards hould free it completely.
- dissociation between prevention and reparation, or the lack of coordination between these two components, appear to us to be likely to diminish the employer's responsibility to take

care of the health of his workers.

The current Belgian system of compensation for occupational diseases is blind to risk: the veil of ignorance, a necessary condition fort he principle of equality and for justice in the system of reparation (Lois coordonnées du 3 juin 1970 relatives à la réparation des dommages résultant des maladies professionnelles; Rawls).

In the framework of the list system, it is in fact enough that the victim was exposed to the risk of occupational disease–insofar as this exposure is, according to generally accepted medical conditions, likely to cause the disease –for him to be eligible for compensation. Moreover, as soon as the occupational disease is at least a partial cause of disability, the disability is appreciated completely and not only for its occupational part. This principle, called the principle of indifference to the prior state, further reinforces the veil of ignorance with regard to possible genetic predispositions (Demet et al., 1996).

B. Genetic tests and occupational diseases

Is the use of genetic tests in the context of occupational diseases likely to have an influence on the decision to compensate for the disease? This is the question of the limit of the veil of ignorance as regards genetic data.

As the system is blind to risk and allows access to compensation as soon as there is occupational exposure, genetic predisposition to an occupational disease is currently not an obstacle to its compensation, nor a cause of reduction of allowances.

We think however that the question deserves to be discussed for several reasons:

- Thus, earlier, at the time of determination of the occupational character of a disease in view of its inscription on the list, the causal factors between the occupational exposure and the disease are analyzed. Aren't genetic factors likely to "scramble" this bond and to prevent certain diseases from being indexed on the list of occupational diseases?

As A. Thébaud-Mony emphasizes, there exists within the legal system for occupational diseases an irreducible contradiction: "the law exempts the victim from the burden of proof by the application of the presumption of origin, but the expert charged with applying it is constantly in search of 'objective' technical or medical criteria proving causality between work and the disease" (Thébaud-Mony, 1991).

- Moreover, the problem of causality emerges again in the open system which, in Belgium, coexists with the list system. In this case, the victim must prove that the affliction from which he suffers has its direct and determining cause in his occupation. The exercise of the occupation must have been the real, dominant and decisive cause of the disease (Vandeweert, 2002). Is this always the case when a factor of individual predisposition comes to be intercalated between the disease and the exposure?

- Lastly, a political and social choice governst he adoption of this system. We know however that the operation of the legal system for occupational diseases could be improved not ably by are duction in under-declaration and by better visibility. These changes would involve a major increase in costs. The prospects for privatisation of certain risks and the contamination of the system of solidarity by reasoning specific to private insurance are likely to harm the principle

of ignorance (in the United States, the insurance system depends in broad measure on the employer, who is inclined to practise many medical tests).

By pushing the reasoning further, one can imagine a system based on individual liability for each person with regard to his health. The idea has been defended many times since the discovery of genetics, that each person has a duty of knowledge, that is to say to know "his state of health" in order to manage it with due diligence. Pushed to the extreme, this reasoning could lead to a recognition of the individual responsibility of each person to choose his occupational environment, hazardous if necessary, taking into account his health, and to take the consequences. This would result in replacing the social security system for occupational diseases by a system of individual liability in a freely negotiated market for risk. This point inevitably raises the question of the autonomy of the person at work.

3°- Genetic tests and the role of autonomy in the system of prevention and protection of health in the workplace

To achieve the goal of disease prevention and protection of health in the workplace, the legislator can choose an interventionist model, based on imperative or mandatory provisions which are imposed on the will of the parties, or a model which leaves the field open to individual negotiation (*Self Regulation*). This dual conception of health in the workplace can be seen in the question of consent to at-risk work.

To the question of consent to the carrying out of genetic tests, the answer of the trade unions is ambiguous (Lebeer at al., 1997). When it comes to traditional medical examinations, the trade unions come down in favour of a primacy of the protection of health. They would accept a mandatory protection system which would be imposed on their will. As for genetic tests, opinions are divided: some feel that personal freedom must remain intact. It is up to the worker to make the choice between his health and access to work. For others, this freedom is illusory ,for the choice is too often dictated by economic need, at the expense of health.

On the question of the free choice of working in a hazardous sector, or of freedom of choice between health and employment, some, holding to *Self Regulation*, consider that the State should not intervene and that it is up to the individual to freely make his choice (Viscusi, 1984; Posner; Epstein, 1994).

For these authors, the choice to work in an at-risk environment must be a freely negotiable choice within the framework of a market balancing risks against wages, and based on a good distribution of information. Thus, a balance will be achieved when a worker agrees to work in a hazardous post in exchange for a certain wage. This is what is known as "hazard pay".

Conversely, other authors maintain that the freedom of the workers is but a fiction and that the State must intervene to allow a true autonomy. Indeed, these note that in reality, considering the state of economic dependence of the job applicant (as well as the worker), he is not on an equal footing with the employer. On the contrary, he is very often forced to accept prejudicial conditions to preserve his employment (Nelkin & Brown, 1984).

Various arguments seem to us, following this study, able to be raised in favour of this thesis:

- It is the very basis of employment and social security law that it is constructed from an interventionist point of view, resulting from the observation of the inadequacy of the common

law fictions of freedom and equality. Resulting from the social question, employment and social security law is founded on the observation of a deep inequality between the worker and the employer, and is given the mission of resolving it by protecting the worker (unequal law in favour of the worker). In this context, the question arises of the value of consent and the reality of the freedom of consent, revived by the current neo-liberal conception of society, and by the coercion which financial markets, unemployment and mass precariousness place on the employee (Coutrot, 1999). This basis is however questioned by some authors, who observe an evolution of the responsabilisation of the social policy-holder from the point of view of an active Welfare State.

- Economic dependence, resulting in particular from the current context of the labour market, which makes freedom of choice illusory. Thus, Nelkin and Brown show that external circumstances, such as economic instability or employment opportunities, make the choice of working in a hazardous sector far from voluntary (Nelkin & Brown, 1984). According to Draper: "*Industrial workers are unable to choose alternative protection policies, and instead are given the dubious choice between their health and their jobs—a choice that is so heavily constrained by economic pressures that it is not a real choice at all*" (Draper, 1986; Basset-Stanford, 1981).

- It is erroneous to believe that information relating to risk at work is distributed perfectly, for various reasons: this information isn't always known, it is often complex and not easily communicable, it is often not in the employer's interest to communicate it, … (Draper, 1984; Nelkin & Brown, 1984; Draper, 1986)

- Lastly, the system of workers' free and informed choice is based on the idea that he is able, by balancing interests as divergent as health, employment, the economic future of his family, etc…, to make a rational choice, which in our opinion is debatable (Duraffourg, 1985).

Thus, West adds that it can be necessary to correct the imbalance of power between employers, who may sometimes find it beneficial to maintain a hazardous work environment, and workers, who seldom have other choices or the opportunity to demand better working conditions (West, 1985 ; Lemmens, 1997).

Following the example of these authors, we think that the question must be: how to transform, to improve working conditions so that workers no longer have to choose between their work and their health?

4°- Analysis of the law of January 28, 2003

The Belgian legislature recently intervened to prohibit certain types of genetic tests, that is to say preventive genetic tests, in the occupational context. Analysis of these provisions, however, has shown us that they have not settled the question of the use of genetics in the workplace.

Among the various questions raised by this legislation, we will cite only one: the scope of application of the law, or the type of genetic tests targeted by the prohibition. An analysis of the law and preliminary works make it possible to think that the tests concerned are tests of susceptibility to non-occupational diseases. We regret however that the text does not specify this more clearly and, if this definition is correct, we deplore such a limited scope of the legislation.

In spite of that, the law envisages an exception to this prohibition. Indeed, a genetic or HIV test could be carried out given the adoption of a royal decree debated in the Council of Ministers. However, these are precisely the situations which will carry risks of discrimination and which would deserve to be discussed in parliament, because they relate not only to fundamental rights (right to respect of the private life, of physical integrity) but also because they are likely to reverse the order of priority of our principles of prevention, namely the adaptation of work to the man and not the reverse.

CONCLUSION

As a conclusion, we can present some syntheses which could be useful in the context of the adoption of a legal regulatory framework for genetic tests.

A- Aptitude, protection of the health of the worker and third parties

A first examination can be made under the terms of three legitimate purposes (aptitude, protection of the health of the person, protection of the health of third parties) which could be used as justification for carrying out genetic tests, and of the principles of legality and proportionality (Convention de sauvegarde des droits de l'homme et des libertés fondamentales, 1950).

1/ Tests of susceptibility to non-occupational diseases

Within the framework of employment relations, the tests of genetic susceptibility to non-occupational diseases are not justified by the determination of aptitude(current aptitude),since, at the time of the test, the person does not present the symptoms of the disease (**principle of utility**).

Neither do they serve the goal of protection of the health of the person tested, because the disease does not have a link to the working conditions. To refuse him access to his work will not have any beneficial effect on the evolution of his health (**principle of utility**).

The frequently quoted example of the pilot carrying the gene for Huntington's disease shows that the safety of third parties remains the principal justification for exemption from the prohibition of this type of test. However, if these people - we mean here all workers in hazardous posts and not only certain genetically identified workers - are regularly subjected to health monitoring examinations, they will not carry any more risk of suddenly displaying symptoms of Huntington's disease, or another genetic disease, than of any other possible health problems.

For these reasons, we think that tests of susceptibility to non-occupational diseases must be prohibited both before and after recruiting.

2/ Tests of susceptibility to occupational diseases

For the same reasons, neither aptitude nor the protection of third parties can be called upon to justify carrying out tests of genetic susceptibility to occupational diseases in the context of employment relations.

On the other hand, the question arises of knowing if one can expose a person to a substance when he has an increased susceptibility to the occupational disease caused by this exposure? Can the protection of the health of the worker be called upon to justify at this stage either a refusal to recruit, or a preventive suspension? Can one expose people who one knows - or who one will know-to run a much higher than average risk? What would be the consequences? Is it necessary to inform the worker? We are confronted here with the limits of this framework of analysis.

3/ Genetic monitoring tests

In the same manner, monitoring tests make it possible to observe the early effects of certain mutagenic substances. Such effects seem to be precursors to the appearance of occupational diseases. Here also, an objective of protection of the health of the person justifies carrying out these tests. However, questions identical to those posed above remain outstanding.

B- Monitoring of health at work

A second reflection is thus at the heart of the system of workplace health protection in a prospect for identification of the issues and impact of the application of genetics.

Thus, let us consider an Occupational Health doctor confronted with a test of genetic susceptibility to an occupational disease on the one hand, and an early detection of genetic lesions following an exposure to mutagens on the other.
In both cases, he will be in possession of anticipated information on the risk of appearance of an occupational disease. Indeed, the test of susceptibility reveals a weakness, an increased risk of developing such an occupational disease after an exposure to a toxic substance.
In the same way, monitoring tests make it possible to highlight the early effects of certain mutagenic substances which seem to herald the appearance of occupational diseases. The essential difference from tests of susceptibility to occupational diseases lies in the fact that in monitoring tests, a pathological process is observed, if at an early stage, whereas in the case of tests of susceptibility, it is only a matter of increased probability.

We think that it is at the heart of occupational medicine that these questions must be solved. They necessarily bring us back to the capabilities and limits of the Occupational Health doctor in his role of prevention and monitoring of health. The issue relates in particular to weighting between collective risks and individual risks, as well as identifying the appropriate moment to intervene.
When is the Occupational Health doctor likely to intervene: at the discovery of a simple susceptibility, at the appearance of the first early effects, or is it necessary to wait until the first symptoms are expressed?
Which type of information must a decision to suspend, or to refuse to recruit, be based on: is a simple susceptibility enough, or is it necessary to await the start of an inexorable process?
What answers can the Occupational Health doctor provide to such a discovery: modification of the post, lower limits of exposure, temporary or permanent suspension, possibilities of redeployment?
Is it technically and economically possible to lower the thresholds of exposure to harmful substances, in order to allow predisposed people to be able to work under suitable conditions from the point of view of their health?

The importance of these questions is recalled by Murray: "*The danger is that we blame the person and exonerate the environmental or workplace conditions that precipitated the disease. All disease depends on the interaction of organism and environment. Which of the two we focus on is a social and political choice with important ethical consequences*" (Murray, 1983).
On the answers to these questions will depend the construction of our system of workplace health protection. Today, schematically we can summarize it as follows: this system is mandatory and not subject to agreement by the parties concerned. It is based on multiple and interdependent risks factors. It privileges collective measures over individual measures, and advocates the reduction of exposure levels to the lowest possible.
In our model, we think that it would be coherent to prohibit all tests of susceptibility before recruitment, because they proceed from a rationale of selection and not of prevention.
After recruitment, we think that tests of susceptibility must remain prohibited. Indeed, they allow only probabilistic information which means that, even if predisposed, some people will never develop the disease. They are likely thus to be wrongfully excluded from their posts. In addition, even those not predisposed can still catch the disease. However, application of tests of susceptibility, and exclusion of people with predispositions, will undoubtedly make it possible to maintain the amounts of exposure at a certain threshold, or will not encourage their reduction to levels any lower than necessary. The policy of reduction of exposure levels to the lowest possible would consequently be endangered.
As for monitoring tests, we think that there is a risk of contradiction with the principle of priority of collective measures over individual measures if these tests lead only to measures of suspension of predisposed workers. We think however that they must be able to be used by the Occupational Health doctor insofar as they allow a more accurate interpretation of measurements of exposure to substances, a better comprehension of pathological mechanisms, and a more effective protection of health at work. But safeguards must be in place in order to give the Occupational Health doctor work the competences, the means, and the autonomy necessary to use these new technologies in the service of health protection and to avoid the subversion of protection for selective ends.

ELABORATION

The results obtained within the framework of the legal component of the project have been the object of various elaborations by means of publications and participations in conferences and colloquia. Meetings with specialists allowed a comprehension of certain concepts and a critical approach to the results.

Promotor: Dr. Ch. Laurent
Scientific collaborator: C. Roncancio

University of Liège
Laboratory of Oncology, Radiobiology
and Experimental Mutagenesis

1. Introduction

The statement that human quality of life has become more and more improved by the emergence of several industrials processes can obviously be done. Simultaneously, the rapid progress of Science has emphasized that many compounds present in our general, professional, and/or even cellular environment have been shown to display genotoxic properties. Effect of mutagens is rarely immediately toxic for the cells and their mechanisms of action are more insidious ; their cell target molecules being DNA, RNA and proteins. Most DNA damages, once established, may persist unless the cell die or a reverse mutation occurs. Moreover, a cell with induced mutation can accumulate additional DNA damages increasing the probability to develop hazardous gene alterations.
Exposed human populations to mutagenic carcinogens may be monitored using different chemical and biological endpoints. Biomonitoring falls in the latter category ; it consists in detecting, characterizing and evaluating the effect produced in an individual exposed to potential mutagens and/or carcinogens.

The purpose of this challenge was not only to provide laboratory data but also to illustrate the rationale and the significance surrounding the use of biomarkers for assessing exposure to genotoxicants and to describe how the scientific community has a important role to play to heighten public awareness and to popularise, share and disseminate results of ongoing researches in the field of public health and sustainable development.

2. Objectives of the research

The objective of our team is to develop and contribute to this project by transferring know-how as well as molecular and cytogenetic technologies developed and validated in our laboratory. The ultimate goal being to contribute to characterize and evaluate genetic effects associated with occupational exposures to genotoxic agents.
Our expertise will also contributes to answer the questions that we formulated and that refers to the capability of measuring variability parameters? The scientific usefulness to implement these tests?, their public health relevance?, and if these tests are potentially useful, to identify if they are applicable in occupational medicine.
The rationale followed a sequential mode including several distinct phases performed in parallel or simultaneously. It comprises :

Action 1: Validation of the relevant parameters for the detection of chromosomal aberrations using the chromosome painting technique "FISH" (Fluorescent *in Situ* Hybridization).

Chromosomal aberrations in human lymphocytes have been used as a measure of dose to individuals for many years. The initial development of a methodology for studying chromosomal aberrations has established the dicentric as the aberration of choice for biological dosimetry because of its low yield in control persons and its ease of scoring. However, this system has one disadvantage: the yield of dicentrics decreases with time following exposure and so the biological indicator of damage declines. The half-life of this loss has been suggested to be about 3 years, but it must be dependent on the person and his or

her state of health. In addition, some evidence suggests that a decrease in dicentric yield is much more rapid after exposure to a high dose. However, the stable translocation, is lost much more slowly over the time and persists many years after exposure. The initial methodology for its detection required karyotyping procedures, and more recently the technique of highlight specific chromosomes using molecular probes called FISH or (Fluorescent *in situ* hybridization) chromosome painting, has been used.

However, it is necessary to optimize this procedure, to determine its sensitivity, its limitations and generally attempt to validate the method for practical use. Most of the information has been obtained from exposure to ionizing radiation, in *in vitro* and in occupationally exposed individuals.

Analyzing the scientific literature for ionizing radiation and FISH, it can be observed that each laboratory used its own probe combination, in general, they paint 3 chromosomes (monocolour and / or tricolour), which accounts for about 20% of the DNA content of the whole genome and, on each case a counterstain is used (DAPI, propidium-iodide). In our case, we paint chromosomes 2, 4 and 8 (tricolour), which accounts for 7,90; 6,29 and 4,80% of the DNA content (Total 18,99% of the DNA content). In addition, the centromeres should be painted in another colour to distinguish between translocations and dicentrics.
Once it has been decided which chromosomes to paint it is important to establish how we will score the aberrations. Two systems of scoring can be found in the literature. One is that proposed by Simpson and Savage (S&S) which strictly applies when only one chromosome is painted and is based on mechanistic aspects of aberration formation.
The second scoring system is known as Protocol for Aberration Identification and Nomenclature Terminology (PAINT). This simply records each coloured fragment or object containing a coloured junction (interphase between two colours) using simple rules.

Scoring criteria:

Aside from the implicit criteria that chromosomes should be unambiguously stained and free of overlaps and artifacts that would interfere with the analysis, the nomenclature system described here involves the following basic assumptions.
1. The term painting is defined to mean chromosomes that are labeled with probe along their entire length, from the *p* terminus to the *q* terminus. This restriction is necessary because probes that label only a portion of a chromosome will by definition, have color junctions (interphase between two colors) that may be indistinguishable from chromosome rearrangements. Relatively minor exceptions are allowable, such as the diminished labeling that is sometimes seen over centromeric heterochromatin (i.e. 1, 3, et 9).
2. The identification and enumeration of chromosome exchanges is limited to those events that result in the conjunction of chromosomes of different colors, or chromosomes of substantially different staining intensities. Exchanges between different chromosomes of the same color or intensity, such as mono-color dicentrics, are formally ignored since painting is not instrumental in their detection. Similarly, symmetrical intrachanges (inversions) are also formally ignored.
3. The identification and enumeration of acentric fragments is limited to those that are at least partially painted. Unpainted fragments are ignored.
4. The centromeres should be readily identifiable. This can be accomplished with a suitable counter-stain (propidium-iodide or DAPI) or by simultaneous hybridization of a pan-centromeric probe.

It is recognized that a calibration curve is set up by scoring cells at the first metaphase after irradiation, whereas a real sample would be taken several years after irradiation. This means that the metaphases seen would generally be derived from stem cell irradiated, that it would be in their second or subsequent division. Thus, with regard to calibration the main problem is to decide the application of translocation measurements and therefore what calibration is required. Acute whole body doses exceeding about 2 Gy will cause deterministic effects which means the exposure will be discovered fairly rapid. For this time period, the conventional dicentric method of biological dosimetry can be used and there is a little need to use FISH. However after a large dose, dicentric yields decrease over periods of a few months. Therefore FISH dosimetry may have a role to play in some recent accidents where it is necessary to confirm if the dicentric yield is representative of the dose received. Doses of a few Gy received over a period of years will not cause deterministic effects and may pass undetected. Under these circumstances FISH would be used and the crucial calibration factor is the linear dose coefficient.

In order to measure this linear coefficient *in vitro* many thousands of cells need to be scored at low doses. Several laboratories have produced calibration curves at doses ranging from 1 to 6 Gy. At lower doses the amount of scoring done has generally been insufficient to obtain a good estimate of the linear term. For high energy gamma rays such as ^{60}Co or ^{137}Cs which are the main radiations for which the technique would be used the best estimate of the linear term actually, is probably derived from the value for full genome dicentrics. At higher doses the yield for the sum of one and two way translocations is 10-30% higher than for full genome dicentrics. Two way translocations alone are about 70-80% of the dicentric yield. The linear coefficient for full genome dicentrics is about 2 per 100 cells per Gy and is known to within a standard error of about 15 –20%.

It has been observed a large variation in control translocation yields, both within a laboratory and between laboratories. However, a clear age related effect is evident. From 20 years to 70 years the translocation yield increased by a factor of 3 or more. At 20 years old the mean full genome two-way translocations yield was about 2 per 1000 cells. At 60 years and over, the yield was about 10 per 1000 cells. The corresponding figures for one and two-way translocations combined were about 3 and 15 per 1000 cells.

Action 2: The use of FISH technique for biological dosimetry in the nuclear power plant workers.

After having identify and validate the relevance and impact of confounding parameters associated with the « FISH » technique for the detection of chromosomal rearrangements, the analysis of the frequency of the translocations present in peripheral human lymphocytes, has been performed in order to assess «a posteriori» ionizing radiation doses to which subjects have been exposed.

THEORETICAL FRAME

It has been largely described the obvious carcinogenic and genotoxic properties of Ionizing Radiation (IR). Ionizing radiation can randomly cause damage to all cellular components and are able to induce numerous types of DNA lesions by complex direct or indirect mechanisms.

Such molecular lesions range from base and sugar alterations to single- and double-strand breaks and nucleoprotein-DNA cross-links. If cellular mechanisms do not act efficiently to control and regulate the cell cycle and allow more time for completion of the repair of DNA damage prior to DNA duplication and subsequent mitosis, multiple mutagenic events may occur and lead to numerical and/or structural chromosome aberrations. It has been proposed that carcinogenic process requires successive and cumulative changes in the genome.
Therefore, radiation induced genetic lesions - that are not repair or misrepaired – and may be responsible for the occurrence of mutations that will accumulate. This will lead individuals that carry them to be at higher risk of developing some cancers in the future.
Methods allowing **the early detection or evaluation of induced genetic damages in human are proven to be contributive for identifying heavily (or more susceptible?) exposed subjects.** Biological tests have been developed that allow biological retrospective dosimetry after exposure to IR. The frequency of chromosomal aberrations (dicentric chromosomes) was used to detect or quantify radiation-induced effects. However due to the recent introduction of the fluorescent *in situ* hybridization (FISH) technique using DNA chromosome-specific DNA probes has increased the sensitivity of the technique and eased the detection limit of stable chromosome aberrations (especially translocations) in human.
The present study was conducted to detect - using the FISH technique - if genetic effects could have been induced in workers involved in the recycling of nuclear fission products and therefore potentially exposed to IR. Our study concerned also and simultaneously unexposed administrative staff members.

MATERIALS AND METHODS

The study comprises 30 male workers involved in the recycling of nuclear fission products, in a nuclear power plant in Belgium; the mean age is 48.13 years (SD= 10.94).
A group of 20 healthy unexposed men from the administrative area, aged 39.45 years (SD= 11.64) was used as a control.
Whole blood cultures were set up as follows: 500 µl whole blood was added to 5 ml MEM (Life technologies) supplemented with 15 % fetal calf serum (Life technologies), 2 % phytohaemagglutinin (Life technologies), 1 % penicillin-streptomycin (Life technologies), 1 % L-glutamine (Life technologies) and 4 % BrDU (25 % w/v in MEM) (Sigma). The cultures were incubated for 52 h at 37°C including a 1 h 15 min colcemid (Life technologies) treatment (0.06 µg/ml) before harvest. Consequently the cells were treated with a hypotonic solution (0.075 M KCl) for 10 min and fixed three times with 3:1 methanol: acetic acid (Merck-Belgolabo).
FISH analysis of the chromosome aberrations was performed with a cocktail of chromosome-specific probes for chromosomes WCP 2 spectrum green, WCP 2 spectrum orange, WCP 4 spectrum green, and WCP 8 spectrum orange used according to the manufacturer (Vysis, Downers, Ill,USA) WCP chromosome painting system.
Each slide was treated with 100 µg/ml RNAse (Boehringer) followed by a pepsin digestion (0.005 % solution Boehringer) before denaturation at 70°C in a mixture containing 70 % formamide in a 2 X SSC buffer. The DNA mixture probe was also denaturated at 72°C and 10 µl DNA probe were deposited per slide. Hybridization was performed overnight at 37°C in a humidified chamber. The slides were then washed three times at 45°C in a 50 % formamide (Sigma)/2X SSC solution and one time in 2 X SSC/0.1% NP-40. Finally DAPI I (10 µl/slide) was applied to the slides for chromosome counterstaining. The slides are stored at 4° C before

observation. For the scoring a Diaplan Leitz microscope with 630 x magnification was used. The PAINT was used as nomenclature system for describing the structural aberrations detected by the chromosome painting.
Between 200 and 300 metaphases were analyzed by person and they were scored only if they appeared to be complete and all parts of the six painted chromosomes appeared somewhere in the cell. A bi-coloured chromosome with one centromere was classified as a translocation. If both reciprocal counterparts of the translocation were present, the translocation was termed as a two ways (complete) translocation, and as a one way (incomplete) translocation if only one bicoloured monocentric was present. Cells containing visible complex rearrangements were taken into account (Cells arising from the interaction of >=3 breaks on >=2 chromosomes).
We extrapolated the frequencies of translocations for chromosomes 2, 4 and 8 to a frequency of translocations for the whole genome following the formula of Lucas

$$\mathbf{Fp = 2.05\ X\ (fp_2\ X(1-\ fp_2) + fp_4\ X(1-\ fp_4) + fp_8\ X(1-\ fp_8) - fp_2\ X\ fp_4 - fp_2\ X\ fp_8 - fp_4\ X\ fp_8)\ X\ FG}$$

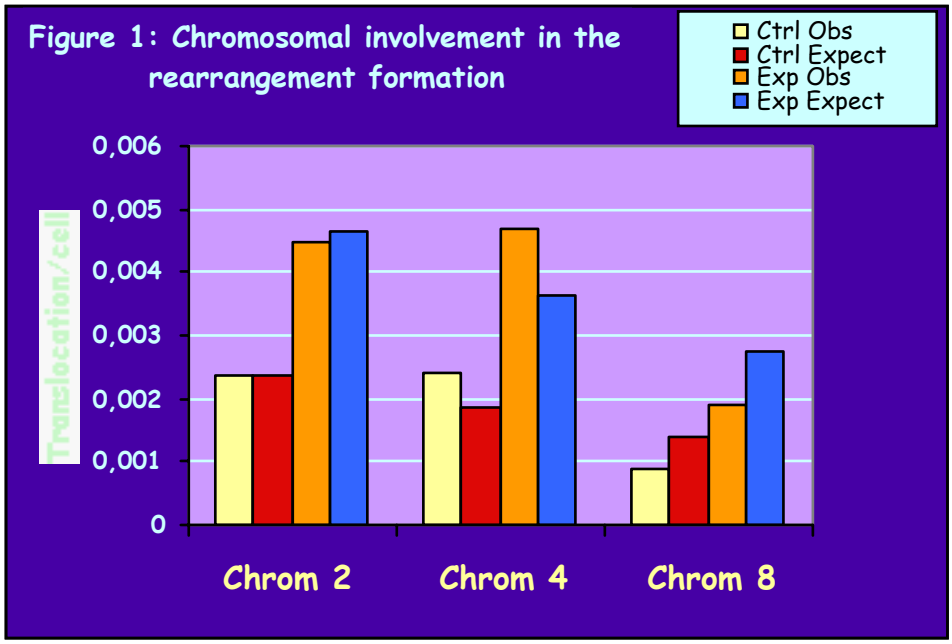
where Fp represents the frequency of translocations observed for the three chromosomes and fp_x the length of each individual painted chromosome relative to the whole genome. For the chromosomes 2, 4 and 8 a fp value of respectively 7.976%, 6.338% and 4.828% was adopted from Mortons.
For the determination of the equivalent total-body dose a calibration curve was used based on the translocation frequencies in *in vitro* Co gamma-ray curve obtained from irradiated blood samples.

$$FG = (0.050 \pm 0.013)D^2 + (0.031 \pm 0.004)D + (0.002 \pm 0.007)$$

RESULTS AND DISCUSSION

In the pooled data set, the mean translocation frequency of the worker group was significantly elevated compared with the control group. The number of one and two ways translocations in the worker group is increased by a factor of 1.7 and 2.6 respectively, when it is compared with the control group. The number of one way translocations is higher in both groups, however this finding is in contrast with previous studies on which it has been observed a higher number of two way translocations (Finnon et al., 1999).
This prevalence of one way can be explained by the fact that some apparently one-way exchanges may be caused by incomplete rejoining of broken DNA or by the joining of material to an intact terminal end of a chromosome; the majority are thought to be reciprocal involving a small telomeric region which is beyond the limits of detection by FISH. Evidences in favor of this idea comes from the findings in this study and previous ones (Finnon et al., 1995; Tucker et al., 1995), on which there is a higher number of “incomplete exchanges” which have a FITC signal on the end of an unpainted chromosome t(Ab), than “incomplete exchanges” which have a DAPI signal on the end of a painted chromosome, t(Ba). The proportion of truly incomplete exchanges is reflected by the extremely small numbers of cells with a bicoloured chromosome and an acentric fragment. In this study only one t(Ba) + ace(b) was observed out of a total of 61 translocations.
In the present study an average estimated dose of 0.31 ± 0.20 Gy (range 0.07 up to 0.61 Gy) was obtained in the worker population. Previous studies have been assessed doses in workers exposed to IR at specific exposure conditions; Salassidis obtained dose estimates between 1.6

and 4.9 Gy 5-8 years after exposure, Snigiryova estimate doses between 0.32 up to 1.0 Gy for Chernobyl clean-up workers (Salassidis et al. 1994; Snigiryova et al., 1997).
If chromosome 4 appears to be more frequently involved in chromosomal rearrangements than expected, this finding is not statistically significant, however contradictory results on the involvement of target chromosomes in translocations analyzed by painting techniques are reported in the literature (Stephan et al., 1995; Lambert et al., 2001; Natarajan et al., 1992).Figure 1
The translocation yields in the worker population vary with age but smoking has only a marginal effect; in this case the number of chromosomal aberrations per 1000 cells did not differ when the cigarettes/year were compared, this finding has also been published elsewhere (Littlefield et al., 1998; Pressl et al., 1999).Tables 1 and 2.



Within the battery of biomarkers available today for detecting occupational exposure to physical genotoxicants, the conventional chromosome aberration methodology has been proven to be an efficient qualitative and quantitative tool for detecting exposure to IR. Nowadays, the FISH technology makes possible to detect stable chromosomal aberrations and rearrangements that could not be observed when conventional approach is used. This offers advantages to detect biological effects relevant in term of health risk. Therefore, the Chromosome Painting Methodology sounds to be one of the most sensitive approaches in case of chronic - or chronic like – occupational exposure to IR.

Table 1. Number of translocations according to the age of worker population
Expressed in number of aberration per 1000 cells

Age	Cells obs.	One way	Two ways	No. trans	No. dic	No. ins	Ace	Ring
30-39 (n=2)	324	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00
40-49 (n=12)	2952	4,07	2,03	**6,10**	0,34	0,34	1,02	0,00
50-59 (n=13)	3586	3,63	2,79	**6,41**	1,12	1,12	0,00	0,28
>60 (n=1)	300	3,33	3,33	**6,67**	0,00	0,00	6,67	0,00

Table 2. Number of translocations according to the smoking habits of the workers
Expressed in number of aberration per 1000 cells

Cigarette/year	Cell. obs.	One way	Two ways	No. trans	No. dic	No. ins	Ace	Ring
0 (n=11)	2536	3,55	2,76	**6,31**	0,00	0,39	1,58	0,00
1-300 (n=9)	2612	4,21	1,15	**5,36**	1,53	0,77	0,77	0,38
300-750 (n=8)	2038	3,43	3,43	**6,87**	0,49	0,98	0,00	0,00

Action 3: New mechanisms of action : endocrine disruption.

Beside of the activities performed in the laboratory within the framework of the project, related with the study of effects of ionizing radiations in human population, we also have focused our attention to the potential role of the new called "endocrines disruptors" and their relevance to mutagenicity, carcinogenicity and reprotoxicity
The *in vivo* effect of a given toxic agent on the cellular machinery and its consequence on human health are dependent on many interactions between the agent and the different levels of physiology. Before a chemical toxicant reaches its final target(s), its systemic concentration can be modulated by varying absorption rates and its chemical structure can be modified by activation or detoxification enzymes. Once the agent reaches its target(s) the lesion induced can be directly fixed, repaired or transformed into a mutation or a functional deficiency. Alternatively the toxicant can induce necrosis or the damaged cells can be blocked at a given stage of the cell cycle and triggered to apoptosis or can induce, for example, the cell to divide many times inducing the overexpression of the genes.

Endocrine disrupters are chemicals known to interfere with the synthesis, transport, metabolism and elimination of natural hormones (estrogens, androgens, thyroid hormones) , in addition they can interfere with physiological systems dependant of hormones such as reproduction and sexual development, as well as the hypothalamic-pituitary axis. These type of substances -estimated 87,000 kinds - are introduced into the environment by industrial detergents, fertilizers, pesticides, and pulp and paper effluent, and they may be present in the water, food and air.

We have been analyzing this extremely complex problematic under many different aspects, first the fact that populations can be **exposed continuously to a low dose** of this type of substances. The exposure is not only due to a single type of substance; the presence of a **mixture of compounds** is the general rule. This exposure can happen in an inadvertent way and specific **susceptible populations** can be exposed to similar and/or higher doses, for example the fetus, newborns, children and elderly population. Second, **different types of human health effects** due to a potential exposure to endocrine disruptors has been described in the scientific literature mainly during the last 10 years, however some reports of adverse health effects in rats exposed to endocrine disruptors have been published on the 60s and 70s. Third, the difficulty to **establish a methodology** for the study of this type of substances, because the interaction between the possible affected systems –endocrine, reproductive- are extremely complex and that, it can affect very different types of populations –marine and

terrestrial wildlife, mammals and especially humans-. A general procedure for the study of this type of substances should include:

- The collection of the available data from the toxicological literature, considering: the epidemiological studies, mechanistic studies, obtained by *in vitro* data and i*n vivo* data.
- The evaluation of these data using an approach that considers:
    - The endpoint that has been measured and its relevance to endocrine effects,
    - How it has been measured, the reliability and confidence in the specific assay,
    - The quality of the study and its protocol and
    - The coherence of the data for each endpoint and in the cellular response chain.
- Finally, the development of a risk assessment framework for endocrine disruptors provides a robust tool with which to evaluate the impacts of natural and synthetic substances on the wildlife and human health. This framework balances exposure characterization versus effects characterization, taking into accounts the need for test validation, data acquisition, and field monitoring.

Evaluation and analysis of reported environmental endocrine disruption phenomena should be examined from a risk assessment perspective. Generally, quantitative risk assessment includes estimation of levels of exposure to a toxic substance that leads to specified increases in lifetime incidence rates or in the probable occurrence of an undesirable consequence.

The ecological risk assessment framework is conceptually similar to the approach used for human health risk assessment, with a few distinctions. Ecological risk assessment considers effects beyond individuals of a single species and may examine population, community, or ecosystem-level risks. The framework consists of three major phases: problem formulation, analysis (which includes exposure and effects assessment), and risk characterization. The end points for ecological risks most often considered are survival, growth, and reproduction of individuals of a few representative species and populations. Although not specific to endocrine disruption effects, some limited inferences about endocrine-controlled processes may be made.

Hazard characterization focuses on the qualitative evaluation of the adverse effects of an agent on human and animal health and ecological well-being. Health end points of particular concern with environmental hormones are reproductive (including developmental) effects, cancer, and neurological and immunologic effects.

For human health, relevant and adequate epidemiologic studies and case reports for the agent(s) are preferable. In the absence of this information, pertinent test animal toxicology studies should provide useful information. *In vitro* studies may provide useful data for elucidating mechanisms of toxicity but are not sufficient by themselves to characterize a hazard. Important factors to consider in the evaluation of a hazard include inherent toxicity, route of exposure, dose level, timing and duration of exposure, body burden, susceptible populations and interspecies differences, and all of the assumptions and uncertainties in the data.

Dose-response assessment is the process of characterizing the relationship between the dose of an agent and the incidence/degree of an adverse effect. Factors to consider in the dose-response assessment are the intensity or frequency of the response with increasing dose, the shape and slope of the dose-response curve, pharmacokinetics (uptake, distribution, metabolism/detoxification, elimination), and the methods used for extrapolation of data from surrogate or sentinel species to ecological end points or to humans.

The exposure assessment component of the paradigm attempts to measure the intensity, frequency, and duration of exposure to an agent in the environment or to estimate hypothetical exposures that might arise from the release of new chemicals. Factors to consider in the exposure assessment include the amount of the agent in the environment; reactivity; half-life; environmental fate and disposition of the agent; the magnitude, duration (acute, subchronic, lifetime), schedule (timing), and route of exposure (oral, inhalation, dermal, aquatic); the size and nature of the exposed population; and all of the uncertainties and assumptions in the estimates.

Exposure assessment for potential endocrine disruptors is important in directing specific-effects testing, such that risk assessment and risk management can proceed. Exposure assessment may be defined as the contact between the bioavailable fraction of the compound of interest and the organisms of concern. A tiered approach to exposure assessment whereby conservative assumptions in the estimate are progressively refined is likewise appropriate for endocrine disruptors as well as compounds that may be active via other mechanisms.

Potential for exposure should drive the selection of appropriate test organisms in hazard assessment. For example, where a pesticide is sprayed directly onto crops, it is reasonable to expect potential exposure to aquatic organisms and birds via spray drift. The expected environmental concentrations should then be compared with the toxic concentration to aquatic organisms and birds to determine the potential ecological risk. For natural or synthetic substances discharged via wastewater into rivers, aquatic organisms are expected to be exposed if the substance is not degraded during wastewater treatment. In addition, the bioaccumulation and biomagnification potential of the substance should be assessed to determine if fish-eating birds and mammals might also be at risk.

Once potential for exposure is determined, suitable effects tests should then be selected and the hazard assessed. In comparing the degree of toxicity with the level of exposure, the risk of the compound may then be characterized and any necessary risk management can be conducted.

Risk characterization is the process of estimating the incidence of a health or ecological effect under various conditions of human and biotic exposure. It draws together the hazard, dose-response, and exposure assessments. It discusses the assumptions, uncertainties, and limitations of all of the data.

With respect to recent reports of hazard (i.e., endocrine disruption causing human health or ecological effects), a critical element for risk assessment is the exposure assessment component. Without a clear understanding as to the magnitude and distribution of exposure and the potency and nature of endocrine activity, development of a credible risk assessment for specific endocrine-disrupting agents is not feasible. Another factor to consider in the evaluation of possible risk is whether testing paradigms in past or current use are capable of adequately identifying an agent as an environmental endocrine disruptor.

Some of the effects that has been associated with potential exposure to endocrine disruptors includes:

Female Reproductive System. A variety of chemicals have been shown to disrupt female reproductive function throughout the lifespan in laboratory animals and humans (e.g.,

diethylstilbestrol). These effects include the disruption of normal sexual differentiation, ovarian function (i.e., follicular growth, ovulation, corpus luteum formation and maintenance), fertilization, implantation, and pregnancy. Only a few agents are associated with direct interference with the endocrine reproductive axis. Examples are those with estrogenic activity or the potential to interact with the aryl hydrocarbon (Ah) receptor. Exposure to toxicants during development is of particular concern because many feedback mechanisms functioning in the adult are absent and adverse effects may be noted at doses lower than those observed in the adult. Additionally, studies that include multigenerational exposure should be conducted, followed by time of exposure and dose level required for effect.

Endometriosis is a painful reproductive and immunologic disease of women characterized by aberrant location of uterine endometrial cells. It affects approximately 5 million women in the United States from 15 to 45 years of age and often causes infertility. The etiology of this disease is unknown. In a single study with a small number of animals, research has suggested a link between dioxin exposure and the development of endometriosis in rhesus monkeys. The severity of this lesion was dependent on the dose administered. Recently, a small pilot study to test the hypothesis that serum dioxin concentrations have an association with human endometriosis has been reported. No statistically significant correlations between disease severity and serum levels of halogenated aromatic hydrocarbons were found. These preliminary data, admittedly for a limited population, suggest that serum dioxin concentrations may not be related to human endometriosis. There is a need to develop and validate nonprimate laboratory animal endometriosis models for testing chemicals and xenobiotics. Several novel models for predicting potential causative agents of endometriosis are available.

Human **breast cancer** is a major health problem in the United States. Although considerable information is available on risk factors for human breast cancer, the mechanisms of mammary gland carcinogenesis and the precise role played by chemical carcinogens, physical and biological agents, varied lifestyles, genetic susceptibility, and developmental exposures have yet to be elucidated. It has been hypothesized that exposure to organochlorines, some pesticides, and/or polyaromatic hydrocarbons (PAHs) might play a role in the etiology of mammary gland neoplasms via an endocrine disruption pathway, perhaps via an estrogen-mimetic route or alternative estrogen pathways. With respect to the possible role of hormone disruption by chemicals in the occurrence of breast cancer, there is a lack of sufficient evidence implicating organochlorines in this disease. Clearly, there is a need for research on the etiology of breast cancer. With respect to chemically induced breast cancer, there is a need to develop and validate both *in vitro* short-term tests and *in vivo* animal testing models for predicting human breast cancer risk following chemical insult. Finally, given the wealth of human epidemiologic data on human breast cancer but limited specific agent exposure data, it is not possible to assign a specific chemical or physical cause and effect at this time. Further epidemiologic investigations to address the issue are needed, as well as complementary mechanistic studies.

Male Reproductive System. Convincing evidence exists in rodents that exposure to chemicals that have estrogenic activity, reduce androgen levels, or otherwise interfere with the action of androgen during development can cause male reproductive system abnormalities that include reduced sperm production capability and reproductive tract abnormalities. Results obtained from observation of men exposed to diethylstilbestrol (DES) *in utero* demonstrate a limited potential of exogenous estrogens to disrupt the reproductive system during development in human males compared with that observed in rodents. Further intense research on the population exposed to DES might allow stratification of effects by timing and level of

exposure. Apparently, no increased incidence of reproductive system cancer has been found in those men.

Controversy persists as to the allegation that human sperm production has decreased over the past 50 years. However, the firm data indicating an increase in human testicular cancer, as well as apparent occurrence of other plausibly related effects, support the concept that an adverse influence has occurred or still exists. Whether these effects in humans can be attributed to an endocrine disruption by environmental chemicals is unknown at present, and research into the cause(s) is needed. It is possible that the mechanism by which estrogenic chemicals impair development of the male reproductive system is via antiandrogenic properties rather than or in addition to activity related to estrogen receptor activation.

Leydig cell hyperplasia and tumors are a significant problem in testing with laboratory species. Scientific evidence shows that human incidence of the tumors is low relative to that in rodents and that not all modes of induction in test species are relevant to humans. However, occurrence of Leydig cell tumors in test species may be of concern with certain modes of action if the potential exists for sufficient exposure.

Testing for endocrine-disrupting potential of environmental chemicals should include the ability to detect antiandrogenic activity in addition to estrogenic activity. Testing also should be able to detect alteration in androgen receptor and Ah receptor function as reflected in genome expression.

Little is known about the causes of human prostatic cancer, but age, genetics, diet, endocrine status, and environmental risk factors have been proposed. With respect to the cause(s) of human prostate cancer, a single retrospective epidemiology study has established a weak but statistically significant association between acres sprayed with herbicides and prostate cancer deaths. Furthermore, an occupational study of coke-oven workers has found an association between coke-oven emission and significant excess mortality from cancer of the prostate. Whether herbicide or polyaromatic hydrocarbon exposure contributes to the increasing incidence of human adenocarcinoma of the prostate and whether the mechanism is triggered by an endocrine disruption remain to be determined. More research is required before assigning a specific endocrine disruption (or any other) mechanism as a specific cause of human prostate cancer.

Hypothalamus and Pituitary. There are a number of ways that environmental agents may alter neuroendocrine function both during development and in the sexually mature organism. Exposure during development may be of particular concern because many of the feedback functions of the endocrine system are not operational during this period, permanent changes in endocrine function may be induced at levels of exposure to a toxicant that have no effect in the adult animal, and compounds that are considered antiestrogenic in the adult (i.e., tamoxifen, dioxin) may act as estrogens in the developing organism. Similarly, exposure to such agents in the adult can modify the feedback of endogenous hormones as well as behavior (i.e., libido, appetite, aggression) of the individual. Because of the complex role that the central nervous system plays in regulating endocrine function, cells within the brain are potential targets for environmental chemicals that have no impact on steroid hormones directly but yet will lead to a disruption of endocrine function. There is a substantial need to better characterize the role of the brain and pituitary when evaluating suspected reproductive toxicants in both the male and female.

Thyroid. Numerous environmental agents have been found to alter thyroid hormone levels (e.g., urea derivatives, polyhalogenated biphenyls, and chlorinated dibenzo-*p*-dioxins). Thyroid hormones are critical to normal growth and development; thus, developmental exposures may have lasting adverse effects.

Sensitive sub-populations

Current tests used for the detection of this type of substances, have underestimated toxic effects due to the fact that some groups in the population may be unusually sensitive to these kind of substances. The concern is largely based on the hypothesis put forward by Spearow and co-workers (1999), suggesting that strains of mice commonly used in toxicity testing are insensitive to oestrogenic compounds.

The study of Spearow was done only using immature mice of different strains, which were compared without an assessment of the effects in mature mice; the observed effects may well reflect differences in maturation of the distinct mouse strains rather than the effects of oestrogens.

The mouse strain commonly used in toxicity testing (CD1 mouse) is generally recognised as highly effective in detecting a wide variety of reproductive effects and remains the strain of choice for reproduction tests in mice (Chapin et al., 1997).

Studies on *in utero* exposure to the estrogenic drug diethylstilbestrol (DES) have served as an important model for delineating problems associated with exposure to estrogenic compounds in both animal models and in humans; DES-induced effects on the male and female reproductive tracts strongly support the endocrine-disruptor hypothesis Bisphenol A, an important intermediate in the production of polycarbonates, is a weakly estrogenic industrial compound in most, but not all, assays. For example, bisphenol A induces mammary gland growth in Noble rats at doses as low as 0.1 mg/kg/day; this is similar to the dose required for the potent estrogen DES to induce the same response. The estrogenic activity of bisphenol A in CF-1 mice has also generated controversy; vom Saal *et al*. reported that foetal exposure to low doses of bisphenol A (2 or 20 µg/kg/day) resulted in increased prostate weight in the male offspring. Results for bisphenol A, E2, and DES all gave low dose inverted U-shape dose-response curves for this effect; at higher doses, decreased prostate weight was observed in the offspring. In contrast, Cagen *et al.* did not observe this low dose effect for bisphenol A or DES in CF-1 mice. Thus, the "low dose" hypothesis for this response should be resolved for bisphenol A and other estrogenic compounds.

It has been also observed an age-dependent difference in susceptibility to endocrine disruptors. In adult ovariectomized C57BL/Tw mice, three daily doses of 100 µg of clomiphene, tamoxifen or nafoxidien or 1 µg of estradiol but not keoxifene exhibited decreased uterine and vaginal weights at 60 days of age. Similarly, while TCDD can inhibit certain estrogenic effects in adults, weanling Sprague-Dawley female rats are apparently insensitive to the antiestrogenic effects of TCDD.

For human health risk assessment two-generation reproduction studies and the 2 year cancer bioassay should be able to detect many adverse effects, however these were not designed specifically to identify mechanisms of action of endocrine disruption, subtle functional deficits or transplacental carcinogenesis that might result after exposures at critical stages of development not currently included in testing protocols.

GENERAL DISCUSSION.

Our research focused on the social implementation of the acceptability of occupational technological risks. The aim included to identify the risks and hazards perception of subjects having already been involved in previous biomonitoring studies performed in the framework of "occupational welfare" granted by the SSTC.

Issued from a multidisciplinary approach, the results gained are intended to contribute to a better risk and/or hazard identification, evaluation, information, communication and perception.

The integration of the knowledge related to the « occupational health welfare » into a sustainable development requires not only the development of sophisticated biological technologies allowing detection of early biological effects but also to take into account others variables inherent to health welfare such as social, psychosocial, environmental and legislative parameters.

Therefore, it appears essential to keep in mind that every human and industrial activity includes risks for which the evaluation and characterization are obviously associated to uncertainties inherent to the technological issues. Identify the respective and relative fraction of uncertainties constitutes a new challenge that requires a total decompartmentalization of skills in order to gain proactively a novel and real interdisciplinary approaches. Such integrated approach will allow the adjustment of transversal decisions by combining specific points of view from different disciplines : respective views of the same reality.

Crossing and interlinking skills and knowledge between partners will contribute to help stakeholders to take knowledge based decisions with regards of technological, scientific, economical and social parameters.

In some way biomonitoring studies are inspired by the precautionary principle as well as by the recognition of scientific uncertainties. It aims to identify any health threats as early as possible and consists therefore of precautionary measures to health protection. The lack of certainties should, in this case, not be considered as a pretext for postponing measures aiming to prevent health effects. In this sense, coordination and communication steps are essentials both between respective concerned scientist, between scientists and public authorities and between the scientific community and the society. For us, it seems obvious that the scientific community has a important role to play to heighten public awareness and to popularise, share and disseminate results of ongoing researches in the field of public health and sustainable development.

Annexes

Test	Determination of the opioids in air samples					
Biomarker of	External exposure	Internal exposure	Early effects	Genetic effects	Susceptibility: genotype	Susceptibility: phenotype
	X					
Developed by	KUL	UCL	UG	ULg	VUB	
	X					
Principle	During production and formulating activities, workers can be exposed to potent opioid narcotics through inhalation of opioid containing dusts or aerosols. The airborne concentration of the opioid compounds can be determined by personal or environmental air sampling. Airborne opioid are collected by drawing a known quantity of air through a 25 mm glass fiber filter mounted in an IOM sampler at a flow rate of 2L/min. The analytes are subsequently extracted from the filters and are analyzed using a highly specific and sensitive gas chromatographic mass spectrometric GC-MS method.					
Choice	ADVANTAGES Air sampling remains a rapid and simple standard tool in the assessment of external occupational exposure. The results of the measurements can be compared with in-the-house established occupational exposure limits (OELs) for airborne concentrations.			DISADVANTAGES Attention should be drawn to the fact that air-sampling focuses on one possible route of exposure, namely through inhalation. When the possibility exists of opioid absorption through the skin, following dermal contact, biomonitoring should be evaluated as a complementary technique.		
Importance	A rapid and reliable quantitative assessment of external occupational exposure to potent opioid analgesics, in which airborne concentrations can be compared with established OELs.					
Applied in		Cobalt	Styrene	Ionizing Radiation	Opioid Analgesics	
	In vitro human lymphocytes					
	In vivo rat lymphocytes rat pneumocytes					
	Biomonitoring human lymphocytes					
	External monitoring workers breathing air				**X**	
	Patients					

Test	**Urinary mandelic acid**					
Biomarker of	External exposure	Internal exposure	Early effects	Genetic effects	Susceptibility: genotype	Susceptibility: phenotype
		X				
Developed by	KUL	UCL	UG	ULg	VUB	
	X					
Principle	Mandelic acid is one of the major urinary excretion products after styrene absorption. The activation and deactivation of Styrene occurs mainly through the intermediate formation of styrene oxide and further styrene glycol. Mandelic acid is related to styrene glycol. As a consequence mandelic acid is the preferred metabolite to be sampled for biomonitoring purposes. Mandelic acid in the urine is detected through High-pressure liquid chromatography (RSD: < 10%). The detection limit is 50 mg/l					
Choice	ADVANTAGES • Mandelic acid is a metabolite derived from styrene oxide (the reactive metabolite of styrene) • It takes into account absorption by all routes • Mandelic acid refers to recent exposure • Availability: simple urine sample collection • Automatisated • Cheap method			DISADVANTAGES • Possible interference (smoking)		
Importance	The level of mandelic acid in the urine provides a measure for the internal dose after styrene exposure. Mandelic acid is easy to analyse through HPLC. Mandelic acid is an metabolite of styrene oxide. Styrene oxide is the reactive metabolite of styrene which is possibly responsible for the carcinogenic effects of styrene. Intensive research executed by the KUL has shown that urinary mandelic acid is better biomarker than other metabolites as e.g. phenylglyoxylic acid. As a consequence urinary mandelic acid is the recommended biomarker for the evaluation of internal dose of styrene exposure.					
Applied in		Cobalt	Styrene	Ionising Radiation	Opioid Analgesics	
	In vitro					
	In vivo					
	Biomonitoring urine		**X**			
	Patients					

<table>
<tr><td>Test</td><td colspan="6">Adducts (DNA and Haemoglobin)s</td></tr>
<tr><td rowspan="2">Biomarker of</td><td>External exposure</td><td>Internal exposure</td><td>Early effects</td><td>Genetic effects</td><td>Susceptibility: genotype</td><td>Susceptibility: phenotype</td></tr>
<tr><td></td><td>Biological effective dose</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Developed by</td><td>KUL</td><td>UCL</td><td>UG</td><td>ULg</td><td>VUB</td><td rowspan="2"></td></tr>
<tr><td></td><td>X</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Principle</td><td colspan="6">Adducts are covalent bounds between genotoxic agents and nucleophilic molecules, e.g. DNA (target site adduct) and Haemoglobin, Albumin (nontarget site adduct). The formation of stable carcinogen-DNA adducts are considered critical events in the initiation of the carcinogenic process. DNA adducts correlate with tumor incidence. Haemoglobin and Albumin adducts are used as surrogates for DNA adducts when they correlate quantitatively with target site adducts (DNA adducts). Adducts determination occurs through GC-MS analysis or other techniques (^{32}P-postlabeling, HPLC,…) depending on the agent and the type of adduct.</td></tr>
<tr><td>Choice</td><td colspan="3"><u>ADVANTAGES</u>
DNA is a target molecule in carcinogenesis
It takes into account absorption by all routes
It integrates exposure from all sources
Adducts show individual exposure, metabolic capacity, and DNA repair capacity.
Adduct forming chemicals, e.g. many anticancer agents, are potentially dangerous.
Adducts can show active ingredients in complex mixtures
Data obtained may be directly related to adverse effects
Haemoglobin adducts refer to the exposure of the previous 4 months
DNA adducts refer to recent exposure
Haemoglobin and DNA of lymphocytes are easily available through venous bloodpunction</td><td colspan="3"><u>DISADVANTAGES</u>
DNA adducts are subjected to repair
labour intensive
Possible interference
Existence of background adduct levels</td></tr>
<tr><td>Importance</td><td colspan="6">Assays for the level of carcinogen-adducts provide a measure of the biologically effective dose for certain carcinogens.
Human adduct formation is a promising biomarker for molecular cancer epidemiology. Satisfactory analytical techniques now exist for the measurement of many DNA and protein adducts caused by human exposure to genotoxic agents. These will likely lead to improved risk assessment for groups of exposed individuals and may indicate opportunities for chemoprevention.</td></tr>
</table>

Applied in		Cobalt	Styrene	Ionising Radiation	Opioid Analgesics	
	In vitro human haemoglobin rat haemoglobin mouse haemoglobin		 **X** **X** **X**			
	In vivo rat haemoglobin mouse haemoglobin		 **X** **X**			
	Biomonitoring human haemoglobin		 **X**			
	Patients					

Test	**CYP2E1 genotyping**					
Biomarker of	External exposure	Internal exposure	Early effects	Genetic effects	Susceptibility: genotype	Susceptibility: phenotype
					X	
Developed by	KUL	UCL	UG	ULg	VUB	
		X				
Principle	Detection by restriction fragment length polymorphism (RFLP) of the 3 most frequent allelic variants of CYP2E1 (CYP2E1*1B or A1/A2, CYP2E1*5A or c1/c2, CYP2E1*6 or D/C) on a PCR amplification product obtained from a blood sample.					
Choice	ADVANTAGES Simple Does not necessitate expensive equipment			DISADVANTAGES Time consuming		
Importance	Detection of variant genotypes with possibly increased or decreased metabolic activity					
Applied in		Cobalt	Styrene	Ionising Radiation	Opioid Analgesics	
	In vitro human lymphocytes		**X**			
	In vivo rat lymphocytes rat pneumocytes					
	Biomonitoring human lymphocytes		**X**			
	Patients		**X**			

Test	**HLADR Glu^{69} genotyping**					
Biomarker of	External exposure	Internal exposure	Early effects	Genetic effects	Susceptibility: genotype	Susceptibility: phenotype
					X	
Developed by	KUL	UCL	UG	ULg	VUB	
		X				
Principle	Detection of the presence of glutamic acid on position 69 of the ß chain of the DPß1molecule (GAG sequence of codon 69 of exon 2 of the *DPB1* gene (reverse-sequence specific oligonucleotide (SSO) probe line-blot method).					
Choice	ADVANTAGES Simple and accurate			DISADVANTAGES Expensive		
Importance	Detection of variant genotypes with possibly increased or decreased metabolic activity					
Applied in		Cobalt	Styrene	Ionising Radiation	Opioid Analgesics	
	In vitro human lymphocytes					
	In vivo rat lymphocytes rat pneumocytes					
	Biomonitoring human lymphocytes	**X**				
	Patients					

Test	**GST genotyping**					
Biomarker of	External exposure	Internal exposure	Early effects	Genetic effects	Susceptibility: genotype	Susceptibility: phenotype
					X	
Developed by	KUL	UCL	UG	ULg	VUB	
		X				
Principle	Detection by restriction fragment length polymorphism (RFLP) of the allelic variants of glutathione S-transferases isoforms (GSTT1, GSTM1 and GSTP) on a PCR amplification product obtained from a blood sample.					
Choice	ADVANTAGES Simple Does not necessitate expensive equipment			DISADVANTAGES Time consuming		
Importance	Detection of variant genotypes with possibly increased or decreased metabolic activity					
Applied in		Cobalt	Styrene	Ionising Radiation	Opioid Analgesics	
	In vitro human lymphocytes		**X**			
	In vivo rat lymphocytes rat pneumocytes					
	Biomonitoring human lymphocytes		**X**			
	Patients		**X**			

Test	**Epoxide hydrolase genotyping**					
Biomarker of	External exposure	Internal exposure	Early effects	Genetic effects	Susceptibility: genotype	Susceptibility: phenotype
					X	
Developed by	KUL	UCL	UG	ULg	VUB	
		X				
Principle	Detection by restriction fragment length polymorphism (RFLP) of the allelic variants of microsomal epoxide hydrolase (at residues 113 and 139) on a PCR amplification product obtained from a blood sample.					
Choice	ADVANTAGES Simple Does not necessitate expensive equipment			DISADVANTAGES Time consuming		
Importance	Detection of variant genotypes with possibly increased or decreased metabolic activity					
Applied in		Cobalt	Styrene	Ionising Radiation	Opioid Analgesics	
	In vitro human lymphocytes		**X**			
	In vivo rat lymphocytes rat pneumocytes					
	Biomonitoring human lymphocytes		**X**			
	Patients		**X**			

Test	**OGG1 genotyping**					
Biomarker of	External exposure	Internal exposure	Early effects	Genetic effects	Susceptibility: genotype	Susceptibility: phenotype
					X	
Developed by	KUL	UCL	UG	ULg	VUB	
					X	
Principle	Detection by restriction fragment length polymorphism (RFLP) of the most frequent allelic variants of OGG1 (Ser/Cys) on a PCR amplification product obtained from a blood sample.					
Choice	ADVANTAGES Simple Does not necessitate expensive equipment			DISADVANTAGES Time consuming		
Importance	Detection of variant genotypes with possibly increased or decreased metabolic activity					
Applied in		Cobalt	Styrene	Ionising Radiation	Opioid Analgesics	
	In vitro human lymphocytes					
	In vivo rat lymphocytes rat pneumocytes					
	Biomonitoring human lymphocytes	**X**	**X**	**X**		
	Patients					

Test	**XRCC1/3 genotyping**					
Biomarker of	External exposure	Internal exposure	Early effects	Genetic effects	Susceptibility: genotype	Susceptibility: phenotype
					X	
Developed by	KUL	UCL	UG	ULg	VUB	
					X	
Principle	Detection by restriction fragment length polymorphism (RFLP) of the most frequent allelic variants of XRCC1 (codon 194 , Arg/Trp ; codon 399, Gln/Arg; codon 280, Arg/His) and XRCC3 (codon 241, Thr/Met) on a PCR amplification product obtained from a blood sample.					
Choice	ADVANTAGES Simple Does not necessitate expensive equipment			DISADVANTAGES Time consuming		
Importance	Detection of variant genotypes with possibly increased or decreased metabolic activity					
Applied in		Cobalt	Styrene	Ionising Radiation	Opioid Analgesics	
	In vitro human lymphocytes					
	In vivo rat lymphocytes rat pneumocytes					
	Biomonitoring human lymphocytes	**X**	**X**	**X**		
	Patients					

Test	**COMET assay**					
Biomarker of	External exposure	Internal exposure	Early effects	Genetic effects	Susceptibility: genotype	Susceptibility: phenotype
			X			**X**
Developed by	KUL	UCL	UG	ULg	VUB	
					X	
Principle	Single cell gel (SCG) electrophoresis or 'Comet assay' is a rapid and very sensitive fluorescent microscopic method to examine DNA damage and repair at individual cell level. This technique is used for detecting various forms of DNA damage (*e.g.*, single- and double-strand breaks, oxidative DNA base damage, and DNA-DNA/DNA-protein/DNA-Drug crosslinking) and DNA repair in virtually any eukaryotic cell.					
Choice	ADVANTAGES cell/cell approach, applicable on many cell types, no obligation in vitro cultivation step required, possible estimation of DNA repair capacity after in vitro challenging, low cost, fast, simple, gives some indication of cell death			DISADVANTAGES detected DNA damage ≠ fixed mutations, low specificity, need for internal standard to minimise experimental variation, controversial confounding factors (age, smoking,...)		
Importance	Detection of recent DNA damage (alkali labile sites) and is applicable on many cell types This assay has critically important applications in fields of toxicology ranging from aging and clinical investigations to genetic toxicology and molecular epidemiology.					
Applied in		Cobalt	Styrene	Ionising Radiation	Opioid Analgesics	
	In vitro human lymphocytes	**X**	**X**	**X**		
	In vivo rat lymphocytes rat pneumocytes	**X** **X**				
	Biomonitoring human lymphocytes	**X**	**X**	**X**		
	Patients					

Test	**Sister Chromatid exchange Assay**					
Biomarker of	External exposure	Internal exposure	Early effects	Genetic effects	Susceptibility: genotype	Susceptibility: phenotype
				X		
Developed by	KUL	UCL	UG	ULg	VUB	
Principle	The Sister Chromatid Exchange assay is sensitive but not specific method to examine the effects induced at a cytological level by chemical DNA lesions (that were not repaired during the GO/G1 phase) and that persist up to the S-phase. This assay is used to screen molecular DNA damages such as DNA-adducts, alkylation or methylation of DNA bases. Under some instances, it may also give some indications of DNA repair sensitivity: cells of patients with DNA repair defects (Xeroderma Pigmentosum) show a larger induced SCE frequency than "normal" individuals.					
Choice	ADVANTAGES Theoretically applicable on any cells able to divide twice (2 S-phases) in culture; this assay is mainly used in human lymphocytes and cell lines. Every cell display a SCEs number, therefore, the mean value expressed is based on many observations. Also, this methodology requires BrdU and gives to possibility to check cytotoxicity by scoring the proliferation rate index. Low cost. Biomarker of exposure or early biological effects. Gives insights in potentially damaged subpopulation of cells.			DISADVANTAGES Low specificity, time consuming, controversial confounding factors (age, smoking,...) but that may be under control. Absence of cancer risk predictivity.		
Importance	Detection of non repaired DNA damages applicable on many cell types This assay has applications in fields of toxicology, genetic toxicology and molecular epidemiology.					
Applied in		Cobalt	Styrene	Ionising Radiation	Opioid Analgesics	
	In vitro human lymphocytes		**X**			
	In vivo rat lymphocytes rat pneumocytes					
	Biomonitoring human lymphocytes		**X**			
	Patients					

Test	**P53 hotspots**					
Biomarker of	External exposure	Internal exposure	Early effects	Genetic effects	Susceptibility: genotype	Susceptibility: phenotype
				X		
Developed by	KUL	UCL	UG	ULg	VUB	
		X			**X**	
Principle	Restriction site mutation (RSM) assay based on the PCR amplification of a fragment of a selected gene mutated on a specific restriction site.					
Choice	ADVANTAGES Specific, very sensitive, rapid Detects gene mutations and therefore complements other genotoxicity assays (CA, MN, comet, …)			DISADVANTAGES Cannot detect unsuspected mutations		
Importance	Detection of variant genotypes with possibly increased or decreased metabolic activity					
Applied in		Cobalt	Styrene	Ionising Radiation	Opioid Analgesics	
	In vitro human lymphocytes	**X**				
	In vivo rat lymphocytes rat pneumocytes					
	Biomonitoring human lymphocytes	**X**	**X**			
	Patients					

Test	**Conventional Chromosome Aberration Assay**					
Biomarker of	External exposure	Internal exposure	Early effects	Genetic effects	Susceptibility: genotype	Susceptibility: phenotype
				X		
Developed by	KUL	UCL	UG	ULg	VUB	
Principle	Methods allowing the early detection or evaluation of induced genetic damages in human contributive for identifying heavily (or more susceptible?) exposed subjects to ionising radiations. These biological tests allow a biological retrospective dosimetry after exposure to IR. Up to recently, the frequency of chromosomal aberrations (dicentric chromosomes) was used to detect or quantify radiation-induced effects. *In vitro* cultured lymphocytes are "harvested " at the first metaphase and the structural and/or numerical chromosome aberrations are scored during microscopic evaluation.					
Choice	ADVANTAGES When used in human lymphocytes it gives a clear indication of chromosomal rearrangements. Biomarker of exposure and early biological effects. Low cost, high specificity. Indicator of cancer risk predictivity when used in biomonitoring studies included in molecular epidemiological studies. Indicator of mutagenic/ clastogenic properties when used in screening chemicals. Gives indication of both stable or unstable aberrations types.			DISADVANTAGES Time consuming, controversial confounding factors in biomonitoring studies (age, smoking,...). Requires very experienced scorer and a large number of cells to be scored.		
Importance	Detection of non repaired DNA damages applicable on many cell types This assay has applications in fields of toxicology, genetic toxicology, and molecular epidemiology.					
Applied in		Cobalt	Styrene	Ionising Radiation	Opioid Analgesics	
	In vitro human lymphocytes			**X**		
	In vivo rat lymphocytes rat pneumocytes					
	Biomonitoring human lymphocytes			**X**		
	Patients					

Test	**FISH Chromosome Aberration Assay**					
Biomarker of	External exposure	Internal exposure	Early effects	Genetic effects	Susceptibility: genotype	Susceptibility: phenotype
				X		
Developed by	KUL	UCL	UG	ULg	VUB	
Principle	Methods allowing the early detection or evaluation of induced genetic damages in human contributive for identifying heavily (or more susceptible?) exposed subjects to ionising radiations. The fluorescent *in situ* hybridisation (FISH) technique using DNA chromosome-specific DNA probes has increased the sensitivity of the technique and eased the detection limit of stable chromosome aberrations (especially translocations) in human. FISH analysis of the chromosome aberrations is generally performed with a cocktail of chromosome-specific probes for different sets of chromosomes (e.g. WCP 2 spectrum green, WCP 2 spectrum orange, WCP 4 spectrum green, and WCP 8 spectrum orange). These biological tests – need some more extensive data - to allow a precise biological retrospective dosimetry after exposure to IR.					
Choice	ADVANTAGES When used in human lymphocytes it allows a clear indication of one of the peculiar stable chromosomal rearrangements such as translocations; such translocations are known to be important in carcinogenic processes. Biomarker of exposure and early biological effects. Required less experienced cytogeneticist than conventional technique, automation possible. High sensitivity. Indicator of cancer risk predictivity when used in biomonitoring.			DISADVANTAGES Time consuming, controversial confounding factors in biomonitoring studies (age, smoking,...). Requires experienced scorer and a very large number of cells to be scored. Very high cost. Additional data needed to assess if the fraction of the genome analysed (generally ± 20 % of the genome is considered by using 3 different chromosome sets -ch 2, 4 and 8 for example) is representative of the entire genome. Use a prerequisite that every chromosome is randomly involved in translocation; such clear assessment is still under consideration.		
Importance	Detection of non repaired DNA damages applicable on many cell types This assay has applications in fields of toxicology, genetic toxicology and molecular epidemiology.					
Applied in		Cobalt	Styrene	Ionising Radiation	Opioid Analgesics	
	In vitro human lymphocytes			**X**		
	In vivo rat lymphocytes rat pneumocytes					
	Biomonitoring human lymphocytes			**X**		
	Patients					

Test	**Micronucleus assay**					
Biomarker of	External exposure	Internal exposure	Early effects	Genetic effects	Susceptibility: genotype	Susceptibility: phenotype
				X		**X**
Developed by	KUL	UCL	UG	ULg	VUB	
			X		**X**	
Principle	A micronucleus (MN) is formed during the metaphase/anaphase transition of mitosis (cell division). It may arise from a whole lagging chromosome (aneugenic event leading to chromosome loss) or an acentric chromosome fragment detaching from a chromosome after breakage (clastogenic event) which do not integrate in the daughter nuclei.					
Choice	ADVANTAGES • Cell/cell approach • Simultaneous detection of chromosome + genome mutations • Discrimination between clastogen/ aneugen • Possible co-detection of apoptosis/necrosis • Applicable on many cell types • Rapidity • Cheap • Simplicity • Potential for automation • Statistical power • Discrimination between cells which underwent nuclear division and cells which did not • Enables detection of dicentric bridges as nucleoplasmic bridges • Assessment of cell proliferation (% binucleated cells)			DISADVANTAGES • Does not detect all structural chromosome aberrations (only acentric fragments) • Requires cell division for expression of MN • Possible interference of cyto-B with test chemical; like spindle poisons • Possible interference with other inhibitors of cytokinesis • Cytotoxicity of cytochalasin B itself varies between cell types and sometimes even between subtypes of the same cell type		

Importance	The micronucleus test detects chromosome and genome mutations Scoring of micronuclei can be performed relatively easily and on different cell types relevant for human biomonitoring: lymphocytes, fibroblasts and exfoliated epithelial cells, with/without extra in vitro cultivation step. MN observed in exfoliated cells are not induced when the cells are at the epithelial surface, but when they are in the basal layer. The combination of the micronucleus assay with fluorescence in situ hybridisation (FISH) with a probe labelling the (peri-)centromeric region of the chromosomes (FISH assay) allows discrimination between micronuclei containing a whole chromosome (centromere positive micronucleus) and an acentric chromosome fragment (centromere negative micronucleus).					
Applied in		Cobalt	Styrene	Ionising Radiation	Opioid Analgesics	
	In vitro human lymphocytes	**X**		**X**		
	In vivo rat lymphocytes rat pneumocytes	**X** **X**				
	Biomonitoring human lymphocytes	**X**	**X**	**X**		
	Patients					

<table>
<tr><td>Test</td><td colspan="6">Gene expression analysis with real-time RT-PCR</td></tr>
<tr><td rowspan="2">Biomarker of</td><td>External exposure</td><td>Internal exposure</td><td>Early effects</td><td>Genetic effects</td><td>Susceptibility: genotype</td><td>Susceptibility: phenotype</td></tr>
<tr><td></td><td></td><td></td><td>X</td><td></td><td>X</td></tr>
<tr><td>Developed by</td><td>KUL</td><td>UCL</td><td>UG</td><td>ULg</td><td>VUB</td><td rowspan="2"></td></tr>
<tr><td></td><td></td><td></td><td>X</td><td></td><td></td></tr>
<tr><td>Principle</td><td colspan="6">The RNA is extracted from in vitro irradiated IL-2 cell cultures, followed by a DNase treatment to remove contaminants. This treated RNA is converted in cDNA ,that is used as a template in the RT-PCR.
This technology combines the DNA amplification with the detection of the products. The detection method is based on changes in fluorescence proportional to the increase in product.</td></tr>
<tr><td>Choice</td><td colspan="3"><u>ADVANTAGES</u>
• Less time consuming than gel based analysis
• No need of post-PCR manipulations
• Reduces contamination opportunities
• Good accuracy
• Very sensitive method
• High-throughput capacity
• Applicable on many cell types
• Rapidity
• Simultaneous measurement of gene expression in many different samples
• High degree of potential automation
• Statistical power</td><td colspan="3"><u>DISADVANTAGES</u>
• This technique requires good quality RNA
• Use of different housekeeping genes to normalize the gene expression data
• More expensive than classical PCR</td></tr>
<tr><td>Importance</td><td colspan="6">Several literature data have shown that a high percentage of breast cancer patients is radiosensitive. As this radiosensitivity may be linked with the expression pattern of genes involved in the processing of DNA damage (DNA repair, cell-cycle checkpoint and stress genes) it would be interesting to investigate differential gene expression. By using the RT-PCR technology it is easy to analyse the gene expression pattern of these specific genes in radiosensitive individuals and compare it with the expression pattern of the non sensitive ones.</td></tr>
</table>

Applied in		Cobalt	Styrene	Ionising Radiation	Opioid Analgesics	
	In vitro human lymphocytes			**X**		
	In vivo rat lymphocytes rat pneumocytes					
	Biomonitoring human lymphocytes			**X**		
	Patients			**X**		

Test	**CYP2E1 phenotyping**					
Biomarker of	External exposure	Internal exposure	Early effects	Genetic effects	Susceptibility: genotype	Susceptibility: phenotype
						X
Developed by	KUL	UCL	UG	ULg	VUB	
		X				
Principle	Real-time quantification of mRNA by RT-PCR in human peripheral mononucleated cells isolated from blood.					
Choice	ADVANTAGES Specific Very sensitive Rapid			DISADVANTAGES Necessitate expensive equipment and reagents Does not measure the protein or the enzyme activity		
Importance	Detection of variant genotypes with possibly increased or decreased metabolic activity					
Applied in		Cobalt	Styrene	Ionising Radiation	Opioid Analgesics	
	In vitro human lymphocytes					
	In vivo rat lymphocytes rat pneumocytes					
	Biomonitoring human lymphocytes		**X**			
	Patients					

Test	**Determination of the opioids and their metabolites in urine**					
Biomarker of	External exposure	Internal exposure	Early effects	Genetic effects	Susceptibility: genotype	Susceptibility: phenotype
		X				**X**
Developed by	KUL	UCL	UG	ULg	VUB	
	X					
Principle	The main metabolic pathway of the opioid analgesics is the oxidative N-dealkylation at the piperidine nitrogen, resulting in the formation of nor-metabolites. Only a few percent of the opioids are excreted unchanged in urine. The opioids and their nor-metabolites are extracted from urine using a simple, one step solid phase extraction procedure (SPE). The analytes are subsequently analyzed using a highly specific and sensitive gas chromatographic mass spectrometric GC-MS method.					
Choice	ADVANTAGES In the assessment of occupational exposure to opioid analgesics, one of the major advantages of biological monitoring is the fact that it takes into account absorption by other routes of exposure than the lungs. In view of the highly lipophilic nature of especially sufentanil and fentanyl and to a less extent of alfentanil, absorption through the skin could present an important concomitant route of exposure. Selecting the appropriate biomarker of exposure could potentially provide additional information on the individual susceptibility of exposed workers.			DISADVANTAGES Especially in a production environment, precautions should be taken to avoid external contamination of the urine samples with the opioid compounds. The use of the nor-metabolites as biomarkers could theoretically circumvent the risk of external contamination but in some production processes these compounds are also present as neat reagents in the working environment. In view of compliance measurements, a quantitative acceptable biological exposure index (BEI) is not yet determined.		
Importance	A rapid and reliable quantitative assessment of occupational exposure to potent opioid analgesics which takes into account absorption by various routes.					
Applied in		Cobalt	Styrene	Ionizing Radiation	Opioid Analgesics	
	In vitro human lymphocytes					
	In vivo rat lymphocytes rat pneumocytes					
	Biomonitoring human urine				**X**	
	Patients				**X**	

<table>
<tr><td>Test</td><td colspan="6">G2 assay</td></tr>
<tr><td rowspan="2">Biomarker of</td><td>External exposure</td><td>Internal exposure</td><td>Early effects</td><td>Genetic effects</td><td>Susceptibility: genotype</td><td>Susceptibility: phenotype</td></tr>
<tr><td></td><td></td><td></td><td>X</td><td></td><td>X</td></tr>
<tr><td>Developed by</td><td>KUL</td><td>UCL</td><td>UG</td><td>ULg</td><td>VUB</td><td rowspan="2"></td></tr>
<tr><td></td><td></td><td></td><td>X</td><td></td><td></td></tr>
<tr><td>Principle</td><td colspan="6">In the G2 assay the cells are in vitro irradiated in the G2 phase of the cell cycle and chromatid breaks are scored in the subsequent metaphases. The short post-irradiation time of 90 min assures you that the metaphases analysed were in G2 phase of the cell cycle when irradiated. Per sample chromatid breaks are scored in 50 metaphases. Although the exact mechanism involved in the formation of G2 chromatid breaks is not completely elucidated yet, processes such as DNA dsb repair and cell cycle control are involved.</td></tr>
<tr><td>Choice</td><td colspan="3"><u>ADVANTAGES</u>
Cell/cell approach
Low background frequencies; low background variability
No age effect on the background and radiation induced chromatid break frequency
Very short post irradiation time (90 min): no interference with radiation induced apoptotic cells
The assay is performed at a low irradiation dose (0.4 Gy)
Applicable on many cell types: lymphocytes, fibroblasts, lymphoblastoid cell lines
Rapidity
Cheap
Simplicity
Automated selection of metaphases is possible; also potential for automated detection of the chromatid breaks
Discrimination between cells which went into mitosis and cells which did not
Assessment of cell proliferation: mitotic index</td><td colspan="3"><u>DISADVANTAGES</u>
Does not detect all structural chromosome aberrations (only chromatid breaks are scored)
Requires stimulation of resting cells; chromatid breaks are scored in metaphases
At the moment the exact mechanism of chromatid break formation is not completely known</td></tr>
<tr><td>Importance</td><td colspan="6">The G2 chromosomal radiosensitivity assay is a cell-cycle based technique which has been used by several groups investigating links between human chromosomal radiosensitivity and increased cancer susceptibility. Although requiring stringent experimental conditions to achieve good reproducibility the G2 assay has potential as a sensitive marker for cancer susceptibility and is particular useful in population studies.</td></tr>
</table>

Applied in		Cobalt	Styrene	Ionising Radiation	Opioid Analgesics	
	In vitro human lymphocytes			**X**		
	In vivo rat lymphocytes rat pneumocytes					
	Biomonitoring human lymphocytes			**X**		
	Patients			**X**		

Test	**DNA Strand Break Repair Phenotype Assay**					
Biomarker of	External exposure	Internal exposure	Early effects	Genetic effects	Susceptibility: genotype	Susceptibility: phenotype
			X			**X**
Developed by	KUL	UCL	UG	ULg	VUB	
					X	
Principle	In vitro repair phenotype based on the Single cell gel (SCG) electrophoresis or Comet assay. The in vitro repair capacity is assessed after in vitro exposure to the challenging mutagen. It is possible to detect with one test, three parameters: initial DNA damage at the base level, DNA vulnerability after in vitro exposure and repair capacity.					
Choice	ADVANTAGES Offers the possibility to estimate whole repair phenotype as a result of the complex interaction of different repair enzymes. Cell/cell approach, applicable on many cell types, low cost, fast, simple, gives some indication of cell death			DISADVANTAGES Not a direct measure of DNA repair but rather a measure of the percentage of unrepaired damage to DNA. The cell types used are not necessarily same as the target organ. For population monitoring, possibility of selection bias. Small sample sizes may affect the power of the tests.		
Importance	Detection of recent DNA damage (alkali labile sites) and is applicable on many cell types. Determination of inter-individual differences in DNA repair capacity. This assay has critically important applications in fields of toxicology ranging from aging and clinical investigations to genetic toxicology and molecular epidemiology.					
Applied in		Cobalt	Styrene	Ionising Radiation	Opioid Analgesics	
	In vitro human lymphocytes	**X**	**X**	**X**		
	In vivo rat lymphocyte rat pneumocytes					
	Biomonitoring human lymphocytes		**X**	**X**		
	Patients					

VUB

Publications:

M. DE BOECK, S. LARDAU, J.P. BUCHET, M. KIRSCH-VOLDERS, D. LISON. Absence of signficant genotoxicity in lymphocytes and urine from workers exposed to moderate levels of cobalt-containing dust: a cross-sectional study. Env. Mol. Mutagenesis, 36(2):151-160, 2000

M. KIRSCH-VOLDERS, M. AARDEMA, A. ELHAJOUJI. Concepts of threshold in mutagenesis and carcinogenesis. Mutation Research 464:3-11, 2000

N. TOUIL, A. ELHAJOUJI, H. THIERENS, M. KIRSCH-VOLDERS. Analysis of chromosome loss and chromosome segregation in cytokinesis-blocked human lymphocytes: non-disjunction is the prevalent mistake in chromosome segregation produced by low dose exposure to ionizing radation. Mutagenesis 15(1): 1-8, 2000

G. SPEIT, H. AUTRUP, R. CREBELLI, L. HENDERSON, M. KIRSCH-VOLDERS, S. MADLE, J.M. PARRY, A.M. SARRIF, H. VRIJHOF. Thresholds in genetic toxicology - concluding remarks. Mutation Research 464:149-153, 2000

M. KIRSCH-VOLDERS, T. SOFUNI, M. AARDEMA, S. ALBERTINI, D. EASTMOND, M. FENECH, M. ISHIDATE Jr., E. LORGE, H. NORPPA, J. SURRALES, W. VON DER HUDE, A. WAKATA. Report from the *in vitro* micronucleus assay working group. Env. Mol. Mutagenesis 35:167-172, 2000

M. DE BOECK, N. TOUIL, G. DE VISSCHER, P. AKA VANDE and M. KIRSCH-VOLDERS. Validation and Implementation of an internal standard in Comet assay analysis.. Mutation Research 469:181-197, 2000

M. KIRSCH-VOLDERS and M. FENECH. Inclusion of micronuclei in non-divided mononuclear lymphocytes and necrosis/apoptosis may provide a more comprehensive cytokinesis-block micronucleus assay for biomonitoring purposes. Mutagenesis, 16(1):51-58, 2002

S. BONASSI, M. FENECH, C. LANDO, Y.P. LIN, M. CEPPI, W.P. CHANG, N. HOLLAND, M. KIRSCH-VOLDERS, E. ZEIGER, R. BARALE, M.P. BIGATTI, C. BOLOGNESI, C. JIA, M. DI GIORGIO, L.R. FERGUSON, A. FUCIC, O. GARCIA LIMA, P. HRELIA, A.P. KRISHNAJA, T.K. LEE, L. MIGLIORE, L. MIKHALEVICH, E. MIRKOVA, P. MOSESSO, W.U. MULLER, Y. ODAGIRI, M.R. SCARFI, E. SZABOVA, I. VOROBTSOVA, A. VRAL and A. ZIJNO. The Human MicroNucleus project. International Data Base comparison for results with the cytokinesis-block micronucleus assay in human lymphocytes. I. Effect of laboratory protocol, scoring criteria and host factors on the frequency of micronulcei. Environ. Mol. Mutagen, 27:31-45, 2001.

M.J. AARDEMA and M. KIRSCH-VOLDERS The *in vitro* micronucleus assay.. in : Genetic Toxicology and Cancer Risk Assessment. 8:161-184, 2001 by M. Dekker, inc. New York-Basel (ed. Wai Nang Choy).

D. LISON, M. DE BOECK, V. VEROUGSTRAETE and M. KIRSCH-VOLDERS.Update on the genotoxicity and carcinogenicity of cobalt comounds. Occupational and Environmental Medicine, 58:619-625, 2001.

M. KIRSCH-VOLDERS, M. DE BOECK et D. LISON.Génotoxicité et activité professionnelle. Encyclopédie Médico-chirurgicale, Paris, 16-537C-10 and 16-537C-11, 2001.

N. TOUIL, P. VANDE AKA, J.P. BUCHET, H. THIERENS and M. KIRSCH-VOLDERS. Assessment of genotoxic effects related to chronic low level exposure to ionising radiation using biomarkers for DNA damage and repair. Mutagenesis, 17(3):223-232, 2002.

M. KIRSCH-VOLDERS, A. VANHAUWAERT, M. DE BOECK and I. DECORDIER. Importance of detecting numerical versus structural chromosome aberrations. Mutation Research 9334:1-12, 2002.

M. FENECH, S. BONASSI, J. TURNER, C. LANDO, M. CEPPI, W.P. CHANG, N. HOLLAND, M. KIRSCH-VOLDERS, E. ZEIGER, M.P. BIGATTI, C. BOLOGNESI, C. JIA, G. DE LUCA, M. DI GIORGIO, L.R. FERGUSON, A. FUCIC, O.GARCIA LIMA, V.V. HADJIDEKOVA, P. HRELIA, A. JAWORSKA, G. JOKSIC, A.P. KRISHNAJA, T.K. LEE, J. SURALLES, A. MARTELLI, M.J. McKAY, L. MIGLIORI, E. MIRKOVA, W.U. MULLER, Y. ODAGIRI, T.T. ORSIERE, M.R. SCARFI, M.J. SILVA, T. SOFUNI, E. SZABOVA, G. TRENATA, I. VOROBTSOVA, A. VRAL and, A. ZIJNO. HUMN Project: Intra- and inter-laboratory variation in the scoring of micronuclei and nucleoplasmic bridges in binucleated human lymphocytes. Results of an international slide-scoring exercise by the HUMN project. Mutation Research 534(1-2): 45-64, 2003

FENECH M, CHANG WP, KIRSCH-VOLDERS M, HOLLAND N, BONASSI S, ZEIGER E. HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. Mutation Research 534(1-2): 65-75, 2003

S. BONASSI, M. NERI, C. LANDO, M. CEPPI, Y. LIN, W.P. CHANG, N. HOLLAND, M. KIRSCH-VOLDERS, E. ZEIGER, M. FENECH and the HUMN COLLABORATIVE GROUP. Effect of smoking habit on the frequency of micronuclei in human lymphocytes : results from the Human MicroNucleus Project. Mutation Research, Mutation Research 543(2): 155-66, 2003

DE BOECK M., LOMBAERT N., DE BACKER S., FINSY R. , LISON D., KIRSCH-VOLDERS M. In vitro genotoxic effects of different combinations of cobalt and metallic carbide particles. Mutagenesis 18(2): 177-86, 2003

KIRSCH-VOLDERS, M., VANHAUWAERT, A., EICHENLAUB-RITTER, U. AND DECORDIER, I. Indirect mechanisms of genotoxicity. Toxicology Letters, 140-141: 63-74, 2003

DE BOECK M, HOET P, LOMBAERT N, NEMERY B, KIRSCH-VOLDERS M, LISON D. In vivo genotoxicity of hard metal dust: induction of micronuclei in rat type II epithelial lung cells. Carcinogenesis. 24(11): 1793-800, 2003

KIRSCH-VOLDERS M., LISON D. Re: Hengstler, J.G., Bolm-Auorff, U., Faldum, A., Janssen, K., Reifenrath, M., Gotte, W., Jung, D., Mayer-Popken, O., Fuchs, J., Gebhard, S., Bienfait, H.G., Schlink, K., Dietrich, C., Faust, D., Epe, B. and Oesch, F. Occupational exposure to heavy metals: DNA damage induction and DNA repair inhibition prove co-exposures to cadmium, cobalt and lead as more dangerous than hitherto expected. Carcinogenesis, 2003, 24, 63-73. Carcinogenesis. 2003 Nov; 24(11): 1853-4, 2003

KIRSCH-VOLDERS M, SOFUNI T, AARDEMA M, ALBERTINI S, EASTMOND D, FENECH M, ISHIDATE M JR, KIRCHNER S, LORGE E, MORITA T, NORPPA H, SURRALLES J, VANHAUWAERT A, WAKATA A. Report from the in vitro micronucleus assay working group. Mutation Research 540(2): 153-63, 2003

DE BOECK M, KIRSCH-VOLDERS M, LISON D. Cobalt and antimony: genotoxicity and carcinogenicity. Mutation Research 533(1-2): 135-52, 2003

LOMBAERT N., DE BOECK M., DECORDIER I., CUNDARI E., LISON D., KIRSCH-VOLDERS K. Apoptosis induced in vitro by hard metal dust (WC-Co), tungsten carbide and metallic cobalt in human peripheral mononucleated cells. Toxicology and Applied pharmacology, submitted

DECORDIER I., AKA P., LOMBAERT N., VANHAUWAERT A., MATEUCA R., KIRSCH-VOLDERS M. Simplicity and complexity of genetic susceptibility in the occupational environment. Archives of Public Health, submitted

AKA P., MATEUCA R., BUCHET J.-P., THIERENS H., KIRSCH-VOLDERS M. hOGG1^{326} and XRCC1^{399} genotypes and the DNA strand break repair phenotype are predictive for genotoxic effects in workers exposed to low dose ionising radiation. Radiation Research, submitted

GODDERIS L., DE BOECK M., HAUFROID V., EMMERY M., MATEUCA R., GARDINAL S., KIRSCH-VOLDERS M., VEULEMANS H., LISON D. Influence of genetic polymorphisms on biomarkers of exposure and genotoxic effects in styrene-exposed workers. Environmental and Molecular Mutagenesis, submitted

MATEUCA R., AKA P., DE BOECK M., KIRSCH-VOLDERS M., LISON D. Influence of hOGG1, XRCC1 and XRCC3 genotypes on biomarkers of genotoxicity in workers exposed to cobalt or hard metal dusts. In preparation.

Presentations:

M. KIRSCH-VOLDERS In vitro Micronucleus Test: EMS Environmental Mutagen Society, Washington D.C., March 1999

M. KIRSCH-VOLDERS Aneuploidy *in vitro* and *in vivo*: EEMS European Environmental Mutagen Society, Copenhagen, July 1999

M. KIRSCH-VOLDERS. Latest issues in the development of genotoxicity test guidelines. Report of the Washington workshops : recommendations on *in vitro* micronucleus test approaches. EEMS : European Environmental Mutagen Society, Copenhagen, July 1999.

M. KIRSCH-VOLDERS The federal OSTC programme for the biomonitoring of workers exposed to mutagens/carcinogens. European Science Foundation meeting on Genetic Susceptibility, Copenhagen, September 1999.

M. KIRSCH-VOLDERS. Apoptosis induced by spindle inhibitors : Annual joined meeting of the French Society of Toxicology and Mutagenesis, Paris, November 1999.

M. KIRSCH-VOLDERS, I. DECORDIER and E. CUNDARI. Activation of apoptosis signalling pathways by microtubule interfering agents. 30th Annual Meeting of the European Environmental Mutagen Society, Budapest (Hungary), August 22-26, 2000.

M. KIRSCH-VOLDERS, N. TOUIL and M. DE BOECK. The global repair phenotype, assessed by the Comet assay, as a measurement of susceptibility. 2nd GENSUT Workshop (European Science Foundation), Cambridge (UK), September 14-17, 2000.

M. KIRSCH-VOLDERS Biomonitoring of Environmental Effects in Children. Conference on the Teplice Programme, Praha (Czech Republic), October 3-5, 2000.

M. KIRSCH-VOLDERS. Test d'aberrations de nombre de chromosomes. DEA National de Toxicologie, Paris, November 29-December 1, 2000.

M. KIRSCH-VOLDERS Perspectives in the application of the *in vitro* micronucleus test for hazard and risk assessment inlcuding IWGTP perspectives. Industrial Genotoxicology Group and Société Française de Toxicologie Génétique. Workshop on the *in vitro* Micronucleus Test, London, December 4, 2000.

M. KIRSCH-VOLDERS. Importance of detecting aneuploidy and polyploidy versus chromosome aberrations. Industrial Genotoxicology Group and Société Française de Toxicologie Génétique. Workshop on the *in vitro* Micronucleus Test, London, December 4, 2000.

M. KIRSCH-VOLDERS Consequences of air pollution on growth and on biomarkers of genotoxicity. Children Genotoxic Exposure Programme, WHO-INCHES, Copenhagen, January 2001.

M. KIRSCH-VOLDERS Micronucleus test as a multi end-point assay to detect apoptosis, necrosis, chromosome breakage and chromosome loss and its relevance to human biomonitoring studies, Turkish Society of Toxicology, Ankara, April 2001.

M. KIRSCH-VOLDERS The detection and hazard evaluation of aneuploidy induction by drugs and environmental chemicals. Department of Pharmacology of the Gazi University of Ankara, Ankara, April 2001.

M. KIRSCH-VOLDERS Relationship between a general repair phenotype and biomarkers of effects (DNA breakage and micronuclei) in low level chronic occupational exposure to ionising radiation. NATO Advanced Research Workshop, France, April 17-20, 2001.

M. KIRSCH-VOLDERS Cytogenetic biomarkers and human cancer risk. European Union Contact Meeting, IARC, France, April 20-21, 2001.

M. KIRSCH-VOLDERS Genotyping/phenotyping of DNA repair polymorphisms. European Union Contact Meeting, IARC, France, April 20-21, 2001.

M. KIRSCH-VOLDERS Micronucleus assay in human monitorng and the HUMN1 project. European Union Contact Meeting, IARC, France, April 20-21, 2001.

M. KIRSCH-VOLDERS Validation of the *in vitro* micronucleus assay for safety evaluation of clastogenic/aneugenic compounds. 8th International Conference on Environmental Mutagens, Shizuoka, Japan, October 21-26, 2001.

M. KIRSCH-VOLDERS Summary of the *in vitro* micronucleus group in the IWGT. 8th International Conference on Environmental Mutagens, Shizuoka, Japan, October 21-26, 2001.

M. KIRSCH-VOLDERS Micronucleus assay in human monitoring and the HUMN project. 8th International Conference on Environmental Mutagens, Shizuoka, Japan, October 21-26, 2001.

M. KIRSCH-VOLDERS Importance of detecting aneuploidy/polyploidy versus chromosome aberrations. 5th International symposium on Chromosomal aberrations - perspectives for the 21st century, Awaji Island, Japan, October 26-28, 2001.

M. KIRSCH-VOLDERS Application of the cytokinesis block micronucleus assay for biomonitoring purposes : inclusion of micronuclei in non-divided mononuclear lymphocytes and necrosis/apoptosis. Shangai satellite meeting for the 8th ICEM - 9th Alexander Hollander Course, Shangai, China, October 30-31, 2001.

M. KIRSCH-VOLDERS Test des Comètes et CBMN. Atelier SFTG, Faculté de Pharmacie, Marseille, France, Mai 29, 2002.

M. KIRSCH-VOLDERS Caractéristiques, place et prédicitivté des tests de génotoxicité au sein du biomonitoring des travailleurs exposés à des mutagènes-cancérogènes. Société Française de Toxicologie Génétique. Marseille, France, 2002.

M. KIRSCH-VOLDERS The micronucleus test : its use *in vitro* as an assay to assess genotoxicity and *ex vivo/in vitro* for biomonitoring purposes. XI Scientific Meeting of the Spanish Society of Environmental Mutagenesis, Bilbao, Spain, July 10-12, 2002.

M KIRSCH-VOLDERS, A. VANHAUWAERT, U. EICHENLAUB-RITTER AND I. DECORDIER "Indirect Mechanisms of genotoxicity." Eurotox meeting 2002, Budapest, Hungary, September 15-18, 2002.

M KIRSCH-VOLDERS, A. VANHAUWAERT, U. EICHENLAUB-RITTER AND , I. DECORDIER "Exemples de génotoxiques indirects: quelques exemples", SFTG meeting, Marseille, France, October 2002.

KIRSCH-VOLDERS M. Importance of genotyping and phenotyping in biomonitoring of occupational exposure. Fedichem meeting, November 2002

KIRSCH-VOLDERS M. Threshold evaluation and biomarkers in risk assessment. 7th Nordic Conference on Toxicology and Environmental Mutagens, Bornholm, June 2003

KIRSCH-VOLDERS M. Participation to the working group for the IARC evaluation of the evidence of carcinogenicity of metallic cobalt particles with or without tungsten carbide. IARC, Lyon, 7-14 October 2003

KIRSCH-VOLDERS M., DECORDIER I., AKA P., LOMBAERT N., VANHAUWAERT A., MATEUCA R., Simplicity and complexity of genetic susceptibility in the occupational environment. OSTC workshop on ethics, October 2003, Brussels

KIRSCH-VOLDERS M. Direct and indirect mechanisms of genotoxicity. 5th International Congress of the Turkish Society of Toxicology, Antalya, November 2003

Ph. D. Thesises:

N. TOUIL
Application of genotoxicity biomarker for chronic occupational exposure to ionsing radiation
PhD Thesis, Brussel, 2001

M. DE BOECK
Genotoxic effects of hard metal dust: possible relevance for lung cancer
PhD Thesis, Brussel, 2002

Reports in the framework of the OSTC project:

Audit summary report: Scientific support programme on the health protection of workers (1998-2003). Genotypic and phenotypic variability, individual susceptibility factors and industrial genotoxicants/neurotoxicants in occupational medicine. *M. KIRSCH-VOLDERS, L. DE RIDDER, C. LAURENT, D. LISON, H. THIERENS, H.VEULEMANS, P. VIELLE.* 2003.

Van gezondheidsrisico's… naar gezondheidsbescherming van de werknemers: 10 jaar federaal onderzoek inzake gezondheid, werk en milieu. *KIRSCH-VOLDERS M., VANHAUWAERT A., LISON D.* 2002

Des risques pour la santé… à la protection des travailleurs en matière de santé: 10 ans de recherche fédérale en santé, travail et environnement. *KIRSCH-VOLDERS M., VANHAUWAERT A., LISON D.* 2002

KUL

Publications:

VIAENE MK, PAUWELS W, VEULEMANS H, ROELS HA, MASSCHELEIN R. Neurobehavioural changes and persistence of complaints in workers exposed to styrene in a polyester boat building plant: influence of exposure characteristics and microsomal epoxide hydrolase phenotype, Occup Environ Med. 2001 Feb;58(2):103-12.

VAN NIMMEN-N, VEULEMANS-H. A highly sensitive gas chromatographic – mass spectrometric screening method for the determination of picogram levels of fentanyl, sufentanil and alfentanil in urine of potentially opioid exposed workers.
In preparation.

VAN NIMMEN-N, VEULEMANS-H. Development and validation of a highly sensitive gas chromatographic – mass spectrometric method for the simultaneous determination of (sub-)nanogram levels of fentanyl, sufentanil and alfentanil in breathing air and hand wipes of opioid exposed workers.
In preparation.

GODDERIS-L, BRAEKMAN-L, VIAENE-MK. Neurobehavioral and clinical effects in workers still exposed and formerly exposed to CS_2 in a viscose rayon industry. A cross-sectional study. In preparation.

GODDERIS-L, VANHAECHT-K, MASSCHELEIN-R, SERMEUS-W, VEULEMANS-H Prevention Pathways: Application of the Critical Path Methodology in Occupational Health Services. In preparation.

N. VAN NIMMEN, K. POELS, H. VEULEMANS. A highly sensitive gas chromatographic – mass spectrometric screening method for the determination of picogram levels of fentanyl, sufentanil and alfentanil and their major metabolites in urine of opioid exposed workers. Accepted for publication in The Journal of Chromatography B.

N VAN NIMMEN, H VEULEMANS. Development and validation of a highly sensitive gas chromatographic – mass spectrometric screening method for the simultaneous determination of nanogram levels of fentanyl, sufentanil and alfentanil in air and surface contamination wipes. Prsently being revised for publication in the Journal of Chromatography A.

GODDERIS L., DE BOECK M., HAUFROID V., EMMERY M., MATEUCA R., GARDINAL S., KIRSCH-VOLDERS M., VEULEMANS H., LISON D. Influence of genetic polymorphisms on biomarkers of exposure and genotoxic effects in styrene-exposed workers. Environmental and Molecular Mutagenesis, submitted

Presentations:

L GODDERIS 'Neurobehavioral and clinical effects in workers still exposed and formerly exposed to CS_2 in the viscose rayon industry: A cross-sectional study.' 8th International Symposium on Neurobehavioral Methods and Effects in Occupational and Environmental Health. Brescia, june 23-26, 2002

N VAN NIMMEN 'Het meten van blootstelling aan opium-achtige stoffen in de farmaceutische industrie'. Symposium Nederlandse Vereniging voor Arbeidshygiëne. Zeist, 20 en 21 maart 2003.

L GODDERIS Monitoring van genotoxische agentia: Een overzicht aan de hand van styreen. Symposium Nederlandse Vereniging voor Arbeidshygiëne. Zeist, 20 en 21 maart 2003.

RUG (promotor H. Thierens)

Papers in international journals:

VRAL A., THIERENS H., BRYANT P. AND DE RIDDER L. The higher micronucleus yield in B versus T lymphocytes after low dose γ-irradiation is not linked with defective Ku86 protein. Int. J. Rad. Biol., 77(3), 329-339, 2001.

LAMBERT V., THIERENS H., MONSIEURS M., RONCACIO C. AND LAURENT C. Translocation frequencies measured in patients one year after radioactive iodine therapy for thyrotoxicosis. Int. J. Radiat. Biol. 77, 679-685 ,2001.

VRAL A., THIERENS H., BAEYENS A. AND DE RIDDER L. The G2 and Micronucleus assays for human blood lymphocytes as biomarkers of individual sensitivity towards ionising radiation: limitations imposed by intra-individual variability. Radiation Research, 157, 472-477, 2002.

TOUIL N., AKA P., BUCHET J.-P., THIERENS H. and KIRSCH-VOLDERS M. Assessment of genotoxic effects related to chronic low level exposure to ionising radiation using biomarkers for DNA damage and repair. Mutagenesis 17, 223-232, 2002.

THIERENS H., VRAL A., BARBE M. and DE RIDDER L. Micronucleus assay reveals no radiation effects among nuclear power plant workers. Health Physics 83, 178-182, 2002.

VOISIN P., BARQUINERO F., BLAKELY B., LINDHOLM C., LLOYD D., LUCCIONI C., MILLER S., PALITTI F., PRASANNA P., STEPHAN G., THIERENS H., TURAI I., WILKINSON D., WOIJCIK A. Towards a normalization of biological dosimetry by cytogenetics. Cellular and Molecular Biology 48, 501-504, 2002.

THIERENS H., VRAL A., BARBE M., MEIJLAERTS M., BAEYENS A. and DE RIDDER L. Chromosomal radiosensitivity study of temporary nuclear workers demonstrates the adaptive response induced by occupational exposure. Int. J. Rad. Biol., 78, 12, 1117-1126, 2002.

BAEYENS A., THIERENS H., CLAES K., POPPE B., MESSIAEN L., DE RIDDER L. & VRAL A. Chromosomal radiosensitivity in breast cancer patients with a known or putative genetic predisposition. British Journal of Cancer, 87, 1379-1385 ,2002.

VRAL A., THIERENS H., BAEYENS A. AND DE RIDDER L. Induction and disappearance of G2 chromatid breaks in lymphocytes after low doses of low LET gamma-rays and high LET fast neutrons. Int. J. Rad. Biol., 78, 4, 249-257, 2002.

THIERENS H., VRAL A., AOUSALAH B., BARBE M. AND DE RIDDER L. A chromosomal radiosensitivity study of a population of radiation workers using the micronucleus assay. International Journal of Low Radiation, 1, 1,102-112, 2003

VRAL A., BAEYENS A., THIERENS H. AND DE RIDDER L. What's the reliability of chromosomal aberration assays as biomarkers of sensitivity towards ionising radiation? International Journal of Low Radiation, 1, 2, 256-265, 2004.

Presentations at scientific meetings and meetings in the framework of the programme:

THIERENS H., VRAL A., MONSIEURS M. AND DE RIDDER L. Patient studies in Ghent: results related to radiosensitivity. In Proceedings of the 10 th Gray Workshop on "Chromosomal Radiosensitivity: Mechanisms and Applications"-St Andrews, Scotland, 13-16 July 2000.

THIERENS H., VRAL A., MONSIEURS M., DIERCKX R. AND DE RIDDER L. Application of the micronucleus assay in the treatment of patients with metabolic radiotherapy. In Proceedings of the 30th Annual Meeting of the European Society for Radiation Biology, p.24- Warszawa, Poland, 27-31 August 2000

VRAL A., BAEYENS A., THIERENS H. AND DE RIDDER L. What's the reliability of chromosomal aberration assays as biomarkers of sensitivity towards ionising radiation? In "The effects of low and very low doses of ionizing radiation on human health." Proceedings of the Second International Symposium held at the Dublin Institute of technology - Dublin, Ireland, 27-29 June 2001, Edited by WONUC, World Council of Nuclear Workers.

THIERENS H., VRAL A., AOUSALAH B., BARBE M. AND DE RIDDER L. A chromosomal radiosensitivity study of a population of radiation workers using the micronucleus assay. In "The effects of low and very low doses of ionizing radiation on human health." Proceedings of the Second International Symposium held at the Dublin Institute of technology - Dublin, Ireland, 27-29 June 2001, Edited by WONUC, World Council of Nuclear Workers.

VRAL A., BAEYENS A., THIERENS H. AND DE RIDDER L. How reliable are chromosomal aberration assays as biomarkers of individual sensitivity towards ionising radiation? Abstractbook, p 77, S9/3 (oral presentation). 31th Annual Meeting of the

European Environmental Mutagen Society. "Genetic susceptibility at low dose exposure". Ghent, 1-5 September 2001.

THIERENS H., VRAL A., BARBÉ M., BAEYENS A. AND DE RIDDER L. Cytogenetic biomonitoring of external workers in the nuclear industry: study of exposure effects and susceptibility. Abstractbook, p 167, P3/11 (poster presentation). 31th Annual Meeting of the European Environmental Mutagen Society. "Genetic susceptibility at low dose exposure". Ghent, 1-5 September 2001.

VRAL A., THIERENS H., BAEYENS A. AND DE RIDDER L. Inter-versus intra-individual variation in G2 assay scores. Oral presentation at the Technical Workshop on the G2 assay, St Andrews University, Bute Medical Buildings, Scotland, 21-23 September 2001.

BAEYENS A., THIERENS H., CLAES K., MESSIAEN L., DE RIDDER L. AND VRAL A. Chromosomal radiosensitivity in lymphocytes of breast cancer patients with a family history of breast cancer. Cancer Detection and Prevention: 2002 Symposium Volume, S-114 , nr. 231 (poster presentation). 6th International Symposium on Predictive Oncology and Intervention Strategies, Pasteur Institute, Paris, France, 9-12 February, 2002.

BAEYENS A., THIERENS H., CLAES K., MESSIAEN L., DE RIDDER L. AND VRAL A. Chromosomal radiosensitivity in lymphocytes of breast cancer patients with a known or putative genetic predisposition. Abstractbook p. 178, III.P.6. (poster presentation). DNA Damage and Repair, Fundamental aspects and contribution to human disorders. 32nd Annual Meeting of European Environmental Mutagen Society, September 3-7, 2002 Warsaw, Poland.

VRAL A., THIERENS H., BAEYENS A. AND DE RIDDER L. Induction and disappearance of G2 chromatid breaks in lymphocytes after low doses of low LET gamma-rays and high LET fast neutrons. Abstractbook p.21 (oral presentation). Proceedings of the 32nd Annual Meeting of the European Society for Radiation Biology (ESRB), September 4-7, 2002, Liège, Belgium.

VRAL A., BAEYENS A., THIERENS H., CLAES K., MESSIAEN L. AND DE RIDDER L. Chromosomal radiosensitivity in lymphocytes of breast cancer patients with a known or putative genetic predisposition. Abstractbook p.80 (oral presentation). Proceedings of the 32nd Annual Meeting of the European Society for Radiation Biology (ESRB), September 4-7, 2002, Liège, Belgium.

THIERENS H., VRAL A., BARBÉ M., BAEYENS A. AND DE RIDDER L. Short term occupational exposure induces *in vivo* adaptive response in PBLs of radiation workers. Abstractbook p.88 (oral presentation). Proceedings of the 32nd Annual Meeting of the European Society for Radiation Biology (ESRB), September 4-7, 2002, Liège, Belgium.

BAEYENS A., THIERENS H., CLAES K., POPPE B., MESSIAEN L., DE RIDDER L. AND VRAL A. Chromosomal radiosensitivity in lymphocytes of breast cancer patients with a known or putative genetic predisposition. Oral presentation at the BEMS meeting held in Brussels, October 3, 2002.

VRAL A., BAEYENS A., THIERENS H., CLAES K., MESSIAEN L. AND DE RIDDER L. Chromosomal radiosensitivity in lymphocytes of breast cancer patients with a known or putative genetic predisposition (oral presentation). In: International Journal of Molecular Medicine, vl 10, suppl. 1, S8, 114, 2002. Proceedings of the abstracts of the 7th world congress on advances in oncology, 5th international symposium on molecular medicine, 10-12 October 2002, Hersonissos, Crete, Greece

VRAL A., THIERENS H., BAEYENS A. AND DE RIDDER L. Induction and rejoining of G2 chromatid breaks in lymphocytes after low doses of low LET gamma-rays and high LET fast neutrons (poster presentation). In: International Journal of Molecular Medicine, vl 10, suppl. 1, S8, 115, 2002. Proceedings of the abstracts of the 7th world congress on advances in oncology, 5th international symposium on molecular medicine, 10-12 October, 2002, Hersonissos, Crete, Greece

THIERENS H., VRAL A., BARBÉ M., MEIJLAERS M., BAEYENS A. AND DE RIDDER L. Chromosomal radiosensitivity is affected by recent exposures in nuclear workers. Abstractbook P17 (poster presentation), International Workshop: Biological basis of sensitivity to ionising radiation, DKFZ Heidelberg, May 9-10, 2003.

BAEYENS A., VRAL A., THIERENS H. AND DE RIDDER L., A comparison of the cytogenetic response of resting peripheral blood lymphocytes, IL-2 stimulated lymphocytes and EBV-transformed lymphoblastoid cells. Abstractbook P16 (poster presentation), International Workshop: Biological basis of sensitivity to ionising radiation, DKFZ Heidelberg, may 9-10, 2003.

BAEYENS A., VRAL A., THIERENS H. AND DE RIDDER L.. A comparison of the cytogenetic response to irradiation of resting peripheral blood lymphocytes, IL-2 stimulated lymphocytes and EBV-transformed lymphoblastoid cells. Abstractbook P59 p.81, From hazard to risk, European Environmental Mutagen Society, 33rd Annual meeting, Aberdeen, Scotland, UK, August 24-28, 2003

VRAL A., BAEYENS A., THIERENS H. AND DE RIDDER L.. Chromosomal aberrations and individual sensitivity towards ionising radiation. In: Toxicology Letters, vol 144 Suppl. 1, S9, 81, p. 25. Abstracts of the 41st Congress of the European Societies of Toxicology, EUROTOX 2003, Florence, Italy, Sept. 28-October 1, 2003., S9, 81

RUG (promotor L. de Ridder)

Publications:

paper 1

S. Bonassi, M. Fenech, C. Lando, Y. Lin, M. Ceppi, W.P. Chang, N. Holland, M. Kirsch-Volders, E. Zeiger, S. Ban, R. Barale, M.P. Bigatti, C. Bolognesi, C. Jia, M. Di Giorgio, L.R. Ferguson, kA. Fucic, O.G. Lima, P. Hrelia, A.P. Krishnaja, T-K. Lee, L. Migliore, L. Mikhalevich, E. Mirkova, P. Mosesso, W-U. Müller, Y. Odagiri, M.R. Scarfi, E. Szabova, I. Vorobtsova, A. Vral and A. Zijno. HUman MicroNucleus project: international database comparison for results with the cytokines-block micronucleus assay in human

lymphocytes: I. Effect of laboratory protocol, scoring criteria, and host factors on the frequency of micronuclei.
Environmental and Molecular Mutagenesis 37: 31-45 (2001).

paper 2

A. Vral, H. Thierens, P. Bryant and L. De Ridder. The higher micronucleus yield in B versus T lymphocytes after low dose γ-irradiation is not linked with defective Ku86 protein. Int. J. Rad. Biol., 77(3), 329-339, 2001.

paper 3

A. VRAL, H. THIERENS, A. BAEYENS AND L. DE RIDDER, The G2 and Micronucleus assays for human blood lymphocytes as biomarkers of individual sensitivity towards ionising radiation : limitations imposed by intra-individual variability. Radiation Research, 157, 472-477, 2002.

paper 4

BRYANT, P.E., GRAY, L., RICHES, A.C., STEEL, C.M., FINNON, P., HOWE, O., KESTERTON, I., VRAL, A., CURWEN, G.B., SMART, V., TAWN, E.J., WHITEHOUSE, C.A., The G2 assay: a technical report. Int. J. Rad. Biol., 78, 9, 863-866 (2002)

paper 5

FENECH M., BONASSI S., TURNER J., LANDO C., CEPPI M., CHANG W.P., HOLLAND N., KIRSCH-VOLDERS M., ZEIGER E., BIGATTI M.P., BOLOGNESI C., JIA C., DE LUCA G., DI GIORGIO M., FERGUSON L.R., FUCIC A., GARCIA LIMA O., HADJIDEKOVA V., HRELIA P., JAWORSKA A., JOKSIC G., KRISHNAJA A.P., LEE T-K., MARTELLI A., MCKAY M.J., MIGLIORE L., MIRKOVA E., MÜLLER W-U., ODAGIRI Y., ORSIERE T.T., SCARFÌ M.R., SILVA M.J., SOFUNI T., SURALLES J., TRENTA G., VOROBTSOVA I., VRAL A., ZIJNO A. Intra- and inter-laboratory variation in the scoring of micronuclei and nucleoplasmic bridges in binucleated human lymphocytes. Results of an international slide-scoring exercise by the HUMN project. Mutation Research. Genetic Toxicology and Environmental Mutagenesis 534: 45-64, 2002.

paper 6

H. THIERENS, A. VRAL, M. BARBE, M. MEIJLAERTS, A. BAEYENS and L. DE RIDDER, Chromosomal radiosensitivity study of temporary nuclear workers demonstrates the adaptive response induced by occupational exposure. Int. J. Rad. Biol., 78, 12, 1117-1126 (2002)

paper 7

A.BAEYENS, H. THIERENS, K. CLAES, B. POPPE, L. MESSIAEN, L. DE RIDDER & A.VRAL, Chromosomal radiosensitivity in breast cancer patients with a known or putative genetic predisposition. British Journal of Cancer, 87, 1379-1385 (2002)

paper 8

A. VRAL, H. THIERENS, A. BAEYENS AND L. DE RIDDER, Induction and disappearance of G2 chromatid breaks in lymphocytes after low doses of low LET gamma-rays and high LET fast neutrons. Int. J. Rad. Biol., 78, 4, 249-257, 2002.

paper 9

STEFANO BONASSI , MONICA NERI , CECILIA LANDO , MARCELLO CEPPI , YI-PING LIN, WUSHOU P. CHANG, NINA HOLLAND, MICHELINE KIRSCH-VOLDERS , ERROL ZEIGER, MICHAEL FENECH, AND THE HUMN COLLABORATIVE GROUP. Members of the HUMN collaborative group are: Sadayuki Ban, Hiroshima, Japan; Roberto Barale, Pisa, Italy; Maria Paola Bigatti, Turin, Italy; Claudia Bolognesi, Genoa, Italy; Cao Jia, Chong Qing, China; Marina Di Giorgio, Buenos Aires, Argentina; Lynnette R. Ferguson, Auckland, New Zealand; Aleksandra Fucic, Zagreb, Croatia; Patrizia Hrelia, Bologna, Italy; Ayyathan P. Krishnaja, Mumbai, India; Tung-Kwang Lee, Greenville, NC, USA; Lucia Migliore, Pisa, Italy; Ludmilla Mikhalevich, Minsk, Belarus; Ekaterina Mirkova, Sofia, Bulgaria; Pasquale Mosesso, Viterbo, Italy; Wolfgang-Ulrich Müller, Essen, Germany; Youichi Odagiri, Kofu, Japan; Maria Rosaria Scarfì, Naples, Italy; Elena Szabova, Bratislava, Slovak Republic; Irena Vorobtsova, St. Petersburg, Russia; Anne Vral, Ghent, Belgium; Andrea Zijno, Rome, Italy. Effect of smoking habit on the frequency of micronuclei in human lymphocytes: results from the Human MicroNucleus Project. Mutation Research 543: 155-166 (2003).

paper 10

H. THIERENS, A. VRAL, B. AOUSALAH, M. BARBE AND L. DE RIDDER. A chromosomal radiosensitivity study of a population of radiation workers using the Micronucleus assay. International Journal of Low Radiation, 1, 1,102-112, 2003

paper 11

A.VRAL, A. BAEYENS, H. THIERENS AND L. DE RIDDER. .What's the reliability of chromosomal aberration assays as biomarkers of sensitivity towards ionising radiation? International Journal of Low Radiation, 1, 2, 256-265, 2004.

Presenations at scientific conferences and seminars in the framework of the programme:

A. VRAL. Relationship between high chromosomal radiosensitivity in B lymphocytes and defective DNA repair(oral presentation). Radiation Research 2000, 10-12/4/2000, Bristol, UK

A. VRAL. Is there a correlation between high chromosomal radiosensitivity in B lymphocytes and defective DNA repair ? Oral presentation at 10th Gray Workshop- "Chromosomal Radiosensitivity: Mechanisms and applications", 13-16 juli 2000, St Andrews, Schotland.

A. VRAL. Is there a correlation between high chromosomal radiosensitivity in B lymphocytes and defective DNA repair ? Oral presentation at 30th Annual Meeting of the European Society for Radiation Biology: "European Radiation Research 2000", 27-31 augustus 2000, Warschau, Polen.

A. VRAL, A. BAEYENS, H. THIERENS AND L. DE RIDDER. What's the reliability of chromosomal aberration assays as biomarkers of sensitivity towards ionising radiation ?
In "The effects of low and very low doses of ionizing radiation on human health." Proceedings of the Second International Symposium held at the Dublin Institute of technology - Dublin, Ireland, 27-29 June 2001, Edited by WONUC, World Council of Nuclear Workers. Oral presentation of the paper during the meeting.

H. THIERENS, A. VRAL, B. AOUSALAH, M. BARBE AND L. DE RIDDER. A chromosomal radiosensitivity study of a population of radiation workers using the micronucleus assay. In "The effects of low and very low doses of ionizing radiation on human health." Proceedings of the Second International Symposium held at the Dublin Institute of technology - Dublin, Ireland, 27-29 June 2001, Edited by WONUC, World Council of Nuclear Workers. Poster presentation.

A. VRAL, A. BAEYENS, H. THIERENS AND L. DE RIDDER. How reliable are chromosomal aberration assays as biomarkers of individual sensitivity towards ionising radiation? Abstractbook, p 77, S9/3 (oral presentation). 31th Annual Meeting of the European Environmental Mutagen Society. "Genetic susceptibility at low dose exposure". Ghent, 1-5 September 2001.

H. THIERENS, A. VRAL, M. BARBÉ, A. BAEYENS AND L. DE RIDDER. Cytogenetic biomonitoring of external workers in the nuclear industry : study of exposure effects and susceptibility. Abstractbook, p 167, P3/11 (poster presentation). 31th Annual Meeting of the European Environmental Mutagen Society. "Genetic susceptibility at low dose exposure". Ghent, 1-5 September 2001.

A. VRAL, H. THIERENS, A. BAEYENS AND L. DE RIDDER. Inter-versus intra-individual variation in G2 assay scores. Oral presentation at the Technical Workshop on the G2 assay, St Andrews University, Bute Medical Buildings, Scotland, 21-23 september 2001.

A. VRAL, H. THIERENS, A. BAEYENS AND L. DE RIDDER Induction and rejoining of G2 chromatid breaks in lymphocytes after low doses of low LET gamma-rays and high LET fast neutrons. Invited speaker at the International Workshop on Biological Effects of Ionising Radiation, Electromagnetic Fields and Chemical Toxic Agents, 2-6 october 2001, Sinaia, Romania.

A. BAEYENS, H. THIERENS, K. CLAES, L. MESSIAEN, L. DE RIDDER AND A. VRAL Chromosomal radiosensitivity in lymphocytes of breast cancer patients with a family history of breast cancer. Cancer Detection and Prevention : 2002 Symposium Volume, S-114 , nr. 231 (poster presentation). 6th International Symposium on Predictive Oncology and Intervention Strategies, Pasteur Institute, Paris, France, 9-12 Februari, 2002.

A. BAEYENS, H. THIERENS, K. CLAES, L. MESSIAEN, L. DE RIDDER AND A. VRAL Chromosomal radiosensitivity in lymphocytes of breast cancer patients with a known or putative genetic predisposition. Abstractbook p. 178, III.P.6. (poster presentation). DNA Damage and Repair, Fundamental aspects and contribution to human disorders. 32nd Annual Meeting of European Environmental Mutagen Society, September 3-7, 2002 Warsaw, Poland.

A. VRAL, H. THIERENS, A. BAEYENS AND L. DE RIDDER, Induction and disappearance of G2 chromatid breaks in lymphocytes after low doses of low LET gamma-rays and high LET fast neutrons. Abstractbook p.21 (oral presentation). Proceedings of the 32nd Annual Meeting of the European Society for Radiation Biology (ESRB), September 4-7, 2002, Liège, Belgium.

A. VRAL, A. BAEYENS, H. THIERENS, K. CLAES, L. MESSIAEN AND L. DE RIDDER. Chromosomal radiosensitivity in lymphocytes of breast cancer patients with a known or putative genetic predisposition. Abstractbook p.80 (oral presentation). Proceedings of the 32nd Annual Meeting of the European Society for Radiation Biology (ESRB), September 4-7, 2002, Liège, Belgium.

H. THIERENS, A. VRAL, M. BARBÉ, A. BAEYENS AND L. DE RIDDER. Short term occupational exposure induces in vivo adaptive response in PBLs of radiation workers. Abstractbook p.88 (oral presentation). Proceedings of the 32nd Annual Meeting of the European Society for Radiation Biology (ESRB), September 4-7, 2002, Liège, Belgium.

A. BAEYENS, H. THIERENS, K. CLAES, B. POPPE, L. MESSIAEN, L. DE RIDDER AND A. VRAL. Chromosomal radiosensitivity in lymphocytes of breast cancer patients with a known or putative genetic predisposition. Oral presentation at the BEMS meeting held in Brussels, October 3, 2002.

A. VRAL, A. BAEYENS, H. THIERENS, K. CLAES, L. MESSIAEN AND L. DE RIDDER. Chromosomal radiosensitivity in lymphocytes of breast cancer patients with a known or putative genetic predisposition (oral presentation). In: International Journal of Molecular Medicine, vl 10, suppl. 1, S8, 114, 2002. Proceedings of the abstracts of the 7th world congress on advances in oncology, 5th international symposium on molecular medicine, 10-12 october, 2002, Hersonissos, Crete, Greece

A VRAL , H. THIERENS, A. BAEYENS AND L. DE RIDDER. Induction and rejoining of G2 chromatid breaks in lymphocytes after low doses of low LET gamma-rays and high LET fast neutrons (poster presentation). In: International Journal of Molecular Medicine, vl 10, suppl. 1, S8, 115, 2002. Proceedings of the abstracts of the 7th world congress on advances in oncology, 5th international symposium on molecular medicine, 10-12 october, 2002, Hersonissos, Crete, Greece

A. BAEYENS, H. THIERENS, K. CLAES, B. POPPE , L. MESSIAEN, L. DE RIDDER AND A. VRAL. Chromosomal radiosensitivity in lymphocytes of breast cancer patients with a known or putative genetic predisposition. Oral presentation presented on " Workshop on Chromosomal radiosensitivtity in breast cancer patients", Universitätsklinikum Ulm, Germany, 25-26 november, 2002.

A. VRAL , H. THIERENS, A. BAEYENS AND L. DE RIDDER. What's the reliability of chromosomal aberration assays as biomarkers of individual sensitivity towards ionising radiation? Oral presentation presented on "Workshop on Chromosomal radiosensitivtity in breast cancer patients", Universitätsklinikum Ulm, Germany, 25-26 november, 2002.

H. THIERENS, A. VRAL, M. BARBÉ, , M. MEIJLAERS, A. BAEYENS AND L. DE RIDDER. Chromosomal radiosensitivity is affected by recent exposures in nuclear workers. Abstractbook P17 (poster presentation), International Workshop: Biological basis of sensitivity to ionising radiation, DKFZ Heidelberg, may 9-10, 2003.

A. BAEYENS, A. VRAL, H. THIERENS AND L. DE RIDDER, A comparison of the cytogenetic response of resting peripheral blood lymphocytes, IL-2 stimulated lymphocytes and EBV-transformed lymphoblastoid cells. Abstractbook P16 (poster presentation), International Workshop: Biological basis of sensitivity to ionising radiation, DKFZ Heidelberg, may 9-10, 2003.

A. VRAL, A. BAEYENS, H. THIERENS, K. CLAES, B. POPPE , L. MESSIAEN AND L. DE RIDDER. Chromosomal radiosensitivity in BRCA1 and 2 patients and healthy carriers. P156 (poster presentation), International Workshop: Biological basis of sensitivity to ionising radiation, DKFZ Heidelberg, may 9-10, 2003.

A. BAEYENS, A. VRAL, H. THIERENS AND L. DE RIDDER. A comparison of the cytogenetic response to irradiation of resting peripheral blood lymphocytes, IL-2 stimulated lymphocytes and EBV-transformed lymphoblastoid cells. Abstractbook P59 p.81, From hazard to risk, European Environmental Mutagen Society, 33rd Annual meeting, Aberdeen, Scotland, UK, August 24-28, 2003

A.VRAL, A. BAEYENS, H. THIERENS, K. CLAES, B. POPPE , L. MESSIAEN AND L. DE RIDDER. Chromosomal radiosensitivity in BRCA1 and 2 patients and healthy carriers. Abstractbook P60, p.81, From hazard to risk, European Environmental Mutagen Society, 33rd Annual meeting, Aberdeen, Scotland, UK, August 24-28, 2003

A. VRAL, A. BAEYENS, H. THIERENS AND L. DE RIDDER. Chromosomal aberrations and individual sensitivity towards ionising radiation. In: Toxicology Letters, vol 144 Suppl. 1, S9, 81, p. 25. Abstracts of the 41st Congress of the European Societies of Toxicology, EUROTOX 2003, Florence, Italy, Sept. 28-October 1, 2003., S9, 81

A. VRAL, A. BAEYENS, H. THIERENS, K. CLAES, B. POPPE, L. MESSIAEN AND L. DE RIDDER. Chromosomal radiosensitivity in BRCA1 and 2 patients and healthy mutation carriers (oral presentation). In: International Journal of Molecular Medicine, vol 12, suppl. 1, S71, 366, 2003. Proceedings of the abstracts of the 8th world congress on advances in oncology, 6th international symposium on molecular medicine, 16-18 october, 2003, Hersonissos, Crete, Greece

UCL (promotor D. Lison)

Publications:

M. DE BOECK, S LARDAU, JP BUCHET, M. KIRSCH-VOLDERS, D. LISON (2000) Absence of significant genotoxicity in lymphocytes and urine from workers exposed to moderate levels of cobalt-containing dust : a cross-sectional study. Environ Mol Mutagen 36: 151-160.

LISON D. – Composés métalliques. In "Les cancers professionnels – Tome 1 – J.C. Pairon, P. Brochard, J.-P. Le Bourgeois, R. Ruffié. Edition Margaux
Orange. chapitre 6, 81 –95, 2000.

HAUFROID V, GARDINAL S, LICOT C, VILLALPANDO ML, VAN OBBERGH L, CLIPPE A, LISON D (2000) Biological monitoring of exposure to sevoflurane in operating room personnel by the measurement of hexafluoroisopropanol and fluoride in urine. Biomarkers 5:141-151

D. LISON (2000) Lung fibrosis reported in a dental technician (letter). Am Industrial Hygiene Association J 61 : 158.

D. LISON (2000) letter to the Editor. Toxicol appl Pharmacol 168:173-175.

HAUFROID V, BUCHET JP, GARDINAL S, GHITTORI S, IMBRIANI M, LISON D (2001) Importance of genetic polymorphisms for enzymes involved in the biotransformation of styrene in humans and interest for interpretation of biomarkers of exposure. Biomarkers 6:236-249

T NOMIYAMA, V. HAUFROID, J-P BUCHET, H MIYAUCHI, S TANAKA, T YAMAUCHI, S IMAMIYA, Y SEKI, K OMAE, D LISON (2001) Insertion polymorphism of CYP2E1 and urinary N-methylformamide after N,N-dimethylformamide exposure in Japanese workers. Int Arch Occup Environ Health 74(3):224-228 .

HAUFROID V., TOUBEAU F., CLIPPE A., BUYSSCHAERT M., GALA JL., LISON D. (2001) Real-time quantification of cytochrome P4502E1 (CYP2E1) mRNA in human peripheral blood lymphocytes by RT-PCR : Method and practical application. Clin Chem 47:1126-1129

D LISON, M DE BOECK, V VEROUGSTRAETE, M KIRSCH-VOLDERS (2001) Update on the genotoxicity and carcinogenicity of cobalt compounds. Occup Environ Med 58:619-625

LIGOCKA D., LISON D., HAUFROID V. (2001) Quantitative analysis of 5-hydroxy-N-methylpyrrolidone in urine for biological monitoring of N-methylpyrrolidone exposure. J Chromat B 778:223-230.

M KIRSCH-VOLDERS, M DE BOECK, D LISON (2002) Génotoxicité et activité professionnelle- l'Encyclopédie médico-chirurgicale (Paris). 16-537-C10, 9 pages.

KIRSCH-VOLDERS M, DE BOECK M, LISON D (2002) Tests de génotoxicité. Encyclopédie médico-chirurgicale 16-537-C11, 4 pages.

*NEMERY B, BAST A, BEHR J, BORM PJA, BOURKE SJ, CAMUS PH, DE VUYST P, JANSEN HM, KINNULA VL, LISON D, PELKONEN O, SALTINI **C*** (2001) Interstitial lung disease induced by exogenous agents : factors governing susceptibility. Eur Respi J 18:30s-42s.

V. HAUFROID, J.-P. BUCHET, S. GARDINAL AND D. LISON. (2002a) Cytochrome P4502E1 phenotyping by the measurement of the chlorzoxazone metabolic ratio: evaluation of its usefulness in workers exposed to styrene. Int Arch Occup Environ Health 75:453-458

HAUFROID V, JAKUBOWSKI M, JANASIK B, LIGOCKA D, BUCHET JP, BERGAMASCHI E, MANINIP, MUTTI A, GHITTORI S, ARAND M, HANGEN N, OESCH F, HIRVONEN A, LISON D (2002b) Interest of genotyping and phenotyping of drug-metabolizing enzymes for the interpretation of biological monitoring of exposure to styrene. Pharmacogentics 12:691-702.

HAUFROID V AND LISON D. (2002c) Genotyping and phenotyping of metabolic enzymes relevant for the interpretation of biomarkers of exposure. Arch Public Health, 60, 187-202

DE BOECK M., LOMBAERT N., DE BACKER S., FINSY R. , LISON D., KIRSCH-VOLDERS M. (2003) In vitro genotoxic effects of different combinations of cobalt and metallic carbide particles. Mutagenesis 18:177-186

LIGOCKA D., LISON D, HAUFROID V. (2003) Contribution of CYP2E1 to N-Methyl-2-Pyrrolidone metabolism. Arch Toxicol 77: 261-266

HAUFROID V., LIGOCKA D., BUYSSCHAERT M., HORSMANS Y., LISON D. (2003) Cytochrome P4502E1 (CYP2E1) expression in peripheral blood lymphocytes : Evaluation in hepatitis C and diabetes. Eur J Clin Pharmacol 59: 29-33

L. GODDERIS, M. DE BOECK, V. HAUFROID, M. EMMERY, R. MATEUCA,, S. GARDINAL, M. KIRSCH-VOLDERS, H. VEULEMANS, D. LISON. (2004) Influence of genetic polymorphisms on biomarkers of exposure and genotoxic effects in styrene-exposed workers. (submitted)

V. VEROUGSTRAETE, A. MALLANTS, J.-P. BUCHET, B. SWENNEN, D. LISON (2004) Determinants of lung function changes in workers exposed to cobalt compounds: a 13-year follow-up" (in revision).

Oral presentations:

D. LISON. Health risks associated with exposure to cobalt-containing dusts. First Uruguayan Congress of Clinical Toxicology Montevideo April 1st 2000.

D. LISON. Genotoxicity study in workers exposed to cobalt-containing particles. Tripartite meeting VUB-UCL-Université Maastricht – Woluwé 14 April 2000.

V. HAUFROID AND D. LISON. Individual variations in the expression of enzymes involved in the biotransformation of styrene and influence on the interpretation of biomarkers of exposure. 26th international congress on occupational health. Singapore 27 August-1 September 2000.

D. LISON. Intégration du génotype dans le monitoring biologique d'exposition au styrène. Journées nationales de médecine du travail. Bruxelles, le 5 octobre 2000.

V. HAUFROID ET AL. Real time RT-PCR quantification of CYP2E1 expression as a tool for biomonitoring. Fifth International Symposium on Biological Monitoring in Occupational and Environmental Health, 18-21 September, Banff, Alberta, Canada.

D. LISON. Determinants of the genotoxicity of metallic compounds. European Environmental Mutagenesis Society - Gent, 5 September 2001.

D. LISON. Metals and toxicology. British Association for Lung Research - Leuven, 8 September 2001.

V. HAUFROID ET D. LISON. Outils de biologie moléculaire et biomonitoring d'exposition Ulg « Les jeudis de Médecine du travail » 28 février 2002

D. LISON. Applications du génotypage pour une meilleure interprétation de l'exposition professionnelle. Journée d'étude SSTC- 30 octobre 2003.

Verslagen in het kader van het DWTC project:

Audit summary report: Scientific support programme on the health protection of workers (1998-2003). Genotypic and phenotypic variability, individual susceptibility factors and industrial genotoxicants/neurotoxicants in occupational medicine. *M. KIRSCH-VOLDERS, L. DE RIDDER, C. LAURENT, D. LISON, H. THIERENS, H.VEULEMANS, P. VIELLE*. 2003.

Van gezondheidsrisico's… naar gezondheidsbescherming van de werknemers: 10 jaar federaal onderzoek inzake gezondheid, werk en milieu. *KIRSCH-VOLDERS M., VANHAUWAERT A., LISON D.* 2002

Des risques pour la santé… à la protection des travailleurs en amtière de santé: 10 ans de recherche fédérale en santé, travail et environnement. *KIRSCH-VOLDERS M., VANHAUWAERT A., LISON D*. 2002

UCL (promotor P. Vielle)

Publications:

- « La génétique comme outil de prévention de la santé au travail : perspectives et dangers », en préparation

- « Tests génétiques et risques professionnels : impact sur la prévention et l'indemnisation des maladies professionnelles », Archives of Public Health , à paraître, juin 2004

- « Les examens médicaux liés à la relation de travail : bref commentaire de la loi du 28 janvier 2003 », T. Gez. / Rev. Dr. Santé, à paraître

- « Les tests génétiques dans la relation de travail : nouvelle forme de contrôle ou amélioration de la santé au travail ? », Recherches sociologiques, Du contrôle à la responsabilisation. 2002, vol. XXXIII, n°1, pp. 49-66

- « Secret, génétique et emploi,… », Ethica Clinica, septembre 2001, n° 23, p. 19-21

- « Tests génétiques préalables à l'embauche et droit au respect de la vie privée », Annales d'études européennes, Bruxelles, Bruylant, 2000, pp. 275-318

- « L'application des techniques génétiques à la médecine contemporaine au regard de la Convention de biomédecine », T. Gez. / Rev. Dr. Santé, novembre-décembre 1999-2000

Presentations in the framework of colloquia, conferences and seminars:

« Tests génétiques et risque professionnel : impact sur la prévention et l'indemnisation des maladies professionnelles », présentation dans le cadre du colloque *Enjeux de la susceptibilité génétique en milieu professionnel*, SPF Politique scientifique, Bruxelles, le 30 octobre 2003

- Exposé relatif à la réglementation internationale et européenne concernant les examens génétiques prédictifs et tests VIH dans le cadre des relations de travail et le statut du consentement, Comité Consultatif de bioéthique, le 4 septembre 2001
- Séminaire pluridisciplinaire sur les modes de contrôle et éthique du compromis, Faculté des Sciences économiques, sociales et politiques, U.C.L. Présentation sur les tests génétiques en droit social, 14 février 2001

- Conférence sur « Les aspects juridiques des tests génétiques », 11ème journée médicale, organisée par le CHR, Liège, le 2 décembre 2000

- Conférence sur les tests génétiques en médecine du travail, le 17 octobre 2000 dans le cadre du cycle de formation continue des médecins du travail organisé par l'UCL

- Participation à un groupe de travail dans le cadre de la recherche d'orientation sur la relation entre la recherche épidémiologique concernant la santé au travail et la législation récente en matière de protection de la vie privée, SSTC, Faculté de médecine de la KUL, octobre 2000- mars 2001

Organisation of colloquia or seminars:

- Co-organisation et participation au colloque *Enjeux de la susceptibilité génétique en milieu professionnel*, SPF Politique scientifique, Bruxelles, le 30 octobre 2003

- Organisation d'un séminaire de présentation des résultats interdisciplinaire et interuniversitaire, le 6 novembre 2002, Louvain-la-Neuve

VUB

Ahsan H., Rundle A.G. (2003) Measures of genotype versus gene products: promise and pitfalls in cancer prevention. *Carcinogenesis* 24: 1429-1434.

Aka P., Mateuca R., Buchet J.-P., Thierens H., Kirsch-Volders M. hOGG1^{326} and XRCC1^{399} genotypes and the DNA strand break repair phenotype are predictive for genotoxic effects in workers exposed to low dose ionising radiation. *Radiation Research*, submitted.

Anard D., Kirsch-Volders M., Elhajouji A., Belpaeme K., Lison D. (1997) In vitro genotoxic effects of hard metal particles assessed by alkaline single cell gel and elution assays. *Carcinogenesis* 18(1):177-184.

De Boeck M. (2002) Genotoxic effects of hard metal dust: possible relevance for lung cancer. Proefschrift voorgelegd tot het behalen van de wettelijke graad van Doctor in de Wetenschappen.

De Boeck M., Lardau S., Buchet J.P., Kirsch-Volders M., Lison D. *(*2000a) sence of significant genotoxicity in lymphocytes and urine from workers exposed to moderate levels of cobalt-containing dust: a cross-sectional study. *Env. Mol. Mutagenesis* 36(2): 151-160.

De Boeck M., Touil N., De Visscher G., Vande P.A., Kirsch-Volders M. (2000b) Validation and implementation of an internal standard in comet assay analysis. *Mutat Res.* 469(2): 181-97.

Decordier I., Aka P., Lombaert N., Vanhauwaert A., Mateuca R., Kirsch-Volders M. Simplicity and complexity of genetic susceptibility in the occupational environment. *Archives of Public Health,* submitted.

Dypbukt J.M., Costa L.G., Manzo L., Orrenius S., Nicotera P. (1992) Cytotoxic and genotoxic effects of styrene-7,8-oxide in neuroadrenergic Pc 12 cells. *Carcinogenesis* 13:417-424.

Farah S.B. (1983) The *in vivo* effect of cobalt chloride on chromosomes. *Rev Brasil Genet VI* 3: 433-442.

Girre C., Lucas D., Hispard E., Menez C., Dally S., Menez J.F. (1994) Assessment of cytochrome P4502E1 induction in alcoholic patients by chlorzoxazone pharmacokinetics. *Biochem Pharmacol.* 47(9): 1503-8.

Godderis L., De Boeck M., Haufroid V., Emmery M., Mateuca R., Gardinal S., Kirsch-Volders M., Veulemans H., Lison D. Influence of genetic polymorphisms on biomarkers of exposure and genotoxic effects in styrene-exposed workers. *Environmental and Molecular Mutagenesis,* submitted.

Goode E.L., Ulrich C.M., Potter J.D. (2002) Polymorphisms in DNA repair genes and associations with cancer risk. *Cancer Epidemiol Biomarkers Prev* 11:1513-1530.

Halliwell B. and Gutteridge J.M. (1999) Free Radicals in Biology and Medicine. Oxford University Press, third edition.

Hartwig A. (2001) Zinc finger proteins as potential targets for toxic metal ions: differential effects on structure and function. *Antioxidants and Redox Signaling* 3(4): 625-34.

Hartwig A., Snyder R.D., Schlepegrell R., Beyersmann D. (1991) Modulation by Co(II) of UV-induced DNA repair, mutagenesis and sister-chromatid exchanges in mammalian cells. *Mutation Research* 248(1): 177-85.

Hartwig A, Dally H, Schlepegrell R. (1996) Sensitive analysis of oxidative DNA damage in mammalian cells: use of the bacterial Fpg protein in combination with alkaline unwinding. *Toxicol Lett.* 88(1-3):85-90.

Haufroid V., Jakubowski M., Janasik B., Ligocka D., Buchet J.P., Bergamaschi E., Manini P., Mutti A., Ghittori S., Arand M., Hangen N., Oesch F., Hirvonen A., Lison D. (2002) Interest of genotyping and phenotyping of drug-metabolizing enzymes for the interpretation of biological monitoring of exposure to styrene. *Pharmacogenetics* 12: 691-702.

IARC (1991) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Cobalt and Cobalt Compounds, vol 52., IARC Scientific Publications, Lyon.

Kasten U., Mullenders L.H., Hartwig A. (1997) Cobalt(II) inhibits the incision and the polymerization step of nucleotide excision repair in human fibroblasts. *Mutation Research* 383(1): 81-8.

Kirsch-Volders M., Fenech M. (2001) Inclusion of micronuclei in non-divided mononuclear lymphocytes and necrosis/apoptosis may provide a more comprehensive cytokinesis block micronucleus assay for biomonitoring purposes. *Mutagenesis* 16:51-58.

Lison D. (1996) Human toxicity of cobalt-containing dust and experimental studies on the mechanism of interstitial lung disease (hard metal disease). *Critical Reviews in Toxicology* 26(6): 585-616.

Lison D, Lauwerys R. (1990) *In vitro* cytotoxic effects of cobalt-containing dusts on mouse peritoneal and rat alveolar macrophages. *Environmental Research* 52(2): 187-98.

Lison D., Lauwerys R. (1995) The interaction of cobalt metal with different carbides and other mineral particles on mouse peritoneal macrophages. *Toxicology in vitro* 9(3): 341-347.

Lison D., Carbonnelle P., Mollo L., Lauwerys R., Fubini B. (1995) Physicochemical mechanism of the interaction between cobalt metal and carbide particles to generate toxic activated oxygen species. *Chem Res Toxicol.* 8(4): 600-6.

Lu A.L., Li X., Gu Y., Wright P.M., Chang D.Y. (2001) Repair of oxidative DNA damage: mechanisms and functions. *Cell Biochem Biophys* 35:141-170.

Lunn R.M., Langlois R.G., Hsieh L.L., Thompson C.L., Bell D.A. (1999) XRCC1 polymorphisms: effects on aflatoxin B1-DNA adducts and glycophorin A variant frequency. *Cancer Res.* 59(11): 2557-61.

Mateuca R., Aka P., De Boeck M., Kirsch-Volders M., Lison D. Influence of hOGG1, XRCC1 and XRCC3 genotypes on biomarkers of genotoxicity in workers exposed to cobalt or hard metal dusts. In preparation.

Moulin J.J., Wild P., Romazini S., Lasfargues G., Peltier A., Bozec C., Deguerry P., Pellet F., Perdrix A. (1998) Lung cancer risk in hard-metal workers. *American Journal of Epidemiology* 148(3): 241-8.

Resende de Souza-Nazareth H. (1976) Efeito do cloreto de cobalto em nao-disjunçao. *Cience e Cultura* 28(12): 1472-5.

Shen M.R., Jones I.M., Mohrenweiser H. (1998) Nonconservative amino acid substitution variants exist at polymorphic frequency in DNA repair genes in healthy humans. *Cancer Res.* 58(4): 604-8.

Shield A.J., Sanderson B.J. (2001) Role of glutathione S-transferase mu (GSTM1) in styrene-7,8-oxide toxicity and mutagenicity. *Environ Mol Mutagen* 37:285-289.

Touil N. (2001) Application of genotoxicity for chronic occupational exposure to ionising radiation. Proefschrift voorgelegd tot het behalen van de wettelijke graad van Doctor in de Wetenschappen.

Touil N., Vande Aka P., Buchet J.P., Thierens H., Kirsch-Volders M. (2002) Assessment of genotoxic effects related to chronic low level exposure to ionising radiation using biomarkers for DNA damage and repair. *Mutagenesis* 17(3):223-232.

Tuimala J., Szekely G., Gundy S., Hirvonen A., Norppa H. (2002) Genetic polymorphisms of DNA repair and xenobiotic-metabolizing enzymes: role in mutagen sensitivity. *Carcinogenesis* 23:1003-1008.

Van Goethem F., Lison D., Kirsch-Volders M. (1997) Comparative evaluation of the in vitro micronucleus test and the alkaline single cell gel electrophoresis assay for the detection of DNA damaging agents: genotoxic effects of cobalt powder, tungsten carbide and cobalt-tungsten carbide. *Mutation Research* 392(1-2): 31-43.

Wood R.D., Mitchell M., Sgouros J., Lindahl T. (2001) Human DNA repair genes. *Science* 291:1284-1289.

Wormhoudt L.W., Commandeur J.N., Vermeulen N.P. (1999) Genetic polymorphisms of human N-acetyltransferase, cytochrome P450, glutathione-S-transferase, and epoxide hydrolase enzymes: relevance to xenobiotic metabolism and toxicity. *Crit Rev Toxicol.* 29(1): 59-124.

KUL

ACGIH. 2003. TLVs and BEIs; Threshold Limit Values for Chemical Substances and Physical Agents & Biological Exposure Indices. Cincinnati.
Arand M, Muhlbauer R, Hengstler J, Jager E, Fuchs J, Winkler L, Oesch F. 1996. A multiplex polymerase chain reaction protocol for the simultaneous analysis of the glutathione S-transferase GSTM1 and GSTT1 polymorphisms. Anal Biochem 236:184-186.

Blasiak J, Kowalik J. 2000. A comparison of the in vitro genotoxicity of tri- and hexavalent chromium. Mutat Res 469:135-145.

Brenneman MA, Weiss AE, Nickoloff JA, Chen DJ. 2000. XRCC3 is required for efficient repair of chromosome breaks by homologous recombination. Mutat Res 459:89-97.

Christakopoulos A, Bergmark E, Zorcec V, Norppa H, Maki-Paakkanen J, Osterman-Golkar S. 1993. Monitoring occupational exposure to styrene from hemoglobin adducts and metabolites in blood. Scand J Work Environ Health 19:255-263.

De Boeck M, Lardau S, Buchet JP, Kirsch-Volders M, Lison D. 2000a. Absence of significant genotoxicity in lymphocytes and urine from workers exposed to moderate levels of cobalt-containing dust: a cross- sectional study. Environ Mol Mutagen 36:151-160.

De Boeck M, Lison D, Kirsch-Volders M. 1998. Evaluation of the in vitro direct and indirect genotoxic effects of cobalt compounds using the alkaline comet assay. Influence of interdonor and interexperimental variability. Carcinogenesis 19:2021-2029.

De Boeck M, Touil N, De Visscher G, Vande PA, Kirsch-Volders M. 2000b. Validation and implementation of an internal standard in comet assay analysis. Mutat Res 469:181-197.

Farmer PB. 1995. Monitoring of human exposure to carcinogens through DNA and protein adduct determination. Toxicol Lett 82-83:757-762.

Fustinoni S, Colosio C, Colombi A, Lastrucci L, Yeowell-O'Connell K, Rappaport SM. 1998. Albumin and hemoglobin adducts as biomarkers of exposure to styrene in fiberglass-reinforced-plastics workers. Int Arch Occup Environ Health 71:35-41.

Gambelunghe A, Piccinini R, Ambrogi M, Villarini M, Moretti M, Marchetti C, Abbritti G, Muzi G. 2003. Primary DNA damage in chrome-plating workers. Toxicology 188:187-195.

Goromaru T, Matsuura H, Yoshimura N. 1984. Identification and quantitative determination of fentanyl metabolites in patients by gas chromatography- mass spectrometry. Anesthesiology. 61: 73 - 77.

Haufroid V, Clippe A, Knoops B, Bernard A, Lison D. 1998. Genotyping in urine: an interesting tool for epidemiological studies. Clin Chem 44:2210-2211.

Hu JJ, Mohrenweiser HW, Bell DA, Leadon SA, Miller MS. 2002. Symposium overview: genetic polymorphisms in DNA repair and cancer risk. Toxicol Appl Pharmacol 185:64-73.

Ikeda M, Koizumi A, Miyasaka M, Watanabe T. 1982. Styrene exposure and biologic monitoring in FRP boat production plants. Int Arch Occup Environ Health 49:325-339.

Kharasch ED, Russell M, Mautz D, Thummel KE, Kunze KL, Bowdle TA, Cox K. 1997. The role of cytochrome P450 3A4 in alfentanil clearance: Implications for interindividual variability in disposition and perioperative drug interactions. Anesthesiology. 87 (1): 36 - 50.

Kogevinas M, Ferro G, Andersen A, Bellander T, Biocca M, Coggon D, Gennaro V, Hutchings S, Kolstad H, Lundberg I, . 1994. Cancer mortality in a historical cohort study of workers exposed to styrene. Scand J Work Environ Health 20:251-261.

Kolstad HA, Lynge E, Olsen J, Breum N. 1994. Incidence of lymphohematopoietic malignancies among styrene-exposed workers of the reinforced plastics industry. Scand J Work Environ Health 20:272-278.

Labroo RB, Paine MF, Thummel KE, Kharasch ED, Evan D. 1997. Fentanyl metabolism by human hepatic and intestinal cytochrome P450 3A4: implications for interindividual variability in disposition, efficacy, and drug interactions. Drug Metabolism and disposition. 25 (9): 1072 - 1080.

Lauwerys RR, Hoet P. 2001. Industrial Chemical Exposure; Guidelines for Biological Monitoring. Florida: CRC Press.

Luceri F, Fattori S, Luceri C, Zorn M, Mannaioni P, Messeri G. 2001. Gas chromatography-Mass Spectrometry of 6-β-OH-Cortisol/Cortisol ratio in human urine: a specific marker of enzymatic induction. Clin Chem Lab Med 39: 1234-1239.

Maki-Paakkanen J, Walles S, Osterman-Golkar S, Norppa H. 1991. Single-strand breaks, chromosome aberrations, sister-chromatid exchanges, and micronuclei in blood lymphocytes of workers exposed to styrene during the production of reinforced plastics. Environ Mol Mutagen 17:27-31.

Marczynski B, Peel M, Baur X. 2000. New aspects in genotoxic risk assessment of styrene exposure--a working hypothesis. Med Hypotheses 54:619-623.

Meuldermans W, Van Peer A, Hendrickx J, Woestenborghs R, Lauwers W, Heykants J, Vanden Bussche G, Van Craeyvelt H, Van der Aa P. 1988. Alfentanil pharmacokinetics and metabolism in humans. Anesthesiology. 69(4): 527-534.

Mohrenweiser HW, Wilson DM, III, Jones IM. 2003. Challenges and complexities in estimating both the functional impact and the disease risk associated with the extensive genetic variation in human DNA repair genes. Mutat Res 526:93-125.

Mutti A. 1999. Biological monitoring in occupational and environmental toxicology. Toxicol Lett 108:77-89.

Severi M, Pauwels W, Van Hummelen P, Roosels D, Kirsch-Volders M, Veulemans H. 1994. Urinary mandelic acid and hemoglobin adducts in fiberglass-reinforced plastics workers exposed to styrene. Scand J Work Environ Health 20:451-458.

Stanley T,H, Hague B, Mock DL. 1989. ral transmucal fentanyl citrate (lollipop) premedication in human volunteers. Anesth. Analg. 69: 21-27.

Sumner SJ, Fennell TR. 1994. Review of the metabolic fate of styrene. Crit Rev Toxicol 24 Suppl:S11-S33.

Tuimala J, Szekely G, Gundy S, Hirvonen A, Norppa H. 2002. Genetic polymorphisms of DNA repair and xenobiotic-metabolizing enzymes: role in mutagen sensitivity. Carcinogenesis 23:1003-1008.

Valaer AK, Huber T, Andurkar SV, Clark CR, DeRuiter J. 1997. Development of a gas chromatographic-mass spectrometric drug screening method for the N-dealkylated metabolites of fentanyl, sufentanil and alfentanil. J Chromatogr Sci 35: 461-466.

Vodicka P, Bastlova T, Vodickova L, Peterkova K, Lambert B, Hemminki K. 1995. Biomarkers of styrene exposure in lamination workers: levels of O6-guanine DNA adducts, DNA strand breaks and mutant frequencies in the hypoxanthine guanine phosphoribosyltransferase gene in T-lymphocytes. Carcinogenesis 16:1473-1481.

Vodicka P, Soucek P, Tates AD, Dusinska M, Sarmanova J, Zamecnikova M, Vodickova L, Koskinen M, de Zwart FA, Natarajan AT, Hemminki K. 2001. Association between genetic polymorphisms and biomarkers in styrene-exposed workers. Mutat Res 482:89-103.

Vodicka P, Stetina R, Koskinen M, Soucek P, Vodickova L, Hlavac P, Kuricova M, Necasova R, Hemminki K. 2002. New aspects in the biomonitoring of occupational exposure to styrene. Int Arch Occup Environ Health 75 Suppl:S75-S85.

Vodicka P, Tvrdik T, Osterman-Golkar S, Vodickova L, Peterkova K, Soucek P, Sarmanova J, Farmer PB, Granath F, Lambert B, Hemminki K. 1999. An evaluation of styrene genotoxicity using several biomarkers in a 3-year follow-up study of hand-lamination workers. Mutat Res 445:205-224.

Willens JS, Myslinski NR. 1993. Pharmacodynamics, pharmacokinetics and clinical uses of fentanyl, sufentanil and alfentanil. Heart & Lung. 22(3): 239-251.

RUG (promotor L. de Ridder)

Baria K., Warren C., Roberts S.A., West C.M. & Scott D. (2001) Chromosomal radiosensitivity as a marker of predisposition to common cancers? *Br J Cancer* 84: 892-896.

Bryant P.E. (1998)The signal model: a possible explanation for the conversion of DNA double-strand breaks into chromatid breaks. *Int J Radiat Biol* 73: 243-251.

Burrill W., Barber J.B.P., Roberts S.A., Bulman B. & Scott D. (2000) Heritability of chromosomal radiosensitivity in breast cancer patients: a pilot study with the lymphocyte micronucleus assay. *Int J Radiat Biol* 76: 1617-1619

Fenech M. (1993) The cytokinesis-block micronucleus technique: a detailed description of the method and its application to genotoxicity studies in human populations. *Mutat Res* 285: 35-44.

Papworth R., Slevin N., Roberts S.A. & Scott D. (2001) Sensitivity to radiation-induced chromosome damage may be a marker of genetic predisposition in young head and neck cancer patients. Br J Cancer 84 (6): 776-782.

Riches A.C., Bryant P.E., Steel C.M., Gleig A., Robertson A.J., Preece P.E. & Thompson A.M. (2001) Chromosomal radiosensitivity in G2-phase lymphocytes identifies breast cancer patients with distinctive tumour characteristics. *Br J Cancer* 85: 1157-1161.

Roberts S.A., Spreadborough A. R., Bulman B., Barber J.B.P., Evans D.G.R. and Scott D. (1999) Heritability of cellular radiosensitivity: a marker of low-penetrance predisposition genes in breast cancer? *Am J Hum Genet* 65: 784-794.

Scott D., Barber J.B.P., Levine E.L., Burrill W. and Roberts S.A. (1998) Radiation induced micronucleus induction in lymphocytes identifies a high frequency of radiosensitive cases among breast cancer patients: a test for predisposition? *Brit J Cancer* 77: 614-620.

Scott D., Barber J.B.P., Spreadborough A.R., Burrill W. and Roberts S.A. (1999) Increased chromosomal radiosensitivity in breast cancer patients: a comparison of two assays. Int J Radiat Biol 75: 1-10.

Terzoudi G.I., Jung T., Hain J., Vrouvas J., Margaritis K., Donta-Bakoyianni C., Makropoulos V., Angelakis P.H. and Pantelias G.E. (2000) Increased G2 chromosomal radiosensitivity in cancer patients: the role of cdk1/cyclin-B activity level in the mechanisms involved. *Int J Radiat Biol* 76: 607-615.

Vral A., Verhaegen F., Thierens H. and De Ridder L. (1994) The *in vitro* cytokinesis-block micronucleus assay: a detailed description of an improved slide preparation technique for the automated detection of micronuclei in human lymphocytes. *Mutagenesis* 9: 439-443.

Vral A., Louagie H., Thierens H., Philippe J., Cornelissen M., De Ridder L. (1998) Micronucleus frequencies in cytokinesis-blocked human B lymphocytes after low dose gamma-irradiation. *Int J Radiat Biol* 73(5): 549-555.

UCL (promotor D. Lison)

Godderis L., De Boeck M., Haufroid V., Emmery M., Mateuca R.,, Gardinal S., Kirsch-Volders M., Veulemans H., Lison D.. (2004) Influence of genetic polymorphisms on biomarkers of exposure and genotoxic effects in styrene-exposed workers. (submitted)

Haufroid V., Toubeau F., Clippe A., Buysschaert M., Gala JL., Lison D. (2001) Real-time quantification of cytochrome P4502E1 (CYP2E1) mRNA in human peripheral blood lymphocytes by RT-PCR : Method and practical application. Clin Chem 47:1126-1129

Haufroid V., Buchet J.-P., Gardinal S. and Lison D.. (2002a) Cytochrome P4502E1 phenotyping by the measurement of the chlorzoxazone metabolic ratio: evaluation of its usefulness in workers exposed to styrene. Int Arch Occup Environ Health 75:453-458

Haufroid V, Jakubowski M, Janasik B, Ligocka D, Buchet JP, Bergamaschi E, ManiniP, Mutti A, Ghittori S, Arand M, Hangen N, Oesch F, Hirvonen A, Lison D (2002b) Interest of genotyping and phenotyping of drug-metabolizing enzymes for the interpretation of biological monitoring of exposure to styrene. Pharmacogentics 12:691-702.

Haufroid V., Ligocka D., Buysschaert M., Horsmans Y., Lison D. (2003) Cytochrome P4502E1 (CYP2E1) expression in peripheral blood lymphocytes : Evaluation in hepatitis C and diabetes. Eur J Clin Pharmacol 59: 29-33

Ligocka D., Lison D, Haufroid V. (2003) Contribution of CYP2E1 to N-Methyl-2-Pyrrolidone metabolism. Arch Toxicol 77: 261-266

Nomiyama T, Haufroid V., Buchet J-P, Miyauchi H, Tanaka S, Yamauchi T, Imamiya S, Seki Y, Omae K, Lison D (2001) Insertion polymorphism of CYP2E1 and urinary N-methylformamide after N,N-dimethylformamide exposure in Japanese workers. Int Arch Occup Environ Health 74(3):224-228 .

UCL (promotor P. Vielle)

Bassett-Stanford M. « Genetic Testing in Employment : Employee Protection or Threat ? », *Suffolk University Law Review*, 1981, vol. XV, p. 1194

Convention de sauvegarde des droits de l'homme et des libertés fondamentales, Rome, le 4 novembre 1950, *M.B.*, 19 août 1955

Coutrot T. *Critique de l'organisation du travail*, Paris, la découverte, 1999, p. 76

Demet F., Manette R., Delooz P., Kreit D. *Les maladies professionnelles*, Bruxelles, De Boeck, 1996, p. 25 et 54

Draper E. « Risk by choice? Knowledge and power in the Hazardous Workplace », *Contemporary Sociology*, 1984a, vol. 213, November, p. 689

Draper E. « High-Risk Workers or High-Risk Work? Genetic Susceptibility Policies in the Hazardous Workplace », *International Journal of Sociology and social Policy*, 1986

Duraffourg J. « La relation santé-travail : une question complexe », in B. CASSOU (sous la direction de), *Les risques du travail. Pour ne pas perdre sa vie à la gagner*, Paris, la Découverte, 1985, p. 22

Epstein R.A. « The Legal Regulation of Genetic Discrimination: Old Responses to New Technology », *Boston University Law Review*, 1994, 74(1) : 1-23

Ewald F. « Génétique médicale, confidentialité et assurance », in *Ethique et génétique humaine*, 2ème symposium du Conseil de l'Europe sur la bioéthique, Strasbourg, 30 novembre – 2 décembre 1993, p. 159

Gostin L. O., Hodge J.G. « Genetic Privacy And The Law : An End to Genetics Exceptionalism », J*urimetrics*, 40 (1999), p. 23

Groupe européen d'éthique, Avis du groupe européen d'éthique des sciences et des nouvelles technologies auprès de la Commission européenne, *Aspect éthiques des tests génétiques dans le cadre du travail*, 28 juillet 2003

Lazzarini L. « What Lessons Can We Learn from the Exceptionalism debate (Finally)?, The journal of Law, Medicine and Ethics, vol. 29 :2, 2001, p.150

Lebeer G., Moulin M., Schmitz P., Vermiglio M. « Contribution à l'analyse des positions syndicales et patronales à l'égard des tests génétiques à l'embauche », *Médecine du travail et ergonomie*, vol. XXXIV, N°1-1997, p. 25

Lemmens T. « What About Your Genes ? Ethical, legal, and Policy Dimensions of Genetics in the Workplace », *Politics and the Life Sciences*, March 1997, p. 65.

Lemmens T., Austin L. « Les défis posés par la réglementation de l'utilisation de l'information génétique », *ISUMA*, vol. 2, n°3, Automne 2001, p. 5 (http://isuma.net/v02n03/lemmens/lemmens_f.shtml)

Lois coordonnées du 3 juin 1970 relatives à la réparation des dommages résultant des maladies professionnelles, *M. B.*, 27 août 1970

Murray T.H. « Warning: Screening Workers for Genetic Risk », *The Hastings Center Report*, February 1983, p. 6

Murray T. « Genetic secrets And future Diaries : is Genetic Information Different from Other Medical Information ? », dans M. A. Rothstein, ed. *, Genetic secrets : protecting Privacy and Confidentiality in the genetic Era*, New Haven : Yale University Press, 1997, p.71

Nelkin D., Brown M.S. *Workers at Risk : Voices from the Workplace*, Chicago, Chicago University Press , 1984

Nelkin D. « The Social Power of genetic Information », in D. J. Kevles and L. Hood, *The Code of codes. Scientific and Social Issues in the Human Genome Project*, Cambridge, Harvard University Press, 1992, p. 189

Posner N.A. « The Ethical Significance of Free Choice: A Reply to Professor West », *Harvard Law Review* 99: 1431-1448

Rawls, J. *Théorie de la justice*, Paris, Seuil, 1997, p. 168 et sv.

Ross L.F. « Genetic Exceptionalism vs. Paradigm Shift : lessons from HIV », *The Journal of Law, Medicine & Ethics*, vol 29 :2, summer 2001, pp. 141- 148

UNESCO, Projet de déclaration internationale sur les données génétiques humaines, Conférence générale, 32ème session, Paris, 2003

Viscusi W.K. *Risk by Choice : Regulating Health and Safety in th Workplace*, Cambridge, MA, Harvard University Press, 1983

Vogel L. *L'organisation de la prévention sur les lieux de travail, Un premier bilan de la mise en œuvre de la directive cadre communautaire de 1989*, Bruxelles, Bureau technique syndical européen pour la Santé et la Sécurité, 1994, p. 29

West R. « Authority, Autonomy and Choice : The Role of Consent in the Moral and politic vision of Franz Kafka and Richard Posner », *Harvard law Review*, 1985, 99 :384-428

Législation

1. DROIT INTERNATIONAL :

1.A. O.I.T. :

Convention N° 111 du 25 juin 1958 concernant la discrimination (loi 16/02/1977)
Convention N°139 du 24 juin 1974 sur le cancer professionnel – ratifiée par la Belgique (11/10/96)
Recommandation R147 du 24 juin 1974 sur le cancer professionnel
Convention C155 du 22 juin 1981 sur la sécurité et la santé des travailleurs (non ratifiée par la Belgique)
Recommandation N° 164 du 22 juin 1981 sur la sécurité et la santé des travailleurs
Convention N° 159 du 20 juin 1983 sur la réadaptation professionnelle et l'emploi des personnes handicapées
Convention C161 du 25 juin 1985 sur les services de santé au travail (non ratifiée par la Belgique).
Recommandation N° 112 du 24 juin 1959 sur les services de médecine du travail
Recommandation N° 171 du 26 juin 1985 sur les services de santé au travail
Recueil de directives pratiques sur la protection des données personnelles des travailleurs, adopté par l'OIT lors de la 267e session en novembre 1996

1.B. UNESCO

- Déclaration universelle sur le génome humain et les droits de l'homme, adoptée lors de la Conférence générale de l'UNESCO, 29ème session, 11 novembre 1997
- Projet de déclaration internationale sur les données génétiques humaines, Conférence générale, 32ème session, Paris, 2003

1.C. OMS

- Proposed interational guidelines of ethical issues in medical genetics and genetic services
- Déclaration commune adoptée par l'OMS et le BIT à l'issue de la consultation sur le sida et le lieu de travail

1.D Commission internationale de la médecine du travail

- Code international d'éthique à l'usage des professionnels de la santé du travail

2. DROIT EUROPEEN

2.A. Conseil de l'Europe

Recommandation R 89 (2) sur la protection des données à caractère personnel utilisées à des fins d'emploi
Recommandation 89 (14) sur les incidences éthiques de l'infection VIH dans le cadre sanitaire et social
Recommandation 92 (3) sur le test et le dépistage génétiques à des fins médicales
Recommandation R94 (11) sur le dépistage comme instrument de médecine préventive
Recommandation R (97) 5 relative à la protection des données médicales

Proposition de lignes directrices européennes – Comité d'experts sur l'utilisation des examens médicaux, Conseil de l'Europe
Conclusions du Conseil et des ministres de la santé des Etats membres, réunis au sein du conseil le 15 décembre 1988 concernant le sida et le lieu de travail
Convention de sauvegarde des droits de l'homme et des libertés fondamentales, adoptée le 4 novembre 1950
Charte sociale européenne, adoptée le 18 octobre 1961
Convention du Conseil de l'Europe pour la protection des droits de l'homme et de la dignité de l'être humain à l'égard des applications de la biologie et de la médecine ou Convention sur les droits de l'homme et la biomédecine , adoptée le 19 novembre 1996 (+ rapport explicatif) - non ratifiée par la Belgique

2.B Union européenne

- Directive 89/391/CEE du Conseil du 12 juin 1989 concernant la mise en œuvre de mesures visant à promouvoir l'amélioration de la sécurité et de la santé des travailleurs au travail, *J.O.C.E*., n°L138 du 29 juin 1989, pp. 1-8
- Directive 90/394/CEE du Conseil du 28 juin 1990 concernant la protection des travailleurs contre les risques liés à l'exposition à des agents cancérigènes au travail, *J.O.C.E*., N°L196 du 26 juillet 1990, p. 1-7 modifiée par la directive1999/38/CEE du Conseil du 29 avril 1999 modifiant pour la deuxième fois la directive 90/394/CEE concernant la protection des travailleurs contre les risques liés à l'exposition à des agents cancérigènes au travail, et l'étendant aux agents mutagènes, *J.O.C.E*., n°L138 du 1 juin 1999, pp. 66-69. .
- Directive 98/24/CEE du Conseil du 7 avril 1998 concernant la protection de la santé et de la sécurité des travailleurs contre les risques liés à des agents chimiques sur le lieu de travail, *J.O.C.E*., n°L131 du 5 mai 1998, pp. 11-23.
- Directive 2000/78/Ce du 27 novembre 2000 portant création d'un cadre général en faveur de l'égalité de traitement en matière d'emploi et de travail.
- Avis du groupe européen d'éthique des sciences et des nouvelles technologies auprès de la Commission européenne, *Aspect éthiques des tests génétiques dans le cadre du travail*, 28 juillet 2003

3. DROIT BELGE

1. A.R. 28 mai 2003 relatif à la surveillance de la santé des travailleurs, *M. B*., 16 juin 2003
2. Loi du 25 février 2003 anti discrimination
3. Loi du 28 janvier 2003 relative aux examens médicaux dans le cadre des relations de travail, *M. B*., 9 avril 2003
4. Loi du 4 août 1996 relative au bien-être des travailleurs lors de l'exécution de leur travail, M. B.,
5. Code sur le bien-être au travail
6. Loi du 8 décembre 1992 modifiée par la loi du 11 décembre 1998
7. AR du 3 février 2001 portant exécution de la loi du 8 décembre 1992
8. Règlement général pour la protection du travail, approuvé par les arrêtés du régent des 11 février 1946 et 27 septembre 1947 (RGPT)
9. Conventions collectives N°38, 38 *bis*, 38 *ter* et 38 *quater* concernant le recrutement et la sélection de travailleurs
10. Convention collective N°9 du 9 mars 1972 coordonnant les accords nationaux et les conventions collectives de travail relatifs au Conseil d'entreprise conclu au sein du CNT
11. Convention collective de travail N° 39 du 13 décembre 1983 concernant l'information et la concertation sur les conséquences sociales de l'introduction de nouvelles technologies
12. Proposition de loi relative aux examens médicaux dans le cadre des relations de travail, *Doc. parl*., Sén., sess. ord 1995-1996, n° 1-127/1
13. Proposition de loi relative aux examens médicaux dans le cadre des embauchages, *Doc. parl*., Sén., sess. ord. 1998-1999, n° 1-1411/1
14. Proposition de loi relative aux examens médicaux dans le cadre des relations de travail, *Doc. parl*., Sén., sess. extraord. 1999, n° 2-20/1

Avis et recommandation du CNT
15. Recommandation n°10 du 25 juillet 1995 dans le cadre de l'égalité de traitement des travailleurs séropositifs.
16. - Avis n°1.160 du 23 juillet 1996 concernant le recueil de directives pratiques de l'OIT sur la protection des données personnelles.

Doctrine

PARTIE I : Compréhension scientifique et sociale du rôle de la génétique dans le processus pathologique

Ouvrages

- AMSELEK P., *Science, déterminisme, éthique et liberté*, Paris, Puf, 1988 (à lire)
- ANDORNO R., *La bioéthique et la dignité de la personne*, Paris, PUF, Médecine et société, 1997, 127 p.
- ATLAN H., *La fin du « tout génétique » ? Vers de nouveaux paradigmes en biologie*, Paris, INRA, 1998, 91 p.
- ATLAN H., *Les étincelles de hasard*, Paris, seuil, 1999, 393p. (à lire)
- BUCHANAN A., BROCK D.W.,DANIELS N.,WILKER D., *From Chance to Choice. Genetics and Justice,* Cambridge, cambridge University Press, 2000, 398p. (à lire)
- CAYLA O., THOMAS Y., *Du droit de ne pas naître, A propos de l'affaire Perruche*, Paris, Gallimard, 2002, pp. 170.
- CHARLESWORTH M., *Bioethics in a Liberal Society*, Cambridge, 1993 (à lire)
- DOUAY S., *Travail et génétique. De la sélection préventive à la prévention sélective ?*, Paris L'Harmattan2003, 738
- DRAPER E., *Risky business – Genetic testing and Exclusionary practices in the Hazardous Workplace,* Cambridge, Cambridge University Press, 1991 (à lire)
- ELGER B., *Médecine prédictive et décisions procréatives et prénatales*, Chêne-Bourg (Suisse), éd. Médecine et hygiène, 1998, 112 p.
- FOX KELLER E., *Le siècle du gène*, Paris, Gallimard, 2003, 169 p.
- FRYDMAN R., *Dieu, la médecine et l'embryon*, Paris, O. Jacob, 1999, 358p.
- HENNAU-HUBLET C. et KNOPPERS B.-M. (dir.), *L'analyse génétique à des fins de preuve et les droits de l'homme*, *Aspects médico-scientifique, éthique et juridique*, Bruxelles, Bruylant, 1997, 509 p.
- HERMITTE M., *Travail, assurance : l'analyse du génome humain : liberté et responsabilités*, Paris, Association Descartes, 1992 (à lire)
- HOTTOIS G. et MISSA J.-N., *Nouvelle encyclopédie de bioéthique*, Bruxelles, De Boeck, 2001, pp.922
- HUBBARD R., WALD E., *Exploding the Gene Myth : how genetic information is Produced and Manipuled by Scientists, physicians, employers, insurance Companies, Educators and Law Enforcers*, Boston : Beacon Press, 1999, 225p.
- KAHN A. et ROUSSET D., *La médecine du XXIè siècle. Des gènes et des hommes,* Paris, France Loisirs, 181 p.
- KAHN A., *Et l'Homme dans tout ça ? Plaidoyer pour un humanisme moderne*, Paris, Nil, 2000376 p.
- KEMP P.,.RENDTORFF J., MATTSSON JOHNSEN N., Bioethics and Biolaw. Judgement of life, vol. 1 & Four ethical principles, vol. II, Copenhagen, 2000, 313 p., 177 p. (à lire)
- KEVLES D.J., *Au nom de l'eugénisme. Génétique et politique dans le monde anglo-saxon*, Paris, PUF, 1995 (à lire)
- KEVLES D.J., HOOD L., *The Code of Codes, Scientific and Social issues in The Human Genome Project,* Cambridge, Hervard University Press, 1992, 397 p.
- KNOPPERS B.-M., *Le génome humain : patrimoine commun de l'humanité* ?, Québec, Fides, 1999, 41 p.
- KNOPPERS B.-M., CADIET L., LABERGE C.-M., *La génétique humaine : de l'information à l'informatisation*, Litec, 1992,
- MALAUZAT M.-I., *Le droit face aux pouvoirs des données génétiques*, Presses universitaires d'Aix-Marseilles, 2000, 497 p.
- MILLIEZ J. , *L'euthanasie du fœtus Médecine ou eugénisme ?*, Paris, O. Jacob, 1999, p.218
- Mc GLEENAN T., WIESING U. and . EWALD F., *Genetics and insurance*, Bios, 1999, 125 p.
- RUFFIÉ J., *Naissance de la médecine prédictive*, Paris, O. Jacob, 1993, 477 p.
- SOUMASTRE S. (dir.), *Droit et génie génétique, Premier bilan international et européen des réglementations et des nouvelles politiques*, Paris, Elsevier, 1994, 162 p.
- TESTART J., *Des hommes probables. De la procréation aléatoire à la reproduction normative*, Paris, Seuil, 1999, 280 p.
- TILMANS-CABIAUX C. et DUCHENE J., *Les tests génétiques aujourd'hui. Destin ou liberté ?*, Namur, presses universitaires de Namur, 2002, 220 p.
- TILMANS-CABIAUX C. et DUCHENE J., *Risquer de naître. Médecine prénatale et tests génétiques*, Namur, presses universitaires de Namur, 2002, 214 p.

-VANDELAC L., *Recoder le monde et s'échapper de l'humanité…ou démocratiser le génie génétique*, Paris, Futuribles, 2001
- *La nouvelle génétique médicale*, Bruxelles, éd. De l'Université de Bruxelles, Science, santé et société, 1998, 142 p.
- Comité Consultatif de Bioéthique, CASSIERS L., VERMEERSCH E. (éd), *Hérédité tests génétiques et Société*, Bruxelles, De Boeck & Larcier, 2001, 139 p.
- *Conférence internationale du Conseil de l'Europe sur les questions bioéthiques soulevées par l'application de la biotechnologie*, Actes , Oviedo, 16-19 mai 1999, 368 p.
- *Ethique et génétique humaine*, acte du 2è symposium du conseil de l'Europe sur la bioéthique, Strasbourg, 30 novembre – 2 décembre 1993, 332 p.
- Basic ethical principles in european bioethics and biolaw, Autonomy, dignity, integrity and vulnerability, vol. 1, Partner's research, vol. II, Report to the european Commission of the BIOMED-II Project, Copenhagen, Barcelona, 2000, 426 p.372 p.
- The troubed Helix : Social and Psychological Implication of The New Human Genetics, Cambridge University ress, 1996 (à lire)

Thèses

- ABBAS K., *La génétique humaine au miroir du droit*, lilles 2 (2001 ?).
- BABIN M., Étude critique du risque professionnel, Nantes, décembre 2003

Articles

Génétique et emploi

- BASSETT-STANFORD M., « Genetic testing in Employment : Employee Protection or Threat ? », *Suffolk university Law Review*, 1981, vol. 15, pp. 1187-1217 (à lire)
- BICH M.-F., « Information génétique et emploi- droit science et conscience » , in *Droit de la personne « Les bio-droits », Actes des journées d'études strasbourgeoises de l'I.C.E.J.Sup*., sous la dir. De J.-L ; BAUDOUIN et S. LEBRIS, éd. Y. Blais, 1997, pp. 231-308
- DARBY A., « The individual, health hazardous Lifestyles, Diseases and Liability », *DePaul journal of Health Care Law*, (1999) 4, 787 ? ? (à lire)
- DIBIE D., « Discriminations biologiques et droit des contrats », in C. LABRUSSE-RIOU, *Le droit saisi par la biologie*, Paris, L.G.D.J., 1996, pp. 147-178.
- DRAPER E., « High-Risk Workers or High-Risk Work ? Genetic Susceptibility Policies in the Hazardous Workplace », *International Journal of Sociology and social Policy*, 1986, pp. 12-28
- DRAPER E., « Genetic Secrets : Social Issues of Medical Screening in a Genetic Age », Hasting Center report, 1991, 22(4) : S15-18
- EPSTEIN R.A., « The legal regulation of Genetic Discrimination : Old Responses to New technology », *Boston University law review*, jan. 1994, 74(1) :1-23
- GEVERS S., « Use of Genetic data : Employment and Insurance : An International Perspective », *Bioethics*, 7(1993) : 126-134
- GOSTIN L., « Genetic discrimination : The Use of Genetically Based and Pronostic tests by Employers and Insurers », *American Journal of law and medicine*, 1991, 17, 109
- GOSTIN L.O. & HODGE J.G., « Genetic Privacy and The Law : An End to Genetics Exceptionalism », *Jurimetrics*, 1999, 40, 21-31.
- GUAY H., KNOPPERS B.-M. et PANISSET I., « La génétique dans les domaines de l'assurance et de l'emploi », *Revue du barreau*, 1992, 183, pp. 335-
- HENDRIKS A., « Genetics, Human Rights and Employment. American and European perspectives », *MedLaw* (1997) 16 :557-565
- LAPPE M., « Ethical Issues in Testing for Differential Sensitivity to Occupational Medicine », *Journal of Occupational Medicine*, November 1983, 25(11) : 797-808
- LEBEER G., MOULIN M., SCHMITZ P.,. VERMIGLIO M., « Contribution à l'analyse des positions syndicales et patronales à l'égard des tests génétiques à l'embauche », *Médecine du travail et ergonomie*, vol. XXXIV, N°1-1997, pp. 21-27
- LE BRIS S., « Donnes-moi ton ADN, je te dirai qui tu es…ou seras- Questionnements autour de l'utilisation de l'information génétique en Europe », *Isuma*, vol.2, n°3, automne 2001, 19 p.

- LEMMENS T., « What About Your Genes ? Ethical, Legal and Policy Dimensions of genetics in the Workplace », Politics and Life Sciences, 1997 16(1) : 57-75
- LEMMENS T. et AUSTIN L., « Les défis posés par la réglementation de l'utilisation de l'information génétique, *Isuma*, vol.2, n°3, automne 2001, 20 p.
- LYON-CAEN G., « Génétique et droit du travail », *R.I.D.E*., 1993, pp. 61-74
-MILLER P.S., « Genetic Discrimination in the Workplace, Genetic Alliance », http://www.geneticalliance.org/geneticissues/eeoc.html
- MURRAY T.H., « Warning : Screening Workers for Genetic Risk », *The Hastings Center Report*, February 1983, pp.5-8.
- MURRAY T.H., « The Social Context of Workplace Screening », *The Hastings Center report*, October 1984, pp. 21-23.
- NATOWICZ, MARVIN R., ALPER J.S., JANE K., « Genetic Discrimination and The law », *American journal of Human Genetics*, 50(3) : 465-475, march 1992 (à lire)
- ROTHSEIN M.A., « Protecting Genetic Privacy : Why It is So Hard To Do » , *Human genome News*, http://www.ornl.gov/hgmis/publicat/hgn/v10n1/14roth.html
- ROTHSEIN M.A., « Employee Selection Based on Susceptibility to Occupational Illness », *Michigan Law Review* 81 :1379-1496
- SCHULTE P.A., LOMAX G.P., WARD E.M., COLLIGAN M.J., « Ethical Issues in the Use of Genetic Markers in Occupational Epidemiologic research », *JOEM*, vol. 41, 8, August 1999, p. 639-646.
- STULIC M., « Genetic non discrimination, Privacy and Property Rights, *E Law – Murdoch university Electronic journal of law*, vol. 7, N°2, June 2000, 19 p.
- SWELTZ E.L., « Genetic Testing in the Workplace : An Analysis of the Legal Implications », *Forum*, 19 :23-32, 1984.
- THÉBAUD-MONY A., « Vers la sélection génétique des travailleurs ? », *Le Monde Diplomatique*, avril 1999.
- VAN DAMME K., CASTELEYN L., CHELLINI E., « General considerations : Accuracy, relevance, « Need/Necessity », and Consequences of testing Practices in Occupational Medicine », *Int. J. Occup. Environ. Health*, vol.2, N°3, jul.-sept 1996, supplement S57-S61.
- VAN DAMME K., CASTELEYN L., Pertinence et aspects socio-éthique des tests génétiques en médecine du travail
- VAN DAMME K., CASTELEYN L., COLLARD A. et VAN DEN BERGHE H., « Tests génétiques sur le lieu de travail », *Travail et Bien-être*, mars 2002, pp. 25-28.
- VOGEL L., « Le point de vue de la Confédération Européenne des Syndicats sur l 'utilisation de tests génétiques dans le cadre des rapports de travail » http://www.etuc.org/tutb
- WERTZ D.C., « Professional Perspectives : A survey of Canadian Providers », *Health Law Journal*, 3 :59-130 (à lire)
- ZEITZ K., « Employer Genetic testing : A legitimate Screening Device or Another Method of discrimination ? », *Labor Law journal*, 1991, 230
- « problématique éthique posée par les tests génétiques dans un contexte professionnel touchant à l'emploi », Comité d'éthique de la Fédération des maisons médicales et collectifs de santé francophones, ss la présidence de P. de LOCHT, *Santé conjuguée*, janvier 1998, n° 3, pp. 18-25
- Council on Ethical and Judicial Affairs, American Medical Association, « Use of Genetic testing by Employers » , *JAMA*, October 2, 1991 – vol. 266, n°13, pp. 1827-1830

Génétique

- J.S. ALPER and J. BECKWITH, « Distinguishing Genetic From Non Genetic Medical Tests : Some Implications for Antidiscrimination Legisation », *Sci. Eng. Ethics*, 4 : 141-150
- R. BAYER, « Public Health Policy and the Aids Epidemic. N End to HIV Exceptionalism ? », *N. Engl. J. Med.*, 324 (1991) : 1500-04
- M.-H. BOULANGER et J. DHONT, « Protection de la vie privée et données génétiques : quelques considérations », *Cahiers du CRID*, N°16, pp. 163-185
- F. BOURGUIGNON et J. M. DUBY, « Médecine prédictive : nouvelles inégalités, nouvelles solidarités », *Risques*, n° 21, 1995, pp.
- C. BYK, « La déclaration universelle sur le génome humain et les droits de l'homme », *J.D.I.* 3, 1998, pp. 675-695
- J. DAVEY, « Future Imperfect : human Géenetics and Insurance », *Journal of Business Law*, 2000, pp. 587-617
- C. de SOLA, « Privacy and Genetic data. Cases of Conflict », *Law & Hum Gen rev*, 1/1994 : 173-184

- K. DIERICKS, « The belgian perspective on genetic screening », in R. CHADWICK et al., *The Ethics of genetic Screening*, Netherlands, Kluwer, 1999, pp. 43-53
- X. DIJON, « Le normal et l'absolu en génétique humaine », …, pp. 138-144
- H. DOUCET, « Le développement de la génétique – Quelles tâches pour l'éthique ?, *Isuma*, vol.2, n°3, automne 2001, 12 p.
- B . EDELMAN, « Génétique et liberté », *Droits*-13, 1991, pp. 31-42
- R. A. EPSTEIN, « The Legal regulation of Genetic discrimination Old resonses to new technology », *Boston University Law Review*, 1994, 74(1) : 1-23 (à lire)
- H. GUAY et B.-M. KNOPPERS, « Information génétique : qualification et communication en droit québécois, *R.G.D.*, 1990, 21, 545-606
- R. GOLD, « Hope, fear, and genetics, judicial responses to biotechnology », *Judicature genes and justice. The Growing Impact of the new Genetics on the Courts*, *AJS,* november-december 1999, vol. 83(3), 10 p.
- L. O. GOSTIN, J.G. HODGE, « Genetic Privacy And The Law : An End to Genetics Exceptionalism », J*urimetrics*, 40 (1999), p. 21-58.
- GRAEME T. L., « The most personal information of all : an appraisal of genetic privacy in the shadow of the human genome project », *International Journal of Law, Policy and the Family*, 10 (1996), 74-101
- C. LABERGE, « L'information génétique : Aspects scientifiques et médicaux », in B.-M. - KNOPPERS, L. CADIET, C.-M. LABERGE, *La génétique humaine : de l'information à l'informatisation*, Litec, 1992, pp. 9-22
- C. LABRUSSE-RIOU, « Les implications juridiques de la génétique », *Rev. dr. publ*., 1990, n°5, 1367-1384
- M. A. LAPPÉ, « Justice and the Limitations of Genetic Knowledge », dans T.F. MURPHY et M.A. LAPPÉ (dir.) *Justice and the Human Genome Project*, Berkeley, University of california Press, 1994, p. 156 (à lire)
- V. LAUNIS, « Genetic Discrimination », in *Genetic and Insurance*, ed. T. Mc. GLEENAN, U. WIESING & F. EWALD, Oxford, BIOS Scientific Publishers Ltd, 1999, pp. 35-47
- G. T. LAURIE, « The Most Personal infrmation of All : an Appraisal of genetic Privacy in the Shadow of The Human genome project », *International journal of Law, Policy and the Family* 10, (1996), 74-101
- Z. LAZZARINI, « What Lessons Can We learn from The Exceptionalism Debate (finally) ?, *The Journal of Law, Medicine & Ethics*, vol. 29 :2, sumer 2001, pp. 149-151
- T. LEMMENS, « Selective Justice, genetic Discrimination and Insurance : Should We Single Out Genes in Our Laws ? », *Revue de droit de Mc Gill*, vol. 45, 2000, p. 375
- T. LEMMENS, L. AUSTIN, « Les défis posés par la réglementation de l'utilisation de l'information génétique », *ISUMA*, vol. 2 N°3, 2001, 20 p.
- T. LEMMENS, L. AUSTIN, « Of Volume, depth and Speed : The challenges of Genetic Information », february 2001, 38 p.
- D. LOCHAK, « Diagnostic prénatal : le difficile passage de l'éthique au droit », in , pp.451-478
- T. MURRAY, « Genetic secrets And future Diaries : is Genetic Information Different from Other Medical Information ? », dans M. A. ROTHSEIN, ed. *, Genetic secrets : protecting Privacy and Confidentiality in the genetic Era*, New Haven : Yale University Press, 1997, pp.60-73
- D. NELKIN, « The Social Power of genetic Information », in D. J. KEVLES and L. HOOD, *The Code of codes. Scientific and Social Issues in the Human Genome Project*, Cambridge, Harvard University Press, 1992, pp.177-190.
- B. RENAUD, « le diagnostic postnatal de prédiction », in… …
- L.F. ROSS, « Genetic Exceptionalism vs. Paradigm Shift : lesssons from HIV », *The Journal of Law, Medicine & Ethics*, vol 29 :2, summer 2001, pp. 141- 148.
- M. A. ROTHSEIN, « Genetic Insurance and the Ethics of Genetic Counseling », *Molecular Genetic Medicine*, vol. 3, 1993, p. 169
- A. SOMMERVILLE V. ENGLISh, « Genetic Privacy : Orthodoxy or Oxymoron ? », *Journal of Medical Ethics*, 1999, 25 :144-150
- K. TAYLOR et R. MYKITIUK, « La génétique, la normalité et l'incapacité », *Isuma*, vol.2, n°3, automne 2001, 12 p.
- F. TERRÉ, « Génétique et sujet de droit »,
- M. TIBON-CORNILLOT, « Crise de la biologie, crise du droit : du code génétique à la biologisation des normes », *Droits* –18, 1993, pp. 119-134
- I. VACARIE, « Examens génétiques et médecine prédictive », *Rev. dr. sanit. soc*., 1993, n°3, pp. 429-
- E. WAGNER, « Genotechnologie, identité personnelle et intégrité physiue,. La biogénétique et les droits de l'Homme, Institut Grand-Ducal, section des sciences Morales et Politiques, 1997, pp. 1-20
- R.L.ZIMMERN, « Genetic testing : a Conceptual Exploration », *Journal of Medical Ethics* 1999, 25 : 151-156

Jurisprudence

- Norman Bloodsaw v. Lawrence, U.S. 9th Circuit Court of Appeals,

Génétique et droit au respect de la vie privée

- BLONDIAU P., « Protection de la personne et vie privée du travailleur au moment de la rupture du contrat », *Orientations*, juin/juillet 1992, p. 165-180
-BOSSU B., « Droits de l'homme et pouvoirs du chef d'entreprise : vers un nouvel équilibre », *Droit social*, 1994, p. 747.
-CLAUWAERTS H., « Le respect de la vie privée lors de la recherche d'un emploi et la sélection du personnel », *Rev. trav*., avril-mai-juin 1997, p.13-19
- DE SCHUTTER O., « La vie privée entre droit de la personnalité et liberté », *Rev. trim. dr. h*., 1999,
- DE SCHUTTER O., « La vidéosurveillance et le droit au respect de la vie privée », *Journal des procès*, N° 296, janvier 1996
- de TISSOT O., « La vie privée du salarié », *Droit social*, 1995, p. 224.
- DIERICKX K, *Genetische screening, gezondheid en ethiek. Een historisch, filosophisch en moraaltheologisch onderzoek naar de vooronderstellingen en mogelijkheidsvoorwaarden van een genetische screening* , Leuven, K.U.L., 1998, p. 291 et suiv.
- DRZEMCZEWSKI A., « La Convention européenne des droits de l'homme et les rapports entre particuliers », *Cah. dr. eur.*, 1980, p. 3-24
- JACQUES P., « Rol van de arbeidsgeneesheer », in B. OVERSTEYN, *Gezondheid en onderneming. Aids, alcohol, drugs en roken*, Brugge, Die Keure, 1989, p. 59
- JAMOULLE M., « La vie privée et le droit du travail », *Ann. Dr. Louv*., 1984, p. 51
- JUVIGNY P., « La protection des travailleurs face aux nouvelles techniques d'enregistrement et d'informatique », *Rev. Int. Trav*., vol. 114, n°3, nov.- déc. 1976, p. 275-290
- LABBE C., *Sida et assurances. Aspects médicaux, assurances de personnes, responsabilités et assurances de responsabilités*, Les dossiers du J.T., Bruxelles, Larcier, 1994, p.
- LAGASSE F., « La vie privée et le droit du travail », *Chr. D.S*., 1997, p. 417-435.
-LAMBERT P., « La Convention européenne des droits de l'homme et la protection de la vie privée », *Privacy*, Séminaire des 26 et 27 novembre 1998- Palais d'Egmont
- GANSHOF VAN DER MEERSCH W.J., « L'ordre public et les droits de l'homme », *J.T*., 1968, p. 658-663.
- HENDRICKX F., *Privacy en arbeidsrecht*, Brugge, Die Keure, 1999, p
- MAINGAIN B., « Vie privée, vie familiale, vie professionnelle. Chronique d'une privatisation annoncée », in *Droit comparé des personnes et de la famille*, *Liber Amicorum M.-T. MEULDERS-KLEIN*, Bruxelles, Bruylant, 1998, p. 429 et suiv
- NYS H., « Recht en AIDS », *Panopticon*, 1988, p. 19
- OVERSTEIN B., « Het recht op eerbiediging van het privé-leven », *R.W*., 1988-1989, (p. 490)
- PELES S., «L'informatique et la protection de la vie privée des travailleurs : approche juridique », *Chron. D. S*., 1990, p
- PIRENNE P., « Respect de la vie privée et travail sous surveillance », *Rev. trav*., avril-mai-juin 1997, p. 3-8
- RAUWS W., *Civielrechtelijke beëindigingswijzen van de arbeidsovereenkomst : nietigheid, ontbinding en overmacht*, Kluwer, 1987, 162
- RIGAUX F., *La protection de la vie privée et des autres biens de la personnalité*, Bruxelles, Bruylant, 1990,
- RIGAUX F., *La vie privée. Une liberté parmi les autres ?*, Bruxelles, Larcier, 1992, p.
- RIVERO J., « Les libertés publiques dans l'entreprise », *Droit Social*, 1982, p
- RUSSO C., « Article 8§1 », *in La convention européenne des droits de l'homme. Commentaire article par article*, sous la direction de L.-E. PETTITI E. DECAUX et P.-H. IMBERT, Paris, Economica, 1995, p.
- SCHYVENS H., « C.A.O. Nr 38 betreffende de werving en de selektie van werknemers : een stap in de richting van een volwaardig aanwervingsrecht ? », *Chron. D. S.,* 1984, 61-81
- SUDRE F., « Les obligations positives dans la jurisprudence européenne des droits de l'homme », *Rev. trim. D. H*., 1995, (p. 363)
- TREBILCOCK A.-M., « Le sida et le lieu de travail. Les orientations que suggèrent les normes de l'OIT », *Rev. int. trav*., 1989, n°1, p.37
.VANACHTER O., « Une convention collective de travail concernant le recrutement et la sélection de travailleurs », *Orientations*, janvier 1984, p. 18-19

- VAN EECKHOUTTE W., « Juridische aspecten bij aanwerving », Actes de la journée d'études « Privacy en anderen grondrechten in de onderneming. Naar een verantwoord ondernemingsbeleid », B.V.V.A. & ASSOCIARE, R.U. Gand, 26 novembre 1993, p. 25
- VANSWEEVELT T., *Le sida et le droit*, Bruxelles, 1990, n°34
- VAN VAERENBERG R., « Recrutement et sélection des travailleurs », *Orientations*, mars 1990, p. 65-70
- VELU J. et ERGEC R., *La convention européenne des droits de l'homme*, Bruxelles, Bruylant, 1990,
- VELU J., *Notes de droit public*, T.II, Presses Universitaires de Bruxelles, 1979-1980, p.863
- VELU J., *Le droit au respect de la vie privée*, Namur, Presses universitaires de Namur, 1974,p.
- VINCINEAU M., « Assurance et vie privée. Du vide légal à l'illicite », *R.B.D.I.*, 1994/2, p. 486
- VOGEL-POLSKI E., « Droit du travail comparé et sida », in *Le sida, un défi aux droits ?*, réf…, p. 708 et 709
- WARREN S. et BRANDEIS L., « The right to privacy », 4 *Harvard Law Review*, (1890), 193-220

PARTIE II : La notion de risque professionnel à l'épreuve de la génétique

1- Cadre théorique

Lecture de base :

- EWALD F., *L'Etat Providence*, Paris, Grasset, 1986, 608 p.

Du même auteur :

- EWALD F., *L'accident nous attend au coin de la rue, Les accidents de la circulation, Histoire d'un problème*, Paris, la documentation française, 1982.
- EWALD F., « Responsabilité-solidarité-sécurité, La crise de la responsabilité en France à la fin du Xxème siècle », *Risques* n°10, avr.-juin 1992, pp. 9-24
- EWALD F., « Génétique médicale, confidentialité et assurance », *Risques* n° 18, avr.-juin 1994, pp. 111-120
- EWALD F., « Génétique médicale, confidentialité et assurance », *Ethique et génétique médicale, Actes du 2ème symposium du Conseil de l'Europe sur la bioéthique, Strasbourg, 30 novembre – 2 décembre 1993*, Strasbourg, Les éditions du Conseil de l'Europe, 1994, pp. 155-172
- EWALD F., « L'expérience de la responsabilité », in *Qu'est-ce qu'être responsable ?*, Ouvr. Coll., Auxerre, Science humaine, Paris, mécénat seita, 1997, pp. 55-81
- EWALD F., « Génétique et assurance », *R.G.D.A.*, 1999, p. 539-555

Lectures complémentaires :
Ouvrages

- BEAUCHAMP A., *Gérer le risque, vaincre la peur*, Montréal, Bellarmin, 1996
- BERNSTEIN P.L., *Against the gods : The remarkable story of Risk*, New York, Wiley, 1998, p.
- BUCHANAN A., BROCK D.W. et al., *From Chance to Choice, Genetic and Justice*, Cambridge University Press, 2000 - CASSOU B. (sous la direction de), *Les risques du travail. Pour ne pas perdre sa vie à la gagner*, Paris, la Découverte, 1985, 640 p.
- CHIAPPORI P.-A., *Risque et assurance*, Paris, Flammarion, 1997, 122 p.
- DELMAS-MARTY M., *Pour un droit commun*, Paris, seuil, 1994, 306 p.
- DOUGLAS M. et WILDANSKY A., *Risk and Culture, an essay on the selection of technical and environental dangers*, University of california Press, 1982.
- DAHL RENDTORFF J. and KEMP P., *Basic Ethical Principles in European Bioethics and Biolaw, vol. I, Autonomy, Dignity, Intergity and vulnerability*, Report of the European Commission of the BIOMED-II Project, 1995-1998, Center for Ethics and Law, Copenhagen, Denmark and institut Borja de Bioetica, Barcelona, Spain, 2000, 428 p.
- DUCLOS D., *La santé et le travail*, Paris, la découverte, 1984, 123p.
- DUCLOS D., *La peur et le savoir*, *La société face à la science, la technique et leurs dangers,* Paris, la découverte, 1989, 307p.
- DUPEYROUX J.-J., *Droit de la sécurité sociale*, Paris, Dalloz, 13è éd., 19998, 1228 p.

- FISCHOFF B. et al., *Acceptable Risk*, Cambridge University Press, 1981
- GODARD O., Principe de précaution et responsabilité, une révision des relations entre science, décision et société, in *Qu'est-ce qu'être responsable ?*, Ouvr. Coll., Auxerre, Science humaine, Paris, mécénat seita, 1997, pp. 95-127.
-KEFER F., *Le droit pénal du travail*, Bruges, la Charte, 1997, pp.552
- LEBRETON D., *La sociologie du risque*, Paris, PUF, 1995.
- LEBRETON D., *Passions du risque*, Paris, Métailié, 1996.
- MEYER F. (Dir.), *L'évaluation des risques professionnels*, Strasbourg, Presses Universitaires de Strasbourg, 1995, 226 p.
- ROSANVALLON P. , *La crise de l'Etat-providence*, Paris, seuil, 1992, 184 p.
- SUPIOT A., *Critique de droit du travail*, Paris, PUF, 1994,
- THÉBAUD-MONY A., *La reconnaissance des maladies professionnelles*, Paris, la documentation française, 1991, 284.
- VAN LANGENDONCK J., *The new Social Risks*, London, The Hague, Boston, Kluwer Law International, EISS Yearbook, 1996, 261 p.
- *Quand le travail nuit à la santé, 50 ans de sécurité sociale… Et après ?,* Vol. 5, Bruxelles, Bruylant, 1995, 155 p.

Articles

- CADET B., « L'évaluation du risque : une construction subjective ? », *Risques*, n° 39, sept. 1999, pp. 72-78
-DE GERLACHE J., « Maîtriser dangers et risque : entre proportion et précaution », communication lors de la réunion SSTC le…….
- DE KEYSER V., « Le risque acceptable », *Revue de médecine du travail*, tome XX, n°5, 1993, pp. 237-245
- DUMOULIN A., « le support économique de l'assurance ou premiers pas vers une «Economie de la sécurité » , Bull. Ass., 1969, pp. 337-358
- MATTEI B., « La normalisation des accidents du travail : l'invention du risque professionnel », *Les temps modernes*, N° 354, janvier 1976, pp.
- MURRAY T.H., « Warning : Screening Workers for Genetic Risk », *Hastings Center Report*, 1983, 13(1) : 5-8
- TUNC A., « Risques et sécurité », J.T., 1972, pp. 457-461
- VAN DAMME K. et CASTELEYN L., « Ethical, Social and Scientific Problems Related to the Application of Genetic Screening and Genetic Monitoring for Workers in the Context of a European Approach to Health and Safety at Work », *La medicina del lavoro*, 1998, 89, supplément 12
- VAN DER VORST P., « Esquisse d'une théorie générale du « Risque professionnel » et du « Risque juridique » », *J.T.*, 1975, pp. 373-394

Presse :

- CASTEL R., « « Risquophiles », « risquophobes » : l'individu selon le Medef, *Le monde*, 7 juin 2001

Lectures de base en droit social

- ARNSPERGER C. et VAN PARIJS P., *Ethique économique et sociale*, Paris, la découverte et Syros, 2000, 123 p.
- BOLTANSKI L. et CHIAPELLO E., *Le nouvel esprit du capitalisme*, Paris, Gallimard, 1999, p. 843
- BOURDIEU P., *Les structures sociales de l'économie*, Paris, Seuil, 2000, p. 289
- CHLEPNER B.S., *Cent ans d'histoire sociale en Belgique*, Bruxelles, Éd. Université Libre de Bruxelles, 1972,
- CORNIL, M., *La sécurité sociale ou l'anti-responsabilité*, JT, 1964, pp. 181-182
- DE COSTER M., PICHAULT F., *Traité de sociologie du travail*, Bruxelles, De Boeck, 1994, 551 p.
- DENIS P., *Droit de la sécurité sociale*, Bruxelles, Larcier, 1993, 350 p.
- DUPEYROUX J.-J., *Droit de la sécurité sociale*, Paris, Dalloz, 1998 (13ème édition), p. 1228
- EYMARD-DUVERNAY F., MARCHAL E., Façons de recruter. Le jugement des compétences sur le marché du travail, Paris, Métailié, 1997
- ESPING-ANDERSEN G., *Les trois mondes de l'Etat-Providence, Essai sur le capitalisme moderne*, Paris, PUF, 1999, p. 310
- FITOUSSI J.P., ROSANVALLON P., *Le nouvel âge des inégalités*, Paris, Seuil, 1996
- GANGUILHEM G., *Le normal et le pathologique*, Paris, 1966,

- GORZ A., *Misères du présent richesses du possible*, Paris, Galilée, 1997
- JAMOULLE M., *Le contrat de travail*, Tome I et II, Faculté de Droit et d'Economie et des Sciences sociales de Liège, 1982
- NEUVILLE J., *La condition ouvrière au XIXème siècle, Histoire du mouvement ouvrier en Belgique*, Bruxelles, Editions vie ouvrière, 1976, T. 1 (L'ouvrier objet, p. 240) et T. 2 (L'ouvrier suspect, p. 271)
- POLANYI K., *La grande transformation, Aux origines politiques et économiques de notre temps*, Paris, Gallimard, 1983, p. 419
- SAINT-JOURS M., *La faute dans le droit général de la sécurité sociale, thèse et la réparation des risques professionnels*
- ROSANVALLON P., *La crise de l'Etat-providence*, Paris, seuil, 1992, p.184
- ROSANVALLON P., *Le capitalisme utopique : histoire de l'idée de marché*, Paris, Seuil, 1999
- ROSANVALLON P., *La nouvelle question sociale : repenser l'État Providence*, Paris Seuil, 1995
- SOURNIA J. C. ? *Ces malades qu'on fabrique, la médecine gaspillée*, Paris, Seuil, 1977 (le citoyen protégé devient-il irresponsable ?)
- SUPIOT A., *Critique de droit du travail*, Paris, PUF, 1994,
- SUPIOT A., *Le travail en perspective*, Paris, Librairie générale de droit et de jurisprudence, 1998, 640 p.
- SUPIOT A., *Au-delà de l'emploi,, transformations du travail et devenir du droit du travail en Europe, Rapport pour la Commission des Communautés européennes avec la collaboration de l'Université Carlos III de Madrid*, Paris, Flammarion, 1999, 321 p.
- P. VANDERVORST (dir.), *cent ans de droit social belge*, Mélanges offerts à Louis Duchatelet, Bruxelles, Bruylant, 1988, 923 p.
- J. VAN LANGENDONCK, *The new social risks*, EISS, kluwer int., série N°1, 1997
- *Le bel avenir du contrat de travail. Appel des économistes pour sortir de la pensée unique* (ouvrage collectif), Paris, Syros, 2000, 226 p.
- « A l'enseigne du droit social belge », *Revue de l'Université de Bruxelles*, sous la direction de P. Van Der Vorst, Edition de l'Université de Bruxelles, 1978/1-3

Lectures complémentaires droit social

- LUSSATO A., *Les tests de recrutement*, Que sais-je ?, PUF, 1998, p.

1. Le risque professionnel dans le secteur des maladies professionnelles

- CAVIGNEAUX A., AUTISSIER R., PORCHER B., *La réparation des accidents et des maladies professionnelles*, Paris, Masson, 1982
- CHARLES P., *Les particularités du régime de réparation des maladies professionnelles* , LLN ? UCL, 1974
- DE BROUWER C., *La médecine dans l'entreprise*, Bruxelles, de Boeck, 1997, 332 p.
- DELOZ P. et MANETTE P., Les maladies professionnelles du secteur privé, Chroniques de droit à l'usage du Palais, tome IV, Risque professionnel-Droit social et fiscal, 1989, Bruxelles, Story-Scientia, pp 1-117
- DEMET F., MANETTE R., DELOOZ P. et KREIT D., *Les maladies professionnelles*, Bruxelles, De Boeck et Larcier, 1996, 324 p.
- DESOILLE H., *Médecine du travail et maladies professionnelles*, Paris, Flammarion, 1957
- HAGUENOER J.-M., FRIMAT P., BONNETERRE J., VENNIN P, *Les cancers professionnels*, Paris, Lavoisier, 1982
- SOCHJER-ROUSSELLE M., *Droit de la sécurité et de la santé de l'homme au travail*, Bruxelles, Bruylant, collection droit social, 1979, 439 p.
- THÉBAUD-MONY A., *La reconnaissance des maladies professionnelles*, Paris, la documentation française, 1991, 284
- *Quand le travail nuit à la santé, 50 ans de sécurité sociale… Et après ?,* Vol. 5, Bruxelles, Bruylant, 1995, 155 p.

Encyclopédies

- *Encyclopédie médico-chirurgicale*, 2 tomes, Intoxications, Paris, Ed. Techniques

- *Encyclopédie de médecine, d'hygiène et de sécurité du travail*, 2 tomes, Genève, Bureau international du travail, 1974

Articles

- BETEMP J.-M., « L'amélioration de la réparation des accidents du travail et des maladies professionnelles », *Droit soc*., 1993, pp. 129-133
- BOLLAND M., « Lésions corporelles, accidents de travail et ordre public », *R.G.A.R*., 1994, 12311, 1-3
- BOLLAND M., « Etat antérieur et accidents du travail », *R.G.A.R*., 1993, 12113 -
- COLIN D. et DELFOSSE V., « Le champs d'application rationae materiae de la loi du 10 avril 1971 », *Chronique de droit à l'usage du palais*, t. VI, Risque professionnel – Droit social et fiscal, C.U.P., Story-Scientia, 1989, p. 1-25
- DAL A., « Les maladies professionnelles dans le secteur public », *R.G.A.R*., 1994, p. 12326, 1-14
- DE BRUCQ D., « maladie professionnelle hors liste, condition de causalité, arrêt de la Cour de cassation, 2 février 1998, FMP c/ V », *R.B.S.S*., 1999, p. 573-588
- DE BRUCQ D., « Une vaste enquête : Les maladies professionnelles en Europe, Déclaration, reconnaissance et indemnisation », *R.B.S.S*.,
- DELVAUX P.-H., Les immunités civiles créées par la loi sur les accidents du travail en liaison avec l'article 18 de la loi du 3 juillet 1978 et les principes régissant le cumul des responsabilités », *R.G.A.R*., 1984, n° 10812, 1-9
- ELST R., « Beschouwingen bij de nieuwe ziekenfondswet », *R.D.S*., 1990, p. 321-330
- F.M.P., « Le système ouvert », *R.B.S.S*., , p. 435-440
- HIRSCH J.L., « Récentes modifications législatives en matière d'accidents du travail et de maladies professionnelles, *R.G.A.R*., 1997, p. 12708, 1-2
- JANVIER R., « Aspects juridiques de 'causalité'et d''imputabilité' dans le cadre de l'expertise médico-légale », *R.B.S.*S., 1990, p. 3-37
- LAHAYE D., « Strategie van de beroepziekteverzekering in belgïe ten opzichte van de uitbreiding van het beroepziektebegrip », *Tijdschrift voor verzekeringskeneeskunde*, 1992 (30), 7-9(à lire)
- LIETAERT B. « Is de immuniteit van de arbeidsongevallenwet en de beroepsziektenwet aan eenieer tegenwerpelijk », *Rev. dr. soc*., 1996, 2, pp. 143-185
- MATHIEU R., « Le cumul des indemnités accidents du travail avec d'autres prestations sociales ou une réparation de droit commun », *R.B.S.S*., 1982, p. 163-199
- MORGENTHAL L., « Quelques aspects de l'influence de la crise sur le droit du travail et sur la dérégularisation de la sécurité sociale sensu lato », *J.T.T*., 1985, p. 141-148
- PAROTTE D., « La définition du préjudice réparé et le jeu de la responsabilité civile dans la loi sur les accidents du travail, *Chr. D. S*., 1983, p. 477-490
- SAINT-JOURS Y., Les cancers professionnels : identification, réparation, prévention, Dr. Soc., mai 1995, n°5, p. 520
- SAKABE H., « Quelques réflexions sur les limites d'exposition à des substances dangereuses en suspension dans l'air », *Rev. Int. Trav*., vol. 117, n°5, sept-oct. 1978, p. 601-613
- SEILLAN H., « Réflexions sur la signification et la portée juridique des valeurs limites en matière de santé dans le travail, *Dalloz Sirey*, 1990, p. 38-42
- SIMOENS D., « Herscholing na ziekte of ongeval », *J.T.T*., 1983, p. 17-22
- SOCHJER-ROUSSELLE M., « La responsabilité civile limitée en matière de maladies professionnelles », in *Accidents du travail et maladies professionnelles,* Bruxelles, Larcier, coll. Les Novelles, t. IV, 1975, pp. 736-755
- VANDEWEERT M., « Le 'système ouvert' dans l'assurance contre les maladies professionnelles », *R.B.S.S*., 1994, p. 1015-1030
- VANDEWEERT M., « Nécessité de fixer des critères d'exposition pour l'application de la loi sur les maladies professionnelles, *R.B.S.S*., 1989, p. 271-282
- VANDEWEERT M., « Medische en juridische causaliteit » in D. LAHAYE, *Sociale zekerheid in beweging*, Liber Amicorum J. VIAENE en R. VANDEN DRIESSCHE, Antwerpen, Maklu, 1993, pp. 307-329
- VANDEWEERT M., « Beroepziekten : begripsvorming en rechtspraak », *Rev. dr. soc*., 1996, N° spécial, pp. 575-622
- VANDEWEERT M., « La causalité dans l'assurance contre les maladies professionnelles », *Rev. dr. soc*., 1995, n°4, pp ; 419-448
- VAN REGENMORTEL A., « Actuele knelpunten bij de uitoefening van de arbeidsgeneeskunde », in M. Rigaux, *Actuele problemen van het arbeidsrecht*, Antwerpen, Maklu, 1993, pp. 183-

- VIAENE J. et D. LAHAYE, « medische en juridische causaliteit. De betekenis van de door de wetgever 'erkende' beroepziekte », *R.W.*, 1975, p. 479-488
- VIAENE J., « Le problème de l'octroi des soins de santé pour des raisons préventives en matière d'assurance maladies professionnelles », , p. 215-216
- VIAENE J., « L'assurance contre les maladies professionnelles en mutation », *R.B.S.S.*, 1994, p. 223-246
- WESTRADE M., « Inédits de droit de la sécurité sociale, II, Maladies professionnelles », *J.L.M.B.*, 1987, p. 595-605, 941-944, 1321- 1329

Le risque professionnel dans le secteur de la prévention

Ouvrages

- ABECASSIS P., ALBOUY J., BROM M., CREN S., EHSTER J. M., SANDRET N., TRAVERSE G., *« L'aptitude médicale » en question. Réflexion d'un groupe de travail de l'Inspection médicale du travail et de la Main-d'œuvre*. Direction régionale du travail, de l'emploi et de la formation professionnelle d'Île-de-France , 2002
- ABEL G. et LAGASSE P., *Code industriel belge*, Bruxelles, Bruylant, 1926 ? ? ?, 2 tomes, 636 p. et 654 p.
- ANTONA - J.-P. et BRUNOIS R., *Hygiène et sécurité dans l'entreprise, préventions et sanctions*, Paris, Dalloz, 1991, 355 p.
- CASSOU B. (sous la direction de), *Les risques du travail. Pour ne pas perdre sa vie à la gagner*, Paris, la Découverte, 1985, 640 p.
- DUCLOS D., *La santé et le travail*, Paris, la découverte, 1984, 123p.
- HAIDANT P., *Précis de législation industrielle et sociale*, 2ème édition, Bruxelles, 1939, 347 p.
- JUFFÉ M., *A corps perdu. L'accident du travail existe-t-il ?*, Paris, 1980
- KÉRAVEL P., BEAUMONT F., *Les droits des travailleurs à la santé*, Éd. Ourières, 2 vol., 1980
- LAZAR P., *Pathologies industrielles : approche épidémiologique*, Paris, Flammarion Médecine, 1979.
- LE RICOUSSE G, *L'établissement des valeurs limites d'exposition aux substances toxiques dans les atmosphères de travail : un tournant dans la prévention des maladies professionnelles*, Paris, INRS, 1985, 50 p.
- LEVENSTEIN C., WOODING J., *Work, Health and Environment. Old Problems, New Solutions*, New York : The Guilford Press, 1997
- NELKIN D. & BROWN M. S., *Workers at Risk. Voices From the Workplace*, Chicago, The University of chicago Press, 1984, 220 p.
- OUDIZ A., LE GALÈS C., *Prévention des cancers professionnels*, Paris, Inserm, 1989, 295 p.
- OVERSTEYNS B., *Gezondheid en onderneming*, Brugge, Die Keure, 1989,
- SCHERRER J., *Précis de physiologie du travail*, Paris, Masson, 1981.
- SOCHJER-ROUSSELLE M., *Droit de la sécurité et de la santé de l'homme au tra*vail, Bruxelles, Bruylant, Collection droit social, 1979, 439 p.
- SUPIOT A., *Critique du droit du travail*, Paris, PUF, Les voies du droit, 1994, 280 p.
- THÉBAUD-MONY A., L'industrie nucléaire. Sous-traitance et servitude, Paris, Inserm, 2000 272p.
- VELGE H., *Éléments de droit industriel belge*, Bruxelles, librairie A. Dewit, 1927, 2 tomes, 368 p. et 431 p.
- VERKINDT P. Y., FRIMAT P., ELOY E. et CUVELIER R., *Aptitude physique et contrat de travail*, Paris, Liaisons, 1990, 219p.
- VILLAGGI J.–P., *La protection des travailleurs, L'obligation générale de l'employeur*, Cowansville, Les éditions Y. Blais, 1996, 475 p.
- VISCUSI, W.K., *Risk by choice : regulating Health and Safety in the Workplace*, Cambridge, MA : Harvard University Press, 1983
- VOGEL L., *L'organisation de la prévention sur les lieux de travail, Un premier bilan de la mise en œuvre de la directive cadre communautaire de 1989*, Bruxelles, Bureau technique syndical européen pour la Santé et la Sécurité, 1994, 425p**.**

Articles :

- BAYER R., « Women, Work and Reproductive Hazards », *Hastings Center Report*, 1982,vol. 12, N°5, October, pp. 14-19
- BERLINGUER G., FALZI G., « Ethical Problems in the Relationship between Health and Work », *Int. J. Health Services*, 1996, 26 :147-171

- BERRA D., « L'inaptitude médicale du salarié et le contrat de travail », *Sem. Soc. Lamy*, 1985, n° 274, D3
- BLEUS P., « La loi du 4 août 1996 : réforme du droit de la sécurité au travail », *Orientations*, janvier 1997, pp. 1-12
- BLEUS P., « Les entreprises et la sécurité des intérimaires », *Orientations*, février 1998, pp. 36-48
- CHAUMETTE P., « Le médecin du travail, l'employeur et l'inspecteur du travail », *D. Soc*., 1983, pp.
- CHU P., « Les conceptions modernes du bien-être dans la vie professionnelle », *Rev. Int. Trav*., vol. 135 (1996), n°3-4, pp. 407-418
- COMBETTE J.-M., « L'état des lieux : le domaine de l'hygiène et de la sécurité du travail », *Rev. sc. crim*., 1992, pp. 503-504
- DASSA S., « Travail salarié et santé des travailleurs, *Sociologie du travail*, N° spécial sur le corps du pauvre, 1976, n° 4, pp. 394-410
- DE BERNIS G., « Prévenir : du jugement sur l'organisation sociale à l'alternative politique », in *Prévenir*, 1981, n° 3
- DE COCK E., LAMOTTE J.-M., PONNET G, VAN DE LAER M., VAN HAMME L., « Dispositions spécifiques relatives à des circonstances particulières », *Rev. Trav.*, juil.-août-sept. 1997, pp. 24-26
- DE COCK E., PONNET G., « L'analyse des risques et la réglementation », *Rev. Trav*., juil.-août-sept., 1995, n°19, pp. 34-40
- DEGRE S., « Physiologie du travail d'hier et d'aujourd'hui », in *Cent ans de droit social belge*, Bruxelles, Bruylant, Droit social, 1988, pp. 657-662
- DELAMOTTE Y., « Les pouvoirs publics et la qualité de vie de travail, Quelques voies suivies en Europe occidentale », *Rev. Int. Trav*., vol. 114, n° 2, sept.-oct. 1976, pp. 153-170
- DESSORS D. , SCHRAM J., VOLKOFFS S., « Du « handicap de situation » à la sélection exclusion. Une étude des conditions de travail antérieures aux licenciements économiques », *Travail et emploi*, n° 48, 1991, pp.
- DRAPER E., « Risk by Choice ? Knowledge Power in The Hazardous Workplace », *Contemporary Sociology*, 1984a, vol. 213, November
- DURAND C. , « La carence fautive de l'État en matière de la protection de la santé au travail. De l'enrichissement mutuel du droit du travail et des principes de prévention et de précaution », *RD sanit. soc.* 38 (1), janv.-mars 2002, pp. 1-19
- HESELMANS M., « Les nouvelles orientations et les alternatives en matière de services de prévention, » *Rev. Trav*., juil.-août-sept., 1994, n°15, pp. 45-47
- HESELMANS M., « La politique de prévention dans une perspective européenne, » *Rev. Trav*., juil.-août-sept., 1995, n°19, pp. 27-33
- JAVILLIER J.-C., « Le statut des médecins du travail », *Dr. soc*., 1980, S40
- LAMOTTE J.-M., « La santé des travailleurs : une dimension sociale européenne », *Rev. Trav*., juil.-août-sept., 1991, n°3, pp. 30-34
- LAMOTTE J.-M. et VAN EMELEN J., « La prévention, essence de la médecine du travail », *Rev. Trav*., juil.-août-sept., 1994, n°15, pp. 33-44
- LENAERTS H.-F., « La loi du 4 août 1996 : le comité pour la prévention et la protection au travail », *Orientations*, 1997, pp. 13-17
- LYON-CAEN A., « Les répercussions des orientations des médecins du travail », *Dr. Soc*., 1980, N° spéc., la médecine du travail, pp.
- OLIVIER Y., « Les directives européennes et la médecine du travail, *Rev. Trav*., juil.-août-sept., 1991, n°3, pp. 35-39
- PONNET L., « La portée de la loi sur le bien-être », *Rev. Trav*., juil.-août-sept. 1997, pp. 8-14
- KRYNEN B., « Le droit des conditions de travail. Droit des travailleurs à la santé et à la sécurité », *D. Soc*., 1980, p. 523
- PONNET L., VAN DE LAER M., VAN HAMME L., « Les organes de prévention et de protection », *Rev. Trav*., juil.-août-sept. 1997, pp. 15-23
- PONNET L., « Travaux avec des tiers », *Rev. Trav*., juil.-août-sept. 1997, pp.27-32
- SAKABE H., « Quelques réflexions sur les limites d'exposition à des substances dangereuses en suspension dans l'air », *Rev. Int. Trav*., vol. 117, n° 5, sept. – oct. 1978, pp. 601-613
- SAVATIER J., « le médecin du travail et le sort du salarié », *Dr. Soc*., 1987, pp. 604-612
- SCHEPERS J., « Welzijnrecht : een nieuwe tak van het sociaalrecht », *Rev. dr. soc*., 1994, n°3, pp. 253-291
- SEILLAN H., « Réflexions sur la signification et la portée juridique des valeurs limites en matière de santé dans le travail », *D*., 1990, chron., VIII, pp. 38-42
- SHORT J. F. Jr, « The Social Fabric at Risk : Toward the Social Transformation of Risk Analysis », *American Sociological Review*, 1984, vol. 49, december, pp. 711-725
- SOCHJER-ROUSSELLE M., « Prévention et réadaptation », in *Accidents du travail et maladies professionnelles,* Bruxelles, Larcier, coll. Les Novelles, t. IV, 1975, pp. 771-800

- SOCHJER-ROUSSELLE M., « La médecine du travail après dix années d'institutionnalisation », *J.T.T.*, 1978, pp. 97-100 et 117-125
- VANDELAC L., BARALDI R., BACON M.-H., « Quand l'État confie la protection de la santé aux entreprises », *Éthique publique – Revue internationale d'éthique sociétale et gouvernementale*, La vérité en politique, L'avenir des services publics, vol. 1, N°1, Montréal, Editions Liber, INRS, 1999, pp ; 102-115
- VAN EMELEN J., LAMOTTE J.-M., « L'analyse des risques, nouveau pilier de la politique de prévention », *Rev. Trav*., juil.-août-sept., 1995, n°19, pp. 8-21
- VAN VAERENBERG R., « Recrutement et sélection des travailleurs », *Orientations*, 1990, pp. 65-70
- WERNEKE D.,. BROADFIELD R, « Une politique de la main-d'œuvre orientée vers la satisfaction des besoins dans les pays avancés à économie de marché », *Rev. Int. Trav*., vol. 116, n° 2, sept.-oct. 1977, pp. 189-201

Bibliographie électronique :

- Une introduction au risque cancérogène en milieu professionnel, http:/ www.inrs.fr :dossiers/riscanmut.htm

Revues, brochures :

- Commissariat général à la promotion du travail, Administration de l'hygiène et de la médecine du travail, *Valeurs limites d'exposition*, Ministère de l'emploi et du travail, 52 p.
- Ministère de l'emploi et du travail, Commentaire de la loi du 4 août 1996 sur le bien-être au travail

Chapitre III Liens entre les régimes de prévention et de réparation

- CHAUMETTE P. « L'activation du lien réparation-prévention », *D. Soc*., N°9/10, 1990, pp. 727-728

Divers

- CASSOU B. et SCHIFF M., *Qui décide de notre santé ? le citoyen face aux experts*, Paris, Syos, 1998 , 266 p.
- CRESPIN R. et LASCOUMES P., « Régulation de la carrière d'un instrument de santé. Le parcours de l'usage du test VIH dans l'emploi en France et aux Etats-Unis », *Sociologie du travail*, 2000, 42, pp. 133-157
- FAIGMAN'S D.L., *Insecurity With Science. Legal Alchemy : The Use and The Misuse of Science in the Law*, W.H. Freeman and Cy Publisherss, 1999, 256 p.

- MEULDERS-KLEIN M.-T. et MAINGAIN B., « Le droit de disposer de soi-même » *in Licéité en droit positif et références légales aux valeurs*, Dixième journées juridiques Jean Dabin organisée par l'Unité de Droit pénal, Bruylant, Bruxelles, 1982, pp.14 et s.
- PETRELLA R., *Le bien commun : éloge de la solidarité*, Bruxelles, Labor, 1996
- THUILLIER P., *Les passions du savoir : essai sur les dimensions culturelles de la science*, Paris, fayard, 1988
- VACQUIN M., Main basse sur les vivants, paris, Fayard, 1999, 276 p.
- VANNES V., « La relation de travail et le sida », in *Le sida, un défi aux droits* , Bruxelles, Bruylant, 1991, pp. 331-
- WILKIE A. D., « Mutuality and Solidarity : Assessing Risks and Sharing Losses », *Br. Actuarial J*., 1997, 3 : 986

Rapports, avis,…

17. The Nuffield Trust Genetics Scenario Project, genetics and health, http://www.official-documents.co.uk/document/nuffield/policyf/gen-ak.htm#gen0
18. *Symposium Les cancers d'origine professionnelle*, Fédération Belge contre le Cancer, Bruxelles, le 12 juin 2002
19. *Journée d'études Enjeux de la susceptibilité génétique en milieu professionnel*, SPF Politique scientifique, Bruxelles, le 30 octobre 2003
20. Le bien-être des travailleurs lors de l'exécution de leur travail. Commentaire juridique de la loi du 4 août 1996, Ministère de l'emploi et du travail

Presse

Le Soir, 3 février 2003, A. KAHN, « Le clonage thérapeutique est illusoire ».

JURISPRUDENCE

VIE PRIVÉE

Cour eur. D. H., arrêt *Sunday Times* du 26 avril 1979, Série A, n°30,
Cour eur. D.H., arrêt *Airey* du 9 octobre 1979, Série A, n° 32, p. 17
Cour eur. D. H., arrêt *Dudgeon c. Royaume-Uni* du 22 octobre 1981, Série A, n° 45
Cour eur. D.H., arrêt *X. et Y. c. Pays-Bas* du 26 mars 1985, Série A, n° 91, p. 11
Cour eur. D.H., arrêt *Olsson c. Suède* du 24 mars 1988, Série A, n° 130, p. 31-32
Cour eur. D. H., arrêt *Niemitz c. Allemagne* du 16 décembre 1992, *Rev. trim. D. H.*, 1993, p. 468
Cour eur. D. H., *arrêt C. c. Belgique* du 7 août 1996
Cour eur. D.H., arrêt *Laskey, Jaggard et Brown c. Royaume-Uni* du 19 février 1997, *Rev. trim. D. H.*, 1997, p. 733-745, note M. LEVINET
Cour eur. D.H., arrêt *Guerra et autres c. Italie* du 19 février 1998, *Rev. trim D. H.*, 1998, p. 808-833, note P. FRUMER.
Cass., 26 septembre 1978, *Arr. Cass*., 1978-1979, 116, *Pas.*, 1979, I, 126
Cass., 23 juin 1980, *Pas*., 1980, I, 1303
Cass. 10 mai 1985, *Arr. Cass*., 1984-1985, 1230 Concl. Av. Gén. TILLEKAERTS, *J.T*., 1986, p. 75
Cour eur. D. H., arrêt *Niemitz c. Allemagne* du 16 décembre 1992, Série A, n° 251-B, par. 29
Cour Eur. D. H., affaire *Peters c. Pays-Bas* du 6 avril 1994, Req. N°21132/93, *D.R*., vol. 77-A, p. 75 ;
Cour Eur. D. H., affaire *A.B. c. Suisse* du 22 février 1995, Req. N° 20872/92 , *D.R*., vol. 80-A, p. 66 ;
Cour Eur. D. H., affaire *J.R., G.R. et Y.R. c. Suisse* du 5 avril 1995, Req. N° 22398/93, *D.R*., vol. 81-A, p. 61 ;
Cour Eur. D. H., affaire *G. Jehl-Doberer c. Suisse* du 1er septembre 1993, Req. N° 17667/91.
Comm. D. H., 10 avril 1961, Req. N° 911/60, *Ann. Conv*., IV, p. 199 ;
Comm. Eur. D. H., 16 janvier 1963, Req. N° 1449/62, *Ann. Conv*. VI, p. 263 ;
Comm. Eur. D. H., 23 mai 1966, Req. N° 2916/65, *Ann. Conv*. IX, p. 437 ;
Comm. Eur. D. H., 6 février 1968, Req. N° 2822/66, *Rec*. N° 26, p. 42.
C. J. C. E., 5 octobre 1994, *X. c. Commission,* C-404-92-P, note O. DE SCHUTTER, « Les examens médicaux à l'embauche de la Commission européenne », *Rev. trim. D. H*., 1995, p. 101-122 ; également sur le droit de ne pas savoir :;.
Cass. 21 décembre 1962, *Pas*., 1962, I, 499
Cass. 27 mai 1971, *Pas*., 1971, I, p. 886
Cass. 4 avril 1977, *J.T*., 1977, p. 554, *Pas*., 1977, I, p. 831
Cass., 26 septembre 1978, *Pas*., 1979, I, p. 126.
Cass., 28 février 1979, *Pas*., 1979, I, 774
Cass., 9 mars 1987, *J.T.T*., 1987, p. 128 ; *Chron. Dr. Soc*., 1987, 210.
Cass. 23 janvier 1991, *Pas*., 1991, I, 491
Cass., 1er octobre 1997, *J.L.M.B*., 1998, p. 796, note C. MEUNIER.
C. trav. Anvers, 14 janvier 1981, *J.T.T*., 229 a jugé que le fait de taire ses études universitaires et quelques employeurs précédents était constitutif de motif grave, *contra* :
Trib. Trav. Gand, 18 mai 1981, *R.W*., 1981-1982, 1426, *J.T.T*., 1981, 300 a jugé que le fait de taire son état de grossesse bien que fautif n'est pas constitutif de motif grave ; sur l'état de santé
C. trav. Anvers, 4 juin 1981, *J.T.T*., 1981, 297, *R.W*., 1981-1982, 1414, *Jura Falconis*, 1981-1982, 283.
Cass., 8 septembre 1982, *Pas*., 1983, I, p. 43
C. trav. Liège, 17 mai 1985, *J.T.T*., 1985, p. 472
Liège, 22 septembre 1988, *Pas*., 1989, II, 47
C. trav. Bruxelles, 26 mars 1990, *Chron. D. S*., 1992, 154
Trib. Trav. Bruxelles, 24 juin 1991, *Chron. D.S*., 1995, 225
Trib. Trav. Bruxelles, 23 mars 1992, *R.W*., 1992-1993, 652 qui reconnaît un droit au mensonge au candidat pour des questions non pertinentes lors de la procédure de recrutement.
Trib. Trav. Charleroi, 19 avril 1993, *Chron. D.S*., 1994, 372
Trib. Trav. Gand, 21 avril 1993, *R.W*., 1993-1994, 92.
C.trav. Mons, 16 mars 1995, *J.T.T*., 1996, 147
Trib. Trav. Bruges, 14 mars 1996, *Chron. D .S*., 1997, 26, note P. HUMBLET ;

Gand, 20 mars 1996, *R.W.*, 1996-1997, p. 1257, note P. LEMMENS, *T. Gez.* / *Rev. dr. santé*., 1996-1997, p. 35
C. trav. Bruxelles, (4ème ch.), 5 novembre 1997, *J.T.T.*, 1998, 18.
Cass., 6 mars 1986, *Arr. Cass*., 1985-1986, 937

ULg

Cagen SZ, Waechter JM Jr, Dimond SS, Breslin WJ, Butala JH, Jekat FW, Joiner RL, Shiotsuka RN, Veenstra GE, Harris LR. Normal reproductive organ development in CF-1 mice following prenatal exposure to bisphenol A. *Toxicol Sci* 50:36-44 (1999).

Chapin, R.; Sloane, R.A. Reproductive assessment by continuous breeding: Evolving study design and summaries of ninety studies. *Env. Health Persp*. 105, no. Suppl. 1, pp. 199-395, 1997.

Colborn T, vom Saal FS, Soto AM. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect 101:378-384 (1993).

Finnon, P. *et al.* Fluorescent in situ hybridization detection of chromosomal aberrations in human lymphocytes: applicability of biological dosimetry. *Radiat. Biol.* (1995) 68, 429-435.

Finnon, P. *et al.* The ^{60}Co gamma ray dose-response for chromosomal aberrations in human lymphocytes analyzed by FISH: applicability of biological dosimetry. *Radiat. Res*. (1999) 75, 1215-1222.

Kavlock RJ, Daston GP, DeRosa C, Fenner-Crisp P, Gray LE Jr, Kaattari S, Lucier G, Luster M, Mac MJ, Maczka C, Miller R, Moore J, Rolland R, Scott G, Sheehan DM, Sinks T, Tilson HA. Research needs for the assessment of health and environmental effects of endocrine disruptors: a report of the U.S. EPA-sponsored workshop. Environ Health Perspect 104(Suppl 4):715-740 (1996).

Lambert V, *et al.* Translocation frequencies measured in patients one year after radioactive iodine therapy for thyrotoxicosis. *Intl. J. Radiat. Biol.* (2001) 77, 679-685.

Littlefield, L.G. *et al.* Do recorded doses overestimate true doses received by Chernobyl cleanup workers? Results of cytogenetic analyses of Estonian workers by FISH. *Radiat Res.* 150 (1998) 237-249.

McLachlan JA, Korach KS, Newbold RR, Degen GH. Diethylstilbestrol and other estrogens in the environment. Fundam Appl Toxicol 4:646-691 (1984).

Natarajan. A.T. *et al.* Frequencies of X-ray-induced chromosome translocations in human peripheral lymphocytes as detected by in situ hybridization using chromosome-specific DNA libraries. *Intl. J. Radiat. Biol*. (1992) 61, 199-203.

Pressl, S. *et al.* The influence of age, sex and smoking habits on the background level of fish detected translocations. *Mutat Res*. 442 (1999) 89-95.

Ramsey, M.J. *et al.* The effect of age and life factors on the accumulation of cytogenetic damage as measured by chromosome painting. *Mutat Res*. 338 (1995) 95 –106.

Salassidis, K. *et al.* Dicentric and translocation analysis for retrospective dose estimation in humans exposed to ionizing radiation during thee Chernobyl nuclear power plant accident. (1994) *Mutat Res* 311. 39- 48

Snigiryova G, *et al.* Retrospective biodosimetry of Chernobyl clean-up workers using chromosome painting and conventional chromosome analysis. *Intl. J. Radiat. Biol*. (1997) 71, 119-127.

Spearow, J.L.; Doemeny, P.; Sera, R.; Leffler, R. ;Barkley, M. Genetic variation in susceptibility to endocrine disruption by estrogen in mice. *Science* 285:1259-1261, 1999.

Stephan, G, *et al.* Chromosome aberrations in human lymphocytes analyzed by FISH after in vitro irradiation and in radiation workers 11 years after an accidental radiation exposure. *Intl. J. Radiat. Biol.* (1995) 67, 19-28.

Tucker, J.D. *et al.* Validation of chromosome painting II. A detailed analysis of aberrations following high doses of ionizing radiation in vitro. *Intl. J. Radiat. Biol.* (1995) 67, 19-28.

D/2004/1191/13

Published by the
Belgian Science Policy

For more informations:

Madame E. Bourgeois
Belgian Science Policy
rue de la science 8 Wetenschapstraat
Bruxelles 1000 Brussel
Tel.: +32-2-238.34.94
Fax.: +32-2-230.59.12
E-mail: boug@belspo.be
Internet: http://www.belspo.be