Scientific relevance and irrelevance of genetic susceptibility for standard setting in risk control.

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CONTENT

I   INTRODUCTION

I.1   Context and general frame of the research

The research conducted under this scientific support programme should be considered as a consistent continuation of previous research activities.

The same team has in its subsequent research activities since 1988 till today always adapted its research objectives and methods with a view to maximising its contribution to both the needs and the new possibilities of science and the needs of occupational health prevention, especially in the field of preventing occupation related haematolymphopoïetic disorders.

Quick outputs have therefore never been a goal in setting research priorities. In contrast, the team was always looking for how to take out the maximum of the data it could have access to - some of which are unique world-wide - due to the daily commitment of its members to the world of occupational health. What started as descriptive occupational cancer epidemiology and a study on benzene induced effects on peripheral blood cell counts, has evolved to a step by step process of developing methods and conducting studies which should result in a better understanding of the relationship between occupational risk factors and genetic susceptibility on the one hand, and adverse health effects and exposure specific genomic lesions on the other.

The first study on occupational cancer was based on individual medical records as kept by the occupational health physicians of several industries, because no cancer registry existed in Belgium at that time. The study was a unique demonstration on how existing data in individual medical records could be used for research purposes, even in the absence of reference data. This extremely time consuming cancer study has revealed an excess of Hodgkin's disease in chemical and petrochemical industry. This study has been used as a major argument for setting up regional cancer registry centres in Belgium, before this was imperative at EU level. At the same time, and also based on medical records as kept by the occupational health physicians, a study was undertaken on the effect of low levels of benzene exposure on the peripheral blood cell count. Also this study was extremely time consuming but resulted in the biggest data base ever studied world-wide, and revealed with an exceptional degree of reliability that benzene exposure levels above 0.5 ppm may still lead to possibly adverse effects and that some off these effects (more in particular changes in peripheral blood cell count) may at least in part be

reversible below 0.5 ppm. This study is directly at the basis of setting an occupational exposure limit value at EU level which will not allow occupational exposure above 1 ppm in the future, following a report of the research team to the Scientific Expert Group on Occupational Exposure Limit Values of the European Commission.

At the same time some members of the same research team had set up a European research on ethical, social and scientific issues related to genetic screening and genetic biomonitoring practices in occupational health, a theme which had revealed their concern because it could affect both health protection and right for work. They also conducted a European study on infant leukaemia, a disease which is considered to be a unique experiment by nature to study exposure specific genomic lesions. This is how the previous epidemiological research of the team and genetic aspects of occupational health have met.

In line with the requirements of the next research programme of the Belgian Ministry of Science, and given the still largely controversial and undefined but also largely unexplored scientific hypotheses on exposure specific genomic lesions, a case-referent study was done on Myelodysplastic Syndrome in which causal factors were identified and the association with specific chromosomal anomalies studied and demonstrated.

Indications for a relationship between external exposure and clonal chromosomal aberrations have been observed for a number of years. The idea was a consequence of a paper by Van den Berghe H. et al published in 1974 in 'Nature', entitled: "Distinct haematological disorder with deletion of long arm of No. 5 chromosome." This acquired structural genomic disorder found in Myelodysplasia/Leukaemia opened up the whole research field: Myeloid Leukaemia as an environmentally induced process. Our project aimed to study the relationship between possible environmental causes of Leukaemia/MDS and specific genomic lesions in the cells, as well as the possible relevance of such knowledge for the prevention, the identification and the (medico legal) recognition of occupational diseases. The research moved between two questions, the first of which was answered partly and the second hardly explored. The first question was whether the presence of specific clonal chromosomal anomalies could be indicative of external exposure as the major explanation for the appearance of leukaemia. The second question was whether specific chromosomal anomalies found in malignancies can be directly attributed to specific exposure factors.

For the purpose of this study, a new epidemiological strategy, *in casu* an interviewing strategy and an *a posteriori* exposure categorisation procedure were developed. This was needed because the interviewing method and the exposure assessment had to allow for maximising the accuracy/precision of the information on exposure history, which is crucial in studying associations with chromosomal aberrations, and to take into account latency in the statistical analysis which was possible because all the information collected was put on a time scale and assessed for its consistency. Using this interviewing method, sufficient sensitivity was obtained with a small sample size. The study has revealed a.o. an association between MDS, pesticide exposure and several chromosomal aberrations recognised as environmentally induced.

As an additional objective and as an extension to the study, it was decided during the course of the investigation to develop a research proposal to explore the possible role of genetic susceptibility factors, in particular of polymorphic genes possibly involved in the metabolisation of xenobiotics, in the causation/development of MDS and other haematolymphopoietic disorders. If genetic susceptibility factors may considerably affect the response to genotoxic agents, their identification can be important in studies concerning exposure specific genomic lesions.

Such a project on genetic susceptibility was considered necessary to solve unanswered questions on the possible relationship between specific genetic polymorphism's and the risk for haematological malignances related to specific exposures. Insights concerning this may or may not be a stimulus for the introduction of additional variables into epidemiological research on exposure specific genomic lesions. Moreover, this research may lead to a better insight into the health surveillance of workers exposed to benzene and analogue agents.

The phase I and II projects of the research network in the 'Scientific support programme on workers' were to be considered as a continuation of the above projects.

As the research project on exposure specific genomic lesions and prevention of occupational cancer showed that exposure specific genomic lesions might better be studied in relation to possibly relevant genetic susceptibility factors, we submitted for Phase I of the programme (1998-2003) a valorisation project focusing on susceptibility in relation to standard setting for prevention of occupational cancer and other occupational diseases. This was thought to also be in compliance with a growing societal interest and

need to discuss and understand relevance and acceptability or non-acceptability of genetic susceptibility testing.

The following objectives were accepted for funding under phase I:

(1) a close follow-up of ongoing research world-wide related to genetic susceptibility, including direct contact and collaboration with other research teams.

(2) setting up an information point on genetic susceptibility issues, accessible to policy makers like social partners and public authorities, but also to occupational health professionals, scientists in both human and positive science, etc. with a view to provide scientifically sound information.

The project was called 'Follow-up and dissemination of information on scientific relevance and irrelevance of genetic susceptibility for standard setting in risk control'.

1.2   Research objectives

For the Phase II of the programme we submitted a new proposal of which the following objectives were financed:

(1) to generate hypotheses on the relationship between genetic susceptibility factors known to be involved in the metabolism of xenobiotics and the effect of moderate exposure to benzene on the peripheral blood cell count in healthy workers;

(2) to contribute to understanding the relationship between environmentally induced changes in peripheral blood cell counts and the risk of severe hematolymphopoïetic disorders;

The project was called : 'Scientific relevance and irrelevance of genetic susceptibility for standard setting in risk control'.

This project aims to explore the association between genetic susceptibility factors, in particular of polymorphic genes possibly involved in the metabolisation of xenobiotics, and the risk for severe hematolymphopoietic disorders, with a view of producing reference material for the study of the relationship between peripheral blood cell count alterations found in benzene exposed workers and their genetic susceptibility factors, and the understanding of the possible meaning of blood cell counts and genetic factors for preventing severe haematolymphopoietic disorders amongst benzene exposed workers.

II   THEORETICAL FRAME

For a series of polymorphic genes known or assumed to interfere with the metabolism of genotoxicants, patterns of distribution must be studied and compared in unaffected workers following benzene exposure, in workers affected by several types of reversible changes in peripheral blood cell counts, as well as in persons suffering from severe hematolymphopoietic disorders, which possibly may be benzene related.

Risk assessment procedures and standard setting activities are explicitly or implicitly taking position with respect to the susceptibility of the individuals to whom the standards will apply. Many standards like occupational limit values are set in the best of cases using reliable epidemiological data. However, extrapolation of no observed adverse effect levels from one study population to another may be erroneous. One of the main reasons for this may be that the fraction of susceptible persons might be different between the study population and the population to whom the standard would apply, and therefore the limit values based upon epidemiological data may be inadequate to protect more susceptible persons. With a view to both protecting health and allowing employment of all candidates, it is important that standards offer sufficient protection to all. Increasing the knowledge on susceptibility, and assessing the validity of existing standards for protecting the more vulnerable is thus necessary.

Research on genetic susceptibility factors could contribute to providing answers to some unsolved problems related to current non-genetic testing practices in occupational health surveillance aiming at disease prevention. One of these is the significance of clear but reversible changes in peripheral blood cell counts in benzene exposed workers. Preventing hematolymphopoietic disorders is a major concern in many industries, especially in relation to benzene exposure. Despite dramatic improvement in working conditions in many of those industries, there is at present no guarantee that the risk of severe adverse health effects has completely disappeared. This is why peripheral blood cell counting still is and has to remain a common test as part of periodical medical follow-up procedures for benzene exposed workers. However, little is known on the relationship between reversible changes in peripheral blood cell count in benzene exposed workers and the risk of severe hematolymphopoietic disorders. There is still uncertainty about the significance of some possibly benzene related alterations in peripheral blood cell count as indicators of an increased risk for more severe effects. Better knowledge of genetic

susceptibility factors possibly affecting each of these effects may increase our understanding of that relationship.

During the research project on exposure specific genomic lesions and prevention of occupational cancer it appeared that exposure specific genomic lesions might better be studied also in relation to possibly relevant genetic susceptibility factors. Furthermore the relation between reversible changes in peripheral blood cell count, used as a monitoring tool in the prevention of these cancers in benzene exposed workers, and the risk for severe hematolymphopoietic disorders is not clear.

Some scientific questions need to be addressed, which inevitably require the exploration of related genetic susceptibility issues:

- do a series of particular known polymorphic genes play a role in susceptibility to some types of leukaemia; to bone marrow dysplasia; to pathological changes in peripheral blood cell count; and to alterations in peripheral blood cell counts of healthy individuals who were exposed to benzene?

- Is the distribution of types of polymorphisms the same for all these possible effects? In other words, are these different effects related or not?

- How should alterations in blood cell counts performed as part of health surveillance of exposed workers be interpreted with respect to the risk of severe hematolymphopoietic disorders?

In order to contribute to answering these questions the idea was developed to studying the relationship between traditional testing practices in periodical health surveillance and genetic susceptibility factors of individuals exposed to substances that may enhance a risk for haematolymphopoietic cancer and myelodysplastic syndrome. With a view to generate hypotheses, the relationship between peripheral blood cell count, benzene exposure and particular genetic polymorphisms is studied. Also the distribution of the same specific genetic polymorphisms amongst patients with myelodysplastic syndrome is investigated.

Focusing on susceptibility in relation to standard setting for prevention of occupational cancer and other occupational diseases seemed to be in compliance with a growing societal interest and need to discuss and understand relevance and acceptability or non-acceptability of genetic susceptibility testing for employees exposed tot toxic agents.

III   METHOD

In order to study the relationship between reversible changes in peripheral blood cell counts in benzene exposed workers and the risk of severe hematolymphopoietic disorders, and to learn more about the genetic susceptibility factors possibly affecting these effects, two main populations are to be studied consisting of benzene exposed workers on the one hand and of patients suffering from myelodysplasia/leukaemia on the other.

III.1 Benzene exposed workers

The study could start from an existing base and database on peripheral blood samples as a result of an earlier study on «Cancer incidence in chemical and petrochemical workers» (Impulse programme 'health risk's' Contract NR HH/06/038). The following information was available:

- a database with 13.539 peripheral blood cell counts of 360 benzene exposed workers of a petrochemical industry;

- a historical reconstruction of benzene exposure data for these workers based upon 3867 stationary air samples;

- a database of 13.796 peripheral blood cell counts of 2 589 employees of a chemical plant who had no noticeable occupational benzene exposure;

The database with 13.539 peripheral blood cell counts of 360 benzene exposed workers was selected from the individual medical records archived by the occupational health physician of a petrochemical plant. The data of origin included 17.404 peripheral blood cell counts taken from 1966 till 1989 of 457 potentially benzene exposed workers. Only persons who were first employed before 1978 were included in the selected database. Persons who contributed less than 5 blood samples were not included. Also control blood samples following cell counts out of normal ranges were not included. For each individual included, the following data are available: code number, date of birth, total number of samples, and results for each sample, including: date of sample taking, months since first sample, interval with previous sample in months, haemoglobin, hematocrit, RBC (red blood cell count), platelets, WBC (white blood cell count), and in most cases WBC differentiation in neutrophils, granulocytes, lymphocytes.

These existing database of the benzene exposed workers was completed with results of the peripheral blood cell counts taken from the same persons during the more recent years in the benzene-exposed population, in casu from 1989 till 2000. These data were available in an electronic data base only from 1992 onwards. For the previous years the data had to be transferred from the individual medical records on paper into the electronic data base. The other data were controlled for consistency with the written documents.

As the study base consists of all employees and retirees of a petrochemical plant who had possible exposure to benzene and for whom at least 5 peripheral blood cell counts were collected before the end of 1989 and since, in the petrochemical plant considered, there has been neither a tradition of exclusion of workers for reasons related to the material of interest to this study, nor a tradition of selecting workers for genetic polymorphism, or excluding them for any possible health problem related to benzene exposure, this population can be considered as unselected with respect to the genetic polymorphisms under study.

A particular procedure for selecting individuals from the population was developed based on two complementary approaches: a subjective interpretation of data by experts on the one hand (i), and a mathematical approach (ii) on the other. The process for developing this procedure consists of comparing both approaches with a view to improving mutual consistency of both.

This procedure was considered necessary for studying the relationship between genetic polymorphisms and susceptibility to benzene induced effects, as maximising the reliability of the classification of study base members in effect categories as a function of exposure history is both crucial and complex. Limiting the number of study base members included in these categories, thereby increasing the contrast between effect categories, was done in order to increase the sensitivity of the study.

In order to assess the frequency of genetic polymorphisms in a « reference population », sampling was done amongst all current employees who gave a blood sample for genetic analysis. The sampling size had to be –in so far as possible- in accordance with the expected frequency of occurrence of each polymorphism under study.

Susceptibility related to genetic polymorphisms is to be compared between the categories, by means of a Fisher's exact tests for the hypothesis that each particular category of benzene effects as defined by the health professional experts might be associated with a particular (combination of) polymorphism(s).

Relationships between peripheral blood cell count, benzene exposure and particular genetic polymorphism's must thus be studied, starting with the above described approach. Such an approach was considered the most appropriate with a view to generating hypotheses. Hypothesis testing may be done subsequently. The complexity of the problem and the natural variability or uncertainty of the data being dealt with require a strategy which enables clear hypotheses to be generated before choosing the statistical model to deal correctly with the complexity of the data and thus to test this hypothesis. This cascade method of proceeding enables to limit the number of persons to be tested for genetic susceptibility genes at each step of the decisional cascade. Hypotheses resulting from this procedure can be tested on larger groups of well-selected persons in the database. The whole procedure is called a "cascade exploratory approach".

The whole research team reconsiders all findings and then decides on the next steps.

These may include extension of the study population and the blood samples data base. In this way, genetic polymorphism's may be identified which can be considered as relevant for susceptibility to particular types of reversible effects on peripheral blood cell count.

The frequency of occurrence of these polymorphisms, alone and in combination, in the different categories of benzene exposed workers, can be compared with their frequency in a population of persons suffering from Myelodysplasia/Leukaemia, using both a classical and a Bayesian statistical approach for proportions comparison. Subgroups of Myelodysplasia/Leukaemia patients may be used in the analysis.

The possible relationship between particular types of changes in peripheral blood cell count and the risk of severe hematolymphopoietic disorders may be assessed subsequently.

Categorising the benzene exposed workers

In studying the relationship between genetic polymorphisms and susceptibility to benzene induced effects, maximising the reliability of the classification of study base members in effect categories as a function of exposure history is both crucial and complex. Limiting the number of study base members included in these categories, thereby increasing the contrast between effect categories, must be done in order to increase the sensitivity of the study.

A procedure for selecting individuals from the population was developed based on two complementary approaches: a subjective interpretation of data by experts on the one hand (1), and a mathematical approach (2) on the other. The process for developing this procedure consists of comparing both approaches with a view to improving mutual consistency of both.

(1) *Subjective expert interpretation.*

For every individual included in the study base the cell counts history was interpreted by medical researchers who are trained in occupational health and by the occupational health physician who collected the data and keeps the individual medical records, in order to distinguish different types of changes in blood cell count history, as a function of assumed individual benzene exposure history.

With respect to exposure history, approximately, the following discontinuous periods of benzene exposure were considered:

1. the late sixties and early seventies, when the plant was started up with relatively high benzene exposure. In the same period, benzene was frequently used to wash hands, with skin absorption as a probable consequence (period 1).

2. the mid and late seventies, when benzene exposure was lower than during the early phase, and washing hands in benzene was less common (period 2).

3. the mid eighties[1], when benzene exposure was falling rapidly to reach very low levels as a time weighted average and washing hands in benzene was not done (period 3).

In order to classify individuals, this general assessment of exposure to benzene in different time periods of observation was completed further by individual exposure history assessment, starting from considering historical air sampling data, department and activity altogether, and other possibly relevant information on the individual the

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[1] Samples taken during the early eighties will not be included in the database, because of a temporary lack in accuracy of testing methods.

occupational health physician is aware of such as disease histories of individuals and subsequent medication. The latter is important because it may be a possible confounder in studying the relationship between blood cell counts and exposure to benzene.

In view of these exposure assessments, individuals were in a first exercise be categorised with respect to their cell count histories and in comparison with a reference population as follows:

- Individuals who consistently showed low white blood cell counts in period 1 and/or 2, and higher near average values in period 3.

- Individuals who consistently showed high white blood cell counts in periods 1 and/or 2, and lower near average values in period 3.

- Individuals who hardly showed any variability in WBC counts in periods 1, 2 and 3.

- Individuals who showed a high variability in WBC counts with no apparent relationship with exposure periods.

- Individuals who rather consistently show low lymphocyte counts in period 1 and/or 2, and higher near average values in period 3.

- Individuals who consistently show high lymphocyte counts in periods 1 and/or 2, and lower near average values in period 3.

- Individuals who showed cell count changes for other than white blood cells, and which are more pronounced in the earlier periods.

(2) Mathematical approach.

The results of this classification into effect categories carried out by medical and occupational health experts was then compared with a mathematical approach using the same data. A trial-and-error process was used to develop the approach, starting from a mathematical translation of the initial formulation of the category, and continuing with a comparison between the workers that should have been classified in the category, and the workers actually classified in it, in order to understand the reason for the discrepancies and to correct the criteria for them. The process was stopped as soon as the possible discrepancies between the target list established by the medical experts and the list established using the defined criteria is considered negligible by the medical professionals.

Selection of genetic tests

Since no information was available in this database about genetic polymorphisms, results of tests for genetic polymorphisms were collected from selected members of the study population willing to participate. The analysis of the samples was done at the Finnish Institute of Occupational Health (laboratory of dr. A. Hirvonen).

The following polymorphic genes were initially considered in the study:

- GSTM1 and GSTT1 coding for the glutathione S-transferases M1 and T1, a family of isoenzymes catalysing the conjugation of reactive species to glutathione. Individuals with the GSTT1 null genotype were suggested to have enhanced susceptibility to MDS (Chen et al, 1996);

- CYP 2E1 coding for P450 2E1, an enzyme involved in the metabolisation of several compounds including benzene.

- NQO1 coding for the NQO1 enzyme converting the benzoquinones, which are potent hematotoxic and genotoxic benzene metabolites, back to their less toxic hydroxy metabolites. A higher frequency of NQO1 inactivating polymorphism's has been identified in subjects developing hematotoxicity after benzene exposure.

In order to closely follow recent literature data on this issue, also other polymorphisms were added to this list during the project because of their possible interference with the metabolism of substances that could be toxic to the haematolymphopoietic system and tot DNA repair mechanisms in general. Finally the following series of genotypes were tested for: GSTM1, GSTM3, GSTT1, GSTP1, CYP2E1, CYP2A6, CYP1A1, NQO1, NAT2, XRCC1, XRCC3, XPD.

Sample taking amongst selected individuals in the study population who also agreed

For those selected employees who were still employed, samples were taken by the occupational health physician during the periodical medical examination which is done in accordance with legal requirements. The OHP asked to every selected employee at that

occasion an additional written informed consent (in top of the previous agreement given by the elected workers health and safety committee) (see annex).

A certain number of the selected employees were already retired at the time of the investigation. They were contacted via the association of company retirees, and invited to attend a meeting in which the researchers explained as clear and complete as possible what the research is about, why their population was selected, how the research is conducted, how their privacy will be protected. All volunteers gave a blood sample following written informed consent. It should be stressed that all of the invited persons who were physically able tot attend the meeting were present, and that each one of them contributed with a sample, and were happy to do so. .

All samples (5 cc blood in EDTA tube) were immediately coded. The code is known by the OJP, the nurse who assisted in sample taking, and the MD researchers.

The coded samples were stored at –20°C at the occupational health service in accordance with the strict Belgian labour regulations on confidentiality until they were transported tot the Finnish Lab.

III.2. Patients suffering from Myelodysplasia/Leukaemia

The study base should consist of all adult patients suffering from Myelodysplasia or a type of possibly benzene-related Leukaemia which were – during a precise period - diagnosed and/or treated in the haematology service of one of the hospitals which are part of the network set up during the study on 'Exposure specific genomic lesions in Myelodysplastic Syndromes'- (Programme of scientific support to worker protection in the area of health, contract nr. ST/03/23). These patients are invited to participate. Those who accept are the study population.

IV   RESULTS

Description of the genotypes for the 56 subjects analysed:

CYP1A1:

Wild type (code 0)	49 (87.5%) subjects
MspI variant only (Ile/val = 0 & Msp1=2)	6 (10.7%) subjects (I suppose the 22 in column MspI should be read 2, please check)
exon 7 and MspI variants together (Ile/val = 1 & Msp1=2)	1 (1.8%) subject

CYP2E1	
Wild type (code Rsa I = 0 & Dra I = 0)	53 (94.6%) subjects
Dra1 variant only (code Rsa I = 0 & Dra I = 1)	2 (3.6%) subjects
Rsa1 and Dra1 variants (code Rsa I = 1 & Dra I = 1)	1 (1.8%) subject

GSTM1/T1	
Null (code 0)	25 (44.6%) subjects
+-/++ (code 1)	31 (55.4%) subjects

GSMT3	
Both alleles wild type (coded 0)	33 (58.9%) subjects
One variant allele (coded 1)	21 (37.5%) subjects
Both alleles variant (coded 11)	2 (3.6%) subject

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GSTP1	
Both alleles wild type (coded 0)	27 (48.2%) subjects
One variant allele (coded 1)	25 (44.6%) subjects
Both alleles variant (coded 11)	4 (7.1%) subjects

NAT2	
NAT2*4 (code 0)	5 (8.9%) subjects
NAT2*5 (code 1)	13 (23.2%) subjects
NAT2*6 (code 3)	6 (10.7%) subjects
NAT2*7 (code 2)	1 (1.8%) subject
?? (code 11)	10 (17.9%) subjects
?? (code 12)	15 (26.8%) subjects
?? (code 13)	2 (3.6%) subjects
?? (code 22)	4 (7.1%) subjects

XRCC1-194	
T/T (code 0)	0 (0%) subjects
A/T (code 1)	8 (14.3%) subjects
A/A (code 11)	48 (85.7%)

XRCC1-280	
A/A (code 0)	1 (1.8%) subject
A/G (code 2)	6 (10.7%) subjects
G/G (code 22)	49 (87.5%) subjects

XRCC1-399	
G/G (code 0)	8 (14.3%) subjects

A/G (code 3)	25 (44.6%) subjects
A/A (code 33)	23 (41.1%) subjects

XRCC2-188	
A/A (code 0)	0 (0%) subject
A/G (code 1)	10 (17.9%) subjects
G/G (code 11)	46 (82.1%) subjects

XRCC3-241	
C/C (code 0)	18 (32.1%) subjects
C/T (code 1)	26 (46.4%) subjects
T/T (code 11)	12 (21.4%) subjects

XPD-exon 6	
C/C (code 0)	15 (26.8%) subjects
A/C (code 1)	31 (55.4%) subjects
A/A (code 11)	10(17.8%) subjects

XPD-exon 23	
A/A (code 0)	22 (39.3%) subjects
A/C (code 2)	26 (46.4%) subjects
C/C (code 22)	8 (14.3%) subjects

NQO1	
Wild type (code 0)	39 (69.6%) subjects
Variant allele (code 1)	17 (30.4%)

Connecting the individual results with the effect categories revealed already at the first tentative analysis, that no associations of a particular genetic make-up with a particular benzene-exposure effect on peripheral blood cell counts would come out.

V   Comments

With the number of subjects at hand in each effect category, it would only have been possible to demonstrate such an association, if this were a strong one. The genetic tests showed a huge variability in outcomes. Already at first sight, it was clear that no association could be detected between a particular effect category and a particular genetic property or combination of genetic properties. Given the limited number of persons within each effect-category, any further statistical analysis is highly unlikely to show any association between a particular effect category and a particular genetic property or combination of properties.

As a consequence, a further comparison of the genetic properties of effect categories with the genetic properties of MDS patients could not show any relevant result. The efforts to prepare this part of the research were therefore discontinued. Research on the genetic properties of MDS patients may be very interesting for other reasons. However, in this study it could not add anything to answering to the research question.

From the above may be concluded that in order tot study the association between genetically mediated particular benzene induced peripheral blood cell count changes and the possible risk to severe haematolymphopoietic disorders, much more subjects need to be studied. The problem therewith is that as far as we know, higher numbers of study subjects will be synonymous with a limited number of blood samples per person. This will make any subdivision into effect categories less unreliable. In addition, at the current levels of exposure, the effects will be less pronounced than in this historical database.

The outcome of this study may be illustrative of the fact that genetic susceptibility is a very complex phenomenon, and that in many cases, it cannot easily be demonstrated. As a consequence, it is highly unlikely that within the near future, human epidemiological studies might give the answer to the question to what extent particular benzene induced effects (or the lack of it following benzene exposure) on the peripheral blood cell count might reveal an increased susceptibility to Myelodysplastic Syndrome or haematolymphopoietic cancers following benzene exposure.

For the very same reason, genetic tests might not (yet) constitute an added value in combination with peripheral blood cell counts to identify persons who are considerably more at risk of showing MDS or an haematolymphopoietic cancer following benzene exposure.

The research team therefore reoriented its efforts during the last months of the study to prepare European studies together with other research groups with a similar interest, hoping to be able to contribute with its experience bringing together databases.

It will still be the permanent concern of the research team that the efforts be continued for a better understanding of the relationship between occupational risk factors and genetic susceptibility on the one hand, and adverse health effects and exposure specific genomic lesions on the other. Such understanding may in the long run contribute to allow for:

➢ developing procedures of population observation for unknown or suspected environmentally or occupationally induced cancer risks

➢ the development, for social security systems and in particular for compensation of occupational diseases, of scientifically based criteria for distinguishing occupational induced and non–occupational induced cancer with a view to obtain specific compensation for employees who's cancer is likely to be caused by occupation

➢ setting up accurate risk assessment procedures for new and existing chemicals, in particular for the risk of hematolymphopoïetic cancers, which would allow to do reliable animal experiments using much lower numbers than needed nowadays, because the can be selected according to the similarity of their polymorphisms with those of the more susceptible humans.

Socio-economical, ethical, and legal consequences of the problem under study and ways to validate the study results

The conditions and the context for scientific research on occupational health were and are shifting.

The growing concentration of industrial power in companies that are globally active seems to be coupled with an increase of the grip that the said companies have on

scientific research about health-threatening factors of labour. Epidemiologists are hired by companies to do research with the population of workers that should preferably not lead to conclusions that could be detrimental to the company's economic interests.

A number of reasons can be mentioned:

- The identification of hazardous product properties is governed by international regulations (linked to the elimination of trade barriers). These procedures can lead to officially classifying a product e.g. as being carcinogenic or mutagenic, which has immediate commercial consequences.

- In part of the industrialised world huge indemnification claims are likely to be formulated against companies by potential exposure victims, who can be workers as well as civilians.

- Estimating the level of exposure at which certain effects can occur may lead to stricter exposure threshold limit values, which could increase production costs.

But also: companies who invest in developing or marketing or using genetic tests may have an interest in overestimating its relevance.

As a consequence of this tendency there is growing inequality between partial and non-partial researchers as for the accessibility of research data. In this way data that after research could lead to important knowledge as to the protection of people, are less likely to become a public commodity. Notwithstanding this tendency we have the impression that generally speaking there is relatively more goodwill to put data at the researchers' disposal in certain countries that have a solid system of social protection including social security and a socially controlled and managed occupational medicine. This study may be illustrative in hat respect.

The great financial interests that are coupled with the commercialisation of genetic testing will lead to an expansion of the scope of these tests to areas where they are mostly pointless and unacceptable from a perspective of social protection. Selecting employees based upon genetic tests is an example. When the latter are available potential users will be lulled into using them through a 'scientific' discourse that may or may not be based on grounds that are scientifically plausible but that in many cases is likely to speculate on unscientific deterministic thinking.

For a number of problems urgently needing scientific analysis to actually become the object of a systematic scientific research project does not seem very likely. We mean phenomena such as the significantly lower life expectancy in the lower educated. The fact that there are big and renowned research institutes in Europe, mostly managed on a tripartite basis, does not seem to change that very much. Though phenomena such as these were one of the foundations for justifying the existence and expansion of these research institutes there seems to be a certain reluctance to deal with this kind of subject.

A flexible labour market is tantamount to unstable research populations. The best way to discover the effects of agents on people is studying these effects in people with high exposure levels. For practical reasons the fact that an increasing number of those who have the highest occupational risks are in an unstable occupational situation, will tamper any epidemiological research about the effects on people. The same goes for the identification of occupational diseases in these categories of workers: very many of them – and often the weaker among them – cannot be heard in regular medical feedback research, for the simple reason that they are not working in the same company any longer.

In recent years the concept of privacy protection has gained ground, and it was integrated in law texts that show a lack of consideration as for the balance between the relevance of the concept and other societal interests. A perverse side effect of this is a limitation of the accessibility of data for scientific research. Moreover the actual inequality as for data accessibility between potentially partial and non-partial researchers could be sharpened. It is deplorable that the present legislation uses an all too broad definition of what is to be considered scientific research. Sheer market research with straightforward commercial intentions is put on a par, as it were, with epidemiological research that aims at tracing causes of cancer.

We need more publicly funded research into *genetic susceptibility* to occupational diseases, to make sure that genetic testing is only applied when advisable and acceptable. It is not inconceivable that commercial service centres will come into being that offer genetic tests and medical images of would-be employees to employers. The impartial scientific discourse on the relevance or irrelevance of these procedures is in danger of being suffocated by the immense commercial interests that would be at stake when establishing such centres. One will probably be forced to constantly go against a biased 'scientific' discourse on genetic susceptibility. Without a strong impartial research

network it will be impossible to forward a good defence vis-à-vis such commercial initiatives in a necessary democratic debate. We suspect, however, that such initiatives will offer and apply irrelevant procedures, which are and will be unacceptable for reasons of their irrelevance only.

Publicly funded research should be impartial research. Research organised and financed by the industry itself can be impartial as well, but it is the presence of a well-developed and strong research, based on public resources that will stimulate the impartiality of any research, including research financed by other sources.

The crucial question as to social protection is in which direction we are going on the axis between - on the one hand - adapting humans to labour, i.e. selecting people, and adapting labour to humans on the other hand.

The scientific research that is necessary for underpinning a strategy of social protection will only be generated if governments specifically support such research by starting up research programmes.

Some additional ethical comments

This study allowed to further both accurate and pragmatic approaches to complying with ethical requirements.

The access to the date was strictly organised. Persons with access to the database – limited in accordance with the requirements issuing from there role in the study were:

-the occupational health physician of the company, who is bound by the very strict labour regulations and the medical deontology

-the two researchers who are MD's who together with the OHP had the key to link the name of the employee to the code number.

-the statistician, to the extent necessary

In full agreement and cooperation with the occupational health physician, and after approval by the companies direction to which we are grateful for their openness and respect for impartial research, the legal Committee for Prevention and Protection, in which representatives of employees must meet every month, were fully informed about the study, its aims and methods and possible outcomes, as well as its possible

consequences. Also all privacy considerations were openly discussed, including the informed consent procedure. The Committee has unanimously approved the participation in the project.

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