INTERNATIONAL
AGENCY
FOR RESEARCH
ON CANCER
LYON

1992
BIENNIAL REPORT
1993

WORLD
HEALTH
ORGANIZATION

SC/30/2
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WORLD HEALTH ORGANIZATION

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

BIENNIAL REPORT

1992–1993

FOR THE PERIOD
1 JULY 1991 TO 30 JUNE 1993

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER
LYON, FRANCE

1993

ISBN 92 832 1093 X
PRINTED IN FRANCE

150 cours Albert Thomas, 69372 Lyon, Cédex 08, France

Distributed on behalf of IARC by the Secretariat
of the World Health Organization, Geneva, Switzerland

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INTRODUCTION

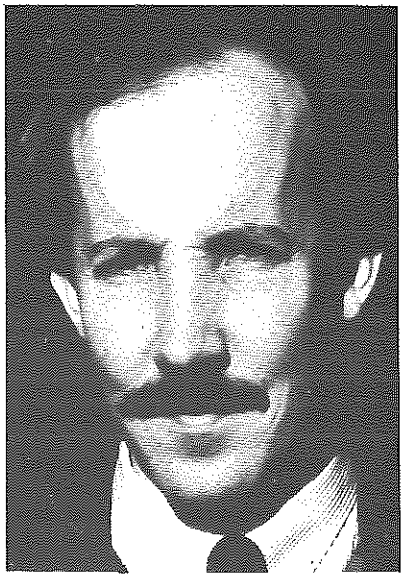
As this is the last occasion on which I will take responsibility for the IARC Biennial Report, I shall take this opportunity to reflect briefly upon some of the trends and changes in orientation in the work of the Agency that have occurred during the 12 years that I have had the honour of directing its activities.

The establishment of both laboratory research and epidemiology was an important and far-sighted policy decision at the launching of IARC in the mid-1960s, but it has only relatively recently become possible to press for a real integration of these two approaches to cancer research into what is now grouped under the still ill-defined term of molecular and biochemical epidemiology. In this area the Agency has been at the forefront with projects in which precise biological laboratory measurements have been made of levels of infection with human papillomavirus and hepatitis B virus, of exposure to aflatoxins and of nitrosation capacity within major epidemiological projects. Such measurements enhance the precision of exposure assessment and thus contribute to responding better to the growing demand for evidence of cancer-causation in humans when the carcinogenicity of agents is evaluated. This is reflected in the increasing weight placed on epidemiological data in the evaluations of carcinogenicity made within the IARC Monographs programme, and in the greater emphasis put on information on mechanisms of action.

Another trend has been the expansion in studies of inherited factors in genetic predisposition to cancer. This has again both laboratory and epidemiological elements, for which it has been hard to find adequate resources with recent financial constraints, but notable successes have been made in a limited range of areas, most particularly those of multiple endocrine neoplasia type 2 and of familial breast cancer.

Advances in etiological research have reached the point where preventive interventions can begin to be planned systematically, but organized interventions tend to require resources beyond the scope of IARC. The Gambia Hepatitis Intervention Study, which was made possible by generous extra-budgetary funding from the Italian Government, has been a prime example of a public-health-oriented research programme conducted at a manageable cost to the Agency for a major benefit to both the population concerned and cancer research in general.

A very encouraging sign has been the joining of five member states, namely Canada, Denmark, Finland, Norway and Switzerland, in the last twelve years, bringing to the Agency additional expertise and reinforcing international commitment to its mission. In spite of this, during the last biennium a number of projects could not be initiated or even had to be discontinued purely because sufficient funds were unavailable. The major factor contributing to this difficulty was the inability of one member state to make its contributions to the Agency's regular budget, a problem that has still not been resolved. The further significant cut in the budget for the forthcoming biennium, decided by the Governing Council at its last meeting, means that much desirable work will have to remain at a standstill, and that more valuable time will probably be devoted to money-chasing from outside sources. In addition, essential maintenance to the Agency's buildings is delayed, with possibly very serious consequences for the functioning of the Agency.



Prof. K.K. Alitalo
(1992–95)

Dr V. Beral
(1993–96)

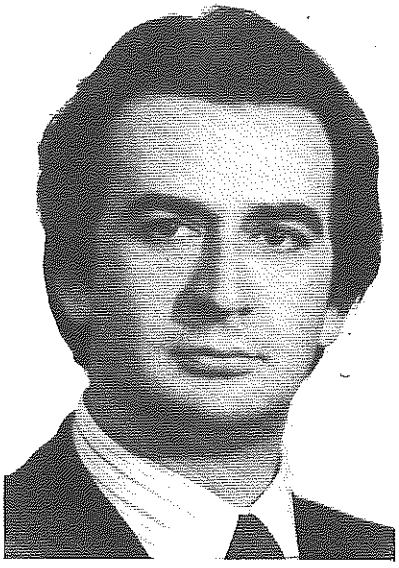
Dr A. Green
(1993–96)

Dr E. Lynge
(1993–96)

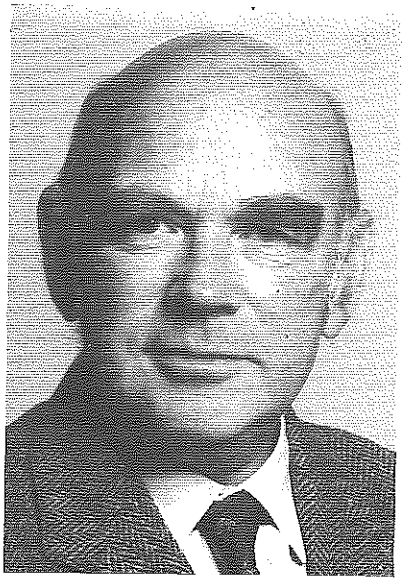
New members of the Scientific Council 1992 and 1993

Prof. H. Marquardt
(1992–95)

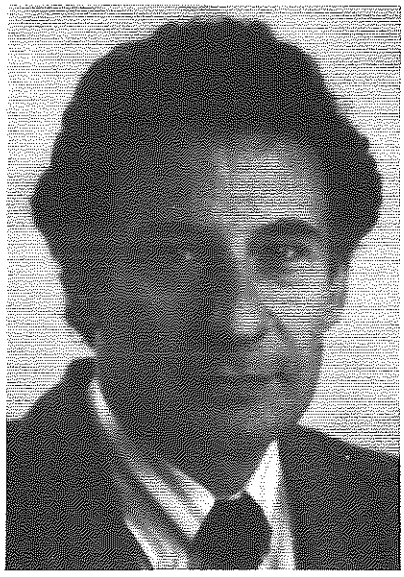
Prof. F. Mitelman
(1992–95)



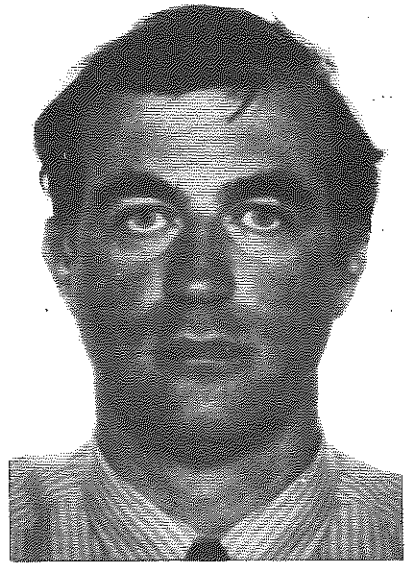
Dr P. Pasquini
(1992–95)



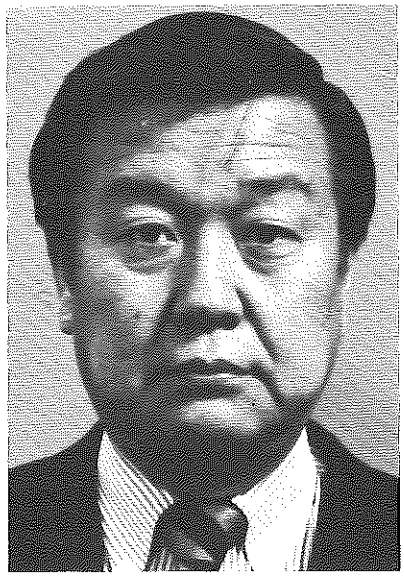
Prof. T. Sanner
(1992–95)



Dr A.R. Sarasin
(1992–97)



Dr B. Standaert
(1993–96)



Dr M. Terada
(1993–96)

A positive feature of the last meeting of the IARC Governing Council in April 1993 was the election of a new Director of the Agency to take over when my mandate terminates at the end of the year. Professor Paul Kleihues, a noted neuropathologist from Zurich, Switzerland, was selected by the Council from among numerous applicants; the Agency's staff as a whole joins me to wish him a warm welcome and a most successful further development of the Agency's activities.

The end of 1993 was also marked by a limited, but nevertheless painful, reduction of the staff and by the departure of two senior scientists. Dr Helmut Bartsch is joining the Deutsches Krebsforschungszentrum in Heidelberg, where he has been appointed head of the Division of Toxicology and Environmental Carcinogenesis. Dr Bartsch, who joined the Agency in 1972, contributed greatly to building up the excellent reputation that the Agency enjoys within the international scientific community. Professor Bruce Armstrong, an epidemiologist of world renown, who has been Deputy Director for the last two and a half years, will take up the position of Director of the Australian Institute of Health and Welfare in December 1993. His contribution to both the management and the scientific work of the Agency during this short period has been outstanding.

An annual memorial lecture to honour Professor Roger Sohier, who died in December 1991, has been established. The first lecture was presented in June 1993 by Professor G. Orth, on "Papillomavirus et cancer humain".

During the 25 years of my association with this Agency, the enthusiasm and commitment of the staff, including scientists, technicians, administrative and general service personnel, have remained at the same very high level and have not weakened even during the difficult period of the closure of the main building for removal of asbestos. Its competence, professional attitude and productivity have been proved over all these years and have substantially contributed to giving the Agency the world-wide recognition it now enjoys.

Alongside the many positive appreciations of the achievements of these 25 years of activity there is, however, the less than positive consideration that the humanitarian and altruistic attitude of the founding fathers of the IARC, that for years inspired the governments of the Agency's Participating States, seems to have been eroded among some by less than altruistic economic reasonings. Although one certainly cannot ignore the constraints that economic recession may impose, the fact remains that the total annual budget of the Agency, shared by the richest countries of the world, is barely equivalent to the cost of a few kilometres of highway or a wing of a military aircraft. It is also pertinent to remember that a sum roughly corresponding to the annual budget of the Agency would be saved in health care costs if about 1000 cancer cases per year could be prevented. If one considers in addition that the health care systems, even in industrialized states, are hardly sufficient to satisfy present demand, despite the increasing share of national budgets that they absorb, the primary prevention of cancer to which the Agency is totally committed appears to be the best and perhaps the only way to actually reduce expenditures while at the same time reducing human suffering. I should like to beseech the Agency and its new Director, together with its Governing and Scientific Councils, to continue to uphold its original commitments in order to fulfil the great expectations that both developed and developing countries have for the role the IARC can play in the control and prevention of human cancer.

Some of the main features of the Agency's scientific activities over the past two years are highlighted below.

Descriptive epidemiology

The most prominent activity in descriptive epidemiology is the quinquennial publication of *Cancer Incidence in Five Continents*, of which Volume VI appeared at the end of 1992, along with diskettes carrying the data of both volumes V and VI. This latest volume includes data for 170 populations in 46 countries, mainly for the period 1983–87, but with a few data-sets up to 1989. For the first time for many years, several registries in Africa have provided data of sufficient quality for inclusion. In addition, the global burden of cancer for 1985 has been estimated, based on known incidence data and calculated extrapolations from mortality, population and survival data. This shows that 7.62 million cases of cancer occurred in that year, divided almost equally between men and women.

The analysis of time trends in cancer, based on the *Cancer Incidence in Five Continents* series and on mortality figures from the WHO data-bank, has been extended to take into account the latest volume of the former publication, and the results have been published. The colour graphs and associated tables document clearly, for example, widespread decreases in stomach cancer and increases in malignant melanoma, and show a variety of patterns for lung cancer that closely reflect known trends in cigarette smoking habits across different populations.

Other descriptive studies include those of Italian migrant populations in about 10 countries, demonstrating how the occurrence of cancer at different sites evolves with time from that in Italy towards the levels usual among the host country's population.

Substantial support continues to be given to cancer registration activities worldwide, by providing active assistance for the setting up and running of registries in 10 African, 6 Asian and 6 South American countries and in the Pacific region, an active participation in the European Network of Cancer Registries funded by the EC "Europe Against Cancer" programme and provision of the secretariat of the International Association of Cancer Registries.

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans

Six meetings were held during the period under review in this report. The first, held in October 1991, examined occupational exposures to acid mists, and decided that there was sufficient evidence that occupational exposure to strong-inorganic-acid mists containing sulfuric acid can cause cancers of the upper respiratory tract. At the same meeting, examination of new epidemiological evidence on the carcinogenicity of 1,3-butadiene led to a reclassification of this substance as probably carcinogenic to humans (Group 2A).

The meeting in February 1992 focused on solar and ultraviolet radiation, particularly in relation to nonmelanocyte skin cancers and malignant melanoma of the skin and eye. Solar radiation was classified as carcinogenic to humans and ultraviolet A, B and C were each considered probably carcinogenic. Use of sunlamps and sunbeds was considered to entail exposures probably carcinogenic to humans, but fluorescent lighting was not classifiable.

A wide selection of naturally occurring substances that are found in foodstuffs, particularly a number of mycotoxins, were examined at a meeting in June 1992. Chinese-style salted fish was evaluated as being carcinogenic to humans, as were naturally occurring mixtures of aflatoxins. Several heterocyclic polycyclic amines, often formed in cooking, were deemed probably or possibly carcinogenic.

The evaluation of epidemiological studies involving hairdressers and barbers, in October 1992, indicated that occupational exposures in these professions probably entail carcinogenic risks. A considerable range of artificial colouring matters was also examined, and a number were felt to be possibly carcinogenic to humans; the evidence that the manufacture of magenta entails carcinogenic risks was considered sufficient.

On the basis of both experimental and epidemiological evidence of lung tumorigenicity, beryllium and beryllium compounds were evaluated as carcinogenic to humans at the meeting in February 1993. A similar conclusion was reached with respect to cadmium and cadmium compounds, whereas for mercury and its compounds, only methylmercury could be classified on available evidence, as possibly carcinogenic.

The meeting in June 1993 broke new ground by evaluating evidence for carcinogenicity of biological agents, namely the hepatitis viruses. It was concluded that both hepatitis B and hepatitis C viruses are carcinogenic to humans; hepatitis D virus could not be classified.

Occupational cancer

A number of long-term studies of occupational cancer in industrialized countries are continuing, including those of workers with phenoxy acid herbicides, styrene, and man-made mineral fibres and in the pulp and paper industry and the lead industry and in biological research laboratories. The study of styrene is pointing to an increased risk for leukaemias and lymphomas due to this substance. New studies have been planned or launched on risks from asphalt vapours and mercury, and those in the wood, leather and textile industries. The initial results of the project on wood workers confirm an elevated risk of nasopharyngeal and sino-nasal cancers.

The major reanalysis of nuclear industry workers in Canada and the UK and USA is largely complete and the results are expected to be released shortly, and will provide an important refinement of understanding of the effects of low doses of ionizing radiation. The study of children in populations exposed to radiation from the 1986 Chernobyl accident is continuing; the first results indicated no increase in leukaemias up to 1988 in relation to estimated exposure.

In parallel, a major effort is being put into identifying occupational risks in developing countries, where exposures are often very high. Studies are beginning on a range of occupational exposures in Brazil, China, Egypt, India, Poland and Uruguay.

Studies are in progress to assess the p53 gene mutation spectra in tumours occurring in workers known to have been exposed to specific carcinogens (aromatic amines and bladder cancer; vinyl chloride and liver haemangiosarcoma).

Diet and cancer

The European Prospective Investigation on Cancer and Nutrition (EPIC) funded by the Europe Against Cancer Programme of the EC has now advanced from the pilot phase of methodological testing to the full prospective study phase of data collection in France, Italy, the Netherlands, Spain and the UK; the full study will begin very soon in Germany and Greece. Dietary questionnaires have been tested, food composition tables prepared where lacking, and logistic problems of handling and storing the very large number of blood samples largely resolved.

Other case–control studies of diet and cancer are in progress. In one, estimates of nitrosamine intake have been compared with gastric cancer risk, yielding supportive evidence for the hypothesis that these agents are involved in the etiology of the disease.

Tobacco and cancer

While there is no reasonable doubt about the ability of tobacco smoking to cause cancer, a number of projects are in progress to improve our understanding of the association, and to assess its consequences and possible preventive measures. The major study of passive smoking is now well advanced, and results are expected in 1994. Possible genetic susceptibility related to polymorphism of carcinogen-metabolizing enzymes is being studied using blood samples from some of the subjects. The roles of such enzymes are also being studied in

samples from patients undergoing lung surgery for various diseases, and other aspects of the biochemistry of the tobacco–cancer link are under investigation in laboratory studies.

Tobacco use in developing countries is being evaluated, since the scale of the problem is largely unknown, although it is clear that the habit is being strongly promoted by the tobacco industry in parallel with the decline now observed in many developed countries.

Viruses and cancer

Although it has not been possible to maintain laboratory work on mechanisms of viral carcinogenesis due to competing demands for resources, viruses form the focus of a range of projects, in addition to the examination of hepatitis viruses in a Monographs meeting (see above) and the anti-hepatitis vaccination study of liver cancer prevention in the Gambia (see below).

The unique collection of biological materials related to Epstein–Barr virus (EBV) and Burkitt's lymphoma is being maintained. Interactions between human immunodeficiency virus (HIV) and EBV have been studied in relation to Burkitt's and other lymphomas, and the epidemiological link between nasopharyngeal carcinoma and EBV and other agents is being explored. Epidemiological study of cancers related to HIV infection has not proceeded far in the absence of adequate funding, but a start has been made in Rwanda and Uganda.

Results of various studies of human papillomavirus and cervical cancer have confirmed the central role of this agent, especially HPV type 16, in the disease. Collection of cervical cancer tissue and serum samples for a major survey of HPV prevalence in 22 countries is now complete; most samples are HPV DNA-positive but the predominant HPV type differs between countries.

Endogenous formation of carcinogens

Nitric oxide (NO), produced enzymatically from L-arginine by the enzyme NO synthase, has been identified as an essential cellular signalling molecule. Experiments with rats have shown that induction of NO synthase by inflammation or infections with bacteria or parasites leads to increased endogenous nitrosamine formation. Measurements of activity of the enzyme are being made in a range of human surgical tissue samples from organs such as liver, bladder, stomach, colon and brain where induction of the enzyme may occur following chronic and/or repeated infections.

Further applications of the nitrosoproline excretion test have provided new evidence on the correlation between an exposure to *N*-nitroso compounds and the risk of cancer of the nasopharynx and oesophagus in China.

Genetics and cancer

Much research has pointed to the role of the cytochrome P450 enzymes in the activation of chemicals into active carcinogenic metabolites. Interindividual phenotypic differences with respect to the various P450 isozymes are therefore being examined in detail in order to clarify mechanisms of action of specific carcinogens and to identify individuals with high susceptibility to their effects. Isozymes that appear to have specificities for particular nitrosamines and aflatoxins are the present focus of attention and are being studied in relation to liver cancer in The Gambia and Thailand, and in a rodent model of nasal carcinogenesis. The hypothesis that liver damage caused by the hepatitis B virus can lead to increased formation of active metabolites of aflatoxins is being investigated.

Genetically controlled DNA repair processes also are thought to affect individual susceptibility to carcinogenesis, and expression of the enzymes involved is being examined in animal models and in human tissue samples. Individual variation in the capacity of human lymphocytes to repair UV-induced DNA photoproducts has been examined in a case–control

study of skin cancer in Australia, but no significant difference between cases and controls has so far been detected.

Linkage analysis has been applied to identifiying genes responsible for the X-linked proliferative syndrome, multiple endocrine neoplasia type 2, neurofibromatosis type 2 and familial breast and ovarian cancer. Although the genetic maps have been refined, the specific genes have not yet been identified, and work towards this goal is continuing. As concerns breast cancer, it appears that although a gene (or genes) on chromosome 17q accounts for the majority of families in which both early-onset breast cancer and ovarian cancer occur, other genes predisposing to breast cancer also exist.

Mechanisms of carcinogenesis

The Agency carries out mechanistic research not for its own academic interest, but insofar as it complements etiological studies, elucidates the origins of cases of cancer not otherwise explained or throws light on individual differences in cancer susceptibility. Similarly, many projects mentioned elsewhere in this summary generate mechanistic information, notably the genetic studies described above.

Various projects have examined the occurrence and role of genetic alterations in oncogenes and tumour-suppressor genes in specific cancers. These may provide information on molecular pathways to cancer and the responsible agents, and may also be usable as molecular biomarkers to identify individuals at elevated risk for cancer in epidemiological research. Mutations of the *p53* tumour-suppressor gene in oesophageal tumour tissue from several countries have been measured, and suggest a different mutation pattern from those in colorectal or lung cancer. The mutations seem to typically occur at least before lesions become invasive, and are sometimes found at very early stages of neoplasia. Mutations or alterations in expression of other genes (*EGFR*, c-*myc*, *Rb*, *mdm2* and *cyclin 91*) are also being examined in order to acquire a better understanding of the natural history of oesophageal cancer. Antibodies against p53 protein in sera of patients have been detected. Mutated *ras* genes have also been found in cell lines established from oesophageal tumours, but not in corresponding tissue samples, and the difference is being explored further. Different frequencies of alterations in both genes between oesophageal adenocarcinomas and squamous cell carcinomas have been identified. *p53* gene mutations have also been found in gastric tumours, exclusively in adenocarcinomas, and appear to be involved in a late stage of carcinogenesis.

Possible involvement of genetic instability in human gastric carcinogenesis has been suggested by the finding of numerous changes in microsatellites (CA repeats) in gastric tumour samples.

Specific *p53* mutations attributable to aflatoxin exposure have been observed in human hepatocellular carcinoma and studies in HBV-transgenic mice show that hepatitis results in an increased expression of P450 enzymes responsible for metabolism of aflatoxins.

The role of gap-junctional intercellular communication in carcinogenesis continues to be investigated. A method for measuring communication in human liver samples, freshly removed at surgery, has been established. It seems that genes coding for connexin proteins that form gap junctions between cells can act as tumour-suppressor genes; more effective intercellular communication when production of connexin-containing gap junctions is increased would be consistent with the hypothesis that such communication provides a control of cell growth and proliferation.

Experiments *in vitro* in which human keratinocytes were irradiated with UVB radiation have suggested that the UV directly induces a mutation of *p53* with a consequent acquired

capacity for selective clonal expansion of cells bearing such mutation. Furthermore it was shown that solar UV radiation induces specific *p53* mutations in normal human skin. Measurement of these mutations could be very useful for assessing UV exposure.

Cancer prevention

Although all its research is aimed at identifying cancer causes so that preventive measures can be adopted, IARC does not have the means to introduce major programmes for the prevention of cancer. Therefore its activities are mainly focused on research into how preventive programmes can be successfully conducted, with limitations dictated by its inability to secure funds for many of the possible projects.

In the Gambia, the vaccination of all newborns against hepatitis B is now routine, and the intervention study's main activities are to follow up the cohort vaccinated during the introductory period. Among vaccinated children tested in 1992, 95% remained free of the infection at age 5 years. Cancer registration is now running efficiently, and the first data from the country were included in *Cancer Incidence in Five Continents*, Volume VI. The intervention study in the Gambia continues to be largely funded by the Government of Italy and a contribution from the Medical Research Council of Sweden.

A trial for an active preventive approach has been set up in Venezuela to examine the effect of anti-oxidants (beta-carotene and vitamins C and E) in interrupting the progression from chronic gastritis to stomach cancer. An additional aim of assessing the effect of eliminating *Helicobacter pylori* infection has been hampered by the difficulty in eradicating this infection using standard therapy; a new small trial is in progress to evaluate the efficacy of other treatments. Associated projects in the same population are a case–control study of stomach cancer and a project to evaluate a screening programme for stomach cancer.

Other evaluations of screening programmes include a major planned study of physical examination of the breasts, in Manila, and assessments of cervical screening programmes in Manila and in southern India.

Fellowships, courses and publications

In the period covered by this report, a total of 23 IARC research training fellowships were awarded, of which 5 were to work in epidemiology, 5 in chemical carcinogenesis, 1 in viral oncology, 8 in cell biology or genetics and 4 in biochemistry or molecular biology. The successful candidates, from among 104 eligible applicants, come from 12 different countries.

Ten training courses were held, attended by a total of 426 participants. Courses on epidemiology and biostatistics were held in Lyon, Florence (Italy), and Ostrava (Czech Republic), on occupational cancer in Ahmedabad (India), on detection of health hazards in human population in Harare (Zimbabwe) and another in Lyon, for the first time, on nutrition. Another well-received innovation was a course on tumour pathology for non-pathologists, held in Oslo.

In the IARC Scientific Publications series, 13 new volumes appeared, among them *Cancer Incidence in Five Continents* Volume VI, as mentioned earlier; four IARC Monographs volumes and six IARC Technical Reports were published.

The approved regular budget for the biennium 1992/93 was US $32 487 000. On 30 June 1993, the Agency's staff consisted of 57 professionals including 51 scientific and 6 administrative staff, 87 laboratory and other scientific support staff and 32 administrative and maintenance support staff.

Lorenzo Tomatis, M.D.
Director

PART 1. COLLECTION, DISSEMINATION AND ANALYSIS OF DATA ON CANCER FREQUENCY AND IMPACT

Knowledge of the size and nature of the cancer problem in the world is fundamental in planning and evaluating appropriate control measures, and the Agency's work on this subject represents an essential complement to the work of WHO Headquarters.

Studies of the variation in the risk of different cancers according to geographical location continue to provide important clues as to possible etiology. Changes in risk over time and between different population subgroups (defined in the terms of ethnicity, socio-economic status, birthplace etc.) provide additional dimensions which enhance the interpretation of the geographical patterns. These descriptive epidemiological studies constitute a major component of the work of the Agency.

Since the value of the descriptive studies depends upon their completeness and quality of the data-sets used, considerable effort is also put into improvement of cancer registration world-wide.

1.1 *Description of cancer incidence and mortality*

1.1.1 *Cancer Incidence in Five Continents*, Volume VI

(D.M. Parkin, S. Whelan and J. Ferlay; in collaboration with C.S. Muir, Edinburgh, UK; Y.T. Gao, Shanghai, China; and J. Powell, Birmingham, UK)

The sixth volume of the *Cancer Incidence in Five Continents* series (Parkin *et al.*, 1992) presents data on the incidence of cancer worldwide for the years 1983–87. One hundred and forty-two registries, covering 170 populations in 46 countries, contributed data to this volume.

For the first time, the data sets submitted were subjected to a series of quality control and validity checks at the Agency. This was made possible because most contributing registries sent their data in the form of a listing of cases (without personal identifying information), which included the topographic site of the tumour, the morphological diagnosis and the basis of diagnosis for each case. This allowed checking for unlikely or impossible site/histology or sex and age versus site and/or histology combinations. After correction, the calculated rates and various indicators of quality were carefully scrutinized by the editors to assess whether each registry was achieving good coverage of its population(s). The aim of the *Cancer Incidence in Five Continents* series is to present data which can be compared with confidence between different geographical areas, and of the 156 registries submitting data for Volume VI, 14 were not accepted for publication.

In the previous volume (Volume V), an asterisk was used beside the registry title to indicate that some caution was needed in the interpretation of the results. Usually this was because of some evidence of underregistration, although this was not of an order to preclude publication of the data. This practice was continued in Volume VI; in addition, for registries whose data

were not verified at the Agency (because the morphological detail required was not available), a flag was used to denote that, although the data may have been checked within the registry, they have not been through the editorial verification process.

The volume contains an extended section on quality and quality control procedures. Several indices of completeness and validity are tabulated: the proportion of cases verified histologically, the number of cases registered on the basis of a death certificate alone, the mortality/incidence ratios by site and by sex, the proportion of the total neoplasms registered for which the primary site was not certain, and the numbers of cases registered for which the age was not known. The importance of accurate information on the population at risk has been underlined by a separate chapter on the denominator. For the much-used world-standardized incidence rates, the standard error of the rate has been included, to assist in evaluating the likely significance of the differences between registries.

A further innovation of Volume VI was the preparation of a diskette containing all published results (population figures, and mortality and incidence at the ICD three-digit level) together with easily used software to permit display of results, or their transfer to a computer file for analysis. This was distributed with the printed volume, along with a diskette containing the data from Volume V of the series. It is now planned to put the data from the first four volumes of *Cancer Incidence in Five Continents* onto diskette.

1.1.2 European cancer incidence and mortality database (EUROCIM)

(D.M. Parkin, J. Ferlay, M.T. Valdivieso and J. Estève; in collaboration with F. Berrino, Milan, Italy; M.P. Coleman, Sutton, UK; T. Hakulinen, Stockholm, Sweden; F. Ménégoz, Grenoble, France; C.S. Muir, Edinburgh, UK; B. Noble, Bristol, UK; R. Otter, Groningen, Netherlands; E. Schifflers and A. de Coninck, Namur, Belgium; H.H. Storm, Copenhagen, Denmark; A.J. Swerdlow, London, UK; H. Tulinius, Reykjavik, Iceland)

The EUROCIM database has been created as one of the activities of the European Network of Cancer Registries (see Section 1.4.2). The objective was to make the data from cancer registries in Europe more easily accessible, hence permitting their use in comparative studies. The software for accessing and analysing the data was developed under contract, and a prototype version containing data from *Cancer Incidence in Five Continents* (Volume V) and limited data sets from Denmark, Varese (Italy) and England and Wales was delivered in 1991.

This prototype will be replaced during 1993 by EUROCIM version 1.0. This will consist of a cancer incidence and mortality database together with software to analyse and display the data. The data are from 35 EC registries and 8 other registries in Europe which have contributed to *Cancer Incidence in Five Continents* Volume VI.

The software will permit access to incidence data by sex, age, site histology combination and year of registration. Cancer registries can use EUROCIM to examine their own data and compare them with data from other registries, while epidemiologists can use them to generate descriptive tables or reports, and to compute rates, trends and projections with the statistical analysis facilities included in the software.

The database of this first version will be updated regularly. Programs for checking and converting incidence data have been produced at the Agency.

1.1.3 Analysis of data from collaborating cancer registries

Several studies have been undertaken in collaboration with researchers working in cancer registries around the world to analyse and present incidence and mortality data sets of special interest.

1.1.3.1 *Africa*

(D.M. Parkin, M.P. Coleman, A. Vizcaino, J. Ferlay and R. Sankaranarayanan; in collaboration with L. Levy and E. Chokunonga, Harare, Zimbabwe; M.E.G. Skinner, formerly Bulawayo; and H. Wabinga, Kampala, Uganda)

The analysis of the material from the cancer registry in Bulawayo, Zimbabwe, from the years 1963–77 was completed, and prepared for publication as an IARC Technical Report: *Cancer in the African Population of Bulawayo, Zimbabwe, 1963–1977* (Skinner *et al.*, 1993). The analysis comprises not only the descriptive epidemiology, including trends in incidence over this period, but an estimate of the importance of certain environmental factors (occupation, tobacco and alcohol consumption, reproductive variables) on the risk of seven different cancers (oesophagus, lung, liver, bladder, breast, cervix and corpus uteri).

The first results from the regenerated cancer registry in Kampala (Wabinga *et al.*, 1993) show interesting changes since the earlier periods of registration (in the 1950s and 60s), including increased incidence of cancers of the oesophagus, lung and prostate in men, and of breast and cervix cancer in women. AIDS-related Kaposi's sarcoma has become the major cancer in men, and is second in frequency in women.

Analysis of the results of the cancer registry in Harare, Zimbabwe from 1989–92 has been started.

1.1.3.2 *Asia*

(D.M. Parkin, J. Ferlay, S. Olivier, P. Boffetta and M. Kogevinas; in collaboration with N.C. Martin, Chiang Mai, Thailand; H.A. Pham, Hanoi, Viet Nam; L. Reyes, Manila, Philippines; S. Sontipong, Bangkok, Thailand; H. Sriplung, Songkla, Thailand; V. Vatanasapt and S. Sriamporn, Khon Kaen, Thailand; and Q.S. Wang, Tianjin, China)

Preparation of a monograph on *Cancer in Thailand 1988–1991* (Vatanasapt *et al.*, 1993) was completed, for publication in the IARC Technical Report series. The analysis is based on the results of three population-based cancer registries (Chiang Mai, Khon Kaen and Songkla), and on an *ad hoc* cancer survey in the Bangkok metropolitan area for 1988–90. Estimates of cancer incidence in the country as a whole are included.

The analysis of data for 1983–87 from three cancer registries in the Philippines (Manila, Rizal and Cebu) was completed by a visiting fellow (Dr Lilia Reyes). The results will be presented in a UICC publication.

The data from the first three years of the newly founded cancer registry in Hanoi, Viet Nam were analysed by an IARC fellow (Dr Pham Hoang Anh). The results show relatively high rates of stomach and lung cancers in men, and surprisingly low rates of cervix cancer in women.

Data from the cancer registry of Tianjin, China, were analysed by a visiting scientist (Dr Q.S. Wang), with emphasis on risks related to occupation and economic status. Proportional mortality ratios for 32 cancer sites according to 33 different occupations or 33 industries have been calculated for presentation in an IARC Technical Report. The results for lung cancer as well as those for socio-economic factors have been prepared for separate publication and include adjustment for smoking prevalence in different occupational groups.

1.1.3.3 *Oceania*

(S. Whelan and D.M. Parkin; in collaboration with F. Bach, Noumea, New Caledonia)

Work has begun on the analysis of data from cancer registries in several Pacific islands (Fiji, Guam, the Islands of Cook and Niue, Mariana, Marshall, Solomon, Kiribati, Nauru, New Caledonia, Palau, Papua New Guinea, Pitcairn, French Polynesia, American Samoa, Western Samoa, Tokelau, Tonga, Vanuatu and Wallis & Futuna). The completed material will comprise the first volume of a new series on *Cancer Incidence in Developing Countries*.

1.1.3.4 *South America*

(D.M. Parkin and C. Bouchardy, Geneva, Switzerland; in collaboration with A.-P. Mirra, São Paulo, Brazil)

The results of an analysis of cancer risk according to social status (measured by educational level) in São Paulo, Brazil, were published (Bouchardy *et al.*, 1993).

1.1.4 Worldwide burden of cancer

(D.M. Parkin, P. Pisani and J. Ferlay)

The annual incidence and mortality of all cancers and of 18 specific sites were estimated for the year 1985 in 24 regions of the world. The estimated age- and sex-specific incidence rates are based, depending on the extent and quality of data available for each country, upon real data reported by cancer registries, regression models of incidence on mortality, and relative frequencies of specific cancer sites. The main sources of data were *Cancer Incidence in Five Continents* Volume VI and the WHO mortality data bank; these were integrated with data from sample surveys from a variety of sources. Mortality data are available for some 30% of the world population, and for the remainder, figures were estimated by combining the incidence previously obtained, with sex- and age-specific survival for each of the cancer sites considered.

Crude and age-standardized rates and numbers of cases and deaths were computed for each of 24 regions of the world as the weighted averages of the component countries. Figure 1 shows estimated world total of incident cases and deaths, by sex and site.

The total number of new cases (excluding non-melanoma skin cancer) was 7.62 million, with an almost equal division (48%:52%) between the developed countries and the developing countries. The most frequent cancer in males was cancer of the lung (accounting for 17.6% of the 3.85 million cases in men), followed by stomach (12.3%), colon/rectum (8.6%) and prostate (7.6%). Cancer of the breast remains the major cancer site in females (19.1% of the 3.77 million cases) followed by cancer of the cervix (11.6%), colon/rectum (9.2%), stomach (7.5%) and lung (5.8%).

In men the risk of developing cancers of the lung, colon/rectum and prostate is highest in the more industrialized areas of the world: North America, Europe and the ex-USSR. Conversely, the risk of gastric cancer is highest in all eastern Asia, Japan showing the highest rates in the world in both sexes: 74.8 (M) and 35.2 (F) new cases per 100 000. In women the risk of breast cancer is highest in the more developed countries, while cancer of the cervix is the most frequent site in females living in the less developed parts of the world.

The mortality pattern parallels that of incidence, although the high frequency of some of the more fatal cancers (such as stomach, oesophagus, liver and lung), together with poorer survival probabilities, place some developing areas higher in the ranking of the risk of dying from cancer. Out of a total of 5 million deaths, 2.8 million (56%) occurred in developing countries and 2.2 million (44%) in developed ones. Lung cancer is the most frequent cause of

death from cancer, with a total of 785 000 deaths worldwide per year; it is followed by stomach cancer in both sexes. In women, cancer of the breast is by far the most frequent cause of death from cancer despite relatively effective therapy (reflected by an overall mortality/incidence ratio of 0.43).

To complete the description of the worldwide burden of cancer, estimates of the prevalence of cancers at the same 18 sites are in preparation. Because of the difficulty in defining "cured" cases among cancer survivors, prevalence is limited to cases diagnosed within the preceding five years. The method of estimation is that of life-table analysis applied to the cohorts of new annual cases previously estimated, and accounting for net survival.

In a previous study (Parkin & Sasco, 1992), the proportion of lung cancer cases in the world in 1980 which could be attributed to tobacco smoking was estimated. Using the new figures for 1985, estimates will be produced of the total burden of cancer morbidity and mortality related to tobacco. A further exercise will determine the proportion of new cancer cases and deaths which are the results of infection with viruses and parasites.

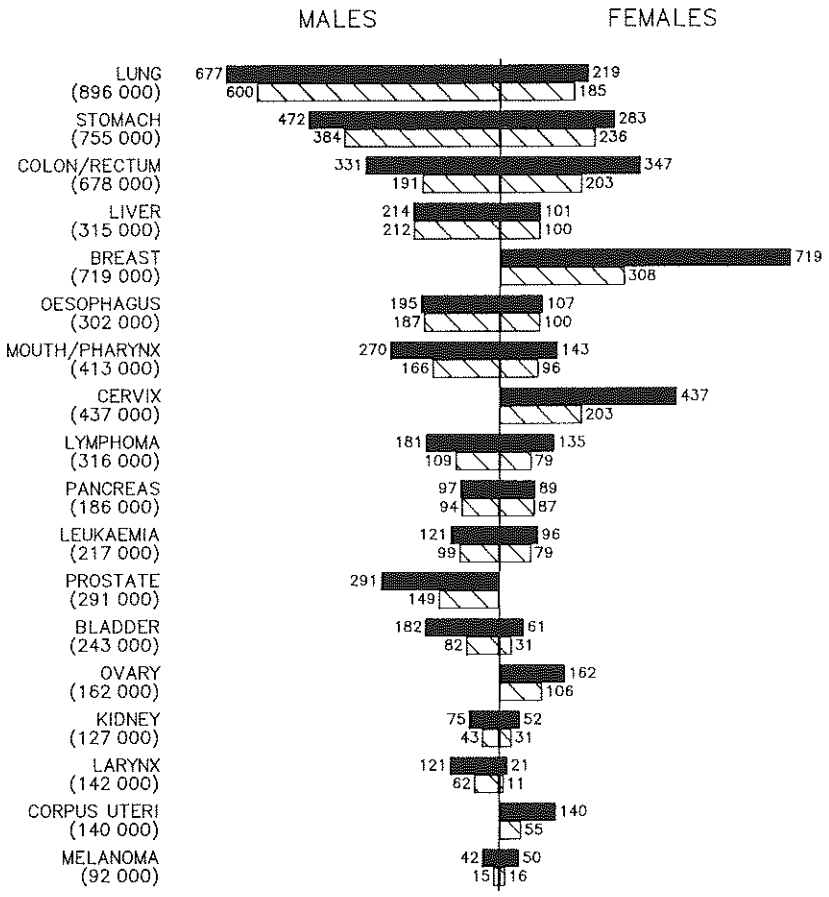


Figure 1. Numbers of incident cancer cases and of cancer deaths worldwide in 1985

1.1.5 Cancer incidence and mortality maps

(M. Smans and J. Estève)

The atlases of cancer incidence in the former German Democratic Republic (1978–82) (Mehnert *et al.*, 1992) and of cancer mortality in the European Economic Community (mainly 1976–80) (Smans *et al.*, 1992) were published in 1992.

Following Dr J. Tyczynski's tenure of a fellowship at IARC, the preparation of an atlas of cancer mortality in Central Europe is now almost complete (Figure 2). At a meeting in Warsaw in June 1993, the material was reviewed by all data contributors. The content of the publication has been approved and it is envisaged that the work will be completed by June 1994.

Dr Tamara Men from the Institute of Carcinogenesis, Cancer Research Centre in Moscow spent two months at the Agency preparing a preliminary cancer incidence and mortality atlas of the former USSR. The geographical patterns revealed are now being analysed.

1.1.6 Time trends in cancer

(J. Estève, P. Damiecki, A. Arslan and H. Renard; in collaboration with M.P. Coleman, London, UK; and E. Schifflers, Namur, Belgium)

The monograph on time trends in cancer (Coleman *et al.*, 1993) is in press; it presents trends in cancer incidence and mortality for 28 sites of cancer using data from 43 registries, which have published data in at least three volumes of *Cancer Incidence in Five Continents* (including Volume VI; see above), and from all countries for which mortality data of reasonable quality are available in the WHO data bank.

The data have been analysed using age-period cohort modelling, and the results are summarized as trends in age-adjusted rate by calendar period of incidence and death

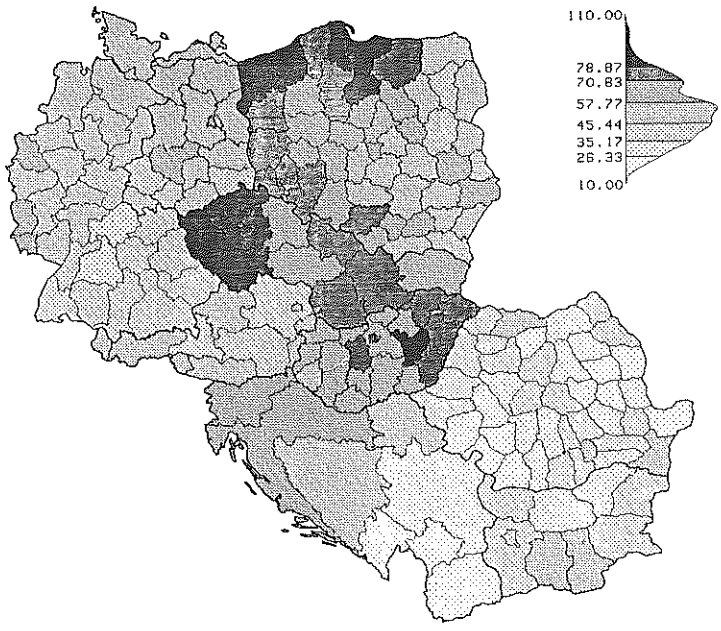


Figure 2. Lung cancer mortality in males in the central and eastern European countries

The data have been analysed using age–period cohort modelling, and the results are summarized as trends in age-adjusted rate by calendar period of incidence and death (1960–85) and trends in cumulative risks by date of birth (1900–40). Linear increase by age group in the more recent period is also included (Figure 3).

This monograph is the first comprehensive analysis of international trends in cancer applying a systematic approach to all available data sets of adequate quality.

1.1.7 Study of survival in European populations

(J. Estève; in collaboration with F. Berrino and M. Sant, Milan, Italy; R. Capocaccia and A. Verdecchia, Rome; M.P. Coleman, Sutton, UK; J. Faivre, Dijon, France; T. Hakulinen, Helsinki, Finland; C. Martinez, Granada, Spain; J.W. Coebergh, Eindhoven, The Netherlands; and S. Wilson, Manchester, UK)

A concerted action (designated EUROCARE) financed by the European Community started in 1989 with the objective of evaluating survival from selected cancers by assembling registry data in a standardized way. The results of this study, coordinated by the Varese Cancer Registry, are being prepared for publication in the IARC Scientific Publication series in early 1994.

1.1 IARC staff publications

Bouchardy, C., Mirra, A.P., Khlat, M., Parkin, D.M., Pacheco de Souza, J.M. & Davidson Gotlieb, S.L. (1991) Ethnicity and cancer risk in São Paulo, Brazil. *Cancer Epidemiol. Biomark. Prev.*, **1**, 21–27

Bouchardy, C., Parkin, D.M., Khlat, M., Mirra, A.P., Kogevinas, M., Dias de Lima, F. & de Carvalho Ferreira, C.E. (1993) Education and mortality from cancer in São Paulo, Brazil. *Ann. Epidemiol.*, **3**, 64–70

Coleman, M.P., Estève, J., Damiecki, P., Arslan, A. & Renard, H. (1993) *Time Trends in Cancer Incidence and Mortality* (IARC Scientific Publications No. 121), Lyon, IARC (in press)

Estève, J. (1993) Background mortality in clinical survival studies (letter to the editor). *Lancet*, **341**, 1416–1417

Paksoy, N., Bouchardy, C. & Parkin, D.M. (1991) Cancer incidence in Western Samoa. *Int. J. Epidemiol.*, **20**, 634–641

Parkin, D.M., Muir, C.S., Whelan, S.L., Gao, Y.T., Ferlay, J. & Powell, J., eds (1992) *Cancer Incidence in Five Continents*, Volume VI (IARC Scientific Publications No. 120), Lyon, IARC

Parkin, D.M. & Sasco, A.J. (1992) Lung cancer: worldwide variation in occurrence and proportion attributable to tobacco use. *Lung Cancer*, **9**, 1–16

Parkin, D.M., Pisani, P. & Ferlay, J. (1993) Estimates of the worldwide incidence of eighteen major cancers in 1985. *Int. J. Cancer*, **54**, 594–606

Pham, H.A., Parkin, D.M., Nguyen, T.H. & Nguyen, B.D. (1993) Cancer in the population of Hanoi, Viet Nam 1988–1990. *Br. J. Cancer* (in press)

Pisani, P., Parkin, D.M. & Ferlay, J. (1993) Estimates of the worldwide mortality from eighteen major cancers in 1985. Implications for prevention, and projections of future burden. *Int. J. Cancer* (in press)

Skinner, M.E.G., Parkin, D.M., Vizcaino, A.P. & Ndhlovu, A. (1993) *Cancer in the African Population of Bulawayo, Zimbabwe, 1963–1977: Incidence, Time Trends and Risk Factors* (IARC Technical Report No. 15), Lyon, IARC (in press)

Smans, M. & Estève, J. (1992) Practical approaches to disease mapping. In: Elliott, P., Cuzick, J., English, D. & Stern, R., eds, *Geographical and Environmental Epidemiology: Methods for Small Area Studies*, Oxford, Oxford University Press, pp. 141–150

Smans, M., Muir, C.S. & Boyle, P. (1992) *Atlas of Cancer Mortality in the European Economic Community* (IARC Scientific Publications No. 107), Lyon, IARC

Vatanasapt, V., Martin, N., Sriplung, H., Sontipong, S., Sriamporn, S, Parkin, D.M. & Ferlay, J. (1993) *Cancer in Thailand 1988–1991* (IARC Technical Report No. 16), Lyon, IARC (in press)

Wabinga, H.R., Parkin, D.M., Wabwire-Mangen, F. & Mugerwa, J.W. (1993) Cancer in Kampala, Uganda, in 1989–91: changes in incidence in the era of AIDS. *Int. J. Cancer*, **54**, 26–36

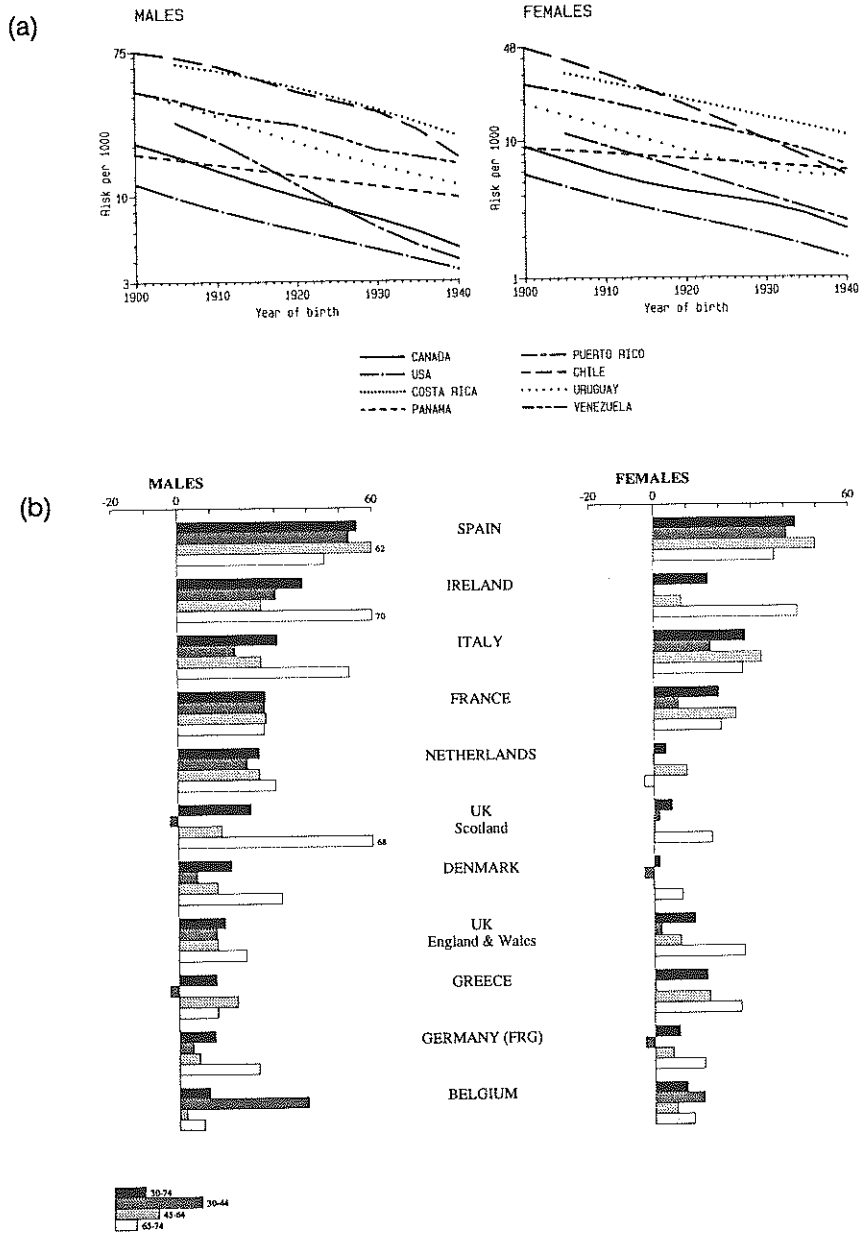


Figure 3. Trends in cancer: (a) mortality from stomach cancer in the Americas – cumulative risk, 30–74 years; (b) mortality from malignant melanoma of the skin in the European Community countries – percentage change per five-year period, 1975–1988, by age-group.

1.2 *Description of cancer incidence and of mortality in relation to particular population characteristics or risk factors*

1.2.1 Cancer incidence and mortality in migrant populations

(D.M. Parkin and E. Masuyer)

Studies of migrant populations are of particular value in estimating the relative contributions of genetic and environmental factors in cancer etiology. In such studies, the risk of cancer in a migrant population is compared with that in persons of the same genetic background (living in the place of origin of the migrants), or with persons in the host country sharing a common external environment. The objective is to see how much the risk of cancer changes from that of the country of origin to that of the host country, and to determine how rapidly such changes occur. The results are useful in formulating hypotheses on the relative importance of environmental factors in etiology, and on the probable stage of carcinogenesis on which they act.

A review of the methodology of migrant studies and the interpretation of their results has been published (Parkin, 1992).

1.2.1.1 *Cancer in Italian migrant populations*

(in collaboration with M. Geddes, E. Buiatti, D. Balzi, Florence, Italy; and M. Khlat Paris, France)

The full results of this study of the risk of cancer in populations of Italian origin in nine countries, drawing upon both incidence and mortality data, were published during 1993 (Geddes *et al.*, 1993).

1.2.1.2 *Cancer in Polish migrant populations*

(in collaboration with W. Zatonski, J. Tyczynski and W. Tarkowski, Warsaw, Poland; E. Matos, Buenos Aires, Argentina; A. Brancker, Ottawa, Canada; J. Iscovich, Jerusalem, Israel; and L. Bernstein, Los Angeles, USA)

The objective of this study is to compare the risk of cancer in Polish migrant populations with that of the host countries. The countries studied include Argentina, Australia, Canada, England and Wales, France, Israel and the USA. For migrants to Australia, Israel and the USA, cancer risk will also be examined in relation to duration of residence in the host country; for Canada, the risk in first-generation migrants (born in Poland) is being compared with that in the Canada-born population of Polish parentage. The results for migrants to France and Australia have been published (Tyczynski *et al.*, 1992, 1993).

1.2.1.3 *Cancer in migrant populations in relation to age at migration or duration of stay*

(in collaboration with M. Khlat, Paris, France; and J. Iscovich, Jerusalem, Israel)

The value of migrant studies is greatly enhanced when it is possible to estimate how the risk of cancer varies in migrants according to the age at which they migrated, or to the duration of their residence in the new environment. These aspects can be studied in data sets in which the date of migration is available. In Australia, death certificates record this information, and the risk of melanoma in migrant populations according to these two variables has been studied (Khlat *et al.*, 1992).

In Israel, the cancer registry records date of migration for all cancer cases. The data set previously used to study cancer in migrants to Israel during 1961–81 is being updated to 1989,

and the effect of age at arrival and duration of stay on the risk of certain major cancers (stomach, large bowel, breast, prostate, melanoma) will be investigated for the principal migrant populations.

1.2.1.4 *Other studies of migrants*
(in collaboration with C. Bouchardy, Geneva, Switzerland; and M. Khlat, Paris, France)

Data sets acquired for the above-mentioned studies have permitted the study of migrant populations for which information was not previously available. They include European migrants to São Paulo, Brazil (Bouchardy *et al.*, 1993), migrants from the Eastern Mediterranean region to Australia (Khlat *et al.*, 1993), and migrants from Africa to France (Bouchardy *et al.*, 1992). A study on cancer in offspring of migrants to Israel is described in Section 1.3.2.

1.2.2 European Childhood Leukaemia/Lymphoma Incidence Study (ECLIS)

(D.M. Parkin and E. Masuyer; in collaboration with J. Augustin, Brno, Czech Republic; L. Barlow, Stockholm, Sweden; B. Bennett, Vienna, Austria; D. Bobev, Sofia, Bulgaria; J.W. Coebergh, The Hague, Netherlands; G.J. Draper, Oxford, UK; H. Hansluwka and H. Friedl, Vienna, Austria; E. Ivanov, Minsk, Belarus; S. Karjalainen, Helsinki, Finland; R. Kriauciunas, Vilnius, Lithuania; F. Langmark, Oslo, Norway; J.-M. Lutz, Meylan, France; V. Merabishvili, St Petersburg, Russian Federation; J. Michaelis, Mainz, Germany; M. Möhner, Berlin, Germany; I. Plesko, Bratislava, Slovakia; V. Pompe-Kirn, Ljubljana, Slovenia; M. Rahu, Tallinn, Estonia; L. Raymond, Geneva, Switzerland; D. Schuler, Budapest, Hungary; J. Sinnaeve, Brussels, Belgium; H.H. Storm, Copenhagen, Denmark; B. Terracini, Turin, Italy; and J. Tyczynski, Warsaw, Poland)

This project was started in 1988 with the support of the Radiation Protection Programme of the European Commission, and involves representatives from cancer registries in 21 European countries. The objective is to follow geographical and temporal trends in the incidence of childhood leukaemia in Europe from 1980 until the mid-1990s, and to evaluate whether any changes can be related to exposure to radioactive material from the accident at Chernobyl in April 1986.

In 1992 the study was extended using the agreed protocol (IARC Internal Report No. 89/002 Rev. 1), to encompass almost all of the western part of the former USSR.

Cancer registries are supplying data on cases of childhood leukaemia and lymphoma and on populations at risk, so that incidence rates by cell type may be calculated for sub-national areas. Collaboration has been established with the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) to obtain estimates of the total body radiation dose attributable to the Chernobyl accident in children under age 15. Estimates are already available for large geographical regions; where appropriate, particularly for more highly exposed regions, the estimates will be recalculated for smaller regions.

The first results, based on leukaemia data to the end of 1988 (8–32 months following the accident) have been published (Parkin *et al.*, 1992). There was no apparent change in incidence in relation to estimated exposure. An updated analysis incorporating data to the end of 1990 (8–54 months after the accident) will be completed during 1993.

1.2.3 UV and skin cancer incidence monitoring network

(B.K. Armstrong, A. Kricker, D.M. Parkin, H. Yamasaki, H. Nakasawa and J. Hall; in collaboration with H.N.B. Gopalan, Nairobi, Kenya; T. Kjellström, Geneva, Switzerland; and E. Weatherhead, Chicago, IL, USA)

Plans for an international research programme on health, solar ultraviolet radiation (UVR) and environmental change (INTERSUN) were approved by the Scientific Council in November 1992. Since that time, the interest in the health effects of solar UVR has been widened from skin cancer to include eye and immunological effects. The programme is being developed in collaboration with the Division of Environmental Health, World Health Organization, and the United Nations Environment Programme.

The general objectives are to evaluate accurately the quantitative relationship between solar UVR at the surface of the earth and human health effects, develop reliable predictions of the health consequences of changes in UVR, provide baseline estimates of the incidence of health effects of UVR in representative populations around the world, and develop practical ways of monitoring change in these effects over time in relation to environmental and behavioural change.

A meeting of experts was held in Lyon in October 1992 to review progress on a draft protocol and make recommendations. The background documents prepared for this meeting have been published as IARC Technical Report No. 13 (Kricker *et al.*, 1993) and a draft outline protocol was sent to interested researchers for their comments and suggestions in early 1993.

A review by the Scientific Council in January 1993 proposed the conduct of feasibility studies and a re-review by the Council. Subsequent to this recommendation, progress has been made in collecting sets of data on trends in UV irradiance at the surface of the earth and development of a protocol for a standardized international analysis of trends in UV irradiance. It has not been possible to undertake the recommended feasibility studies because of lack of resources.

1.2.4 Monitoring of trends in incidence of cancers related to infection with HIV

(D.M. Parkin; in collaboration with V. Beral, Oxford)

Failure to secure external funding for this project has meant that progress has been minimal. Some information on changes in the cancer profile in Africa is beginning to emerge from the cancer registries which are being supported by IARC (see Section 1.4.7).

1.2.5 Other ecological studies

(P. Pisani, B.K. Armstrong)

A study has been planned to correlate the prevalence of exposures, available through routine statistics from international bodies (food disappearance data, tobacco production and consumption, indicators of fertility, WHO MONICA project), at the population level, with cancer incidence and mortality data already assembled covering the same area and time period, has been planned. A review of data sources is in progress.

1.2 IARC staff publications

Armstrong, B.K. (1993) Implications of increased solar UVB for cancer incidence. In: Chanin, M.-L. ed., *The Role of the Stratosphere in Global Change* (NATO Advanced Study Institute Series. I: Global Environmental Change, Volume 8), Berlin, Heidelberg, New York, Springer-Verlag, pp. 517–540

Armstrong, B.K. (1993) The epidemiology of melanoma. Where do we go from here? In: Gallagher, R.P. & Elwood, J.M., eds, *Epidemiology of Melanoma*, Dordrecht, Kluwer (in press)
Armstrong, B.K. & Kricker, A. (1993) Cutaneous melanoma. *Cancer Surveys* (in press)
Armstrong, B.K. & Kricker, A. (1993) How much melanoma is caused by sun exposure? *Melanoma Res.* (in press)
Bouchardy, C., Khlat, M. & Parkin, D.M. (1992) Mortalité par cancer chez les immigrés du Maghreb, d'Afrique noire et d'Asie du Sud-Est en France: quelques résultats préliminaires. *Bull. suisse cancer*, **3**, 90-94
Bouchardy, C., Khlat, M., Mirra, A.P. & Parkin, D.M. (1993) Cancer risks among European migrants in São Paulo, Brazil. *Eur. J. Cancer*, **29** A, 1418–1423
Burton, R.C., Coates, M.S. Hersey, P., Roberts, G., Chetty, M.P., Chen, S., Hayes, M.H., Howe, C.G. & Armstrong, B.K. (1993) An analysis of a melanoma epidemic. *Int. J. Cancer* (in press)
Estève, J., Benhamou, E. & Raymond, L. (1993) *Méthodes statistiques en épidémiologie descriptive*, Paris, Editions INSERM
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Kricker, A., Armstrong, B.K., Jones, M.E. & Burton, R.C. (1993) *Health, Solar UV Radiation and Environmental Change* (IARC Technical Report No. 13), Lyon, IARC
Kricker, A., Armstrong, B.K. & Parkin, D.M. (1993) Measurement of skin cancer incidence. *Hlth Rep.* **5**, 63–66
Matos, E.L., Khlat, M., Loria, D.I., Vilensky, M. & Parkin, D.M. (1991) Cancer in migrants to Argentina. *Int. J. Cancer*, **49**, 805–811
Parkin, D.M. (1992) Studies of cancer in migrant populations: methods and interpretation. *Rev. Epidémiol. Santé Publ.*, **40**, 410–424
Parkin, D.M., Cardis, E., Masuyer, E. & 34 others (1993) Childhood leukaemia following the Chernobyl accident: the European Childhood Leukaemia-Lymphoma Incidence Study (ECLIS). *Eur. J. Cancer*, **29A**, 87–95
Tyczynski, J., Parkin, D., Zatonski, W. & Tarkowski, W. (1992) Cancer mortality among Polish migrants to France. *Bull. Cancer*, **79**, 789–800
Tyczynski, J., Tarkowski, W., Parkin, D.M. & Zatonski, W. (1993) Cancer mortality among Polish migrants to Australia. *Eur. J. Cancer* (in press)

1.3 *Descriptive studies of childhood cancer*

1.3.1 Descriptive studies of particular types of childhood cancer

(D.M. Parkin, J. Ferlay and J. Nectoux; in collaboration with G.J. Draper and C.A. Stiller, Oxford, UK)

The large database collected for the study of international incidence of childhood cancer (Parkin *et al.*, 1988) has been used to complete a detailed review of the geographical and ethnic differences of the common childhood cancers. During the biennium, the results of these analyses for neuroblastoma and bone tumours have been published (Stiller & Parkin, 1992; Parkin *et al.*, 1993), and three others completed (brain and nervous system, soft tissue sarcomas and carcinomas of childhood).

For neuroblastomas, incidence rates are highest in predominantly white Caucasian populations and lower in Africa and Asia. Most of the variation in risk relates to the first year

of life, during which about 30% of cases are diagnosed in white populations (compared with, for example, about 20% in Japan).

Childhood bone tumours are mainly osteosarcomas or Ewing's tumours, and in both cases are most frequent in the last quinquennium (10–14) of childhood (Figure 4). Osteosarcoma appears to be slightly more common in black populations, and its occurrence favours osseous sites (and ages) of maximum growth. In contrast, Ewing's tumour is relatively rare in black populations, and in populations originating in eastern Asia, and is rather more prone to involve the axial skeleton.

Planning has begun for a second edition of *International Incidence of Childhood Cancer*. This will involve collection of data for the 1980s, and minor revisions to the classification scheme.

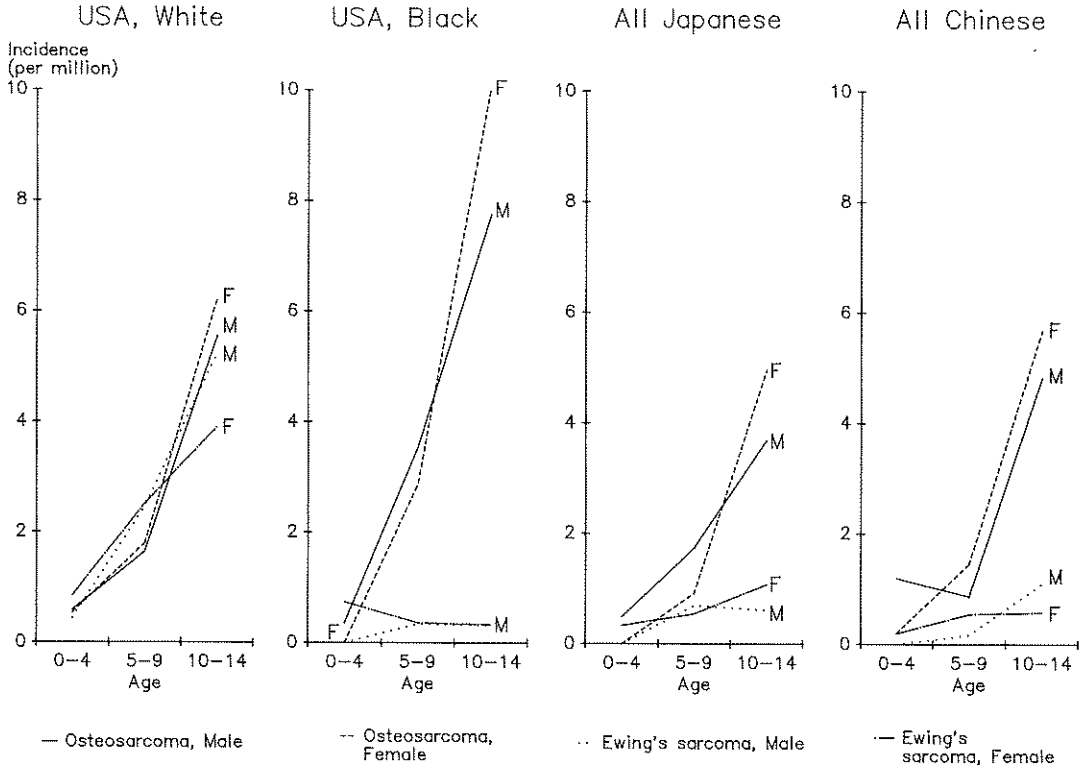

Figure 4. Age-specific incidence rates (per million), by sex, for osteosarcoma and Ewing's sarcoma, in various populations (from Parkin *et al.*, 1993)

1.3.2 Cancer in offspring of migrants to Israel

(D.M. Parkin and E. Masuyer; in collaboration with J. Iscovich, Jerusalem, Israel)

The objective of this study is to examine the risk of cancer in the Jewish population born in Israel according to birthplace of parents, and to compare this with the risk in migrants. The population born in Israel is still quite young, and for this and other technical reasons the analysis is confined to cancers appearing in the young (before age 30). Earlier studies of Jewish migrants (Steinitz *et al.*, 1989) showed large differences in incidence according to

birthplace, and the persistence of a differential in the offspring of such migrants, when compared with individuals whose parents were born in Israel, implies an important hereditary component in etiology.

The data comprise all records of cancer cases aged 0–29 years for the period 1961–89 (10 256 cases). Of these 2660 are migrants, 6554 offspring of migrants, and the remainder 'third generation' (Israel-born, with parents born in Israel). The principal cancers studied are those occurring in a young population: leukaemias, Hodgkin's disease and non-Hodgkin lymphoma, central nervous system neoplasms, malignant bone tumours, soft tissue sarcomas, melanoma and carcinomas of thyroid, breast and testis. Analysis of the results began in 1993.

1.3.3 Neonatal cancers

(A.J. Sasco, J. Little and O. Chatard; in collaboration with D. Frappaz and E. Robert, Lyon, France; D. Satgé, Tulle, France; and B. Terracini, Turin, Italy)

Neonatal cancers, being rare, are exceedingly difficult to assess on a population scale. General cancer registry data are often inadequate sources of detailed information about neonatal cancer or cancer occurring in children under one year of age, and existing specialized childhood cancer registries cover only limited parts of the population (Sasco & Little, 1992). Registries of congenital malformations or birth defects sometimes receive notification of occurrences of tumours, benign or malignant. In order to quantify and study the occurrence of neonatal cancers, we have planned a study to compare data from various such sources within one region, in order to make an exhaustive assessment of all cases and also to compare and ultimately standardize methods of data collection from various sources (Sasco *et al.*, 1993).

1.3 IARC staff publications

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1.4 *Support to cancer registries*

1.4.1 International Association of Cancer Registries

(D.M. Parkin and S.L. Whelan; in collaboration with A. Hanai, Osaka, Japan; and C.S. Muir, Edinburgh, UK)

The International Association of Cancer Registries, which now includes 260 cancer registries from 87 countries in addition to 91 individual members, continues to collaborate

closely with the Agency, which provides the secretariat for the Association. The Agency secretariat publishes a regular Newsletter and information bulletins for Association members. Member registries contribute to many Agency projects and publications, and have been actively involved in the preparation of the IARC Technical Reports *Training Manual for Cancer Registry Personnel* and *Quality Control in the Cancer Registry* (see Section 1.4.4).

Association members work with the Agency in various aspects of cancer epidemiology and cancer control (including acting as expert consultants) and in developing common practices for different aspects of cancer registration methodology. The Association is represented as a non-governmental organization at meetings of the World Health Organization.

Annual scientific meetings of the Association have been held since 1982, and concern both the technical details of cancer registration and the reporting of epidemiological research undertaken using the cancer registry as a point of departure. In 1991 the meeting was held in Quito, Ecuador. The main theme was Poverty and Cancer and encompassed the special problems in developing countries: infection, diet, tobacco and screening. The meeting was preceded by a three-day workshop on cancer registration. The 1992 annual meeting took place in Ottawa, Canada, sponsored by the Canadian Council of Cancer Registries. The topics addressed included cancer and environment, registries and cancer control, cancer and youth, and cancer in special populations.

1.4.2 European Network of Cancer Registries

(D.M. Parkin, J. Estève, J. Ferlay and E. Démaret; in collaboration with F. Berrino, Milan, Italy; E. Grundmann, Münster, Germany; T. Hakulinen, Stockholm, Sweden; F. Ménégoz, Meylan, France; C. Navarro, Murcia, Spain; R. Otter, Groningen, Netherlands; D. Pheby, Bristol, UK; E. Schifflers, Namur, Belgium; H.H. Storm, Copenhagen, Denmark; A.J. Swerdlow, London, UK; and H. Tulinius, Reykjavik, Iceland)

A European Network of Cancer Registries, funded by the EC 'Europe Against Cancer' Programme, was established in 1989, with the aims of improving the quality and comparability of cancer registry data and of making these data more easily and rapidly available.

Members of the Network are currently all general population-based cancer registries in the EC countries. Population-based registries in other countries of Europe, or ones which deal with cancers only in specific sites or age-groups, may be offered associate membership. The activities of the Network are coordinated through regular meetings of a steering committee made up of nominees of cancer registry associations in Europe, and individuals directly elected by the membership. IARC provides the secretariat for this committee.

The main activities of the Network comprise: (1) 'Minifellowships': these are intended to allow staff of the Network registries to visit other registries to study specific methods or operations. One objective is to help reduce the variation in registry results which is due to non-standardized procedures. (2) Consultantships: registries may request the visit of a consultant to advise on a specific topic or problem. (3) Cancer registration courses, of which the first will be held in Copenhagen in January 1994. (4) Publications: a concise publication of 'Facts and Figures on Cancer in the European Community' is being prepared, to present incidence and mortality information on leading sites of cancer in a simple and concise way. (5) EUROCIM: an electronic incidence and mortality database, EUROCIM, has been prepared, as described in Section 1.1.2.

A detailed survey of cancer registration practice in EC registries has been carried out. In the first phase, registries' basic characteristics, sources of data, coding, completeness and

validity of data, notification, etc. have been analysed. The second phase, which deals with coding and classification in more detail, is almost complete and replies are currently being computerized. The survey is carried out by the Danish Cancer Registry.

1.4.3 Cancer registration and cancer epidemiology in Latin countries

(J. Estève and A. Rivoire; in collaboration with A. Tuyns, Lyon; L. Raymond, Geneva, Switzerland; and R. Zanetti, Turin, Italy)

IARC provides support to the 'Groupe pour l'Epidémiologie du Cancer dans les Pays de Langue Latine', in particular by organizing the annual meetings, methodological workshops, and publishing the proceedings of the annual meetings as IARC Technical Reports. The 1992 meeting in Rapallo was organized by Professor Santi, Director of the Genoa Tumour Registry and Professor Amadori, Director of the Tumour Registry of Romagna. A seminar on geographical epidemiology was held on this occasion under the leadership of Dr C. Cislaghi (Milan). The 1993 meeting was held in Dijon at the invitation of Dr J. Faivre, Dr P.M. Carli and Dr G. Chaplain. A seminar on time trends analysis was held before the meeting. Several of the ideas discussed over the years in these methodological seminars are now available in a book published by INSERM (Estève *et al.*, 1993).

1.4.4 Coordination of cancer registry input into ICD revisions and consideration of confidentiality issues

(S. Whelan and D.M. Parkin; in collaboration with M.P. Coleman, London, UK; F. Ménégoz, Meylan, France; C.S. Muir, Edinburgh, UK; and C. Percy, Bethesda, MD, USA;)

The Agency was represented on the committee responsible for the neoplasms chapter of the 10th Revision of the *International Classification of Diseases, Injuries and Causes of Death (ICD-10)*, of which Volume I was printed in 1992. The Index to the 10th Revision will appear in 1994. While it is likely that few, if any, countries will implement ICD-10 for coding mortality before 1995, many cancer registries started using the second edition of ICD-O, which includes the ICD-10 topographic codes, in 1992. The Agency will distribute conversion programs for the different ICD and ICD-O revisions in 1993.

Cancer registries have been closely concerned in the production of a code of confidentiality for the purpose of recording data on cancer (IARC Internal Report No. 92/003; Coleman *et al.*, 1992). The code has been circulated to registries to assist them in formulating procedures to maintain confidentiality. The European Community cancer registries have been active in attempting to have the terms of an EC directive on confidentiality modified to allow the storage of data on individuals with cancer, to permit their use in research into the cause and prevention of disease.

1.4.5 Reliability and validity of cancer registry data

1.4.5.1 *Quality control in the cancer registry*

(D.M. Parkin, S.L. Whelan and J. Ferlay; in collaboration with V. Chen, New Orleans, LA, USA; J. Galceran, Tarragona, Spain; and H. Storm, Copenhagen, Denmark)

The value of cancer registry data in epidemiological research and in planning and evaluation of cancer control programmes is dependent upon the accuracy of completeness of the data recorded on cancer cases and the population at risk. While some registries have

carried out studies on quality control, surveys have shown that completeness or coverage and validity of data are not monitored regularly or with standardized methods, and little information on methodology is available. In order to encourage comparability of definitions and practice between population-based cancer registries worldwide, a manual has been prepared that will give a comprehensive description of methods available to cancer registries for ensuring comparability of data, completeness of registration (including avoidance of duplicates), and the validity of the information recorded.

This manual will be published in 1993 as an IARC Technical Report, and will include a diskette containing the routine checks, primarily of combinations of recorded data items, which were used in the preparation of *Cancer Incidence in Five Continents*. The diskette will also include a series of programs which will permit registries to carry out standard code conversions from the *International Classification of Diseases for Oncology*, 1st and 2nd editions, to ICD-9 and ICD-10.

1.4.5.2 *Training Manual for Cancer Registry Personnel*
(S. Whelan and D.M. Parkin; in collaboration with D. Esteban and A.V. Laudico, Manila, Philippines; D. Badger, Ottawa, Canada; S. Gravestock, Liverpool, UK; and A.L. Maya, Miami, FL, USA)

Existing training and procedure manuals for clerical and technical registry personnel are often far too complex and specialized for the needs of cancer registration in developing countries. A manual in two volumes is being prepared for publication as an IARC Technical Report in early 1994, the first volume comprising a course on medical terminology, and the second describing the various steps involved in registering a case of cancer. Recognition is made of the particular problems faced by registry personnel in developing countries.

1.4.6 Computer software for cancer registries

(D.M. Parkin and S. Olivier)

CANREG consists of a set of microcomputer programs for cancer registration in population-based registries. It is a powerful and integrated software, simple to use and hence suitable for many registries, specially in developing countries where registry personnel may have little or no formal training and experience in computing. Registry personnel are sometimes able to visit IARC for a period of training, but more often a staff member visits collaborating registries to install or modify the CANREG system and train the staff.

The CANREG system permits entry and management of case data in a database specifically designed for cancer registration, and incorporating a variety of in-built controls. The variables to be entered, the codes and definitions to be used for verification, and the format of the data entry screen are adapted to the requirements of the registry. The system also provides for data analysis, both via standard procedures of subset selection and tabulation (by age, sex and site), and via transfer of the master file to EPI-INFO, a well known software package permitting many *ad hoc* analyses.

The CANREG system has been prepared in many languages (English, French, Spanish, Turkish, Thai systems). It has been supplied to many registries in developing countries and several in Europe. Version 2, developed in the last two years, has already been implemented in 25 registries (more than 45 are using CANREG), including several new ones.

CANREG is now installed in the following cancer registries:

Africa:

Algiers (3 centres), Oran and Sétif (Algeria), Fajara (the Gambia), Abidjan (Ivory Coast), Blantyre (Malawi), Bamako (Mali), Rabat (Morocco), Niamey (Niger), Butare (Rwanda), Johannesburg (South Africa), Dar es Salaam (Tanzania), Kampala (Uganda), Harare (Zimbabwe).

America:

Bahia Blanca (Argentina), La Paz (Bolivia), Cali (Colombia), San Jose (Costa Rica), Asuncion (Paraguay), Trujillo (Peru)

Europe:

Mulhouse (France), Luxembourg (Luxembourg), Sta Cruz de Tenerife (Spain)

Southern Asia:

India, Shiraz (Iran), Al Khobar (Saudi Arabia)

Southeast Asia:

Semarang (Indonesia), Kuala Lumpur (Malaysia), Manila (2), Davao and Cebu (Philippines), Bangkok, Khon Kaen and Hat Yai (Thailand), Hanoi and Ho Chi Minh Ville (Viet Nam)

Western Asia:

Izmir and Ankara (Turkey)

South Pacific:

Suva (Fiji), Papeete (French Polynesia), Guam (Micronesia), Noumea (New Caledonia), Port Vila (Vanuatu)

1.4.7 Other support to cancer registries

(D.M. Parkin, P. Pisani, R. Sankaranarayanan, S. Whelan and S. Olivier)

Advice is given both to organizations wishing to set up cancer registries, and to established registries on the methodology of registration and the analysis of data. Staff of the Unit of Descriptive Epidemiology have made visits to several cancer registries in the course of the biennium, and many individuals working in cancer registries have visited the unit for training or discussion.

Collaborating cancer registries in Europe have assisted with training of registry personnel. They include the Mersey Regional Cancer Registry (Mrs S. Gravestock) and East Anglian Cancer Registry (Dr T. Davies and Mrs M. Paige) in the UK, the cancer registries of Bas-Rhin (Dr P. Schaffer) and Isère (Dr F. Ménégoz) in France, the cancer registry of Tarragona (Dr J. Galceran) in Spain, the Varese Cancer Registry (Dr F. Berrino) in Italy and the Danish Cancer Registry (Dr H. Storm).

Abstracts of reports submitted by cancer registries have now been entered onto computer to facilitate retrieval of the information, and to permit searching for specific items by combining parameters of interest and interrogating the system.

Several commonly used computer programs are available to registries free of charge, including verification checks (e.g., tumour site versus age, sex, histology), an ICD-O to ICD-9 conversion program (based on a conversion devised by C. Percy in 1979) and conversion of ICD-O coded cases into the categories of the classification scheme for childhood cancer.

The Unit of Descriptive Epidemiology also provides more direct support and encouragement for cancer registration activities in Africa, Asia, Central and South America, and Oceania.

1.4.7.1 *Africa*

Algeria: Setif (H. Cherif): Results for the period 1986–89 were published in Volume VI of *Cancer Incidence in Five Continents*.

Alger (D. Hammouda): Ms Messaouda Aoun and Ms Lynda Rezzig visited IARC for two weeks to learn cancer registration techniques and to use CANREG software.

Alger (L. Abid): The Registre des Cancers Digestifs d'Alger has been functioning for two years using CANREG.

Burundi (V. Bigirimara and L. Ngendahayo): A permanent cancer registry is being established for the province of Bujumbura. This will have a special role as a base for the study of the association between HIV and cancer (see Section 2.6.5).

Côte d'Ivoire (A. Ahnoux): The principal investigator spent a one-year training period in France, including two weeks at IARC, to prepare for the establishment in 1993 of a population-based registry in Abidjan.

Malawi (L.T. Banda): A cancer registry established in Malawi in 1985 collects information on cases diagnosed histopathologically. During 1993, the decision was made to provide additional support to permit the registry to collect information from all possible sources for a defined region of the country (Blantyre district). The cancer registrar obtained an ICRETT fellowship and spent a training period in UK and Lyon.

Mali (S. Bayo): Financial support for the registry has continued, and the obsolete microcomputer replaced. The registry has now continued active data collection for seven years and the results for 1987–89 were published in *Cancer Incidence in Five Continents*, Volume VI.

Niger (H. Nouhou): The principal investigator returned to the country in 1992 and completed plans for a cancer registry serving the region of Niamey. A Collaborative Research Agreement to provide financial support was signed (November 1992) and data collection began in 1993.

Rwanda (P.-J. Ngilimana and B. Sindikubwabo): Staffing at the registry was completed with appointment of a registrar and interviewer during 1992. A vehicle was also provided to assist data collection from peripheral hospitals. The results for 1992 are complete, and data collection continued during 1993 despite the unstable political situation.

South Africa (F. Sitas): No cancer incidence data from South Africa have been published in *Cancer Incidence in Five Continents* since Volume II, and the National Registry is presently concerned only with recording histopathologically diagnosed cancers. A new network of cancer registries in rural areas is being established and technical assistance has been provided for this project.

Uganda (H. Wabinga and R. Owor): The registry continued to function with manual record-keeping until a computer was provided during 1993. The tumour registrar (Miss S. Nambooze) received training in the UK and in Lyon during 1992. A vehicle was provided to assist in data collection during 1993. The first results of the registry for the period 1989–91 have been published (see Section 1.1.3).

Zimbabwe (L. Levy, E. Chokunonga, B. Mauchaza and M. Bassett): The registry has continued to function well despite staff turnover. The tumour registrar (Mr E. Chokunonga) has received further training in Europe. Computer equipment was acquired to replace the obsolete machine in Harare. Registration in Bulawayo (Mpilo Hospital) was restarted during 1993, and a new computer is ready for installation following training of local personnel. Methods for extension of the current registry to achieve full population coverage were reviewed during a consultant visit in May 1993.

1.4.7.2 *Asia*

India (C.R. Ramachandran and A. Nandakumar): A consultant visit to review the programme of cancer registration activities supported by the Indian Council for Medical Research took place in 1992. Support for re-equipping several registries with new microcomputers, and supplying the CANREG software and training staff in its use (see Section 1.4.5) was agreed. An agreement was made to provide some support to the rural cancer registry project in Barshi (Maharashtra).

Oman (G. Sarfaty): A consultant visit to Oman (on behalf of WHO) advised on changes needed to improve coverage and quality of the national cancer registry. Stomach cancer appears to have a higher than expected incidence in this country.

Philippines (A. Laudico, D. Esteban and B. Talaver): The registries in Manila and Rizal were visited during 1991 and methods reviewed. The results from the cancer registry in Cebu were analysed and suggestions made for improving coverage of registration and quality control of the data. A new cancer registry is being established in Davao (Mindanao Island) following a period of training in England and IARC during 1991 by a WHO fellow, Dr G. Martinez. The combined results of the two registries in the Greater Manila Area (PCS-Manila and DOH-Rizal) are being prepared for publication by UICC (see Section 1.1.3).

Thailand (N. Martin, S. Sontipong, S. Sriamporn, H. Sriplung and V. Vatanasapt) (Figure 5): Regular meetings of registry directors have taken place during the biennium in the context of the project to publish combined results as a monograph (see Section 1.1.3). A cancer survey for the Bangkok Metropolitan Area was executed during 1992. Support for the registry in Songkla continued until 1992; the first results from this new registry are now available.

Turkey (S. Sahin and G. Aydemir): The Izmir Cancer Registry has been set up to centralize cancer data for the Izmir region from various hospitals, and is intended to be developed into a population-based registry. The CANREG system has been installed.

Viet Nam (Pham Hoang Ahn, Nguyen Chan Hung and Le Cao Dai): Registration activities in Hanoi continued despite the absence of the principal investigator on an IARC training fellowship in London. The methodology of the registry was reviewed at a consultant visit during 1992, and support is continuing. In Ho Chi Minh City, some progress was made in extending the hospital registry in the Oncology Centre to become population-based for the entire city. Staff of the registry attended the IARC course on cancer epidemiology in Ahmedabad (see Section 6.5.6). Plans were made, subject to funding being found, to begin a new cancer registry in Bien Hoa, close to Ho Chi Minh City, in an area particularly heavily contaminated by herbicides and dioxins in the late 1960s.

1.4.7.3 *Americas*

In association with UICC and PAHO, and aided by a grant from the American Cancer Society, a three-day seminar/workshop on cancer registration in Latin America was held in Quito, Ecuador, in October 1992. A special session examined potential collaboration in research activities between cancer registries and Latin America and partners in Spain or the United States. Following the workshop, a consultant visited cancer registries in Costa Rica and Honduras to advise on improvements to methodology.

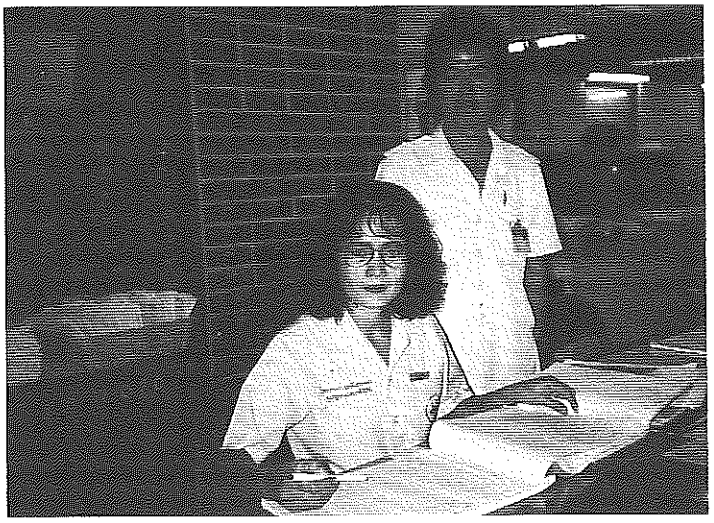

Figure 5. Staff of the Khon Kaen cancer registry regularly visit the local hospitals to abstract data from medical records.

Argentina (E. Laura): A new cancer registry was started in Bahia Blanca, and financial support provided.

Bolivia (J. Rios Dalenz): Support was continued and a consultant visit on behalf of IARC was made (Dr J. Galceran) in May 1993 to advise on registration methods and computing. Results for three years (1988–90) confirm the previously noted elevated incidence of gallbladder and cervix cancer. Overall incidence remains low.

Brazil (M.P. Curado): Support was provided to the cancer registry serving the city of Goiana, the site of an accident involving population exposure to radioactive caesium (of medical origin) in 1987.

Costa Rica (C. Bratti): Following a consultant visit in October 1992 a visit was made to install CANREG (see Section 1.4.6).

Paraguay (P.A. Rolón): Support to the Asuncion registry has continued. The results for the years 1988–89 were published in *Cancer Incidence in Five Continents*, Volume VI. A consultant (Dr J. Galceran) visited the registry in May 1993.

Peru (P.J. Albujar): Support to the registry of Trujillo has continued and the first results, for the period 1984–87, were published in *Cancer Incidence in Five Continents*, Volume VI.

1.4.7.4 *Oceania*

Support for the development of cancer registration in the Pacific region was provided through a Collaborative Research Agreement with the South Pacific Commission (principal investigator Dr F. Bach). This allowed the appointment of local consultants and visiting cancer registrars to assist in data collection. Information from 19 island populations is now available, and is being analysed (see Section 1.1.3).

IARC staff participated in a training workshop for cancer registry staff organized by the South Pacific Commission in Papeete (2–12 July 1991).

1.4 IARC staff publications

Armstrong B.K. (1993) The role of the cancer registry in cancer control. *Cancer Causes Control*, **3**, 569–579

Coleman, M.P., Muir, C.S. & Ménégoz, F. (1992) Confidentiality in the cancer registry. *Br. J. Cancer*, **66**, 1138–1149

PART 2. IDENTIFICATION, ELUCIDATION AND EVALUATION OF ENVIRONMENTAL CAUSES OF CANCER

2.1 *Evaluation of carcinogenic risks to humans*

2.1.1 *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*

(H. Vainio, D. McGregor, H. Møller, C. Partensky, I. Peterschmitt and J. Wilbourn. The following members of other units have contributed to the programme: B.K. Armstrong, H. Bartsch, P. Boffetta, F.X. Bosch, J.R.P. Cabral, E. Cardis, M. Castegnaro, M. Friesen, J. Hall, M. Kogevinas, V. Krutovskikh, J. Little, C. Malaveille, R. Montesano, N. Muñoz, H. Nakazawa, I.K. O'Neill, D.M. Parkin, R. Saracci, D.E.G. Shuker, C.P. Wild and H. Yamasaki)

The *IARC Monographs* Programme aims to identify agents that increase the risk of cancer in exposed humans. Working groups of invited experts in carcinogenesis follow guidelines established during several consultative meetings in formulating their evaluations. During the period 1 July 1991 to 30 June 1993, meetings were held to prepare volumes 54–59 of the *IARC Monographs*.

2.1.1.1 *Volume 54*

A working group convened by IARC evaluated the carcinogenic risks of occupational exposures to mists and vapours from strong inorganic acids, used in a wide range of industries, at a meeting in October 1991. Exposures to mists containing sulfuric acid were found to entail a carcinogenic risk to humans (Group 1). The decision was based on the consistency of reports of cancer of the upper respiratory tract, especially cancers of the lung and larynx, in workers exposed during the manufacture of isopropanol, synthetic ethanol and soap and in pickling operations. On the basis of *sufficient evidence* of carcinogenicity in experimental animals, diisopropyl sulfate was considered to be possibly carcinogenic to humans (Group 2B) and diethyl sulfate to be probably carcinogenic to humans (Group 2A), because of its virtually consistent genotoxicity. The other substances considered that are encountered in acidic occupational environments (sulfur dioxide, metabisulfites, sulfites, bisulfites, hydrochloric acid) were not classifiable as to their carcinogenicity to humans (Group 3). The evaluations made by the Working Group are given in Table 1.

The same Working Group re-evaluated the important monomer, 1,3-butadiene, used principally in rubber. New epidemiological data were considered to provide *limited evidence* of carcinogenicity in humans; there was *sufficient evidence* of carcinogenicity in animals. The overall evaluation based on the results of epidemiological and experimental studies led the group to the conclusion that 1,3-butadiene is probably carcinogenic to humans (Group 2A).

Table 1. Summary of final evaluations — occupational exposures to mists and vapours from strong inorganic acids; and other industrial chemicals

Exposure	Degree of evidence for carcinogenicity		Overall evaluation of carcinogenicity to humans[a]
	Human	Animal	
Bisulfites	Inadequate	Inadequate	3
1,3-Butadiene	Limited	Sufficient	2A
Diethyl sulfate	Inadequate	Sufficient	2A[b]
Diisopropyl sulfate	Inadequate	Sufficient	2B
Hydrochloric acid	Inadequate	Inadequate	3
Metabisulfites	Inadequate	Inadequate	3
Occupational exposure to strong-inorganic-acid mists containing sulfuric acid	Sufficient	Not applicable	1
Sulfites	Inadequate	Inadequate	3
Sulfur dioxide	Inadequate	Limited	3

[a] Group 1: Carcinogenic to humans, Group 2A: Probably carcinogenic to humans, Group 2B: Possibly carcinogenic to humans, Group 3: Not classifiable as to carcinogenicity to humans
[b] Other relevant data were taken into consideration in making the overall evaluation

2.1.1.2 *Volume 55*

In February 1992, a IARC Working Group was convened in Lyon to prepare a monograph on the carcinogenic risks to humans of exposure to solar and ultraviolet (UV) radiation. The main segments of the UV spectrum are UVC (100–280 nm), UVB (280–315 nm) and UVA (315–400 nm). All of the solar UVC radiation is filtered by the ozone layer and other components of the atmosphere; UVB, also known as 'sunburn' radiation, has been considered until now to be biologically the most significant part of the terrestrial UV spectrum. The most frequently used artificial sources of UV radiation are for tanning and phototherapy. Sunlamps used until the 1970s emitted relatively large amounts of UVB and UVC radiation; they were subsequently replaced by lamps that emit primarily UVA radiation and only small amounts of UVB. The lamps used in phototherapy emit primarily UVB; lamps emitting UVA radiation are used in conjunction with psoralens in photochemotherapy. The general population is also exposed to small amounts of UV radiation from fluorescent lighting and tungsten halogen lamps.

The epidemiological data on exposures to solar radiation and artificial sources of UV radiation were considered separately for non-melanocytic skin cancers and malignant melanomas of the skin and eye. The experimental data were evaluated for broad spectrum and individual wavelengths. After consideration of all relevant epidemiological and experimental carcinogenicity data and other information on toxicity and mechanisms, the Working Group concluded that solar radiation is carcinogenic to humans (Group 1), causing cutaneous malignant melanoma and non-melanocytic skin cancers. Ultraviolet A, B and C radiations were each considered to be probably carcinogenic to humans (Group 2A) (genotoxicity data were used in making the overall evaluations), and use of sunlamps and sunbeds was considered to entail exposures that are probably carcinogenic to humans (Group 2A). Exposure to fluorescent lighting was not classifiable as to its carcinogenicity to humans (Group 3).

2.1.1.3 *Volume 56*

An IARC Working Group convened in June 1992 evaluated the carcinogenic risks to humans of some naturally occurring substances, including two food items (salted fish and pickled vegetables), two plant constituents (caffeic acid and *d*-limonene), heterocyclic aromatic amines (IQ, MeIQ, MeIQx and PhIP) and some mycotoxins (aflatoxins, fusarium toxins and ochratoxin A). The conclusions of the meeting are summarized in Table 2. Aflatoxins and Chinese-style salted fish were evaluated as being carcinogenic to humans (Group 1).

2.1.1.4 *Volume 57*

An IARC working group was convened in October 1992 to consider data relevant to the evaluation of carcinogenic risks to humans from occupational exposures of hairdressers and barbers and personal use of hair colourants; the risks entailed by exposures to some individual hair dyes, cosmetic colourings, industrial dyestuffs and aromatic amines were also considered. Table 3 summarizes the Working Group's evaluations; those which involved consideration of human data are described below.

Although the occupational exposures of hairdressers and barbers are multiple and therefore difficult to define, limited evidence was provided by epidemiological studies of an excess risk for bladder cancer among male hairdressers and barbers. The Group thus concluded that occupation as a hairdresser or barber entails exposures that are probably carcinogenic (Group 2A). Personal use of hair colouring products was not classifiable as to its carcinogenicity (Group 3).

Magenta, a variable mixture prepared from aniline and *ortho*-toluidine, has been used since the nineteenth century to dye textiles and in printing inks and biological stains. The Group confirmed a previous evaluation that manufacture of magenta entails exposures that are carcinogenic (Group 1). Since a known component of magenta, CI Basic Red 9, is clearly carcinogenic to experimental animals, the Group additionally concluded that magenta containing this compound is possibly carcinogenic to humans (Group 2B).

The aromatic amine 4,4′-methylene bis(2-chloroaniline) (MOCA) is used widely in plastics manufacture. Although the available epidemiological evidence for its carcinogenicity is inadequate, the sufficient evidence for its carcinogenicity in experimental animals and the strong similarities in its properties in experimental systems and in human studies led the Group to conclude that MOCA is probably carcinogenic to humans (Group 2A).

2.1.1.5 *Volume 58*

A working group of experts met in February 1993 to discuss the carcinogenic risks to humans of exposures to three metals — beryllium, cadmium and mercury — and their compounds, and of exposures in the glass manufacturing industry, where lead, arsenic and other metal oxides may occur.

The carcinogenicity of beryllium and its compounds in humans was assessed on the basis of extended analyses of mortality among cohorts of workers in beryllium extraction and production plants and follow-up of deaths of workers with beryllium-related diseases showing an excess of lung cancer. Lung tumours have also been induced in experimental animals. The commonality of this target site suggests a common mechanism for the carcinogenesis of beryllium in humans and experimental animals. The overall evaluation was that beryllium and beryllium compounds are carcinogenic to humans (Group 1).

Table 2. Summary of final evaluations — some naturally occurring substances

Exposure	Degree of evidence for carcinogenicity		Overall evaluation of carcinogenicity to humans[a]
	Human	Animal	
Chinese-style salted fish	Sufficient	Limited	1
Pickled vegetables, traditional Asian	Limited	Inadequate	2B
Caffeic acid	Inadequate[b]	Sufficient	2B
d-Limonene	Inadequate[b]	Limited	3
IQ	Inadequate	Sufficient	2A[c]
MeIQ	Inadequate	Sufficient	2B
MeIQx	Inadequate	Sufficient	2B
PhIP	Inadequate	Sufficient	2B
Naturally occurring mixtures of aflatoxins	Sufficient	Sufficient	1
Aflatoxin B1	Sufficient	Sufficient	
Aflatoxin B2		Limited	
Aflatoxin G1		Sufficient	
Aflatoxin G2		Inadequate	
Aflatoxin M1	Inadequate	Sufficient	2B
Toxins derived from *Fusarium sporotrichioides*	Inadequate[b]		3
T-2 Toxin		Limited	
Toxins derived from *Fusarium graminearum*, *F. culmorum* and *F. crookwellense*	Inadequate		3
Zearalenone		Limited	
Deoxynivalenol		Inadequate	
Nivalenol		Inadequate	
Fusarenone X		Inadequate	
Toxins derived from *Fusarium moniliforme*	Inadequate	Sufficient	2B
Fumonisin B_1		Limited	
Fumonisin B_2		Inadequate	
Fusarin C		Limited	
Ochratoxin A	Inadequate	Sufficient	2B

[a] See footnote to Table 1
[b] No data
[c] Other relevant data were taken into account in making the overall evaluation

Consistent evidence has also been provided that occupational exposure to cadmium increases the risk for lung cancer. Early studies reported an increased risk for prostate cancer, but the results of later studies were not consistent. The finding that lung tumours are also induced in experimental animals exposed by inhalation led to an overall evaluation that cadmium and cadmium compounds are carcinogenic to humans (Group 1).

Table 3. Summary of final evaluations – occupational exposures of hairdressers and barbers and personal use of hair colourants: some hair dyes, cosmetic colourants, industrial dyestuffs and aromatic amines

	Degree of evidence for carcinogenicity		Overall evaluation of carcinogenicity to humans[a]
	Human	Animal	
Occupational exposures as a hairdresser or barber	Limited		2A
Personal use of hair colourants	Inadequate		3
2-Amino-4-nitrophenol	Inadequate[b]	Limited	3
2-Amino-5-nitrophenol	Inadequate[b]	Limited	3
para-Chloroaniline	Inadequate[b]	Sufficient	2B
CI Acid Orange 3	Inadequate[b]	Limited	3
CI Acid Red 114	Inadequate[b]	Sufficient	2B
CI Direct Blue 15	Inadequate[b]	Sufficient (technical grade)	2B
CI Pigment Red 3	Inadequate[b]	Limited	3
D & C Red No. 9	Inadequate[b]	Limited	3
1,4-Diamino-2-nitrobenzene (2-nitro-*para*-phenylenediamine)	Inadequate[b]	Limited	3
2,6-Dimethylaniline (2,6-xylidine)	Inadequate[b]	Sufficient	2B
N,*N*-Dimethylaniline	Inadequate[b]	Limited	3
HC Blue No. 1	Inadequate[b]	Sufficient	2B
HC Blue No. 2	Inadequate[b]	Inadequate	3
HC Red No. 3	Inadequate[b]	Inadequate	3
HC Yellow No. 4	Inadequate[b]	Inadequate	3
Magenta (containing CI Basic Red 9)	Inadequate		2B
Magenta		Inadequate	
CI Basic Red 9	Inadequate	Sufficient	2B
Manufacture of magenta	Sufficient		1
4,4′-Methylene bis(2-chloroaniline) (MOCA)	Inadequate	Sufficient	2A[c]

[a] See footnote to Table 1
[b] No data
[c] Other relevant data were taken into account in making the overall evaluation

The epidemiological studies of exposure to metallic mercury and organomercury compounds and of mercury miners were judged to provide inadequate evidence for carcinogenicity. There was considered to be sufficient evidence for the carcinogenicity of methylmercury chloride in experimental animals. Methylmercury compounds were thus evaluated as possibly carcinogenic to humans (Group 2B), whereas metallic mercury and inorganic mercury compounds could not be classified as to their carcinogenicity to humans (Group 3).

A monograph was prepared on occupational exposures in glass manufacture. Although the type of glass industry studied was usually poorly defined, the available epidemiological

studies provided enough information to allow the Group to conclude that the manufacture of art glass, glass containers and pressed ware entails exposures that are probably carcinogenic to humans (Group 2A). Owing mainly to lack of information, occupational exposures in flat-glass and specialty glass manufacture were considered to be unclassifiable as to their carcinogenicity to humans (Group 3).

The evaluations are listed in Table 4.

Table 4. Summary of final evaluations — beryllium, cadmium, mercury and exposures in the glass manufacturing industry

	Degree of evidence for carcinogenicity		Overall evaluation of carcinogenicity to humans[a]
	Human	Animal	
Beryllium and beryllium compounds	Sufficient	Sufficient	1[b]
Cadmium and cadmium compounds	Sufficient		1[b]
Cadmium compounds		Sufficient	
Cadmium metal		Limited	
Mercury and mercury compounds			
Methylmercury compounds	Inadequate		2B[b]
Methylmercury chloride		Sufficient	
Metallic mercury and inorganic mercury compounds	Inadequate		3
Metallic mercury		Inadequate	
Mercuric chloride		Limited	
Occupational exposures in the glass industry:			
Art glass, glass containers and pressed ware, manufacture of	Limited		2A
Flat glass and specialty glass, manufacture of	Inadequate		3

[a] See footnote to Table 1

[b] Other relevant data were taken into account in making the overall evaluation

2.1.1.6 *Volume 59*

In 1991, an ad-hoc advisory group convened by IARC agreed unanimously that viruses and other biological agents should be included in the *IARC Monographs*. Thus, in June 1993, IARC convened a working group consisting of experts in epidemiology, experimental carcinogenicity, internal medicine, pathology, communicable diseases and molecular biology to discuss three viruses given high priority by the advisory group: hepatitis B virus (HBV), hepatitis C virus (HCV) and hepatitis D virus (HDV, or the delta agent).

Chronic infection with HBV is highly prevalent in many human populations, particularly in developing countries: it has been estimated that over 300 million people are chronically infected. In studies in which hepatitis B surface antigen (HBsAg) was used as a marker of chronic infection, the proportion of primary liver cancers attributable to chronic infection with HBV ranges from a few per cent in most developed countries to over 50% in parts of Africa and Asia.

The review by the Working Group of the epidemiological evidence on the relationship between hepatocellular carcinoma (HCC) and chronic HBV infection showed a strong

association, and potential confounding by aflatoxin, concurrent infection with HCV, cigarette smoking and alcohol drinking appeared to have been excluded in those studies in which they were evaluated. Therefore, evidence from studies in experimental animals was not required for the Group to reach the overall evaluation that chronic infection with hepatitis B virus is carcinogenic to humans (Group 1).

Hepatitis C virus is an RNA virus with wide genetic diversity. It causes most non-A, non-B, post-transfusion hepatitis and a variable proportion of non-A, non-B hepatitis that is unrelated to transfusion. The prevalence of HCV infection worldwide shows less variation than that of HBV: in most populations, 0.5–2% of individuals have serological evidence of past or current infection.

The epidemiological evidence showed a clear association between chronic infection with HCV and the development of HCC, and control of confounding by concomitant infection with HBV, smoking and alcohol consumption did not materially alter the relationship. The Working Group therefore concluded that chronic infection with hepatitis C virus is carcinogenic to humans, leading to a classification in Group 1.

HDV exists as a satellite agent of HBV — it is a parasitic RNA virus requiring a simultaneous HBV infection. As HDV occurs only in the presence of HBV infection, the prevalence of HDV infection is low in countries where the endemicity of HBV is low, except among parenteral users of illicit drugs and recipients of blood products. In areas of intermediate and high endemicity of HBV, however, the prevalence of HDV infection is highly variable.

No epidemiological study has shown clearly an association between HDV infection and HCC, and the Working Group therefore concluded that infection with hepatitis D virus is not classifiable as to its carcinogenicity to humans, placing it in Group 3.

2.1.2 International Network of Carcinogenicity Testing

(J.D. Wilbourn, H. Vainio, E. Cardis and J.R.P. Cabral)

National laboratories in Hungary, Lithuania, the Russian Federation and Sweden are collaborating with the Agency in a small network to undertake carcinogenicity tests on agents identified as priorities in *IARC Monographs*. Studies are in progress on acetochlor, trichlorfon and sulfuric acid mists. Tests have been completed on ethanol in isocalorific diets, which showed negative findings for tumorigenicity, suggesting that cancer risks in humans associated with the drinking of alcoholic beverages may be dependent on additional factors as well as modifying effects produced by ethanol.

In a study on the transplacental effects of diethylstilbestrol in mice, transmission of increased carcinogenic risk in the female progeny of treated male mice was shown (Turusov *et al.*, 1992). No skin tumour promoting activity was observed in mice exposed to 50 Hz alternating magnetic fields (Rannug *et al.*, 1993).

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2.2 *Occupational causes of cancer*

Occupational cancers have long been a focus of attention among epidemiologists because individual exposures, and therefore risks, in the work environment tend to be higher than in the general environment. In addition, the exposed population can be relatively easily defined, and exposures estimated from measurements or known characteristics of the work environment.

Studies at IARC focus on both industrialized countries, where very detailed information on exposures and on other life-style factors can be obtained, and developed countries, where high levels of exposure are often encountered.

2.2.1 Occupational cancer in industrialized countries

2.2.1.1 *International Register of Workers Exposed to Phenoxy Acid Herbicides and Contaminants*

(R. Saracci, M. Kogevinas, R. Winkelmann, P. Boffetta and G. Ferro; in collaboration with E. Johnson, Research Triangle Park, NC, USA; H. Becher, Heidelberg, Germany; P.A. Bertazzi, Milan, Italy; H.B. Bueno de Mesquita, Bilthoven, The Netherlands; D. Coggon, Southampton, UK; M. Fingerhut, Cincinnati, USA; L.M. Green, Toronto, Ontario, Canada; T. Kauppinen, Helsinki, Finland; M. Littorin, Lund, Sweden; E. Lynge, Copenhagen, Denmark; J.D. Mathews, Casuarina, NT, Australia; L. Needham, Atlanta, USA; M. Neuberger, Vienna, Austria; J. Osman, Bootle, UK; and N. Pearce, Wellington, New Zealand)

Chlorophenoxy herbicides, widely used since the mid-1950s, may be contaminated during the production process with polychlorinated dioxins and furans, including tetrachlorodibenzo-*p*-dioxin (dioxin, TCDD). An international study of 18 910 production workers and sprayers exposed to chlorophenoxy herbicides, chlorophenols and contaminants (principally dioxins and dibenzofurans) is being conducted in collaboration with the US National Institute of Environmental Health Sciences (NIEHS). It includes workers from Australia, Austria, Canada, Denmark, Finland, Italy, the Netherlands, New Zealand, Sweden and the United Kingdom, and has recently been enlarged with four cohorts from Germany, and 12 cohorts from the USA. The first mortality follow-up has been completed. An excess risk was observed for soft-tissue sarcoma, but not for non-Hodgkin lymphoma. Risks appeared elevated for cancers of the thyroid, testis, other endocrine glands, nose and nasal cavity, based on small numbers of deaths. Two nested case–control studies on soft-tissue sarcoma and non-Hodgkin lymphoma, to assess the importance of various occupational exposures, are being completed. A further mortality and cancer incidence follow-up and a study to determine serum levels of dioxin and furans in a sample of workers are in progress.

2.2.1.2 *Cohort study on workers exposed to styrene*

(M. Kogevinas, G. Ferro and R. Saracci; in collaboration with A. Andersen and J.E. Bjerk, Oslo, Norway; M. Biocca and C. Galassi, Bologna, Italy; D. Coggon and B. Pannett, Southampton, UK; V. Gennaro and V. Ferraro, Genoa, Italy; S. Hutchings and D. South, Bootle, UK; H. Kolstad, Aarhus, Denmark; I. Lundberg and T. Bellander, Stockholm, Sweden; E. Lynge and A. Astrup-Jensen, Copenhagen, Denmark; and T. Partanen and P. Pfaffli, Helsinki, Finland)

Increased risks for leukaemia and lymphoma have been suggested in studies of workers exposed to styrene in the rubber and plastics industry. An historical cohort study has been conducted in Denmark, Finland, Italy, Norway, Sweden and the United Kingdom involving 40 683 workers employed in the reinforced plastics industry, where high exposure to styrene occurs. Exposure to styrene was reconstructed through job histories, environmental and biological monitoring data and production records of the plants in the study. Among exposed workers, no excess was observed for mortality from all causes, from major epithelial cancers, or from neoplasms of the lymphatic and haematopoietic tissues. The rate of mortality from leukaemias and lymphomas increased with time since first exposure. Among subjects exposed for more than one year, a two-fold risk was observed 20 years after first exposure. A mathematical model of past exposure will be applied to estimate risk of leukaemia and lymphoma according to cumulative styrene exposure. A nested case–control study on leukaemia and lymphoma is planned.

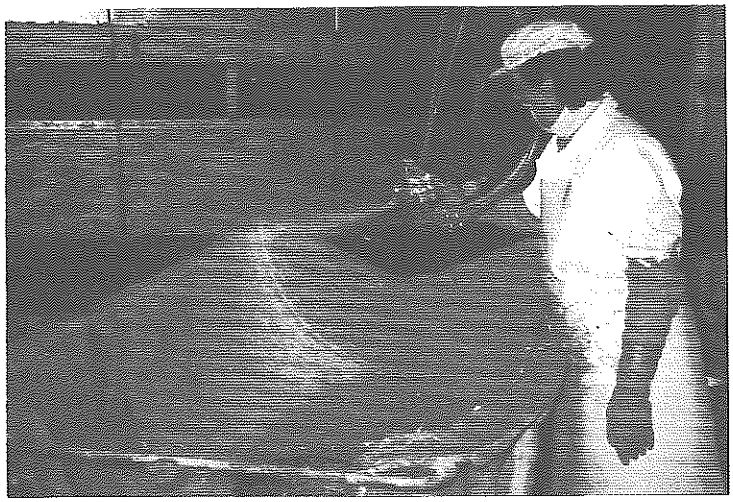

Figure 6. A reinforced plastics worker at a factory in Emilia Romagna, Italy, where risks due to styrene exposure are being studied

2.2.1.3 *Historical prospective study of workers employed in the man-made mineral fibre industry*

(R. Saracci, P. Boffetta, G. Ferro and M. Kogevinas; in collaboration with A. Andersen, Oslo, Norway; P.A. Bertazzi, Milan, Italy; J. Cherrie, Edinburgh, UK; R. Frentzel-Beyme, Heidelberg, Germany; J. Olsen, Copenhagen, Denmark; N. Plato, Stockholm, Sweden; T. Schneider, Copenhagen, Denmark; L. Simonato, Padua, Italy; L. Teppo, Helsinki, Finland; P. Westerholm, Stockholm, Sweden; and P. Winter, Southampton, UK)

An historical cohort study has been conducted since 1977 in 13 factories producing man-made mineral fibres in seven European countries. Data for a new follow-up of this cohort to the end of 1991 have been collected and prepared for both stratified and regression analyses, in particular so as to check the findings of previous follow-up (including an excess of lung cancer in workers employed in the early phases of the rockwool–slagwool production). A mathematical model of past fibre exposure in the rockwool–slagwool industry according to year of employment and factory will be applied to estimate lung cancer risk according to semi-quantitative cumulative fibre exposure (Figure 7).

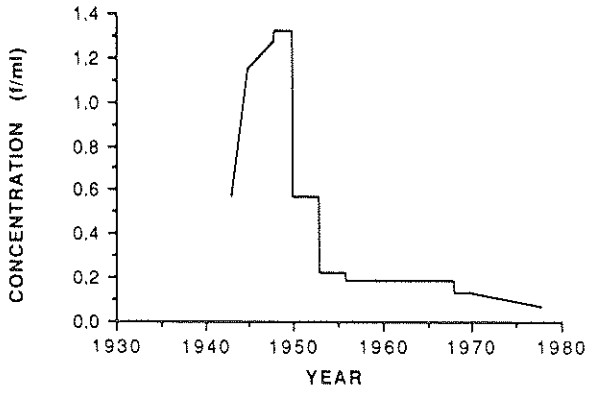

Figure 7. Estimate of past man-made mineral fibre exposure concentration in one of the rockwool–slagwool factories included in the IARC epidemiological study (Krantz *et al.*, 1991)

2.2.1.4 *International cohort study on cancer risk among workers in the pulp and paper industry*

(M. Kogevinas, P. Boffetta, R. Saracci, H. Vainio, R. Winkelmann and G. Ferro; in collaboration with W. Ahrens, Bremen, Germany; A. Andersen, Oslo, Norway; P. Autier, Brussels, Belgium; P. Band and K. Teschke, Vancouver, Canada; A. Bergeret, Lyon, France; W. Boal, Cincinnati, OH, USA; D. Coggon, Southampton, UK; L. Facchini, Pelotas, Brazil; M. Finkelstein, Toronto, Canada; D. Heederik, Wageningen, Netherlands; P.K. Henneberger, Syracuse, NY, USA; P. Jäppinen, Imatra, Finland; T. Kauppinen, Helsinki, Finland; D. Kielkowski, Johannesberg, South Africa; E. Lynge, Copenhagen, Denmark; F. Merletti, Turin, Italy; H. Miyake, Sapporo, Japan; N. Pearce, Wellington, New Zealand; B. Persson, Linköping, Sweden, C. Soskolne, Edmonton, Canada; J. Sunyer, Barcelona, Spain; I. Szadkowska-Stanczyk, Lodz, Poland; G. Thériault, Montreal, Canada; and P. Wild, Vandoeuvre-lès-Nancy, France)

In view of a possible increased risk for cancer at certain sites (lung, gastrointestinal tract, lymphatic tissues) among workers in the pulp and paper industry — an activity employing hundreds of thousands of workers worldwide — a multicentric international cohort study is being developed. Personnel employed in plants producing pulp, paper and paper products and in mills involved in recycling are included. Cohorts are currently being assembled, and it is expected that the international study will include data for more than 100 000 workers. An industrial hygiene study is being conducted. The first results of the study are expected in 1996.

2.2.1.5 *International study of cancer risk in biology research laboratory workers*

(A.J. Sasco and R. Saracci; in collaboration with A. Ahlbom, Stockholm, Sweden; S. Belli, Rome, Italy; F. Berrino, Milan, Italy; S. Benhamou, Villejuif, France; G.J. Bourke, Dublin, Ireland; C. Chilvers, Nottingham, UK; F. Hatton, Le Vésinet, France; O.H. Iversen, Oslo, Norway; T. Kauppinen, Helsinki, Finland; R. Maximilien and M. Tirmarche, Paris, France; J.J. Moulin, Vandoeuvre-lès-Nancy, France; C. Tessier, Strasbourg, France; and F. van Leeuwen, Amsterdam, The Netherlands)

The need to assess cancer risk in research laboratory personnel was based on several considerations (Sasco, 1989): (*a*) the existence of documented health risk in research laboratories such as accidents, infections, occurrence of unwanted reproductive outcomes (spontaneous abortions, perinatal mortality, congenital malformations), increased frequency of chromosomal abnormalities; (*b*) evidence of documented excess cancer risk among chemists (cancer of the lymphohaematopoietic system, brain, pancreas); (*c*) the preliminary evidence of excess cancer risk for research personnel, based on two small pilot studies in France and Italy (Cordier, 1990; Belli *et al.*, 1990); (*d*) the lack of any large, convincing study in this field; (*e*) the recommendations of various bodies that cancer risk be assessed for people handling carcinogens in laboratories or occupationally exposed to potentially oncogenic viruses; and (*f*) the interest and concern among the general public about potential risks linked to genetic engineering.

A feasibility study conducted from 1988 to 1990 at IARC and in eight collaborating countries (Canada, Finland, France, Ireland, Italy, The Netherlands, Switzerland and USA) clearly demonstrated that a study of cancer risk in biology research laboratory workers could and should be carried out. The need for such a study has been reinforced by the final results of a study conducted at the National Institute of Health in Italy (Belli *et al.*, 1992), as well as a recent cancer registry study in the UK (Carpenter *et al.*, 1991), although a Finnish study of persons handling chemical carcinogens gave negative results.

An international retrospective cohort study of mortality is now in progress (Sasco, 1991). Cohorts have been established in parallel in the biomedical and agronomic fields belonging to public European research institutions in eight countries (Finland, France, Ireland, Italy, The Netherlands, Norway, Sweden and United Kingdom) and are being followed up for mortality. The study covers institutions that employ a total of some 70 000 persons. As the cohort will be composed of any person having been employed for at least one year and one day in these institutions during the period 1970 to 1989, it is estimated that more than 1 million person-years will be available. Cancer risk in the whole study population will be compared with that of the general population and will also be evaluated in exposed and non-exposed subjects within the cohort. For the first time the cancer experience of groups defined by job title and type of scientific activity will be compared. Methods to validate some exposure data are being considered (Sasco, 1992; Sasco *et al.*, 1993). Results should be available by 1997.

2.2.1.6 *International collaborative study on workers exposed to lead*

(P. Boffetta, R. Saracci and M. Kogevinas; in collaboration with P.L. Cocco, Cagliari, Italy; J. Davies and G. Kazantzis, London, UK; D. Fanning, Manchester, UK; G. Nordberg, Umeå, Sweden; K. Steenland, Cincinnati, OH, USA; N. Szeszenia-Dabrowska, Lodz, Poland; and O. Wong, Alameda, CA, USA)

Published results of cohort studies of workers exposed to lead suggest an increased incidence of lung and stomach cancers. This project aims to examine in detail the pattern of cancer risk with respect to exposure to lead, by assembling the individual workers' data of existing cohorts and carrying out a reanalysis using uniform procedures.

2.2.1.7 *Cancer risk in the wood and leather industries*

(P. Boffetta, M. Kogevinas, D. Colin, P. Demers, G. Ferro, R. Saracci and H. Vainio; in collaboration with A. Blair, R. Hayes, L. Brinton and B. Miller, Bethesda, USA; U. Bolm-Audorf, Hamburg, Germany; S. Bonassi, Genoa, Italy; P. Comba, Rome, Italy; K. Fukuda, Kurume, Japan; L. Hardell, Orebro, Sweden; A. Leclerc, Paris, France; C. Magnani, Turin, Italy; E. Merler and A.

Seniori-Costantini, Florence, Italy; S. Preston-Martin, Los Angeles, USA; C. Robinson and R. Roscoe and F. Stern, Cincinnati, USA; S. Rodella, Verona, Italy; S. Stellman, New York, USA; T. Vaughan, Seattle, USA; P. Winter, Southampton, UK; and W. Zheng, Shanghai, China)

Employment in the wood and leather industries entails exposures that are carcinogenic to humans. The main target sites in both cases are the nose and the nasal sinuses. However, the role of specific exposures such as wood dust, leather dust, formaldehyde, solvents and preservatives is not clear. Although information on specific exposures was present in many epidemiological studies, this information could not be fully used, because of the relatively small size of each individual study. The raw data of the cohort studies on workers employed in these industries and the case–control studies on nasal cancer have been obtained from the original investigators and transformed into a common format. The complete data-set has been analysed according to specific exposures. Table 5 shows selected results on mortality in the combined cohort. The final results will be available in 1994.

Table 5. Selected results of combined analysis of cohort studies on wood workers

Cause of death (ICD-9 codes)	Number observed	SMR	95% CI
All causes (001-999)	7664	0.77	0.75–0.78
All cancers (140-208)	1725	0.80	0.76–0.83
Buccal cavity and pharynx cancer (140-149)	36	0.67	0.47–0.92
Nasopharynx cancer (147)	9	2.37	1.09–4.51
Sino-nasal cancer (160)	11	3.12	1.56–5.58
Larynx cancer (161)	18	0.66	0.39–1.04
Lung cancer (162)	575	0.80	0.73–0.87
Lymphatic and haematopoietic (200-208)	149	0.88	0.74–1.03
Cardiovascular diseases (390-459)	3698	0.75	0.73–0.78
Respiratory diseases (460-519)	678	0.81	0.75–0.87
External causes (800-999)	550	0.72	0.66–0.78

2.2.1.8 *Cancer risks due to asphalt vapours*

(P. Boffetta, M. Castegnaro, T. Partanen, B. Donvito, H. Bartsch and R. Saracci; in collaboration with A. Benos, Thessaloniki, Greece; A. Bergeret, Lyon, France; P.A. Bertazzi, Milan, Italy; H. Brandt, Amsterdam, Netherlands; R. Frentzel-Beyme, Heidelberg, Germany; D. Heedrick, Wageningen, Netherlands; B. Herity, Dublin, Ireland; B. Jarvholm, Gothenburg, Sweden; T. Kauppinen, Helsinki, Finland; S. Langard, Porsgrunn, Norway; A. Morabia, Geneva, Switzerland; M. Neuberger, Vienna, Austria; B. Pannett, Southampton, UK; J. Sunyer, Barcelona, Spain; O. Svane, Copenhagen, Denmark; N. Szeszenia-Dabrowska, Lodz, Poland; H. Van den Berghe, Leuven, Belgium; and INRS, Vandoeuvre, France; supported by a contract with the European Asphalt Pavement Association, Breukslen, The Netherlands)

The investigation of a possible cancer risk from exposure to asphalt fumes is particularly difficult because of the complex and variable nature of asphalt, the occurrence of

co-exposures (motor engine exhaust, tobacco smoking) and the characteristics of the workforce (seasonal employment, instability, low skill). Recent epidemiological studies from northern Europe suggest an increased risk of cancer from the lung and other organs. In order to obtain sound exposure data with which to test these results, a specific biological marker of exposure to asphalt fumes is needed.

A study has been conducted in 1993 to assess the feasibility of a retrospective epidemiological study on cancer incidence and mortality among workers engaged in road paving and asphalt mixing in 18 European countries. This showed that a full-scale epidemiological study is possible in at least several of these countries.

In a parallel study, rodents will be exposed through skin painting and inhalation to standard asphalt fumes. Initially, fractions generated during the hardening of the bitumen have been obtained which contain the same volatile compounds as the corresponding fumes, but at higher concentrations. One of these has been oxidized to mimic atmospheric oxidation. All have now been tested *in vitro* for their capacity to modify DNA in the presence of a metabolizing system and benzo[*a*]pyrene was tested as a positive control. Anthracene oil, a distillate fraction from coal tar has also been tested. More DNA modifications were obtained with the anthracene oil fraction than from various bitumen fractions, and more DNA modifications were obtained with the lighter fractions than with the higher ones.

2.2.1.9 *Cancer risk among workers exposed to mercury*

(P. Boffetta and R. Saracci; in collaboration with M. Garcia-Gomez, Madrid, Spain; E. Merler, Florence, Italy; V. Pompe-Kirn, Ljubljana, Slovenia; G. Sallsten, Gothenburg, Sweden; and D. Zaridze, Moscow, Russian Federation)

An increase in lung cancer risk among workers exposed to mercury has been found in a number of epidemiological studies. Among the industries entailing, in the present or the past, high exposure levels to mercury are the mining or milling of the metal, thermometer production and felt-hat manufacture, but no large study on workers involved in these occupations has yet been conducted.

A historical cohort study has been started in the four European countries with large mercury mines (Italy, Russian Federation, Slovenia, Spain). Special care is being given to the reconstruction of industrial hygiene data (exposure levels to mercury and co-exposures such as silica and radon). A retrospective cohort study has been conducted among workers compensated for mercury intoxication in an area of central Italy with a large felt-hat manufacturing industry (see Table 6) which showed an excess of stomach and lung cancer; while the former may be explained by local factors, the latter seems related to occupational exposures. A further cohort in a large thermometer production factory in the Russian Federation will be studied.

2.2.1.10 *Feasibility study on cancer risk among workers in the European textile-manufacturing industry*

(M. Kogevinas and P. Boffetta; in collaboration with Chen-Ji-Gang, Shanghai, China; M. Garcia-Gomez, Madrid, Spain; K. Katsouyanni, Athens, Greece; S. Massano-Cardoso, Coimbra, Portugal; J. Osman, Bootle, UK; E. Roman, Oxford, UK; and I. Szadkowska-Stanczyk, Lodz, Poland)

The textile industry is one of the largest employers worldwide, with, for example, around 4 000 000 workers employed in Europe during the mid 1980s. Several epidemiological studies have suggested possible associations of employment in textile manufacturing with oral cancer, intestinal cancer, nasal cancer, laryngeal cancer and bladder cancer. A lack of data on

occupational exposure has been a major obstacle to evaluation of specific risks in this industry. The feasibility of a multicentric industrial hygiene investigation and of a multicentric cohort study in the textile manufacturing industry in China, Greece, Poland, Portugal, Spain and the UK, to evaluate both the overall risk and also to describe the effects of specific exposures, is currently being examined.

Table 6. Mortality of women employed in felt-hat manufacture in Italy and compensated for mercury intoxication

Cause of death	Number of deaths		SMR	95% CI
	Observed	Expected		
All causes	211	329.8	0.64	0.56–0.73
All cancers	72	78.3	0.92	0.72–1.16
Stomach cancer	22	9.7	2.27	1.42–3.43
Lung cancer	10	4.9	2.02	1.97–3.72
Kidney cancer	1	1.1	0.95	0.02–5.31
Brain tumour	2	1.5	1.37	0.17–4.95
Cardiovascular diseases	93	159.4	0.58	0.47–0.71
Respiratory diseases	6	17.6	0.34	0.12–0.74
External causes	8	11.1	0.72	0.31–1.43

2.2.1.11 *Chronic low-dose exposures to ionizing radiation*

(E. Cardis, B.K. Armstrong, J. Estève, I. Kato and C. Lavé ; in collaboration with P. Ashmore, Ottawa, Canada; V. Beral and L. Carpenter, Oxford, UK; J. Bernar Solano, Madrid, Spain; M. Blettner, Heidelberg, Germany; G. Cowper, Deep River, Canada; A. Diez Sacristan, Madrid, Spain; A. Douglas and P. Smith, London, UK; M. Eklöf, Osthammar, Sweden; H. Engels and G. Laleman, Mol, Belgium; J. Fix and E. Gilbert, Richland, WA, USA; S. Fry, Oak Ridge, TN, USA; J. Gray, Menai, Australia; L. Green and G. Howe, Toronto, Canada; M. Hakama, Tampere, Finland; C. Hill, Villejuif, France; Y. Hosoda and M. Kaneko, Tokyo, Japan; J. Kaldor, Darlinghurst, Australia; G. Kendall, B.H. MacGibbon and C. Muirhead, Didcot, UK; L. Kheifets, Palo Alto, CA, USA; H. Malker, Solna, Sweden; M. Moser, Bern, Switzerland; P. Pellerin, Le Vésinet, France; T. Rytömaa, Helsinki, Finland; L. Salmon, Harwell, UK; G. Schüler, Zürich, Switzerland; G. Seitz, Cologne, Germany; R. Shore, New York, USA; G.L. Voelz and L. Wiggs, Los Alamos, USA; and T. Yoshimura, Kitakyushu, Japan)

The aim of these studies is to assess directly the carcinogenic effects of low-dose protracted exposures to low-LET ionizing radiation (predominantly X- and γ-rays).

Three-country study of cancer risks among nuclear industry workers

Combined analyses of mortality data on 95 673 nuclear industry workers who were monitored for external exposure to ionizing radiation in nuclear facilities in the USA (Hanford Site, Oak Ridge National Laboratories and the Rocky Flats Nuclear Weapons plant), the UK (British Nuclear Fuel's Sellafield plant, the Atomic Energy Authority, the Atomic Weapons Research Establishment) or Canada (Atomic Energy of Canada) were

carried out. The majority of workers were exposed to external (whole body) radiation, primarily X- and γ-rays. Cumulative radiation doses ranged from zero to over 1 Sv (mean 36.6 mSv); the dose distribution was very skewed, with 60% of the monitored workers having cumulative doses below 10 mSv and less than 2% over 200 mSv. Most of the dose (98%) was received by men. By the end of the follow-up, 15 825 workers (16.5%) were reported to have died.

Estimates of excess relative risk per Sv were calculated for mortality from all cancers excluding leukaemia and from leukaemia excluding chronic lymphocytic leukaemia. They were then compared with estimates derived from high-dose studies, using three approaches. First, data on male atomic bomb survivors exposed between the ages of 20 and 60, supplied by the Radiation Effects Research Foundation in Hiroshima, were analysed using methods identical to those used to obtain the workers' estimates. Second, the worker-based estimates were compared with the linear excess relative risk estimates derived by the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) that form the basis for the current recommendations of the International Commission for Radiological Protection. Finally, the models used by the US National Academy of Sciences Committee on the Biological Effects of Ionising Radiation (BEIR V) for estimating risk, which incorporate dependencies of the excess relative risk on sex, age at exposure and time since exposure, were applied to the workers' data. The report of these analyses and selected publications are in preparation.

Combining data from seven groups of facilities in three countries has provided the most precise direct estimates to date of the risk of cancer associated with generally low-dose, protracted exposure to ionizing radiation. Since only 16.5% of the workers have been reported to have died, further follow-up of these cohorts and studies of additional groups of workers in these and other countries will be useful to reduce the uncertainties in radiation risk assessment even further.

Biases and uncertainties in occupational radiation dose estimates

A study of past dosimetric and radiation protection practices in the facilities included in the combined analyses described above was carried out to allow comparison of dose estimates across time and facilities.

The accuracy and precision of individual dose estimates was found to depend on the technology used for monitoring in a given facility and time period (detection level, radiation response, precision and accuracy of the dosimeter), on the characteristics of the radiation exposure in the work environment (radiation type and energy, geometry of exposures) and on administrative practices adopted to determine and record dose.

Doses received by the majority of workers resulted primarily from exposure to high-energy (100 keV or more) photons; for these workers, recorded dose estimates were judged to be generally comparable across facilities and time, and to reasonably approximate the "deep dose", or energy absorbed at 1 cm depth in tissue (Hp(10); the quantity currently recommended by the International Commission on Radiation Units and Measurement (ICRU) for radiation protection), but to overestimate dose to the bone marrow by approximately 20%.

Doses from neutrons were underestimated for some subgroups of workers, particularly in the early years of the industry, because of limitations in dosimeter technology, while dose from radioactivity intake other than tritium was not available. Less than 2% of the workers studied received substantial doses from neutrons or internal contamination; efforts were made to identify them and exclude them from selected analyses.

International collaborative study of nuclear industry workers

Results of the feasibility study (Cardis & Estève, 1991; Cardis *et al.*, 1992) indicate that a detailed retrospective study of cancer risk among nuclear industry workers is feasible in all participating countries and will add substantially to the information about the effects of low doses of radiation obtained from the three-country study described above.

Eleven countries (Australia, Belgium, Canada, Finland, France, Germany, Japan, Spain, Sweden, Switzerland, United Kingdom) have confirmed their participation. A common core protocol has been finalized and data collection has started in eight of the countries.

The study population consists of all workers employed for at least one year and monitored for external radiation in public and private nuclear organizations of the participating countries. Individual annual estimates of dose from X- and γ-rays and neutrons are being obtained for each individual in the study cohorts. Flags identifying workers with substantial doses from radioactivity intake will be constructed. Follow-up will be for mortality in all countries, and for cancer morbidity in Australia, Canada, Finland, Sweden and the United Kingdom. Estimates of risk of all cancers excluding leukaemia and leukaemia excluding chronic lymphocytic leukaemia will be derived and compared with those based on high-dose studies.

Data collection is expected to be complete in all countries by late 1996 and the results of the joint analyses should be available at the end of 1998.

Epidemiological studies of the health consequences of the Chernobyl accident

(E. Cardis and B.K. Armstrong; in collaboration with K. Baverstock, Rome, Italy; V. Bebeshko, I. Likhtariov and A. Prisyazhniuk, Kiev, Ukraine; K. Chadwick, A. Karaoglou, M. Lechat and J. Sinnaeve, Brussels, Belgium; G. Howe, Toronto, Canada; V. Ivanov, Obninsk, Russian Federation; A. Kellerer, Munich, Germany; W. Kreizel and I. Riaboukhine, Geneva, Switzerland; J.P. Massué, Strasbourg, France; V. Merabishvili, St Petersburg, Russian Federation; C. Muirhead, Didcot, UK; A. Okeanov and G. Tolochka, Minsk, Belarus; H. Storm, Copenhagen, Denmark; M. Tirmarche, Fontenay-aux-Roses, France; and D. Zaridze, Moscow, Russian Federation)

Many national and international organizations have been contacted by colleagues in the Ukraine, Belarus and Russian Federation to provide assistance in setting up and carrying out epidemiological studies of the health consequences of the Chernobyl accident. IARC's involvement until now has been limited to facilitating communication between these organizations, providing assistance and advice; planning a course in basic cancer and radiation epidemiology in collaboration with a Canada/US initiative, and developing protocols for pilot studies (mainly concerning dosimetry and follow-up) of emergency accident workers in Belarus and the Russian Federation in the framework of a CEC experimental collaboration project. Results of the pilot studies are expected in the summer of 1994.

2.2.2 Occupational cancer in industrially developing countries

2.2.2.1 *Cancer risk among steel workers in Vale do Aco, Minas Gerais, Brazil*

(S. Barreto, P. Boffetta, M. Kogevinas and R. Saracci; in collaboration with A. Swerdlow, London, UK)

More than one third of the adult male worker population of the Vale do Aco, Minas Gerais, is employed in the steel industry. A descriptive analysis of cancer mortality in Vale do

Aco has been conducted, and a study comparing cancer mortality among workers in the largest steelworks with those of the entire valley and of the capital of the State (Belo Horizonte), and related to levels of exposure of the steelworkers to polycyclic aromatic hydrocarbons, has been initiated. A population-based case–control study of lung cancer is planned.

2.2.2.2 *Multi-site case–control study of cancer and occupation in Uruguay*
(M. Kogevinas and P. Boffetta; in collaboration with E. de Stefani, Montevideo, Uruguay)

Uruguay has the highest age-adjusted death rate from cancer of all sites in males in the Americas. A study has been designed to identify possible associations between occupational exposures and cancer risk in Uruguay, estimate the number of deaths from cancer due to working in hazardous occupations, and examine the synergistic effect of certain occupational exposures and tobacco smoking. A questionnaire to request information on demographic factors, full occupational history (including a detailed evaluation of exposure to pesticides and asbestos), blond and black tobacco use and other life-style factors has been pilot-tested.

2.2.2.3 *Multi-site case–control study on occupational risk factors in India*
(P. Boffetta, M. Kogevinas and R. Sankaranarayanan; in collaboration with P. Chattopadhyay and D. Giri, Ahmedabad, India; A. Nandakumar, Bangalore, India; P.B. Desai, Bombay, India; and C. Varghese, Trivandrum, India)

Although the industrial population in India is very large and many hazardous industries are present, virtually no information exists on occupational cancer risk factors. The presence of a network of well organized cancer registries is a favourable condition for conducting multicentric case–control studies.

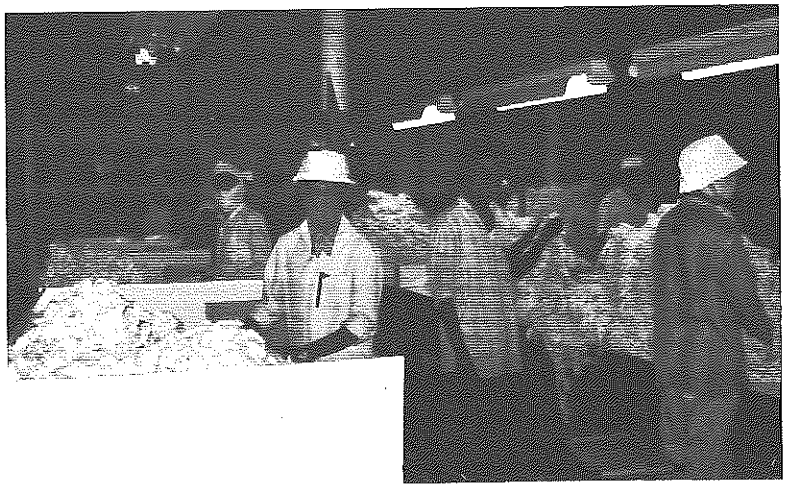

Figure 8. Textile workers in a factory in Ahmedabad, India, included in a study of occupational risk factors for cancer

A preliminary study conducted during 1991–92 in Ahmedabad and Trivandrum showed increased risk of lung cancer among textile, wood, metal and construction workers, and an increased risk of lymphomas and leukaemia among painters and metal and agriculture

workers (Table 7). A five-centre case–control study has been planned, to investigate in detail occupational and other environmental risk factors of lung cancer, lymphomas and leukaemia.

Table 7. Age-adjusted odds ratios for selected sites by occupation

Occupation	Site of cancer						No. of exposed controls
	Lung			Lymphatic and haematopoietic			
	OR	*n*	95% CI	OR	*n*	95% CI	
Agriculture	1.1	40	0.8–1.5	1.6	23	0.9–2.9	79
Textile workers	2.3	5	0.7–7.7	0.9	1	0.1–8.4	5
Wood workers	2.2	7	0.8–6.2	0.4	1	0.1–3.3	9
Metal workers	1.9	4	0.5–7.5	1.2	2	0.2–6.4	6
Construction workers	1.2	3	0.3–5.1	1.1	3	0.3–4.3	9
Painters	–	0		9.3	3	1.0–88.5	1
Transport workers	0.5	3	0.2–1.9	0.8	4	0.3–2.5	17

n = number of exposed cases

2.2.2.4 Schistosoma *infection and occupational and environmental risk factors of bladder cancer in Egypt*

(P. Boffetta and H. Vainio; in collaboration with W. Anwar, Cairo, Egypt; R. Bedwani, Alexandria, Egypt; A. Hirvonen, Helsinki, Finland; C. La Vecchia, Milan, Italy; and C. Rocchi, Rome, Italy)

Bladder cancer incidence is very high in the Nile Delta area (17.5% of all cancers), mainly due to chronic infection with *Schistosoma haematobium*. The area of Alexandria has many industries that may entail risks of bladder cancer, such as rubber, dye and textile manufacture. Therefore, the roles of occupational exposures, dietary factors, tobacco smoking, as well as genetic factors are being studied. A hospital-based case–control study has been planned, to involve approximately 400 male cases and an equal number of controls. History of *Schistosoma* infection will be validated via ELISA tests and egg search in urine. Blood, urine and bladder tissue samples will be collected for glutathione *S*-transferase M1 genotyping and possibly measurements of other markers of genetic susceptibility.

2.2.2.5 *Multicentric historical cohort study of workers employed in the rubber industry in China and in Poland*

(M. Kogevinas and P. Boffetta; in collaboration with Chen-Ji-Gang, Shanghai, China; H. Kromhout, Wageningen, The Netherlands; and N. Szeszenia-Dabrowska, Lodz, Poland)

Cancer risk in workers employed in the rubber industry has been extensively studied and increased risks have been observed for neoplasms of the urinary bladder, stomach, lung and the leukaemias, but it has usually not been possible to associate exposure to specific agents with the excess cancer risks observed. The feasibility of studies of workers in the rubber industry in China and Poland with better methods for exposure assessment is being examined, in order to provide specific guidelines for the implementation of preventive measures.

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2.3 *Diet, nutrition and cancer*

It is now clear that many dietary factors may have causative or protective roles in cancer etiology, but the complexity and variability of human diets make informative epidemiological studies hard to conduct. In addition to the specifically diet-oriented epidemiological studies described in this section, diet and food constituents are factors considered in many other Agency projects, particularly those related to cancer of the digestive tract. Thus the effects of drinks such as alcoholic beverages and mate are being examined in relation to oesophageal cancer (section 2.9), and various laboratory and epidemiological projects are exploring the roles in gastric carcinogenesis of *N*-nitroso compounds formed endogenously from dietary components (section 2.7) and of diet in general, including potentially protective vitamins (sections 2.10 and 4.2.2). Heterocyclic amines formed in cooking and mycotoxins (aflatoxins, ochratoxins, etc.) that contaminate foodstuffs, especially in developing countries, are being studied as etiological factors for liver cancer (section 2.11) and urinary tract tumours (section 2.5), and the carcinogenicity of a range of such substances has been evaluated in the *Monographs* programme (section 2.1.1.3).

2.3.1 European Prospective Investigation into Cancer and Nutrition (EPIC)

(E. Riboli, R. Saracci, R. Kaaks, N. Slimani, C. Casagrande and B. Hémon; supported by the Europe Against Cancer Programme of the European Community)

The EPIC project is a multi-centre prospective cohort study designed to investigate the relation between diet, nutritional status, various lifestyle and environmental factors and the incidence of different forms of cancer (Riboli, 1992a). The cohort will be very large, totalling approximately 350 000 middle-aged men and women. Data on usual current diet are collected by means of detailed dietary assessment methods developed and tested during methodological studies. In addition, full information is obtained, by means of a standardized questionnaire, on physical activity, tobacco smoking, alcohol consumption, occupation and socio-economic status, reproductive history, contraception and hormone replacement therapy, previous illnesses and current drug use. These factors were selected because they may be related to diet and nutritional status or may interact with diet in multifactorial carcinogenic processes.

Basic anthropometric measurements (weight, height, waist and hip circumferences, sitting height) are taken using standard procedures. Blood samples are collected, separated into 28 small aliquots of serum, buffy coat and red blood cells, and stored in liquid nitrogen (−196°C). Later, the samples from subjects who develop cancer and from appropriate control subjects who remain disease-free will be analysed. The range of laboratory analyses carried out will depend on the type of cancer, scientific advances in years to come, and the availability of new techniques for measuring biochemical and molecular markers.

The full-scale project was started in 1991–92 in seven European countries, after completion of methodological feasibility studies conducted between 1989 and 1992 in each of the collaborating centres.

IARC provides the scientific and logistic coordination of the project, which is undertaken with the collaboration of 17 centres in seven countries.

2.3.1.1 *EPIC in France*

(in collaboration with F. Clavel, A. Auquier, N. Andrieu, E. Duquesnel, V. Ezratty, H. Goulard, M. Niravong and M. Pasquet, Villejuif)

Women belonging to the Mutuelle Générale de l'Education Nationale (MGEN), a private health insurance scheme for state school employees, are included in the study. The MGEN has about 500 000 members in the age range 40–65. In June 1990 a first questionnaire on reproductive history, height, weight, etc. was mailed to all female members aged between 40 and 60, and about 100 000 (residing all over France) returned it, agreed to cooperate and gave permission for access to their medical data. A second questionnaire on non-dietary variables, mailed in 1992 to the 100 000 respondents, was returned by about 90 000. A third questionnaire on diet was sent out in July 1993, and collection of blood samples will start in September 1993.

2.3.1.2 *EPIC in Italy*

(in collaboration with F. Berrino and V. Krogh, Milan; B. Terracini and P. Vineis, Turin; D. Palli, F. Cipriani and E. Buiatti, Florence; and L. Gafà and R. Tumino, Ragusa)

The study is based in four regions: two in the north (Turin and Varese), one in the centre (Florence) and one in the south (Ragusa in Sicily). The study will enrol 27 000 women among those attending a breast cancer screening programme or members of associations or leagues against cancer in Varese and Florence, and 8000 women and 25 000 men among regular blood donors, members of the two national associations of blood donors in Turin, Florence and Ragusa.

In Varese 10 700 women have already been enrolled in a prospective cohort study on hormones and diet (ORDET) started by Dr Berrino and his colleagues from the National Cancer Institute in Milan. For the EPIC study, information is obtained mostly by self-administered questionnaires in the north and the centre, while data on current diet are obtained by interview in Sicily. During the first six months, the compliance from blood donors and women attending breast cancer screening was of the order of 70%.

2.3.1.3 *EPIC in Spain*

(in collaboration with C.A. González, M. Torrent and A. Agudo, Mataró; J.R. Quíros and L. Las Heras, Oviedo; C. Martinez and B. Gomez, Granada; M. Dorronsoro and N. Larrañaga, San Sebastian; C. Navarro, D. Fuensanta Gual and M.-J. Tormo, Murcia; A. Barricarte and A. Barcos, Pamplona; and G. López-Abente, Madrid)

The study is coordinated by the Institute for Epidemiological and Clinical Research, Mataró (in the Barcelona Metropolitan area) and is based in five regions of Spain: Asturias, Basque Country, Navarra, Murcia and Granada. It is planned to include in the study about 40 000 blood donors and 10 000 civil servants.

Subjects are invited by letter or by telephone. Information on dietary history is obtained by interview, and on other factors by self-administered questionnaire. Complete data and biological samples had been collected from 8000 subjects by July 1993.

2.3.1.4 *EPIC in the United Kingdom*

(in collaboration with S. Bingham, N.E. Day, K.-T. Khaw, S. Oakes and A. Welch, Cambridge; and T.J.A. Key, D. Forman and L. Cotton, Oxford)

The study in the UK combines two complementary approaches developed by the Imperial Cancer Research Fund (ICRF) Epidemiology Unit in Oxford and the Medical Research Council (MRC) Biostatistics Unit and the University Department of Community Medicine in Cambridge.

In the Cambridge component, 10 000 men and 10 000 women are being recruited in collaboration with local general practitioners (GPs). The GPs are assisted by nurses who take care of interviewing, anthropometric measurements and blood collection.

The Oxford component has based subject recruitment on several hundred GPs from all over Britain, each of whom has been asked to recruit four or five subjects among those agreeing to take part in a national health screening programme that covers a total of 40 000 subjects. The GPs provide each subject with a self-administered questionnaire and collect a blood sample, to be sent by overnight mail to the central laboratory in Norfolk.

The field study started in March 1993 in both centres.

2.3.1.5 *EPIC in The Netherlands*

(in collaboration with H.B. Bueno de Mesquita, H.J.A. Collette, D. Kromhout and P. Peeters, Utrecht)

A methodological study on dietary questionnaires, completed in 1992, indicated that there was fairly good concordance between the self-administered diet history method and the reference method. After minor modifications to the dietary questionnaire, the main study started in May 1993.

This is based on two components: (*a*) a cohort of about 25 000 women from among those attending a breast cancer screening programme at the Preventicon Centre in Utrecht, and (*b*) a cohort of about 25 000 men and women from among subjects participating in the "Monitoring Risk Factors Study" organized by the Dutch National Institute of Public Health and Environmental Protection (RIVM).

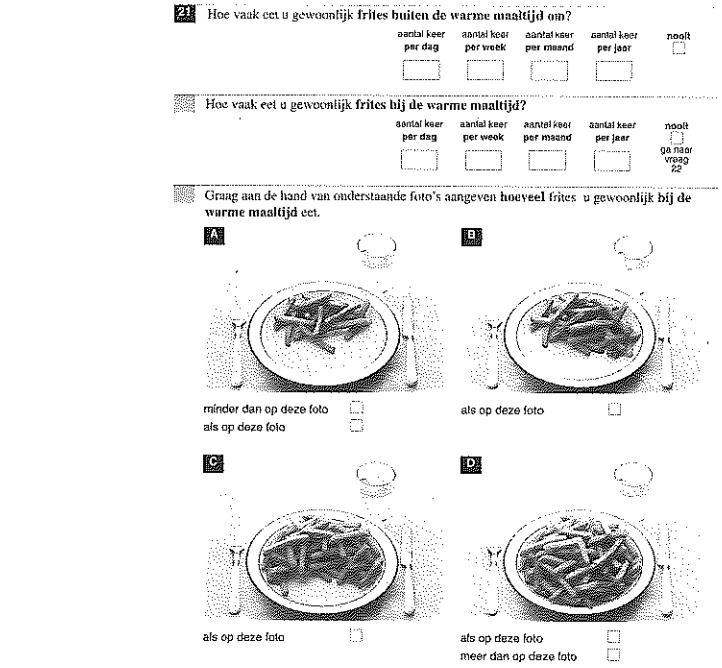
For the collection of data and biological samples, the subjects are invited by letter to visit one of the centres involved in the Monitoring Risk Factor Project or the Preventicon Centre. A questionnaire is enclosed which they are asked to fill in and bring when they come to have a blood sample and anthropometric measurements taken. All blood samples are sent for processing to a central laboratory in Bilthoven.

2.3.1.6 *EPIC in Greece*

(in collaboration with A. Trichopoulou, C. Boulous, G. Gnardelis, K. Katsouyanni, G. Kiriazi, P. Lagiou, P. Mavrika, E. Polychronopoulos and A. Valaora, Athens)

A methodological study to test the dietary questionnaire was completed at the end of 1992. As the list of foods reported by the study subjects was long and detailed, it was necessary to expand the Greek food composition tables in order to perform the statistical analyses.

In the main study it is planned to include about 50 000 civil servants, men and women living in the metropolitan area of Athens. In spring 1993 the first 20 000 letters were sent to employees of the state school system, asking them to send back a card indicating whether they were willing to participate in the project. Enrolment and collection of data and samples will begin in October 1993.



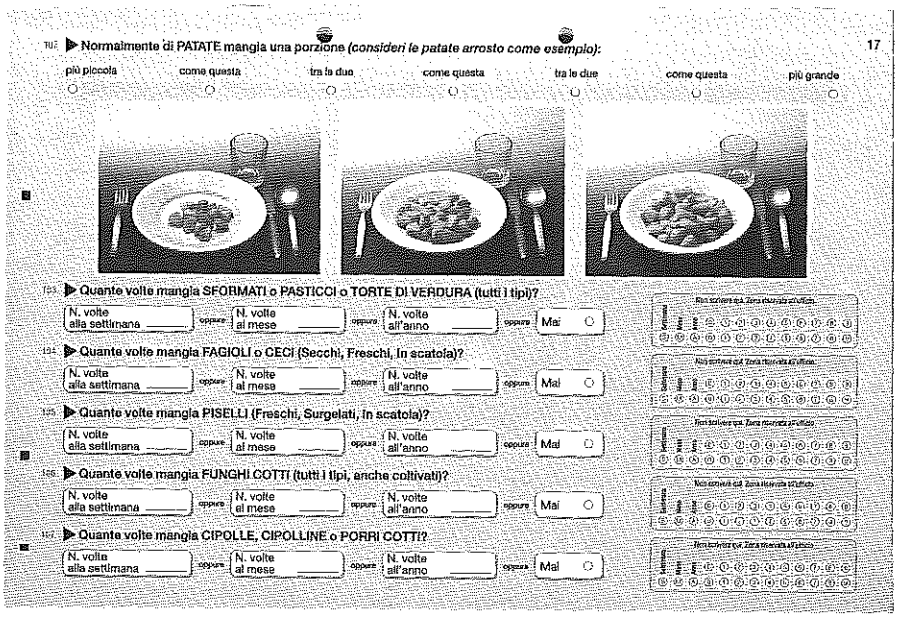


Figure 9. Two examples of the dietary questionnaires used in the EPIC project

2.3.1.7 *EPIC in Germany*

(in collaboration with J. Wahrendorf and S. Bohlscheid, Heidelberg; and H. Boeing, Potsdam)

The methodological study completed in 1992 indicated that the dietary questionnaire measured most of the main nutrients and food groups reasonably well. On the basis of the results, a number of improvements to the questionnaire were made. The main project, originally planned to take place in western Germany, coordinated by the German Cancer Research Centre in Heidelberg, is being extended to an area of eastern Germany, with the collaboration of the Institute for Nutritional Research in Potsdam.

It is planned to recruit about 30 000 men and women in each of the two centres with the collaboration of the health insurance companies which cover the majority of the general population. Study subjects will receive the questionnaires by mail, and will then be invited to the local study centre for blood collection and anthropometric measurements. The field work will start in October 1993 in Heidelberg and in February 1994 in Potsdam.

2.3.2 Methodology for calibration and standardization of dietary measurements in nutritional epidemiology

(E. Riboli, R. Kaaks and N. Slimani)

Prospective cohort studies present many advantages over case–control studies for investigating the relation between diet and cancer. However, if conducted in single areas or populations, they may have limited power to detect exposure–disease associations because the study population may not be large enough, and there may not be sufficient variability in the dietary habits of a single population to cover the range of "exposures" relevant for cancer etiology.

The approach chosen for the EPIC project (see above) is to set up numerous coordinated prospective studies, with the aim of taking full advantage of the prospective approach and at the same time ensuring very large sample size and covering a wide range of dietary exposures in different regions of Europe. The main methodological difficulties of this approach arise from the need to collect dietary data across countries in a comparable and standardized manner (Friedenreich *et al.*, 1992).

For the EPIC project, a novel approach was developed using a questionnaire derived from the diet history method (by interview or self-administered) adapted to local cooking, eating patterns and culture and also to the specific logistic constraints of each region (Figure 9). To correct for possible systematic differences in terms of over- or underestimation of dietary intake, a second measurement of diet will be made in a sub-sample of the cohort with a method, such as 24-hour diet recall, which is much more suitable for strict international standardization than the diet history questionnaire. This sub-sample of 25 000 subjects (about 3000 to 4000 per country) will be interviewed and asked to recall everything they ate or drank during the preceding 24 hours (Kaaks *et al.*, 1993a,b).

An important parallel component of this approach is the standardization of the nutritional methodology for the 24-hour recall interview in different populations (Faggiano *et al.*, 1992). For such interviews, very strict guidelines have been defined on how to proceed, meal by meal, and, within meals, food by food. In practice, for each food which may be reported by the subjects, a sequence of standard questions was defined regarding food identification, appearance, preparation, origin, cooking methods and final mode of consumption.

As it is difficult for the interviewer to keep in mind all the possible questions for any given food out of a list of several thousands which may be reported by the subject, a computer program is being developed to interactively guide the interviewer. Depending on the foods reported by the subject, the computer will prompt the appropriate questions and the possible answers in a multiple-choice, sequential tree structure. Versions of the program will be available in seven languages and incorporate a food data-base (food list, recipes, ingredients, food portions, etc.) specific for each EPIC centre.

An additional methodological component of the standardization of dietary data across countries is to ensure the comparability of the criteria for estimating nutrient intake from dietary questionnaires, in the absence of adequate data on the chemical composition of foods, particularly those consumed in Mediterranean countries. In order to proceed with the analysis of the detailed dietary data collected within EPIC, food composition databases have been prepared at IARC for Italian, Spanish and French foods, to be published as IARC Technical Reports. In addition, a network of collaborators in the field of food chemistry has been set up within the framework of the FLAIR (food-linked agro-industrial research) Eurofoods-Enfant Concerted Action of the European Community and with researchers of the US Department of Agriculture and the US Food and Drug Administration. The aim of this network is to improve the collection and the circulation of food composition data as well as the establishment of international databases linking food to a number of chemical, toxicological and technological characteristics.

2.3.3 The New York University women's health study

(E. Riboli and C. Casagrande; in collaboration with P. Toniolo, B. Pasternack and R.E. Shore, New York, USA)

This prospective study, started in 1985, includes 15 500 women living in the New York metropolitan area who were recruited during 1985–86 while they were attending mammographic screening for breast cancer at the Guttman Institute. Data on diet were collected by means of a self-administered food frequency questionnaire slightly modified from a design by the US National Cancer Institute. The reproducibility of food and nutrient intake estimated by this questionnaire was then tested under real field conditions by asking a subsample of study subjects to fill in the questionnaire on three different occasions. Results indicated fairly good reproducibility in the short term (three months) and still a reasonable concordance of dietary measurements over a longer period (1–5 years). Blood samples were collected in 1985–86 from all women in the cohort. A second sample was collected from about half of the subjects in the cohort on the occasion of subsequent mammographic screening. The blood samples were stored in 10 aliquots of 2 ml each of plasma and buffy coat at –80°C. Results on dietary habits and risk of breast cancer for the first 180 incident cases and 900 matched controls indicate that high consumption of meat and saturated fat doubled the incidence of breast cancer.

2.3.4 Malmö prospective study on diet and cancer

(R. Saracci, E. Riboli and B. Hémon; in collaboration with G. Berglund, L. Janzon, S. Elmstahl, S.A. Larsson and B. Gullberg, Malmö, Sweden)

Collection of data and blood samples from middle-aged men and women living in the Malmö area (Sweden) began at the end of 1991. The target is to include about 40 000 subjects and to follow them up for incidence of cancer and cardiovascular disease. Dietary data are collected by means of a detailed dietary assessment method combining a food frequency questionnaire and a seven-day diet record (Callmer *et al.*, 1993). Blood samples are separated

and aliquotted in serum, plasma and lymphocytes and stored at -80°C. By July 1993, data and blood samples had been collected from about 10 000 subjects, and data collection will continue throughout 1994 and 1995.

2.3.5 Case–control studies on diet and stomach cancer in different populations of Europe

(E. Riboli, A.J. Tuyns, R. Kaaks and B. Hémon; in collaboration with C.A. González, Mataró, Spain; J. Cornée, Marseilles, France; and D. Pobel, Besançon, France)

Various hypotheses on the links between dietary habits and occurrence of gastric cancer have been investigated in case–control studies conducted in Belgium, France and Spain.

The study in Belgium was based in two provinces: Oost-Vlaanderen (Flemish) and Liège (Walloon). It included 449 cases and 3524 population controls (Tuyns *et al.*, 1992).

The French study was based in Marseilles and included 92 cases and 128 hospital controls.

The study in Spain was conducted in four regions: Aragon, Castile, Catalonia and Galicia. Questionnaire data were collected from 354 cases and the same number of hospital controls (Gonzáles *et al.*, 1991, 1993).

The three studies provided very concordant results regarding the inverse association between consumption of fruit and vegetables and risk of gastric cancer. In Spain, a particularly strong protective effect was found for consumption of boiled borage, a vegetable grown in Castile which is rich in dihomo-gamma-linolenic acid, a fatty acid which is suspected of having anticarcinogenic properties (Gonzalez *et al.*, 1991). In the Spanish study an increased risk was also observed for consumption of salted foods, particularly salted fish, while elsewhere, consumption of salted foods was too low to be informative.

In the Spanish study, detailed histological data were available, permitting investigation of the relationship between diet and the different types of stomach cancer (intestinal and diffuse). No substantial differences were found regarding the protection from fruit and vegetables or the increased risk for salted foods. A table on the nitrosodimethylamine (NDMA) content of common foods was used to estimate NDMA intake among cases and controls in the Spanish and French studies. The results indicated that cases had significantly higher intake of NDMA from their usual diet, and in subjects with the highest intake the risk was increased two-fold compared with those with the lowest intake. Notwithstanding the limitations of this type of crude estimation, the results support the theory that intake of nitrosamines or their precursors increases the risk of gastric cancer.

2.3.6 Case–control studies on cancer and polyps of the colorectum and diet in France and Belgium

(E. Riboli, R. Kaaks and C. Casagrande; in collaboration with J. Cornée, G. Macquart-Moulin and M. Guyader, Marseilles, France)

Two parallel case–control studies on diet and tumours of the colorectum were conducted in Belgium and Marseilles during the 1980s. Results on major nutrients and foods were reported earlier. A recent analysis of the dietary data from 389 cases with adenocarcinomas of the colorectum, 252 cases with adenomatous polyps of the colorectum and 641 hospital controls focused on consumption of alcoholic beverages. The results revealed no association between consumption of wine or spirits and risk of cancer or polyps of the colon or rectum. There was, however, an increased risk for cancer of the rectum in men and women in relation to beer consumption, in agreement with previous epidemiological results. This may be related to the relatively high levels of NDMA, characteristic of European and North American beer until the end of the 1970s (Riboli *et al.*, 1991).

The consistent finding of protection by vegetable consumption against colorectal cancer stimulated the review of some mechanistic hypotheses (Miller *et al.*, 1993; Kaaks & Riboli, 1993; Riboli, 1992).

2.3.7 Case–control and family studies of diet and colorectal cancer and polyps in Majorca

(F.X. Bosch, N. Muñoz and J. Estève; in collaboration with E. Benito and M. Mulet, Ciutat de Mallorca, Spain; V. Moreno, Barcelona, Spain; and A. Obrador, Palma de Mallorca, Spain)

A case–control study on diet and colorectal polyps was conducted in Majorca between 1988 and 1990. All newly diagnosed cases at the gastroenterology unit of the largest hospital in the island were included and compared with a population-based sample. A food frequency questionnaire previously used in a study of colorectal cancer was used. The study group included 101 cases and 242 controls.

The statistical analysis of the results has been completed (Benito *et al.*, 1993). A protective effect was found in relation to the consumption of fresh vegetables. Risk estimates for increasing quartiles of consumption were ORs = 1.00, 0.30, 0.37, 0.26 (p for trend < 0.01). The risk was increased for consumption of sugar, ORs = 1.00, 1.44, 1.76, 1.82 (p for trend > 0.05) and pastry, ORs = 1.00, 1.37, 2.40, 2.45 (p for trend < 0.05). Sedentariness and urban residence were identified as non-dietary risk factors for colorectal polyps.

The field part of the family study on diet and colorectal cancer, with 149 cases and 317 sibs of cases used as control group has been completed. The initial results indicate a high risk for increasing consumption of meat and cereals. Consumption of fresh fruits was moderately protective. These results confirm the findings of the population-based case–control study which included 286 cases and 295 controls. The methodological aspects of case–control studies using sibs as controls are being evaluated.

The family project is now being expanded to include screening for colorectal lesions among the members of the families at high risk for colorectal cancer. The screening will include testing for occult faecal blood and endoscopy, with field work starting during the second half of 1993. Samples of colorectal tissue (normal, polyps and carcinomas) and serum will be stored for studies on genetic changes related to carcinogenesis.

2.3.8 Screening through monitoring the effects of macrocomponents of the human diet in producing DNA-damaging agents and DNA adduction

(I.K. O'Neill and A. Ellul; in collaboration with J. Cummings and S.A. Bingham, Cambridge, UK. Supported by the US National Institutes of Health (CA-39417))

This project has the goal of developing a short-term screening procedure for dietary modulators of cancer risk in humans. A procedure for gastrointestinal monitoring with magnetic microcapsules, a set of human diets differing in certain postulated cancer risk factors, and methods for isolation of exfoliated colonic cells have been developed and tested in resident volunteers. The repeatedly observed effects on human colorectal cancer incidence of increased dietary intakes of beef protein and calories or fat, and the beneficial effects of "dietary fibre" polysaccharides, anti-oxidants, aspirin and as-yet unidentified components of fresh vegetables and fruit, formed the basis for a model set of intakes against which to discriminate other modulators of risk. The present work has revealed large biological effects of these ubiquitous components of the diets in developed countries, as summarized in Table 8. A mechanistic basis for effects of "dietary fibre" polysaccharides has emerged with the discovery that their short-chain fatty acid fermentation products have crucial roles in mucosal

regulation; our results show that these polysaccharides also exert an influence on carcinogen metabolism in the colonic mucosa.

Table 8. Comparison of effects in rodents of dietary components and gut microflora on end-points relevant for carcinogenesis measured in the gastrointestinal tract and in the liver[a]

	Independent increase of			Human gut microflora (presence of)
	Fibre NSP 2.5 to 6.4% w/w	Beef 2.7 to 8.4% w/w	Fat	
In microcapsules				
Exogenous carcinogen entrapment[b,c]	↓	↑*	NE	n.d.
Endogenous cross-linking[d,f]	↑↑	↑	↑	n.d.
Enzyme activities of caecal contents[f]				
Nitrate reductase[d]	↓	↑↑**	NE	↑
β-Glucuronidase[d]	↑	NE	↑↑**	↑*
IQ → 7-hydroxy IQ[e]	↑↑**	NE	↑↑*	↑
Hepatic effects[f]				
Endogenous DNA adducts[d]	↑	NE	↑↑*	↑↑**
Microsomal activation of IQ[d]	↑*	NE	↑↑**	↓*
Colonic mucosal effects induced by BaP				
% micronuclei[c]	↓↓*	NE	↓	n.d.
% mitosis[c]	↓↓*	NE	NE	n.d.

[a] Quantified by comparing the effects on each end-point of high versus low intakes of each dietary component in a human diet within normal intake range: result ↑ increased 1.5 to 2.0-fold, ↑↑ increased > 2.0-fold, ↓ decreased to 0.7 to 0.5 and ↓↓ decreased to < 0.5 of value for low intake; n.d. not determined, NE no effect

[b] O'Neill *et al.* (1990), [c] O'Neill *et al.* (1991), [d] Rumney *et al.* (1993b), [e] Rumney *et al.* (1993a)

[f] In rats associated with human microflora

* $p < 0.05$; ** $p < 0.01$ high versus low intake. Human diets were prepared so as to give identical intakes of calories, total protein, calcium, resistant starch and to be adequate for humans for all required macro- and micro-nutrients. Results obtained in male F344 rats after dietary adaptation of separate dietary groups, except for the colonic mucosal end-points in C57/B6 male mice. Comparisons reveal the independent effect of increased consumption of each dietary component.

2.3.9 Modulation of colorectal exposure to protein pyrolysates by human dietary components

(I.K. O'Neill, A. Ellul and C. Malaveille; in collaboration with J. Cummings and S.A. Bingham, Cambridge, UK; R. Turesky and G. Gross, Lausanne, Switzerland; and I. Rowland and C. Rumney, Carshalton, UK. Supported by the US National Institutes of Health (CA-39417))

Magnetic polyethyleneimine microcapsules trap 2-amino-3-methylimidazo-[4,5-*f*]-quinoline (IQ) and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and their metabolites when passing through the colorectal lumen (O'Neill *et al.*, 1992). A switch from putative low- to high-risk human diets (increased fat and beef protein, decreased "fibre" non-starch polysaccharide, NSP) in F344 rats with ambient microflora both increased the gastrointestinal concentration of covalent-binding IQ metabolites and also the systemic exposure to IQ metabolites. Human gut microflora forming the caecal contents of rats consuming a human diet were shown to convert IQ to 7-hydroxy-IQ (assumed to be a detoxification route) much more effectively when the human diet included a high intake of fibre NSP (Rumney *et al.*, 1993a).

Six volunteers were given a series of three diets similar to those consumed by the rodents and differing systematically in the intake of "fibre" NSP (3-fold increase) and fried beef (5-fold increase). Each diet was consumed for a three-week period. Microcapsules modified to include a copper porphyrin moiety for selective trapping (Povey & O'Neill, 1990) were passed through the GI tract and are currently being assayed for PhIP entrapment. The volunteers' phenotypes for *N*-acetylation and *N*-oxidation were assessed using urinary excretion of caffeine metabolites, and both an increase of NSP and a decrease of beef protein were found to significantly induce *N*-oxidation in the liver.

2.3.10 Dietary modulations of cross-linking and reactive oxygen species, and of nitroso compounds in the GI cavity

(I.K. O'Neill, A. Ellul, B. Pignatelli and P. Thuillier; in collaboration with J. Cummings and S.A. Bingham, Cambridge, UK. Supported by the US National Institutes of Health (CA-39417))

Following an earlier finding, through use of $^{14}CH_3$-labelled microcapsules, of cross-linking agents in the rodent gastrointestinal tract (Ellul *et al.*, 1990), such agents were also found in six free-living volunteers (Bingham *et al.*, 1992). A three-fold increase of fibre NSP intake increased both the extent of cross-linking and label loss in microcapsules. However, an inverse correlation was found between cross-linking and faecal weight; with the controlled dietary intake, cross-linking appeared to correlate with starch intake. Anaerobic fermentation of faeces produced both effects on microcapsules *in vitro* (Bingham *et al.*, 1992).

A ten-fold increase of meat protein increased several-fold the excretion of total nitroso compounds, thermo/acid-labile compounds, and ammonia (Bingham *et al.*, 1993). Increased intake of fibre NSP did not alter these meat protein effects. These results are consistent with protein enhancing gastrointestinal nitrosation, as was proposed by Mallett *et al.* (1988) on the basis of *N*-nitrosoproline excretion by rodents.

2.3.11 Development of recoverable microcapsules containing mammalian genes

(I.K. O'Neill, A. Ellul and A. Loktionov; in collaboration with J. Cummings and S.A. Bingham, Cambridge, UK; and R. Neufeld, D. Poncelet, D. Quong and T. Alexakis, Montreal, Canada. Supported by the US National Institutes of Health (CA-39417))

We have previously established that magnetically recoverable microcapsules can trap reactive metabolites of radio-labelled carcinogens in the GI tract (O'Neill *et al.*, 1993a; O'Neill 1993), and the system has been adapted for studying diet-induced chemopreventive changes in exposure to endogenous agents. Gentle conditions have been developed for encapsulation of DNA in a form permitting its gastrointestinal transport (Alexakis, 1992) in an alginate matrix encased in a semi-permeable membrane of polylysine. Using calf thymus DNA, they were shown to entrap a direct-acting carcinogen *in vitro* and benzo[*a*]pyrene metabolites *in vivo*, with significantly higher trapping compared to the same microcapsules without DNA. Using mouse liver DNA, the DNA could be recovered and the K-*ras* oncogene amplified. A polymerase chain-reaction-based method for conversion of adducts to *ras* mutations is being developed, with the goal of detecting one mutation at codons 12/13 in 10^6 DNA molecules.

2.3.12 Development of recoverable microcapsules containing a deoxyguanosine-type target for structural assays of endogenous genotoxins

(I.K. O'Neill and A. Ellul; in collaboration with J. Cummings and S.A. Bingham, Cambridge, UK; and O. Ridgway, Doncaster, UK. Supported by the US National Institutes of Health (CA-39417))

A pseudo-deoxyguanosine target substance was synthesized and tested for covalent entrapment at its guanine reaction sites. This substance contains an anchoring function to attach it to amine groups inside poly(hexamethylene terephthalamide)-polyethyleneimine microcapsules, together with a tetrol chain that permits subsequent gentle, specific cleavage to produce an aldehyde group attached to the guanine adducts. Postlabelling of this aldehyde with sodium boro[^{3}H]hydride allows detection of a wide variety of adducts from exposure *in vivo*. Substantially greater adduct levels were found in rats consuming the putative high-risk human diet (high fat and beef protein, low NSP) compared to the corresponding low-risk diet (O'Neill *et al.*, 1993b).

2.3.13 Characterization of Epstein–Barr virus-inducing substances from foodstuffs associated with high risk of development of nasopharyngeal carcinoma

(M. Hergenhahn, G. Bouvier and H. Bartsch; in collaboration with G.B. de-Thé, Paris, France; and G.W. Bornkamm and A. Polack, Munich, Germany)

The high incidence of nasopharyngeal carcinoma (NPC) in China, and its intermediate and moderate incidences in Greenland and the Maghreb, respectively, are linked to Epstein–Barr virus infection, genetic factors and exposure to environmental factors. Endogenous formation of nitroso compounds has been found to be higher in a Chinese population at high risk than in a low-risk population (Zeng *et al.*, 1993), but levels of nitrosamines and their precursors in foodstuffs epidemiologically associated with a high risk, such as salted dried fish (Chinese-style), were not directly related to the magnitude of the risk. Following our measurement of EBV-inducing activity (i.e., induction of viral replication in cells where the virus is latent) in foodstuffs (Shao *et al.*, 1988), two further short-term assay systems for tumour promoters and EBV inducers have been established, measuring: (*a*) induction of the virally encoded DR promoter in Raji DR-CAT cells, as a surrogate measure of EB virus induction, and (*b*) induction of the oxygen burst in human granulocytes. With the help of these assays, the EBV-inducing factors in a spice mixture from Tunisia, *harissa*, were identified as lignin fractions derived from plant cell walls. Their biological effect was also higher than that of the established EBV inducer and animal tumour promoter 12-*O*-tetradecanoylphorbol 13-acetate (TPA). In addition, acids, red (retinoid) pigments and salt present in the spice can inhibit induction in both systems; such an inhibitory effect was also shown by vitamin C and curcumin (the main flavour ingredient of curry) (Bouvier *et al.*, 1993). Thus, plant lignin fractions, previously thought biologically inert, can have profound effects after activation which might be achieved by cooking or gastrointestinal digestion. The mechanism of action of the lignin fractions in DR-CAT Raji cells and methods for quantitating active lignin fractions in foods require fuller investigation.

2.3 IARC staff publications

Benito, E., Stiggelbout, A., Bosch, F.X., Obrador, A., Kaldor J., Mulet, M. & Muñoz, N. (1991) Nutritional factors in colorectal cancer risk: a case-control study in Majorca. *Int. J. Cancer*, **49**, 161–167

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Bingham, S., Ellul, A., Cummings, J.H. & O'Neill, I. (1992) Novel detection by magnetic microcapsules in the human gastrointestinal tract of cross-linking agents and diet-dependent reactive oxygen species. *Carcinogenesis*, **13**, 683–690

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2.4 *Tobacco and cancer*

Tobacco use in various forms causes by far the most cases of cancer among all identified carcinogens. Research at IARC is little concerned with establishing the risk to smokers of their habit, which is taken as proven, but is examining the effect of smokeing on non-smokers (passive smoking) and the risks of tobacco chewing and snuff-taking. Biochemical studies are being conducted on the carcinogenic compounds responsible for tobacco's effects and on the DNA adducts formed. A methodological study of the effect of smoking on excretion of DNA alkylation adducts is described in Section 5.1.2.1.

Individual susceptibility to the effects of tobacco smoke is being studied in terms of the levels of enzymes that metabolize the substances absorbed by the body into active carcinogens. This susceptibility appears to have a genetic basis.

2.4.1 Case–control study of lung cancer and environmental tobacco smoke in non-smokers

(R. Saracci, P. Boffetta, R. Winkelmann and E. Riboli; in collaboration with W. Ahrens, Bremen, Germany, S. Benhamou, Villejuif, France; S.C. Darby, Oxford, UK; F. Forastiere, Rome, Italy; C.A. González, Barcelona, Spain; A. Hirsch and J. Trédaniel, Paris, France; S.K. Jindal, Chandigarh, India; A. Mendes, Lisbon, Portugal; F. Merletti, Turin, Italy; G. Pershagen, Stockholm, Sweden; L. Simonato, Padua, Italy; D. Trichopoulos, Athens, Greece and Boston, MA, USA; G. Vutuc, Vienna, Austria; H. Wichmann, Munich, Germany; and C. Winck, Porto, Portugal)

An international collaborative case–control study initiated in 1988 is investigating the relationship between exposure to environmental tobacco smoke (ETS) and to other environmental and occupational risk factors and the risk of lung cancer in subjects who have never smoked tobacco. A common questionnaire on exposure to ETS was adopted as well as a common basic protocol. About 500 cases and 1000 controls will be studied in 12 centres in Europe (within the frame of the project "Europass", supported by the EC) and India. Information on exposure to occupational carcinogens, urban air pollution, background radiation and dietary habits, as well as lifelong exposure to ETS, is being collected by personal interview of cases and controls. Self-reported (non-)smoking status is cross-checked by interview of spouses, and cotinine levels in urine, in a subsample of subjects. Data collection will be completed in 1993, and the analysis will take place in 1994. In some centres, active smokers are also enrolled in the study, and a common analysis aimed at evaluating the interactions between tobacco smoking and occupational exposures will be conducted.

2.4.2 Genetic susceptibility to lung cancer and environmental tobacco smoke

(P. Boffetta, M. Lang, M. Friesen, J. Hall and R. Saracci; in collaboration with W. Ahrens, Bremen, Germany; S. Benhamou, Villejuif, France; F. Forastiere, Rome, Italy; K. Hemminki, Stockholm, Sweden; A. Mendes, Lisbon, Portugal; F. Merletti, Turin, Italy; L. Simonato, Padua, Italy; C. Winck, Porto, Portugal; and H. Wichmann, Munich, Germany)

Among lung cancer cases, non-smokers have been exposed on average to lower levels of carcinogens than smokers: genetic susceptibility to lung cancer is therefore likely to play a bigger role in the former group of cases.

In some of the centres participating in the case–control study on lung cancer and environmental tobacco smoke (ETS), blood samples are being collected from non-smoking lung cancer cases, smoking lung cancer cases and non-smoking control subjects, in order to evaluate the genetic polymorphism to some enzymes implicated in the metabolism of lung carcinogens and therefore in determining individual susceptibility to cancer, such as CYP1A1 and 2D6 and glutathione *S*-transferase M1.

2.4.3 Smoking, drinking and drug use among French adolescents

(A.J. Sasco; in collaboration with D. Grizeau, Vanves, France; and G. Freyer and M. Jambon, Lyon, France)

Since 1985, several large-scale surveys have been repeatedly carried out in a representative sample of students (aged 11 to 20) from high schools and colleges in Lyon and the surrounding area, using anonymous self-administered questionnaires. Analysis of the data showed a high prevalence of smokers which increases consistently with age. However, a decrease in smoking prevalence has been observed over time among French adolescents on both local and national scales (Sasco *et al.*, 1991; Sasco, 1993a). A detailed analysis of determinants of smoking behaviour demonstrated the overwhelming influence of peer behaviour (Sasco *et al.*, 1993). Some of these schools were divided into two similar groups, one receiving a health education campaign according to a specified schedule and the other not, but no effect of the health education programme was detected (Sasco, 1992; Sasco & Pobel, 1992).

In 1990, collaboration was established with the Comité français d'éducation pour la santé to study in depth the smoking habits of the French population, both adolescents and adults, on a national scale. Some decrease in smoking rates was noted among men, but prevalence of smoking is still increasing in women (Sasco, 1993b,c). The association of smoking with social class has been evaluated. No decrease in lung cancer has yet been seen (Sasco, 1993d).

2.4.4 Evaluation of the efficacy of various anti-smoking strategies

(A.J. Sasco; in collaboration with J.C. Cêtre, C. Ducos-Mieral, J. Fabry, C. Gindre and B. Laumon, Lyon, France)

At the request of the "Conseil Général du Rhône", IARC is evaluating three large anti-smoking programmes at present being conducted in the Rhône 'département'. Their target populations are respectively: children (aged 9–10), medical doctors and workers in specific factories and work-places in both the public and private sectors (railways, marketing, public administration, asbestos workers, etc.). Preliminary results show that the programme has had a positive impact in the workplace with a reduction in exposure to passive smoking.

2.4.5 Anti-smoking legislation in the EC countries

(A.J. Sasco; in collaboration with P. Dalla-Vorgia, Athens, Greece; and D. Trichopoulos, Boston, USA)

Legislation is an integral part of any national programme against smoking. At the request of the European Commission, a comprehensive survey of existing anti-smoking legislation in the EEC countries was completed (Sasco *et al.*, 1992).

2.4.6 Tobacco use in Africa

(A.J. Sasco and D.M. Parkin)

Although estimates have been produced on lung cancer incidence attributable to tobacco use worldwide (Parkin & Sasco, 1993), little is known for some parts of the developing world. A study is therefore being set up to evaluate the possibility of conducting standardized tobacco surveys in ten African countries and possibly setting up a multicentric case–control study of lung cancer.

2.4.7 Cohort study of tobacco use and mortality in Bombay, India

(D.M. Parkin, A. Sasco and R. Sankaranarayanan; in collaboration with R. Peto, Oxford, UK; P.-C. Gupta, Bombay, India; and A. Lopez, Geneva, Switzerland).

Current estimates of tobacco-attributable mortality in India and other developing countries are mostly speculative. This study aims to assess tobacco cause-specific mortality and incidence of cancer, in relation to use of tobacco, in a cohort of 100 000 subjects aged >35 years in Bombay, India. Enrolment into the cohort started in February 1991 and about 50 000 subjects have so far been interviewed. The list of voters is the main sampling frame. A preliminary analysis of habits in 25 000 subjects revealed that 58.8% are current chewers, 6.7% smokers and 4.9% are both smokers and chewers. Methodological issues related to recruitment, migration, repeatability, follow-up and death ascertainment are being addressed. Cause-specific mortality and the incidence of cancers in this cohort will be assessed based on the data from the municipal death registration systems and the Bombay Cancer Registry. Relative risks using the person-years method will be computed for the different forms of tobacco use for specific cancers and for various categories of cause of death.

2.4.8 Pulmonary carcinogen–DNA adducts, cytochrome P450 enzymes, smoking and occupational exposure in lung cancer patients in Finland

(H. Bartsch, M. Castegnaro, C. Malaveille, K. Alexandrov, A.-M. Camus, A. Schouft, M. Rojas, G. Brun and H. Vainio; in collaboration with E. Hietanen, A. Karjalainen and S. Anttila, Helsinki, Finland)

Asbestos fibre content and DNA adduct levels are being measured in surgical lung tissue samples collected from 91 patients with or without malignant lung disease. Blood and urine samples have been collected from the same individuals, and detailed smoking and occupational histories are being taken through personal interviews.

The main polycyclic aromatic hydrocarbon-inducible cytochrome P450 was studied in lung tissue from 57 lung cancer patients by immunohistochemistry, using a monoclonal antibody that recognizes CYP1A1 and CYP1A2 isozymes. No immunostaining was observed in peripheral lung tissue of non-smokers or ex-smokers, but was seen in the bronchiolar and alveolar epithelium of all patients who were smokers and had a peripheral carcinoma (16/16) and of 60% (10/17) of those who had a bronchial carcinoma. The pulmonary activity of a CYP1A1-dependent enzyme, aryl hydrocarbon hydroxylase (AHH), was positively related to the intensity of immunostaining, and an almost two-fold increase due to smoking was detected in the ratio of caffeine metabolites, a marker of CYP1A2 activity. These results

demonstrate that tobacco smoke induces CYP1A1 in the lung and probably CYP1A2 in the liver, and suggest a role for certain metabolic phenotypes of CYP1A1 in peripheral pulmonary carcinoma (Anttila *et al.*, 1992).

Initial results (Castegnaro *et al.*, 1992), as in the previous study on Italian patients (Geneste *et al.*, 1991), show significantly higher bulky DNA adduct levels in smokers than in ex-smokers and non-smokers. In the ex-smokers, six months after cessation of smoking, the adduct levels had nearly returned to background levels. In 23 patients not exposed to asbestos, there was a correlation between DNA adduct levels and AHH activity. The less steep slope of the regression line ($y = 4.50x + 4.25$) compared to that of the Italian group ($y = 10.51x + 3.22$) may be related to the use of different types of tobacco. The Finnish smoke more light blond (flue-cured) tobacco that contains less tar than black tobacco and thus smoking may produce less aromatic-type carcinogen–DNA adducts in the lung.

An improved high-performance liquid chromatography/fluorometric assay has been established to quantitate benzo[*a*]pyrene (BP) tetrols released after acid hydrolysis of lung DNA from lung cancer patients, as a measure of levels of DNA adducts with BP diol-epoxide (BPDE)–DNA adducts. This assay has a detection limit of 2 pg of *r*-7,*c*-10,*t*-8,*t*-9-tetrahydroxy-7,8,9,10-tetrahydro-BP, requires 100–500 μg of DNA, and can measure 1 adduct per 10^8 unmodified nucleotides, allowing a >90% recovery of BP diol-epoxide–DNA adducts. With DNA samples from non-tumorous lung parenchyma taken from lung cancer patients at surgery, it revealed the presence of DNA adducts of the *anti*-BPDE isomer in 9 of 11 samples from smokers and in both of two ex-smokers, and adducts of the *syn*-isomer in only two samples from smokers. A 15-fold variation in DNA adduct levels was found DNA samples, with a range of 0.6–9.9 adducts of BPDE per 10^8 nucleotides. A highly significant correlation was found between pulmonary microsomal AHH activity and the level of BPDE–DNA adducts. A crude linear correlation between the amounts of these adducts and those of bulky DNA adducts determined by ^{32}P-postlabelling assay was observed in the same samples. Thus this highly sensitive and specific procedure is suitable for measuring BPDE–DNA adducts in human tissues from environmentally exposed subjects and could be adapted to polycyclic aromatic hydrocarbons other than BP.

2.4.9 Efficiency of DNA adducts formed in bacteria from PhIP and 4-aminobiphenyl to produce frameshift mutations

(C. Malaveille, M. Friesen, A. Hautefeuille, L. Garren and H. Bartsch; in collaboration with D.-X. Lin and F. Kadlubar, Jefferson, AR, USA)

Primary aromatic amines and nitrogen-containing heterocyclic amines occur in cigarette smoke (at nanogram/cigarette levels) from black or blond tobacco (Bartsch *et al.*, 1993). We have studied whether the higher mutagenicity of heterocyclic amine food mutagens such as 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), as compared to aromatic amines such as 4-aminobiphenyl (ABP), is attributable to higher mutagenic efficiency of their DNA adducts (Friesen *et al.*, 1992).

PhIP and ABP were incubated with *S. typhimurium* YG1024 in the presence of a metabolic activation system to measure revertant yields, survivors and DNA adduction. The results indicate that the higher mutagenicity of PhIP in bacteria is due to an about 300-fold higher efficiency of its DNA adducts to produce frameshift mutations. If the mutagenic efficiency is similar in mammalian cells, even low levels of PhIP–DNA adducts may be of health significance to humans.

2.4.10 Investigation of substances in human urine strongly inhibiting bacterial mutagenicity of the tobacco-derived mutagen 2-amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine (PhIP) and related heterocyclic amines

(C. Malaveille, A. Hautefeuille, G. Brun and H. Bartsch; in collaboration with P. Vineis, Turin, Italy; and G. Talaska, Cincinnati, OH, USA)

^{32}P-Postlabelling analysis has implicated PhIP as a major DNA-damaging agent present in the urine of smokers of black tobacco (Peluso *et al.*, 1991). When the mutagenic activity of PhIP was measured in *S. typhimurium* TA98 strain under the conditions (liquid incubation assay) used for assaying urinary extracts, substances strongly inhibiting PhIP mutagenicity were detected in urine from both non-smokers and smokers (Malaveille *et al.*, 1992). The percentage inhibition by 5 µl of extract (corresponding to 2.2 ml of 24 h urine) per assay was similar with urine extracts from 10 smokers (mean ± SD: 91.9 ± 4.8) and 10 non-smokers (mean ± SD: 87.6 ± 5.1). The mutagenicity of related amino-imidazoazaarenes, IQ, MeIQ and MeIQx, which are present in urine of smokers and subjects eating fried meat was also strongly inhibited by urine extracts. Similar inhibition experiments were carried out to obtain mechanistic data, with 2-aminoanthracene, 2-nitrofluorene, 2-acetylaminofluorene and 4-nitroquinoline *N*-oxide.

As extracts were prepared from urine of subjects who were coffee drinkers, they could contain caffeine and paraxanthine. These are substrates for CYP1A2, and caffeine inhibits the mutagenicity in *Salmonella* of various heterocyclic amines. However, the urinary concentrations of caffeine and paraxanthine were far too low to account for the inhibitory effect of the urine extracts on PhIP mutagenicity. Unsaturated fatty acids also are inhibitors of the mutagenicity of heterocyclic amines and of various carcinogens, but the levels of even the most abundant (linoleic and oleic acids) were too low to contribute to the inhibitory effect of the urine extracts. Overall, the results suggest that the substances may act through their capacity to non-covalently bind the mutagen or its metabolites and/or their anti-oxidative effects, by reduction of electrophilic species.

An analysis is in progress to see whether these anti-mutagenic substances can reduce the levels of DNA adducts in exfoliated urinary bladder cells (isolated from urine) of smokers, arising from exposure to aromatic and heterocyclic amines.

2.4 IARC staff publications

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2.5 *Urinary tract cancer in relation to Balkan endemic nephropathy and exposure to ochratoxin A and other mycotoxins*

Balkan endemic nephropathy is a non-inflammatory bilateral kidney disease that affects rural populations in certain areas of south-eastern Europe, and has recently been associated with exposure to nephrotoxic mycotoxins. An extremely high incidence of urinary tract tumours in the endemic area suggests a common causative agent, the principal candidate proposed being ochratoxin A. This agent has been classified in IARC Monographs Volume 56 as possibly carcinogenic to humans (see section 2.1.1.3).

2.5.1 Epidemiology of urinary tract tumours in Bulgaria

(M. Castegnaro, J. Estève and H. Bartsch; in collaboration with I.N. Chernozemsky, I. Nikolov and T. Petkova-Bocharova, Sofia, Bulgaria)

This study is a follow-up of a similar one covering the period 1965–74 (Chernozemsky *et al.*, 1977).

Data from 117 villages and towns of the Vratza region have been examined. Altogether 761 urinary tract tumour cases with proven histology were identified and another 121 cases with unspecified kidney tumours are being further analysed.

Eighteen of the villages show a very high incidence of urinary tract tumours, 13 towns or villages show high incidence, while the remaining 86 towns or villages show incidence similar to that in the rest of Bulgaria. Some villages with very high incidence appear newly in the list.

The time trend over the entire period studied (1965–91) is now being examined.

2.5.2 Human biomonitoring for ochratoxin A exposure

(M. Castegnaro and H. Bartsch; in collaboration with E. Creppy, Bordeaux, France; G. Dirheimer, Strasbourg, France; and J.H. Olsen and B. Hald, Copenhagen, Denmark)

The prevalence of ochratoxin A (OA) in blood has been monitored in three areas of France. In the Lyon area, OA was found more often in rural areas (19.4% positive samples, 0.1–4.3 ng/ml) than in small urban areas (13.3% positive samples, 0.2–1.7 ng/ml) or the Lyon urban community (6.1% positive, 0.1–0.7 ng/ml). Similarly, in the Alsace region, there were 2.4 times more positive samples in rural than in urban areas (33.8% versus 14%) and the highest levels are found in the rural areas (2.6% versus 0% above 2 ng/ml; maximum 11.8 ng/ml). Results from the Aquitaine region were less clear-cut. For samples with confirmed origin, the same distribution of contamination was obtained, with 22% of rural blood samples positive (10.4% above 2 ng/ml) versus 17.8% in urban areas (4.7% above 2 ng/ml). However, in another series of samples from this region, contamination levels were similar in rural and urban populations (18.4% versus 18.9%). Further work is required to confirm the origins of these samples. The data confirm previous findings that the prevalence of OA contamination in blood is much lower in France than in Germany (maximum 38% in northern France and 22% in southern France as against 50–65% in Germany), and is the lowest among all the European countries where similar surveys were done.

A study has been initiated in Denmark to compare the geographical pattern of urinary tract cancer incidence (kidney and/or unspecified other urinary tract cancers) with that of epidemic porcine nephropathy.

A meeting has been organized on human ochratoxicosis and related pathologies (July 1993) and the proceedings published (Creppy *et al.*, 1993).

2.5.3 Mechanism of action of ochratoxin A

(M. Castegnaro, J. Michelon and H. Bartsch; in collaboration with M. Goldberg, Guelph, Canada; R. Schulte-Hermann, W. Huber and W. Bursch, Vienna, Austria; U. Mohr, Hannover, Germany; G. Dirheimer and A. Pfohl-Leszkowicz, Strasbourg, France; and E. Creppy, Bordeaux, France)

Levels of nuclear aberrations in exfoliated urinary cells of patients with Balkan endemic nephropathy or urinary tract tumours in Bulgaria and of controls have been measured. The average percentage of nuclear aberrations in patients (5.04%) was not significantly higher than in the controls (4.04%). However, both of these values were much higher than those usually found in control subjects ($\simeq$ 1.5%) or in cyclophosphamide-treated patients (2.63%).

Experiments in which OA was fed to rats as either a single oral administration or three times a week for two years revealed no evidence of oxidative organ damage (histochemical and biochemical determination of aldehydes) or cell death (histological examinations), and there was no statistical difference in peroxisome proliferation level between control and OA-treated animals.

Several DNA adducts have been detected in urinary tract tumours from Bulgarian subjects who may have been exposed to OA, that appear to be related to DNA adducts found in OA-treated mouse kidney. No adducts were found in kidneys from disease-free patients of French origin. Work is in progress to confirm these findings in non-tumorous tissues of patients with known exposure history to OA and in other tumorous tissues.

A two-year rodent experiment has now been completed. OA metabolic ratios in urine have been measured and analysis of kidney function (in Bordeaux) and of pathology (in Hannover) is in progress. First results confirm that (*a*) OA is carcinogenic to the kidney in DA and Lewis rats; (*b*) males are more susceptible to OA carcinogenicity than females in both strains; and (*c*) 2-mercaptoethanesulfonic acid sodium salt (Mesna) did not reduce tumour incidence in either strain.

2.5.4 Genetic polymorphism and Balkan endemic nephropathy

(M. Castegnaro, M. Lang, J.C. Bériziat, O. Geneste and J. Michelon; in collaboration with I.N. Chernozemsky and I. Nikolov, Sofia, Bulgaria; and C.R. Wolf, Edinburgh, UK)

In order to check the correspondence between debrisoquine metabolic phenotype and genotype, blood was collected from subjects living in an area of high incidence of Balkan endemic nephropathy (BEN) and urinary tract tumours in Bulgaria, most of whom have been phenotyped in a previous study (Nikolov *et al.*, 1991). No correlation was seen between debrisoquine genotype prevalence and BEN risk. The genetic analysis allowed us to separate the population into three groups: (*a*) homozygous poor metabolizers; (*b*) homozygous extensive metabolizers; and (*c*) heterozygous extensive metabolizers. While a good correlation between debrisoquine genotype and phenotype is usually found, in this analysis the genotype and phenotype failed to correlate in the heterozygous extensive metabolizers, 25% of whom corresponded to a poor metabolizer phenotype. Work is in progress to confirm and provide an explanation for this finding.

An *in vivo* study has been undertaken to determine the cytochrome P450 isozyme(s) involved in the metabolism of ochratoxin A in rats. No induction of 4-hydroxylation with pyrazole was found, which excludes CYP2E1 and 2A3.

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2.6 *Role of viruses in the etiology of human cancer*

The laboratory activities on viruses within the programme on viral and hereditary factors in carcinogenesis have been phased out during the period covered by this report. Those laboratory investigations linked to epidemiological studies were used to elucidate the role of viruses in the etiology of certain human cancers and to identify the molecular steps involved. The Agency has, since its origin, taken an active part in research on the role of the Epstein–Barr virus (EBV) in the etiology of Burkitt's lymphoma (BL) (a cancer that shows great geographical variation in incidence). A more recent development is the study of lymphomas occurring in immunocompromised individuals, particularly those infected with human immunodeficiency virus (HIV). The role of EBV in nasopharyngeal cancer is also being studied epidemiologically and in terms of substances in certain foodstuffs that have

EBV-inducing activity (section 2.3.13). Other viral agents that are the focus of studies are human papillomavirus, in relation to cervical cancers (section 2.12) and hepatitis B virus, in relation to liver cancer (section 2.11 and 4.1).

2.6.1 Collection of biological material related to Epstein–Barr virus and lymphomas

(G. Lenoir, C. Bonnardel, M. Vuillaume and S. Pauly)

As part of various Agency projects, we have collected, over some 20 years, a large and unique collection of sera, tumour material and cell lines (over 120 BL cell lines established in culture at the Agency, representing one of the largest collections of human tumour cell lines for a given cancer) which are used by institutions all over the world for studies of EBV, BL, nasopharyngeal carcinoma and B-cell neoplasia. On request, lymphoid cell lines, biopsies and sera are sent to institutions in various countries in the form of live cells, frozen cells and samples of DNA. It is intended to maintain this unique collection of material and to distribute samples when requested.

2.6.2 Studies on lymphomas occurring in AIDS patients

(H.J. Delecluse and G. Lenoir; in collaboration with M. Raphaël, Paris; cooperative programme supported by the Agence Nationale de Recherches sur le Sida, Paris)

EBV can cause lymphoproliferative diseases in individuals with immune dysfunction. Most such lymphoproliferations are polyclonal B-cell proliferations classified as diffuse lymphoma, but are not of the Burkitt's type. They are very rare in the general population, but are a frequent cause of death in children with genetically determined immunodeficiencies. They also occur at relatively high incidence in individuals treated with immunosuppressive therapy for organ transplantation. The implication of EBV in these lymphomas is based on detection of markers for the virus within the proliferating cells. The importance of alterations of immune function in their genesis is stressed by the fact that they may regress when the immunosuppressive therapy is reduced or withdrawn. A similar type of lymphoma occurs in AIDS patients with severely altered immune characteristics. Some individuals positive for human immunodeficiency virus (HIV) also develop true Burkitt-type lymphoma, with characteristic chromosomal translocations, but in some cases, no detectable EBV sequences in the malignant cells. This suggests that BL pathogenesis is not directly related to the presence of EBV, nor to the HIV-induced T-cell immunodeficiency.

We have examined 50 AIDS-associated lymphomas at the molecular level in order to better define their biological characteristics. BL was found to be frequent in HIV-infected individuals and these BL contained EBV sequences in about 50% of the cases, detected either by Southern blotting or by *in situ* hybridization. In the context of AIDS, some BL tumours adopt an immunoblastic phenotype, reflecting the morphological evolution of BL cells observed in culture. This switch in phenotype makes morphological diagnosis difficult (Delecluse *et al.*, 1993). The APO-1 antigen was found to be present in lymphomatous cells having a lymphoblastoid phenotype, suggesting that anti APO-1-mediated apoptosis might be considered in the treatment of AIDS lymphomas (Falk *et al.*, 1992).

2.6.3 Molecular aspects of EBV-induced B-cell immortalization and transformation

(M. Cordier-Bussat, G. Lenoir and J. David-Ameline; in collaboration with G. Bornkamm, Munich, Germany; E. Kieff, Boston, MA, USA; and R. Dalla-Favera, New York, USA)

We have completed our studies on the importance of EBV nuclear antigen-2 (EBNA2) and latent membrane protein (LMP) in malignant transformation. Through transfection experiments we were able to demonstrate that the capacity of EBNA2 to upregulate the expression of cell activation molecules, such as CD21 and CD23, requires a suitable cellular context, and that expression of LMP can be independent of EBNA2 expression (Cordier-Bussat *et al.*, 1993a,b).

Since EBV infection of Burkitt's lymphoma cells *in vitro* reproduces many of the activation effects of EBV infection of primary B lymphocytes, a set of our *in vitro* EBV-infected BL cell lines and their non-infected counterparts were used in the laboratory of Professor E. Kieff to identify induced mRNAs by subtractive hybridization. Nine genes were identified, including two novel ones. It was predicted from their cDNA sequences that they encode for G-protein-coupled peptide receptors. They may have important functions in EBV-induced B-cell proliferation (Birkenbach *et al.*, 1993).

Attempts have been made to identify the possible role of anti-oncogenes or tumour-suppressor genes in B-lymphoma genesis by analysis of our collection of Burkitt's lymphoma cells. Deletions involving two distinct regions of chromosome 6q were observed in more than 20% of a series of lymphomas (Gaidano *et al.*, 1992) and deletions identified by allelic losses in a significant proportion of lymphomas were also observed on the long arm of chromosome 17.

2.6.4 Case–control study of NPC in relation to infection with Epstein–Barr virus and exposure to other agents in south-east Asia

(P. Pisani and D.M. Parkin; in collaboration with S. Sriamporn, Khon Kaen, Thailand; H.A. Pham, Hanoi, Viet Nam; and A. Laudico, Manila, Philippines)

A hospital-based case–control study on nasopharyngeal cancer (NPC) was conducted in north-eastern Thailand, a region characterized by incidence rates intermediate between very high observed rates in Chinese populations and the very low rates reported for most other communities. This showed that a high risk of NPC is associated with consumption of salted fish and with exposure to dusts and fumes among agricultural workers (Sriamporn *et al.*, 1992).

A multicentric population-based case–control study, in several countries of south-eastern Asia, to clarify the role of genetic susceptibility, diet, environmental exposures and EBV infection is being planned. Contacts have been established with collaborating centres in Thailand, Viet Nam and the Philippines to prepare the protocol. A pilot study in Viet Nam to assess usual dietary intake in order to design the questionnaire is planned.

2.6.5 Case–control studies of Kaposi's sarcoma, non-Hodgkin lymphoma and carcinoma of the cervix in Africa in relation to infection with HIV

(D.M. Parkin; in collaboration with V. Beral and R. Newton, Oxford, UK; P.-J. Ngilimana and B. Sindikubwabo, Butare, Rwanda; and L. Ngendahayo, Bujumbura, Burundi)

The first study was started in Butare, Rwanda, in 1992. All individuals suspected of having a tumour (malignant or benign) are interviewed with a specially tested questionnaire (Figure 10), and samples of blood taken for antibody studies. In the first phase of the study, the cancer cases of interest will be compared with controls made up of other cancers (unrelated to the agents being studied) and benign lesions. As the study proceeds, recruitment of non-cancer controls (hospital patients without malignancy and hospital visitors) will take place. The main

emphasis of the study is on Kaposi's sarcoma, non-Hodgkin lymphoma and cervix cancer, in relation to likely exposure to infectious diseases through sexual or faeco-oral spread.

In May 1993, agreement was reached to extend this study, using a similar protocol, to Bujumbura (Burundi) and to Kampala, Uganda. Data collection will begin in Burundi in autumn 1993.

2.6 IARC staff publications

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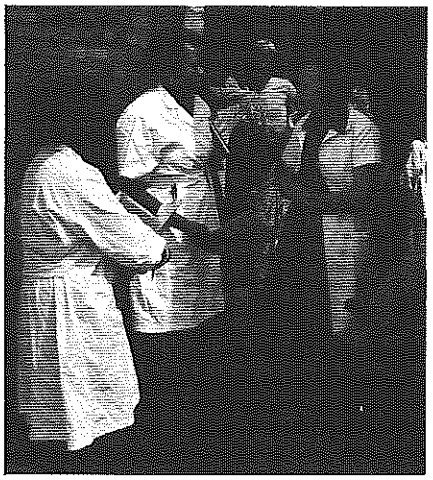

Figure 10. Recruitment of a child with Burkitt's lymphoma, interviewed at the mission hospital in Gakoma, Rwanda

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2.7 *Endogenously formed carcinogens in human cancer etiology*

Humans are exposed to preformed *N*-nitroso compounds (NOC), but also to a wide range of precursors and nitrosating agents which can react *in vivo* to form potentially carcinogenic NOC and diazo compounds. Nitrite, nitrate and nitrosating agents can also be synthesized endogenously in enzymatic reactions mediated by bacteria, activated macrophages and neutrophils. The latter two cell types generate, via the enzyme nitric oxide synthase, the nitrite oxide radical that is involved in cytotoxicity, and is believed to be involved in formation of carcinogenic nitrosamines, DNA base deamination and oxidative damage. Thus endogenous NOC formation, DNA damage and gene mutations in humans could occur at various sites of the body such as the stomach and chronically infected or inflamed organs. Sensitive procedures to estimate the exposure of humans to NOC have been developed and applied in ecological and cross-sectional studies.

2.7.1 Role of nitric oxide synthase in human carcinogenesis

Nitric oxide (NO) has recently been identified as a physiologically essential signalling molecule which mediates various cell functions, but may cause cytotoxic and mutagenic effects when present in excess. NO reacts rapidly with superoxide anion ($O_2^{\bullet -}$) to form peroxynitrite ($ONOO^-$), which may be itself tissue-toxic and may decompose to the highly reactive and toxic hydroxyl radical ($HO^{\bullet}$) and nitrogen dioxide ($NO_2^{\bullet}$).

2.7.1.1 *Purification, characterization and molecular cloning of nitric oxide synthase*

(H. Ohshima, I. Brouet and T. Bandaletova; in collaboration with H. Esumi, Tokyo, Japan)

NO is synthesized enzymatically from L-arginine by NO synthase (E.C.1.14.23). We have purified and characterized immunologically distinguishable constitutive and inducible forms of NO synthase from bovine brain and the liver of rats treated with *Propionibacterium acnes* and lipopolysaccharide (LPS), respectively (Ohshima *et al.*, 1992a,b,c; Oguchi *et al.*, 1992). Complementary DNA for the inducible form from rat liver has been cloned. Northern blot analysis showed that an mRNA species is strongly induced by treatment of rats with *P. acnes* and LPS, a single band at about 4.2 kb being detected 1 h after treatment with LPS and reaching a maximum level at 3 h.

2.7.1.2 *Immunohistochemical localization of an inducible form of nitric oxide synthase in organs of rats treated with* Propionibacterium acnes *and lipopolysaccharide*

(T. Bandaletova, I. Brouet and H. Ohshima)

Immunohistochemical localization of an endotoxin-inducible form of NO synthase was examined using rabbit polyclonal antibody against the enzyme purified from rat liver (Ohshima *et al.*, 1992b). In rats treated with *P. acnes* and LPS, immunostaining was observed in macrophages, occasional lymphocytes, neutrophils and eosinophils in red pulp of spleen, Kupffer cells, endothelial cells and hepatocytes in liver, alveolar macrophages in lung, macrophages and endothelial cells in adrenal glands, and histiocytes, eosinophils, mast cells and endothelial cells in colon. Immunoreactivity was also evident in histiocytes and endothelial cells in kidney; histiocytes and neutrophils in oesophagus; macrophages and eosinophils in duodenum; macrophages, some lymphocytes and mast cells in ileum; histiocytes in thymus; and endothelial cells in heart and aorta. Immunoreactivity was not detected in these organs from untreated rats. Positively staining cells in these rat organs appeared within 2.5 h after LPS administration; their number dramatically increased within the next 2.5 h, remained at high levels for a further 19 h, and then decreased over the following 24 h. The number of positive cells appearing correlated well with the NO synthase activity biochemically determined in the same organs (Bandaletova *et al.*, 1993).

2.7.1.3 *Increased endogenous N-nitrosamine and nitrate formation by induction of nitric oxide synthase in rats with acute hepatic injury caused by* Propionibacterium acnes *and lipopolysaccharide administration*
(Y. Wu, I. Brouet and H. Ohshima)

In rats treated with *P. acnes* followed five days later by LPS, acute hepatic cell necrosis was accompanied by significant induction of NO synthase activity in the liver. Levels of endogenous nitrosation of thiazolidine 4-carboxylic acid (TCA) administered 5 h after LPS injection to the *P. acnes*-treated rats by intravenous, intraperitoneal and oral routes were about 5-, 10- and 8-fold greater than in rats which had not been treated with *P. acnes* and LPS, but received TCA by the same route. Nitrate concentration in plasma and NO synthase activity in the liver, as well as levels of nitrite and nitrate in gastric contents, were increased significantly after LPS administration. The co-administration of *N*-nitro-L-arginine (an inhibitor of NO synthase) and LPS resulted in a marked reduction of urinary levels of nitrate and *N*-nitroso-TCA, indicating that nitrosation is mediated by NO synthase. These results together suggest that induction of NO synthase by infection with bacteria, parasites and viruses could result in increased endogenous nitrosation not only in the infected tissues, but also in the stomach, where nitrosamines would be formed more rapidly under acidic conditions (Wu *et al.*, 1993a).

2.7.1.4 *Nitrosamine formation and* in situ *DNA alkylation in inflamed intra-hepatic bile ducts of liver fluke-infested hamsters*
(H. Ohshima, T.Y. Bandaletova, I. Brouet, G. Kirby, F. Ogunbiyi, H. Bartsch, V. Vatanasapt and C.P. Wild)

A Syrian golden hamster model was utilized to test the hypothesis that increased endogenous nitrosation in human subjects infested with liver fluke *Opisthorchis viverrini* in northeast Thailand, where liver fluke has been associated with an increased risk of cholangiocarcinoma, is mediated by NO synthase induced by the infestation (Srivatanakul *et al.*, 1991). Hamsters experimentally infested with *O. viverrini* liver fluke excreted in the urine significantly greater amounts of nitrate, a stable oxidation product of NO, than untreated hamsters (3.64 ± 0.86 *vs* 2.64 ± 0.60 μmol/hamster/day, $p < 0.001$). When the rapidly nitrosatable TCA was administered orally, the infested hamsters also excreted significantly higher levels of *N*-nitroso-TCA than untreated hamsters (4.27 ± 2.20 *vs* 2.33 ± 1.13

nmol/hamster/day, $p < 0.01$), indicating that endogenous nitrosation is elevated in the animals with liver fluke. NO synthase activity measured in liver cytosol was also induced significantly in the infected hamsters. The inducible form of NO synthase was immunohistochemically localized in the cytoplasm of macrophages and eosinophils in the inflammation zone surrounding the parasite-containing bile ducts. Tissue (cell)-specific DNA damage caused by oxidation and/or deamination and DNA strand breaks in these liver samples is being compared with the NO synthase activity. In order to determine whether over-production of NO leads to nitrosation of aminopyrine to form *N*-nitrosodimethylamine, resulting in alkylation of DNA *in situ* in chronically inflamed tissues, O^6- and 7-methyldeoxyguanosine adducts in liver DNA are being analysed by HPLC followed by radioimmunoassay as well as immunohistochemically.

2.7.1.5 *The presence and role of NO synthase in human cancers*
(H. Ohshima, T.Y. Bandaletova, I. Brouet, S. Calmels-Rouffet and B. Pignatelli; in collaboration with a network of collaborating laboratories)

Surgical tumour specimens from various organs are being collected from various countries. Cancer sites selected are (i) those associated with parasitic, bacterial or viral infection, namely, *Opisthorchis viverrini* and liver cancer (P. Srivatanakul, Bangkok and S. Satarug, Khon Kaen, Thailand), schistosomiasis and bladder cancer (W. Anwar, Cairo, Egypt), *Schistosoma japonicum* infestation and colon cancer (J.S. Zhang, X. Zhong and Y.-T. Gao, Shanghai, China and C. Hsieh, Boston, USA) and *Helicobacter pylori* infection and stomach cancer, (ii) those associated with inflammation, namely, urinary tract tumours (I.N. Chernozemsky, Sofia, Bulgaria), alcoholic cirrhosis (B. Bancel, Lyon, France) and ulcerative colitis and colon cancer (J.F. Jeannin, Dijon, France) and (iii) organs in which presence of a constitutive form of NO synthase and NO toxicity has been reported, namely brain cancer (S. Preston-Martin, Los Angeles, USA) and pancreas cancer (P. Boyle, Milan, Italy). The activity of NO synthase and the expression of its mRNA are being compared in normal, precancerous and tumour parts of the specimens. Pilot studies have been initiated to correlate the presence and induction of NO synthase with precancerous conditions, characteristics of tumours (histological type, invasion, metastasis), prognosis and data on cell proliferation.

The first results indicate that patients with liver cirrhosis ($n = 15$) excreted significantly higher ($p < 0.05$) levels of nitrate (1.29±0.2 *vs* 1.08±0.24 mmol/l) and the sum of four major nitrosamino acids (18.4±12.0 *vs* 6.77±6.15 µg/l) than healthy control subjects ($n = 12$), suggesting that cirrhosis patients produce more NO than controls. The results are being confirmed by measuring NO synthase activity and expression of mRNA in liver samples from these patients.

2.7.2 Endogenously formed N-nitroso compounds in human cancer etiology

2.7.2.1 *Urinary excretion of nitrosamino acids and nitrate by inhabitants of high- and low-risk areas for nasopharyngeal carcinoma in southern China*
(H. Ohshima, G. Bouvier, I. Brouet, P. Roy and H. Bartsch; in collaboration with Y. Zeng, Beijing, China; and G. de Thé, Paris, France)

The hypothesis that endogenous synthesis of nitrosamines from dietary precursors is a risk factor for nasopharyngeal carcinoma (NPC) in China was tested by applying the nitrosoproline (NPRO) test to subjects living in high- and low-risk districts for NPC in Zangwu county, Guangxi region, in southern China. Samples of 12-h urine were collected from 77 subject: (*a*) before any treatment; (*b*) after ingestion of proline; and (*c*) after ingestion

of proline together with vitamin C. NPRO, other nitrosamino acids and nitrate were measured as indices of exposure to preformed and endogenously formed nitrosamines or their precursors. The NPRO level after proline intake was significantly increased in subjects from the high-risk area ($p = 0.012$) and markedly reduced after ingestion of ascorbic acid ($p = 0.007$), but this effect was not seen in subjects from the low-risk area. Levels of *N*-nitrosothiazolidine-4-carboxylic acid and the sum of nitrosamino acids in subjects in the high-risk area were significantly reduced by ascorbic acid ($p < 0.01$), but were not reduced in subjects from the low-risk area. The urinary nitrate level was about twice as high in subjects from the high-risk area and in all subjects was correlated with NPRO level. These results demonstrate a higher potential for endogenous nitrosation in subjects living in the high-risk area of NPC and suggest the occurrence of nitrosation inhibitors in the diet consumed in the low-risk area. Thus, in addition to infection with Epstein–Barr virus and genetic predisposing factors, dietary habits that may entail higher nitrosamine exposure appear to play a role in NPC etiology (Zeng *et al.*, 1993).

2.7.2.2 *Geographical association between urinary excretion of N-nitroso compounds and oesophageal cancer mortality in China*

(H. Ohshima, B. Pignatelli and H. Bartsch; in collaboration with Y. Wu and J. Chen, Beijing, China; T.C. Campbell, Ithaca, NY, USA; and J. Boreham and R. Peto, Oxford, UK)

Two overnight urine samples were collected from each of approximately 60 male adults in each of 69 counties of China, one after a loading dose of proline and ascorbic acid and another after a loading dose of proline only. Levels of *N*-nitrosamino acids and nitrate in the samples were examined in relation to cumulative mortality rates in the same counties for subjects aged between 0 and 64 years in the 1970s. Oesophageal cancer mortality rates were positively and significantly associated with (i) urinary levels of excreted *N*-nitrosoproline (NPRO) (after proline and ascorbic acid loading or proline loading only), (ii) *N*-nitrososarcosine levels, and (iii) nitrosation potential (the decrease in the amount of urinary NPRO or other *N*-nitrosamino acids and that of nitrate. The urinary excretion of nitrate was associated with consumption of various nitrate-rich vegetables. In the present study, a moderate correlation was found between mortality rates for oesophageal cancer in the 1970s and exposure to *N*-nitroso compounds (NOC). Such correlations have low power for assessing causality of an association, but are useful in generating hypotheses and designing future molecular epidemiological studies. When data on cancer mortality rates for 1987–89 become available, the correlation will be reanalysed. Further, multi-variable statistical analysis will be conducted using data from a recently completed cancer mortality survey with indices of NOC exposure, as well as nutritional and other parameters measured in the 1989 survey in the same populations (Wu *et al.*, 1993b).

2.7.2.3 In vivo *nitrosoproline formation and other risk factors in Costa Rican children from high- and low-risk areas for gastric cancer*

(H. Ohshima, B. Pignatelli, C. Malaveille, S. Teuchmann, N. Muñoz and H. Bartsch; in collaboration with R. Sierra and A. Chinnock, San José, Costa Rica)

The hypothesis that intragastric synthesis of NOC in early life plays a role in gastric carcinogenesis was tested by applying the NPRO test to about 50 children living in high- and low-risk areas for stomach cancer in Costa Rica. The median values of excretion in 12-h urine of NPRO and the sum of three nitrosamino acids were 10–20% of those in adults from other high-risk areas for stomach cancer. The urinary NPRO level after proline intake was higher in

children from the high-risk area ($p < 0.04$), and markedly reduced after ingestion of ascorbic acid together with proline ($p < 0.05$). NPRO levels on the day of proline intake were highly correlated with levels of nitrate excretion ($p < 0.001$). The results suggest that intragastrically formed NOC or other nitrite-derived carcinogens could be risk factors for stomach cancer (Sierra *et al.*, 1993).

Mean levels of total NOC in an aqueous extract (pH 2) of cooked beans from the high- and low-risk areas were similar. Acid-catalysed nitrosation of the extract increased the total NOC concentration up to 1000-fold, but there was no difference between samples from the two areas. About 10% of bean extracts from both areas showed weak direct-acting genotoxicity in *E. coli*; after acid-catalysed nitrosation, all samples were genotoxic at similar levels.

The diet of children in the low-risk area satisfied recommended levels of intake of energy and most nutrients except riboflavin and retinol equivalents. Diets from the high-risk area were deficient in energy intake and all nutrients except protein and vitamin C.

Blood samples from 276 children and young adults from the same areas showed no significant difference in the prevalence of serum IgG or IgA antibodies against *H. pylori* between the two regions (Sierra *et al.*, 1992).

2.7.3 Effect of gastric anti-acid secretion drugs on intragastric concentrations of bacteria, carcinogenic *N*-nitroso compounds and their precursors

(B. Pignatelli, P. Thuillier and H. Bartsch; in collaboration with E. Verdu, F. Viani, D. Armstrong, R. Fraser, H.H. Siegrist, A.L. Blum and M. Fried, Lausanne, Switzerland; and J.P. Idstrom and C. Cederberg, Mölndal, Sweden)

Decreased gastric acidity resulting from treatment with antacid drugs has been hypothesized to allow proliferation of nitrite-producing bacteria in the stomach, possibly leading to *in situ* formation of carcinogenic NOC. The effect of inhibition of acid secretion by omeprazole on gastric colonization by nitrate-reducing bacteria and intragastric NOC formation was examined. Fourteen healthy volunteers received a placebo for one week, followed by omeprazole (20 mg/person daily) for two weeks. Treatment with omeprazole led to a higher (median) gastric pH, predisposing to increased gastric bacterial proliferation including nitrate-reducing bacteria, but no consistent trend was observed for elevation of nitrite or total NOC concentrations. A similar study is being performed in patients with duodenal or gastric ulcer or reflux oesophagitis before and after treatment with omeprazole or other antacid drugs.

2.7.4 Levels of *N*-nitroso compounds, direct genotoxins and their precursors in gastric juice of patients with and without precancerous lesions of the stomach and living in areas with contrasting gastric cancer risk

(B. Pignatelli, C. Malaveille, A. Rogatko, N. Muñoz, A. Hautefeuille, P. Thuillier and H. Bartsch; in collaboration with A.T.R. Axon and G. Sobala, Leeds, UK; F. Berger, H. De Montclos, R. Lambert and B. Moulinier, Lyon, France; P. Correa, New Orleans, LA, USA; and B. Ruiz, Cali, Colombia. Supported in part by NIH grant No. CA 47591)

A study has been performed to examine whether elevated risk of gastric cancer is associated with high levels of total NOC, their precursors and nitrosation-dependent direct genotoxins in gastric juice. The subjects were 207 patients with stomach disorders living in three areas with up to eight-fold variations in gastric cancer risk in Colombia (Nariño), where the gastric cancer incidence is among the highest in the world, and in the UK and France, where risks are lower.

In agreement with earlier findings, an acidic gastric pH (< 4) was strongly and significantly associated with normal gastric mucosa or moderate gastritis. Among patients without gastric precancerous lesions, elevated gastric pH was seen much more frequently in Colombia than in the other countries. Colombian patients with precancerous lesions had the highest prevalence of elevated gastric pH. The nitrite concentrations in gastric juice were highest for Colombian patients with precancerous lesions and for subjects with a gastric pH > 4. In contrast, levels of total NOC did not differ between countries nor between gastric juice samples grouped according to histopathological diagnosis or according to a pH below or above 4. Total NOC levels increased with original nitrite concentrations at a greater rate in acidic juices than in those with pH > 4. The data together suggest that acid-catalysed nitrosation contributes at least as much as other nitrosation pathways to intragastric NOC formation.

In vitro nitrosation of gastric juice with excess sodium nitrite at pH 1.5 for 60 min at 37°C increased the concentration of total NOC up to several thousand-fold (maximum 1330 µmol/l). High NOC levels after *in vitro* nitrosation were not associated with higher gastric cancer risk and the NOC concentration in nitrosated gastric juice increased with the original pH only for French samples. These findings suggest that both the level and probably the nature of some of the substances in gastric juice are different between French and Colombian subjects. After acid-catalysed nitrosation, all samples from France and Colombia exhibited genotoxic activity that was more elevated if the original pH was above 4; the highest genotoxicity was seen in Colombian samples. Thus, patients from the area of highest gastric cancer risk had the highest levels of precursors of nitrosation-dependent genotoxins.

These results do not support the idea that total gastric NOC levels are elevated in subjects with precancerous stomach conditions or in those living in a high-risk area for stomach cancer, but are consistent with intragastrically formed nitrite-derived direct mutagens (nitrosamides or diazonium compounds) having a role in gastric cancer etiology (Pignatelli *et al.*, 1993 a,b). Other types of DNA damage may contribute to this risk, such as oxidative damage caused by activated macrophages in chronic *Helicobacter pylori* infection, which is now being studied.

2.7.5 Characterization of nitrite-derived food carcinogens and/or mutagens from high-risk areas for gastric cancer

(B. Pignatelli, C. Malaveille, C.S. Chen, M. Friesen, A. Hautefeuille, P. Thuillier and H. Bartsch)

A high gastric cancer mortality in Fujian province (China) has been associated with the consumption of certain salted fermented fish products such as fish sauce. After nitrosation, samples of the sauce were weakly active in the SOS chromotest. Two phenolic compounds (*para*-hydroxyphenylacetic acid and *para*-hydroxybenzoic acid) have been identified in ethyl acetate extracts of fish sauce samples that upon nitrosation produced direct-acting genotoxins (Chen *et al.*, 1992). Their nitroso derivatives contributed 30% of the genotoxicity of the nitrosated fish sauce samples studied. The nitrosation of these phenolic substances has been shown to produce genotoxic diazonium derivatives.

2.7.6 Bacterial production of nitric oxide mediates nitrosamine production

(S. Calmels-Rouffet, H. Ohshima and H. Bartsch)

Bacterial infections have been epidemiologically associated in humans with an increased risk of cancer of the stomach, urinary bladder and uterine cervix. We have purified the nitrosating enzyme from denitrifying microorganisms isolated from human gastric juice

(Calmels *et al.*, 1990). It appears that a known c,d-haem nitrite reductase is catalysing the formation of carcinogenic *N*-nitroso compounds via the production of NO, since (*a*) polyclonal antibodies raised against the c,d-haem nitrite reductase from *P. aeruginosa* strongly recognized the purified nitrosating enzyme, (*b*) the UV spectrum of the purified nitrosating enzyme is identical with that reported for the oxidized form of the c,d-haem nitrite reductase (*c*) the sequence of *N*-terminal amino-acids of our enzyme shows a strong homology with that of the c,d-haem nitrite reductase from *P. aeruginosa*, and (*d*) EPR studies of the enzyme, in the presence of nitrite and diethyldithiocarbamic acid ethyl ester, gave a characteristic signal for an [Fe-S-NO] complex which has also been reported for the c,d-haem nitrite-reductase from *P. aeruginosa*. Further studies to confirm this identification are in progress.

2.7.7 Role of chronic urinary tract infection and inflammation in the etiology of bladder cancer: development of an animal model

(S. Calmels-Rouffet, H. Ohshima, B. Pignatelli and H. Bartsch)

In order to study the role of infection and inflammation in the etiology of bladder cancer, a model has been developed using Sprague–Dawley rats, with *Proteus morganii* as the infectious agent.

Urinary excretion of nitrite and nitrate was considerably increased in infected rats compared to controls. Treatment of infected rats with *N*-nitroarginine, a known inhibitor of nitric oxide (NO) synthase, significantly reduced the urinary excretion of nitrite and nitrate compared with non-treated infected rats. The difference reflects the production of NO by activated macrophages. When rats were given morpholine, large increases in urinary excretion of *N*-nitrosomorpholine were also observed in infected rats compared with controls.

Bladder tissues were analysed for oxidized proteins and for the expression of antioxidant enzymes (catalase, superoxide-dismutase) and NO synthase. Levels of oxidized proteins were six times higher in bladder tissues from infected rats compared with controls. The expression of antioxidant enzymes and NO synthase was also higher in bladder tissues from infected rats.

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2.8 *DNA damage and its relationship to cancer*

DNA damage has been clearly associated with the process of carcinogenesis; adducts formed between carcinogens and DNA, if not repaired, can lead to mutations in genes that may ultimately lead to loss of control of cellular growth and proliferation. Studies at IARC are particularly focused on obtaining information that will help in identifying specific causes of cancer and on finding markers of early stages in the cancer-forming process for use in epidemiological research and in prevention studies.

Studies of DNA damage to the p53 tumour-suppressor gene in relation to cancers at specific sites are described in Section 2.9 (oesophageal cancer) and Section 2.10 (stomach cancer) and of activation of *ras* oncogenes in Section 2.9 (oesophageal cancer).

Differences in capacity to repair DNA damage may lead to individual variations in susceptibility to effects of some carcinogens, as described in Section 3.2.

Mutations of genes coding for connexin proteins that form intercellular gap junctions are described in section 2.16.1. The formation and methods for detection of various types of DNA adduct are subjects of studies described in Section 5.1.

2.8.1 Detection of carcinogen-specific mutations in oncogenes and tumour-suppressor genes before tumour appearance

(H. Nakazawa, A. Loktionov, O. Bertrand, J.-C. Lozano, N. Martel and H. Yamasaki)

Ample evidence suggests that carcinogen-specific genetic footprints can be detected in tumours (Yamasaki *et al.*, 1993), and these can even be detected before cell transformation occurs (Nakazawa *et al.*, 1992). The detection and quantification of carcinogen-specific

genetic alterations before tumour appearance may not only provide information on the molecular mechanisms of multistage carcinogenesis, but also be usable as a biologically relevant measurement of carcinogen exposure (Yamasaki *et al.*, 1993). We are therefore continuing to develop sensitive methods to detect specific mutations in *ras* and p53 genes, employing cell culture models, animal experiments and human biopsies.

2.8.1.1 *Detection of carcinogen-specific* ras *gene mutations in BALB/c 3T3 cells before and after cell transformation*

After BALB/c 3T3 cells are exposed to 7,12-dimethylbenz[*a*]anthracene (DMBA), we can detect A to T transversion at the 61st codon of H-*ras* as well as K-*ras* genes (Nakazawa *et al.*, 1990). We have shown that cells containing this K-*ras* mutation can transform, since expression of this gene is higher than that of the H-*ras* gene (Nakazawa *et al.*, 1992). We suggested that this kind of mechanism may partially explain why tumours from different tissues exhibit different kinds of oncogenes.

In order to extend this approach, and to link it to the project on second cancers following chemotherapy (see Section 2.8.4), we have started to study the mutation spectra induced by the chemotherapeutic agent cisplatin. In BALB/c 3T3 cells transformed by cisplatin, two out of seven transformed foci contained an A to T transversion mutation at codon 58 of the K-*ras* gene (Lozano *et al.*, 1992). Mutation at codon 58 of the *ras* gene is very rare, but cisplatin-induced mouse skin tumours also contained the same mutation, although in the H-*ras* gene rather than the K-*ras* gene. A method is now being designed to detect mutation at codon 58 of the *ras* gene using polymerase or ligase chain reactions in populations of cells exposed to cisplatin before the appearance of transformation.

2.8.1.2 *Early detection of DMBA-produced* ras *gene mutations* in vivo

Mouse skin tumours induced by DMBA usually contain an A to T transversion at the 61st codon of the H-*ras* gene, regardless of the type of tumour-promoting agent used. However, when DMBA was injected subcutaneously, fibrosarcoma was induced that contained the same mutation but in the K-*ras* gene (Table 9). There are at least two possible explanations for this difference between papillomas and fibrosarcomas: (*a*) DMBA induces H-*ras* mutations in keratinocytes, but K-*ras* mutations in fibroblasts; or (*b*) DMBA induces mutations in both *ras* genes in both cell types, but the different cell types recruit different *ras* genes into carcinogenesis. This hypothesis could be tested if we were able to detect *ras* gene mutations before tumour appearance. Using a method based on a polymerase chain reaction (PCR) with an amprimer specific for the mutant allele, we can detect A to T transversion at the 61st codon of *ras* genes at a frequency of 10^{-4} to 10^{-5}, but we found no mutation in either type of *ras* gene in keratinocytes or fibroblasts from mice exposed to DMBA. We are continuing to improve the sensitivity of our method.

Table 9. Differential *ras* gene mutations found in mouse skin papillomas/carcinomas and fibrosarcomas induced by DMBA

	A^{181} to T mutation in	
	H-*ras*	K-*ras*
	(No. positive/No. tested)	
Papillomas/carcinomas	8/11	0/11
Fibrosarcomas	0/9	9/9

2.8.1.3 *Detection of UV-specific p53 gene mutations in normal human skin*
(with B.K. Armstrong; in collaboration with D. English and P. Randell, Nedlands, Australia)

p53 genes are mutated in many human skin cancers (Brash *et al.*, 1991) and UV-induced mouse skin tumours (Kress *et al.*, 1992), and the mutation pattern appears to be UV-specific. To clarify the link between UV exposure and p53 gene mutation, we have attempted to detect UV-specific mutations before tumour appearance, using a sensitive method to detect CC to TT mutations at codons 245 and 247/8 of the p53 gene. These specific mutations have been reported in skin tumours and can be explained by known mechanisms of UV mutagenesis (Brash *et al.*, 1991). We have developed two methods which specifically amplify mutant alleles, one based on PCR and another based on ligase chain reaction (Figure 11). These methods were used to detect UV-specific CC to TT mutations of the p53 gene in cultured

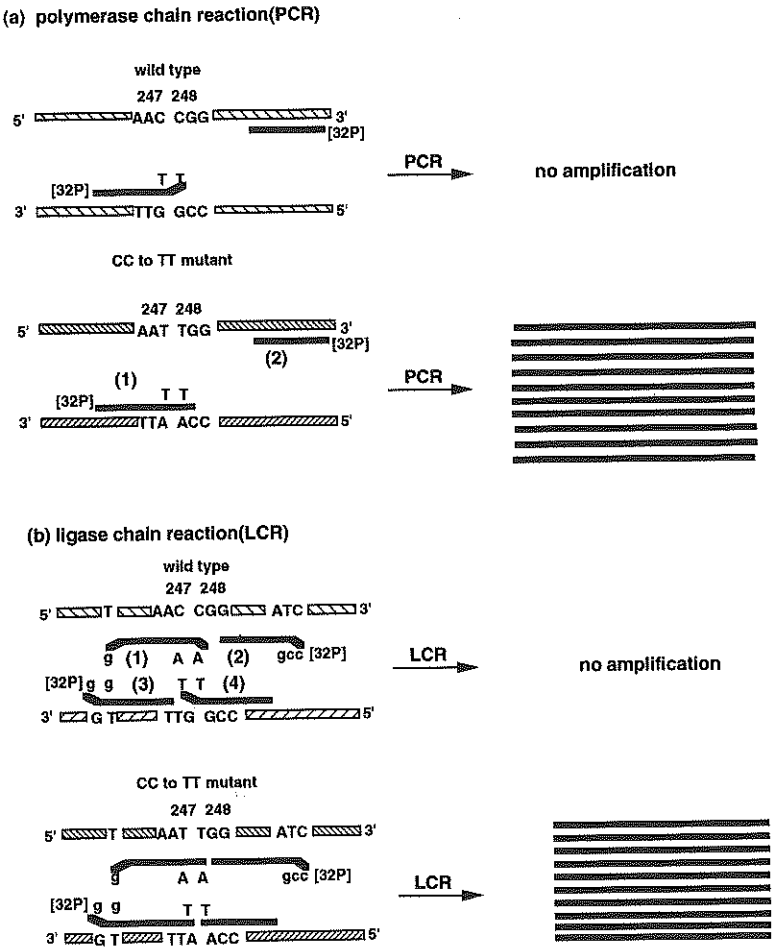

Figure 11. CC to TT mutant allele-specific DNA amplification by (a) polymerase chain reaction and (b) ligase chain reaction. These methods are designed to amplify only the CC to TT mutant alleles.

human skin cells which had been exposed to UV-B. We detected CC to TT tandem base mutations in codons 245 and 247/8 of the p53 gene in UV-B exposed human skin cells in culture. The mutation was induced in a dose-dependent manner in all cell types examined, i.e., skin fibroblasts, keratinocytes and melanocytes. However, the mutations were only detectable in cells passaged a few times after UV exposure (Figure 12), suggesting that selective expansion of p53 mutation-containing cells was necessary for their detection. More mutations were found in XP-A (xeroderma pigmentosum complementary group A) cells exposed to UV than in normal cells. These results suggest that UV directly induces the p53 CC to TT mutation and that cells containing such mutations have the capacity for selective clonal expansion, which would support their role in human skin carcinogenesis.

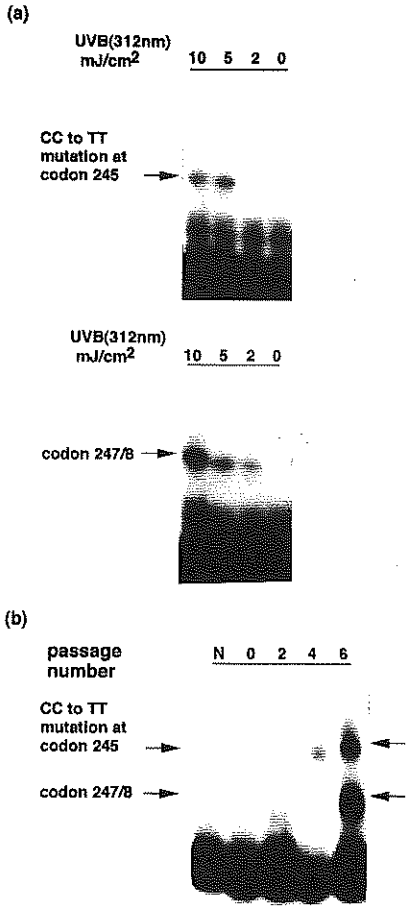

Figure 12. Detection of UV-B-induced p53 CC to TT mutations at codons 245 and 247/8 in cultured human normal keratinocytes.

(*a*) Allele-specific polymerase chain reaction analysis of dose-response.

(*b*) Keratinocytes were exposed to UV-B (10 mJ/m²) at second passage. DNA was isolated after different passage numbers and subjected to allele-specific ligase chain reaction. Note that at least four passages after UV exposure were necessary to detect mutations.

We have applied the same methods to detect p53 gene mutations in normal human skin biopsies from Australian skin cancer patients. In 65% of skin samples from sun-exposed sites, CC to TT mutations were found in one or both of codons 245 and 247/8, and only one of 20 samples from non-exposed sites harboured the mutation. The data from ligase chain reaction studies are presented in Figure 13. None of 15 biopsies of normal skin from non-exposed or intermittently exposed sites in volunteers living in France carried these mutations. Our results suggest that specific p53 gene mutations associated with human skin cancer are induced in normal skin by solar UV radiation. Measurement of these mutations may be useful as a biologically relevant measure of UV exposure in humans and as a possible predictor of risk for skin cancer (Nakazawa *et al.*, 1993).

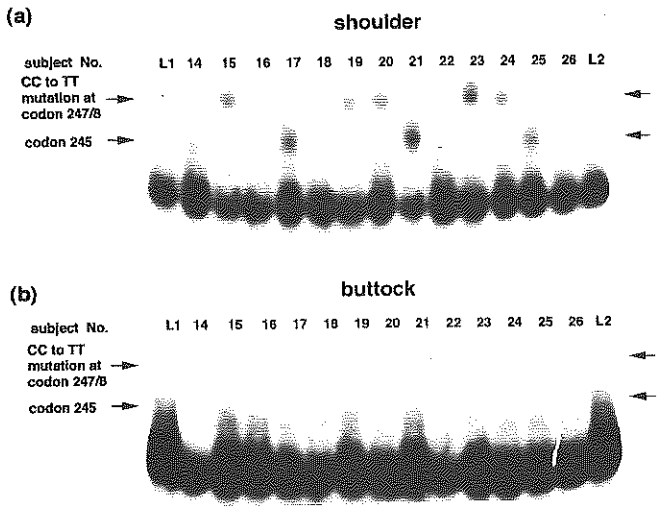

Figure 13. Detection of CC to TT mutations in the p53 gene by allele-specific ligase chain reaction in biopsies of normal skin from sun-exposed and unexposed sites in the same individuals

Two punch biopsies (one from shoulder and the other from buttock) or normal skin were obtained from each of 16 Australian skin clinic patients. Note that many samples from sun-exposed, but not from unexposed sites contained the mutation.

2.8.2 *Ras* and p53 gene mutations in oesophageal tumours induced by *N*-nitrosomethylbenzylamine in rats

(J.-C. Lozano, H. Nakazawa, N. Martel, M.-P. Cros, J.R.P. Cabral and H. Yamasaki)

In order to examine whether the p53 mutations observed in human oesophageal cancers are determined by specific carcinogens, we have analysed genetic alterations in oesophageal tumours induced in rats by a suspected human oesophageal carcinogen, *N*-nitrosomethylbenzylamine (NMBA).

We found a high prevalence of point mutations in H-*ras* and p53 genes in NMBA-induced oesophageal papillomas in BDVI and Fischer 344 rats, with GGA → GAA mutations (expected from the known mechanisms of action of NMBA) at H-*ras* codon 12 in about half of

Table 10. Mutation incidence of H-*ras* gene in NMBA-induced oesophageal papillomas in BDVI and F344 rats

	BD VI rat			F 344 rat		
NMBA dose	M	F	Total	M	F	Total
2.5 mg/kg	7/10	4/10	11/20 (55%)	4/10	6/10	10/20 (50%)
5 mg/kg	5/13	6/13	11/26 (42%)	5/8	7/10	12/18 (66%)
	12/23 (52%)	10/23 (43%)	22/46 (48%)	9/18 (50%)	13/20 (65%)	22/38 (58%)

the papillomas from each strain of rats at doses of 5.0 and 2.5 mg/kg (Table 10). p53 mutations were found in 36% of papillomas in each strain, mainly G to A and C to T transitions. No apparent hot spot codon or exon in the p53 gene was found, and two papillomas contained double mutations in this gene. The high prevalence of G to A mutation in the H-*ras* gene is in contrast to the human tumours, in which no *ras* mutation was found, and suggests that either the biology of oesophageal carcinogenesis differs between human and rat or nitrosamines are not the major etiological risk factor for human oesophageal cancers.

2.8.3 *Ras* gene activation in liver cancers induced by vinyl chloride in humans and rats

(A. Barbin and H. Bartsch; in collaboration with M.-J. Marion, O. Froment, C. Trépo and J.-C. Contassot, Lyon, France. Supported by a contract with the Groupe de Recherche sur les Hépatites, Cirrhoses et Cancers de Foie (INSERM, Lyon) and Elf-Atochem (Paris))

Exposure to vinyl chloride (VC) is associated with the induction of liver angiosarcomas in humans and rodents and a variety of other tumours including hepatocellular carcinomas in rodents.

A previous study of human liver angiosarcomas revealed activation of the K-*ras* gene in six out of seven tumours, through a GC → AT transition at the second base of codon 13 (Marion *et al.*, 1991). Further samples of human liver angiosarcoma associated with VC exposure have been analysed using polymerase chain reaction (PCR) amplification, allele-specific oligonucleotide hybridization and direct sequencing of tumoral DNA. In addition to the GC → AT transition in codon 13, a GC → TA transversion at the second base of codon 12 was found in the K-*ras* gene in several of these tumours. Both types of base-pair substitutioncorrespond to the expected mutational specificity of N^2, 3-ethenoguanine and/or 3,N^4-ethenocytosine, supporting the view that these ethenobases are critical lesions in VC-induced carcinogenesis in humans (see Section 5.1.1).

Ras activation has also been investigated in two hepatocellular carcinomas and five angiosarcomas from Sprague–Dawley rats exposed to VC. PCR-amplified tumoral DNA was analysed for activating mutations in codons 12, 13 and 61 of the H-*ras*, K-*ras* and N-*ras* genes. Using allele-specific oligonucleotide hybridization, no mutation was detected in exons 1 and 2 of K-*ras* nor in exon 1 of H-*ras* in these seven tumours. In contrast, direct sequencing and allele-specific oligonucleotide hybridization revealed an AT → TA transversion at the second base of codon 61 of H-*ras* in the two hepatocellular carcinomas. The presence of an activated H-*ras* gene was confirmed using the NIH 3T3 cell transfection assay and Southern blot analysis.

Depending on the strain, two or three N-*ras* (pseudo) genes, N-*ras* A (homologous to the human N-*ras* gene), N-*ras* B and N-*ras* C, have been reported in rats. These genes exhibit

sequence variations in, among others, exon 1. We used a cloning approach to study N-*ras* sequences in DNA from normal tissues of untreated rats and N-*ras* mutations in tumour DNA from VC-exposed animals. DNA from untreated Sprague–Dawley rats contained N-*ras* A, B and C sequences in 26%, 26% and 14% of the clones, respectively. In addition, a variant N-*ras* B sequence was found in 14% of the clones, indicating that exon 1 of N-*ras* B is polymorphic in Sprague–Dawley rats. Other variant sequences were found at low frequency, implicating nucleotide changes in codons 8, 15, 18 or 24 of N-*ras* A, B and C. Two angiosarcomas contained a mutated N-*ras* A gene, one with a GC → AT transition at codon 13, the other with an AT → CG transversion at codon 36. The presence of a transforming N-*ras* gene in these tumours was confirmed using the NIH 3T3 cell transfection assay followed by Southern blot analysis of transformant DNA.

Other liver tumours contained mutations in codon 18 of N-*ras* B and C. These mutations were not seen in non-tumoral DNA, but their relevance to VC-induced carcinogenesis remains to be established.

2.8.4 p53 alterations in tumours of individuals with industrial exposure to carcinogens and with known smoking histories

(M. Hollstein, G. Martel-Planche and A. Esteve; in collaboration with C.C. Harris and T. Lehman, Bethesda, USA; I. Kusters, Lyon, France; J. Lewalter, Leverkusen, Germany; M.-J. Marion, Lyon, France; and P. Vineis, Turin, Italy)

In some human cancers, mutation patterns in the p53 tumour-suppressor gene appear to be attributable to exposure to a given carcinogen, but the evidence is still limited (Hsu *et al.*, 1991; Ozturk *et al.*, 1991; Brash *et al.*, 1991; Suzuki *et al.*, 1992). Our recent work has focused on two types of cancer for which specific exposure to known human mutagenic carcinogens is associated with high risk: (i) bladder cancer in dye industry workers primarily exposed to benzidine and its analogues, and (ii) haemangiosarcomas in vinyl chloride-exposed factory workers.

2.8.4.1 *Bladder tumours*

As an initial approach to elucidating the role of p53 tumour-suppressor gene damage in the development of bladder tumours, archival specimens of tumours resected from factory workers exposed to benzidine dyes were examined immunohistochemically. Smoking histories have been recorded on these patients, and tumours from smokers not exposed to industrial dyes are available for comparison. In total 30 cases have been screened, of which approximately half show nuclear accumulation of the p53 protein, indicative of an altered gene (Figure 14). Microdissection of tumour cells from histology sections and sequencing of the p53 gene is in progress.

2.8.4.2 *Haemangiosarcoma*

At the time of our study, it was not known whether loss of p53 function can play a role in the development of angiosarcomas in humans, and if so, by what mechanisms this function may be abrogated. In sarcomas (bone and soft tissue forms), an alternative mechanism to loss of function by point mutation has been described that does not appear to occur with any appreciable frequency in tumours of epithelial cancers. This pathway involves amplification of the *mdm2* oncogene, the product of which can bind to and inactivate p53 protein. We examined four angiosarcomas and one hepatocellular carcinoma from factory workers heavily exposed to vinyl chloride for evidence of either mechanism. While the gene copy number of *mdm2* was normal in all instances, we found two angiosarcomas with A to T point mutations within the p53 coding sequence that alter the amino acid sequence of the protein. The analysis of a large number of liver tumours from workers with VC exposure histories to generate

a mutation spectrum for this carcinogen is thus feasible, and would yield valuable information on mechanisms of tumour induction by mutagenic carcinogens.

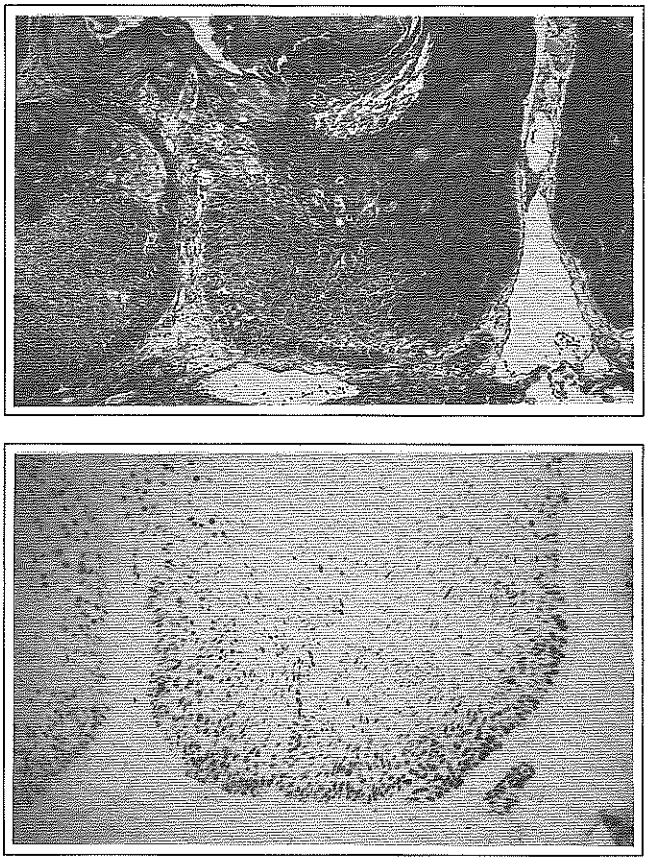

Figure 14. Eosin-stained (upper) and immunohistochemical detection of p53 protein (lower) with polyclonal antiserum CM-1 in bladder carcinoma

2.8.5 Second malignancies following chemotherapy

2.8.5.1 *Epidemiological studies of second malignancies*

(J. Estève, P. Boffetta and A. Arslan; in collaboration with D. Assouline, Lyon, France; P. Band, Vancouver, Canada; J. Bell, Sutton, UK; V. Blair, Manchester, UK; N.W. Choi, Winnipeg, Canada; E.A. Clarke and S.B. Sutcliffe, Toronto, Canada; N.E. Day, Cambridge, UK; P. Fraser, London, UK; C. Garton, Leicester, UK; H. Hakama and S. Karjalainen, Helsinki, Finland; M. Henry-Amar, Caen, France; H. Host and F. Langmark, Oslo, Norway; J. Kaldor, Sydney, Australia; B. Kittelmannand W. Staneczek, Berlin, Germany; M. Koch, Edmonton, Canada; F. Neal, Sheffield, UK; D. Pedersen, Aarhus, Denmark; D. Peters, Leeds, UK; F. Pettersson and B. Zarén, Stockholm, Sweden; V. Pompe-Kirn, Ljubljana, Slovenia; P. Prior, Birmingham, UK; H.H. Storm, Copenhagen, Denmark; and M. Stovall, Houston, TX, USA)

After completion of the studies on leukaemia following treatment for Hodgkin's disease or ovarian cancer and on bladder cancer following treatment for ovarian cancer, a further case–control study on lung cancer has been conducted.

In a study of patients treated for Hodgkin's disease (Kaldor *et al.*, 1992), the risk of lung cancer was increased among those treated with chemotherapy alone (RR 2.1, 95% CI 1.0–4.2), as compared with those treated with radiotherapy alone. Patients treated with both radiotherapy and chemotherapy had the same risk as patients treated only with radiotherapy. There was no increase in risk related to cumulative number of cycles of chemotherapy; among patients treated with radiotherapy only, there was an increase in risk related to estimated radiation dose to the lung.

A pilot study of the risk of endometrial cancer among women treated for breast cancer with tamoxifen has been conducted (see Section 2.13.4).

2.8.5.2 *Markers of DNA damage and risk of second malignancy in Hodgkin's disease patients*

(P. Boffetta, C. Wild, H. Yamasaki, R. Montesano, H. Nakazawa, J. Hall and R. Saracci; in collaboration with P. Boyle, Milan, Italy; T. Cerny, Bern, Switzerland; M. Dicato, Luxembourg; D. English, Nedlands, Australia; K. Hemminki and G. Juliusson, Stockholm, Sweden; M. Henry-Amar, Caen, France; J. Kaldor, Sydney, Australia; S. Karjalainen, Tampere, Finland; K. Katsouyanni, S. Kyrtopoulos and S. Pangalis, Athens, Greece; Y. Kobayashi, Tokyo, Japan; S. Kvinnsland, Oslo, Norway; F. Levi, Lausanne, Switzerland; J. Lopez, Barcelona, Spain; J. Martin-Moreno, Madrid, Spain; I. Plesko, Bratislava, Slovak Republic; F. Rilke, Milan, Italy; E. Sedkackova, Prague, Czech Republic; L. Simonato, Padua, Italy; H. Storm, Copenhagen, Denmark; A. Swerdlow, London, UK; F. Van Leeuwen, Amsterdam, Netherlands; A. Van Oosterom, Edegem, Belgium; L. Vatten, Trondheim, Norway; and J. Walewski, Warsaw, Poland)

A collaborative group of major hospitals treating Hodgkin's disease patients is being established. All patients diagnosed with Hodgkin's disease at a participating hospital will be prospectively followed up for response to therapy and occurrence of second malignancies, particularly leukaemia, non-Hodgkin lymphoma and lung cancer. In parallel to the registration of index and second cancers, blood samples will be taken from each patient, immediately following diagnosis, during and subsequent to therapy, to be analysed for markers of DNA damage.

2.8.5.3 *Studies of DNA damage following chemotherapy for testicular cancer*

(P. Boffetta, J. Hall, C.P. Wild, H. Yamasaki, R. Montesano and R. Saracci; in collaboration with D. Bron, Brussels, Belgium; A.M.J. Fichtinger-Schepman, Rijswijk, Netherlands; A. Horwich, Surrey, UK; J. Kaldor, Sydney, Australia; A. Natarajan, Leiden, Netherlands; P. Roy, Lyon, France; and R. Somers, Amsterdam, Netherlands)

The reaction between alkylating chemotherapeutic agents and cellular DNA is probably the main pathway to both their cytotoxic and their carcinogenic effects. A collaborative study has been set up to measure cis-platinum DNA adducts in testicular cancer patients, and to what extent adduct levels can be used to predict the clinical outcome of chemotherapy.

The study is being performed in conjunction with a clinical trial of cis-platinum in testicular cancer conducted by the genitourinary groups of the European Organization for Research and Treatment of Cancer (EORTC) and the Medical Research Council of the UK. DNA

adducts and haemoglobin adducts are being measured in the testicular cancer patients and will be analysed in relation to the response to chemotherapy.

2.8.6 Detection of DNA methylating adducts following exposure to environmental methylating agents

(C.P. Wild, F. Bianchini and D. Shuker; in collaboration with J. Cuzick, London, UK)

DNA methylation adducts, e.g., O^6-methyldeoxyguanosine (O^6-MedG) and 7-methyldeoxyguanosine (7-MedG) result from exposure to various environmental carcinogens as well as from endogenous cellular processes. The aim of these studies is to improve and apply methods for detecting and quantifying 7-methylguanine (7-MeGua) and other DNA methylation adducts in human and animal tissues. The adduct 7-MeG is selectively hydrolysed from DNA by thermal hydrolysis, immunopurified on affinity columns and then quantitated by HPLC with electrochemical detection. The latter technique provides a detection limit of 0.1 pmol of adduct and complements earlier HPLC–immunoassay approaches.

Initially, human pancreas samples were found to contain high levels of 7-MeGua. However, autolysis in the samples led to contamination of the adduct with RNA-derived 7-methylguanosine. This problem was resolved using hydroxylapatite to purify DNA. The levels in pancreatic DNA of 4.6–6.7 pmol 7-MeGua per mol G were similar to those found in human lung (0.1–7 pmol per μmol G). Thus, the method is sensitive enough to detect 7-MeGua in human tissues and it is now being applied to pilot studies of DNA damage in cancer patients treated with alkylating chemotherapeutic agents (see Section 2.8.5).

It is proposed to use DNA from peripheral blood cells (PBC) to quantitate exposure to methylating agents in human populations at high risk of specific cancers for which methylating agents are suspected etiological risk factors (e.g., oesophageal cancer) (see Section 2.9.2). In order to determine the validity of using PBC, experimental animals were exposed to methylating agents which induce tumours in different organs, namely nitrosodimethylamine (NDMA) (liver), 1,2-dimethylhydrazine (colon), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (lung) and *N*-nitrosomethylbenzylamine (oesophagus). 7-MeGua levels were examined 16 h after treatment in the target organ, as well as in the liver and in PBC DNA, together with the modulation of alkylation-damage-specific DNA repair enzymes in the same tissues. 7-MeGua was detected in PBC DNA with each methylating agent in a dose-dependent manner, but the ratio between target organ and PBC DNA differed markedly between the compounds (Figure 15). The ratio at the highest dose examined for each compound was 3.3 for NNK, 8.6 for 1,2-dimethylhydrazine, 50.5 for NDMA and 297 for *N*-nitrosomethylbenzylamine. These results indicate that the presence of 7-MeGua in PBC reflects the formation of DNA methylation adducts in specific target organs and hence demonstrate that exposure has occurred, but no general relationship has been established between adduct levels in PBC and target organs.

2.8.7 Prediction of carcinogenic potency of genotoxic chemicals

(A. Barbin, J. Nair and H. Bartsch; in collaboration with E. Vogel, Leiden, The Netherlands. Supported by a contract with the Commission of the European Communities (STEP-CT91-0145))

The linear correlation previously found between the carcinogenic potency (TD_{50} values) of monofunctional direct-acting alkylating agents and their Swain-Scott *s* constant (Barbin & Bartsch, 1989; Vogel *et al.*, 1990) is not applicable to procarcinogens. In order to establish a more general relationship that would include both direct-acting agents and procarcinogens,

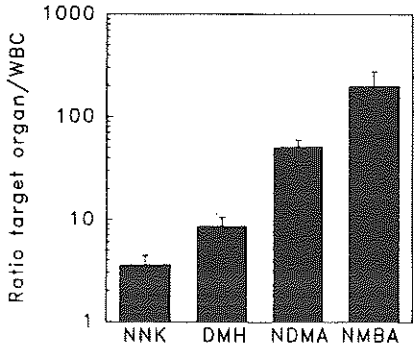

Figure 15. Ratio between 7-methylguanine in target organs and in white blood cells (WBC) after treatment with different methylating carcinogens, at the highest dose level used.

NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; DMH, 1,2-dimethylhydrazine; NDMA, *N*-nitrosodimethylamine; NMBA, *N*-nitroso-*N*-methylbenzylamine

we have calculated covalent binding indices (CBIs) for these compounds. This index is a measure of the covalent binding of a carcinogen to DNA in rodent liver, following a single administration or short-term treatment (Lutz, 1979). Generally, total binding or the level of a major adduct, such as 7-alkylguanine, is considered.

Using published data, we have calculated or recalculated (on the basis of new data) the CBI of a series of 17 monofunctional alkylating agents, including direct-acting chemicals as well as procarcinogens:

N-Nitroso-2-hydroxyethylurea (HNU); *N*-ethyl-*N*-nitrosourea (ENU); *N*-methyl-*N*-nitrosourea (MNU); *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNG); *N*-nitrosodiethylamine (NDMA); *N*-nitrosodimethylamine (NDEA); 1,2-dimethylhydrazine (DMH); streptozotocin (SZT); procarbazine (PC); dimethylsulfate (DMS); diethylsulfate (DES); ethyl methanesulfonate (EMS); methyl methanesulfonate (MMS); 1-phenyl-3,3-dimethyltriazene (PDMT); 2,2-dichlorovinyl dimethyl phosphate (DDVP); ethylene oxide (EO); propylene oxide (PO).

In addition to methylating and ethylating agents, this series includes two hydroxyethylating agents (HNU, EO) and one hydroxypropylating agent (PO). The CBI values for the 7-alkylguanine, calculated as the initial amount of 7-alkylguanine formed in liver DNA, expressed as pmol of adduct.(mg DNA)$^{-1}$.(mmol of compound administered)$^{-1}$.kg bw)$^{-1}$, ranged from 0.42 to 4940.

Because the carcinogenic effects of monofunctional alkylating agents are thought to result from the formation of *O*-alkylated bases in DNA, mainly O^6-alkylguanine, we also calculated a CBI for O^6-alkylguanine. Due to fast repair, the initial level of O^6-alkylguanine formed in liver DNA cannot be measured accurately. Consequently, this level was deduced from the CBI for 7-alkylguanine, using the following linear relationship between the ratio of O^6 to 7-alkylguanine and the *s* value:

$$\log (O^6/N7) = -3.786s + 0.7633$$

This correlation was established on the basis of published data on ENU, MNU, MNNG, DES, EMS, MMS and DMS. The CBI for O^6-alkylguanine ranged, for the series of 17 agents listed, from 0.00176 to 248.

The initial level of 7-alkylguanine in liver DNA correlated poorly ($r = 0.52$) with the carcinogenic potency in rodents of monofunctional alkylating agents. In contrast, a good

correlation ($r = 0.81$) was observed between the CBI for O^6-alkylguanine and the carcinogenic potency of these agents (Figure 16). Therefore, using the CBI for O^6-alkylguanine instead of the s value, a more general quantitative structure–activity relationship, that includes procarcinogens, has been obtained for the class of the monofunctional alkylating agents.

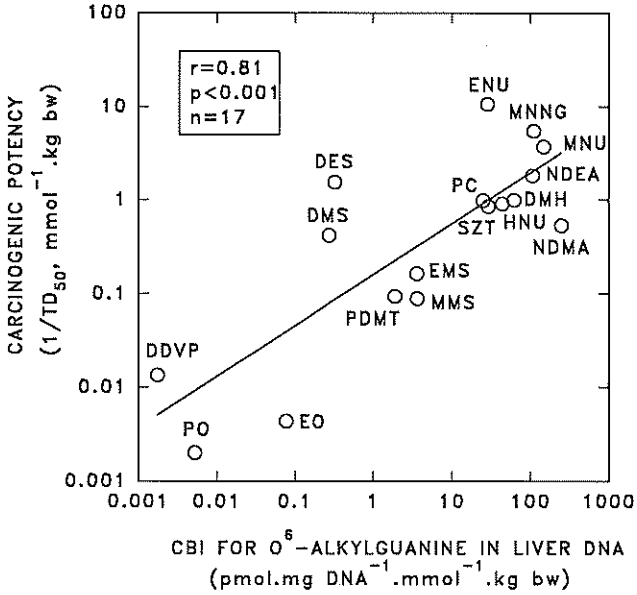

Figure 16. Correlation of carcinogenic potency in rodents with initial level of O^6-alkylguanine in liver DNA for monofunctional alkylating agents. See text for abbreviations

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2.9 *Cancer of the oesophagus*

2.9.1 Case–control studies of oesophageal cancer in high-risk populations of Latin America

(N. Muñoz, X. Castellsagué, M. Rosato and M. Benz; in collaboration with R. Castelletto and J. Iscovich, La Plata, Argentina; E. de Stefani, Montevideo, Uruguay; P.A. Rolón, Asunción, Paraguay; and C. Victora, Pelotas, Brazil)

Five case–control studies have been conducted in Argentina, Brazil, Paraguay and Uruguay (two studies) specifically aimed at studying the role of hot mate drinking in oesophageal cancer. In addition to alcohol and tobacco, identified as the main risk factors for this cancer in all countries, a significant effect of hot mate drinking was detected in Uruguay and Paraguay, a non-significant association in Brazil and no association in Argentina (Victora *et al.*, 1987; de Stefani *et al.*, 1990; Castelletto *et al.*, 1992).

Preliminary findings from a pooled analysis of the five studies including 830 cases and 1779 controls revealed a stronger effect of hot mate drinking among females and among non- or light drinkers of alcohol. No significant differences were detected in the effect of the various risk factors in the different sections of the oesophagus.

2.9.2 Genetic alterations in oesophageal cancer

(M. Hollstein, Y.-Y. Liang, A. Esteve, G. Martel-Planche, N. Lyandrat and M. Laval; in collaboration with C.C. Harris and W.P. Bennett, Bethesda, MD, USA; Li Peri, Montevideo, Uruguay; S.-H. Lu, Beijing, China; A.-M. Mandard, Caen, France; A. Ruol and A. Peracchia, Padua, Italy; and I.B. Weinstein and W. Jiang, New York, USA)

We demonstrated previously the importance of p53 gene mutations in the natural history of squamous cell carcinoma of the oesophagus (Hollstein *et al.*, 1990). Amplification of oncogenes and other genes thought to regulate the cell cycle has also been observed in this malignancy, notably of the *erbB* (coding for the epidermal growth factor receptor) and c-*myc* oncogenes and *cyclin D1* (Hollstein *et al.*, 1988; Jiang *et al.*, 1989, 1992).

Mutations in specific cancer-related genes are of interest not only because they help to identify the molecular pathways to neoplasia, but also because they provide clues about cancer-causing exposures. A clear demonstration of this point is the unique mutation spectrum found in human skin tumours, which reflects the fingerprint of DNA damage left by exposure to the major risk factor for this cancer: ultraviolet light exposure.

In order to determine whether a characteristic mutation pattern is observed in oesophageal tumors of patients exposed to specific risk factors (for example, tobacco and alcohol in Europe; dietary nitrosamines in Linxian, China), we have screened more than 100 cancers from different regions of the world for p53 point mutations (Table 11) (Hollstein *et al.*, 1991). Our analysis shows that the composite mutation spectrum for oesophageal cancer is different from other gastro-intestinal cancers, notably colorectal cancer; the frequency of transitions at CpG nucleotides, a mutation likely to be attributable to endogenous induction of mutation, is much higher in colorectal cancer, for example ($p < 0.01$). The oesophageal mutation pattern is also different from that seen in studies on p53 mutations in lung cancer, also unequivocally a tobacco-related tumour type (Harris & Hollstein, 1992). Since most smokers in our patient groups were also heavy drinkers, this finding suggests that alcoholic beverages, which probably do not contain significant levels of mutagenic contaminants *per se*, may influence mutagenic events originating from exposure to tobacco smoke.

Investigations of the timing of p53 mutations in the natural history of oesophageal cancer were also conducted, using a strategy based on immunohistochemical detection of p53 protein accumulation. While the levels of normal p53 protein are usually too low to be detectable by routine immunohistochemical procedures, the more stable mutant forms accumulate in the cell nucleus, and are easily revealed by staining procedures with the CM-1 polyclonal antiserum to the tumour-suppressor protein. We observed that p53 protein accumulated in dysplastic lesions of the oesophageal squamous epithelium (Bennett *et al.*, 1991, 1992). In one case, microdissection of stained cells and sequencing of amplified regions of the p53 gene confirmed the presence of a single DNA base substitution responsible for the mutant protein. These and other studies showed that mutations in the p53 gene typically occur in oesophageal lesions before invasion, and are occasionally present at early stages of neoplasia.

Table 11. p53 mutations in human oesophageal cancer

					China		
Mutation	Uruguay	Normandy	Lyon	Italy	Linxian	Shenyang	Total
G:C→A:T	2	3	3	3	3	1	15[c]
G:C→T:A	1	3	1	1	1	1	8
G:C→C:G	0	0	0	1	3	0	4
A:T→C:G	3	0	0	0	0	0	3
A:T→G:C	0	0	0	2	1	1	4
A:T→T:A	0	3	0	3	0	2	8
Frameshifts	0	0	1	2	2	0	5
Total mutations	6	9	5	12	10	5	47
Total tumours tested	19	15	14	24	[50][a]	[10][b]	[132]

[a] Analysis in progress (preliminary data)
[b] Pre-selection of these samples by immunochemistry
[c] Seven of the G→A mutations are at CpG sites

Knowledge of the biological processes and biochemical pathways involving the p53 tumour-suppressor protein has advanced considerably in the past two years. One of the many functions of this protein is to monitor the integrity of the genome. Loss of p53 function is expected to increase the burden of DNA sequence changes, including point mutations as well as alterations involving larger segments of the genome such as gene amplifications, which may increase in frequency by several orders of magnitude in cells with damaged or altered p53. We have examined a set of oesophageal cancers for correlations between loss of suppressor gene function (p53 and Rb) and other cellular changes associated with this cancer, including gene amplifications (c-*myc*, *mdm2*, *cyclin D1* and *erbB*) (Estève *et al.*, 1993). In these tumours, an increased copy number of the *erbB* gene was associated with overabundance of the epidermal growth factor receptor gene product, and abnormal levels of the receptor were found more frequently in cells with a mutated p53 gene than in cells where the tumour-suppressor gene was apparently intact. In collaboration with our laboratory, Dr Jiang and co-workers have shown that several oesophageal tumours lack detectable Rb

tumour-suppressor protein, and that this aberration is inversely correlated with amplification of the *cyclin D1* gene, which encodes a protein engaged in cell cycle control (Jiang *et al.*, 1993).

2.9.3 Autoantibodies to p53 protein in the sera of oesophageal cancer patients from Linxian, China

(C.P. Wild, B. Chapot, Y.-Y. Liang, R. Montesano and M. Hollstein; in collaboration with T. Soussi, Paris, France; and S.-H. Lu, Beijing, China)

An ELISA method was developed to detect circulating antibodies to p53 protein in less than 1 µl of serum. For this assay, ELISA plates are coated with p53 protein or with a control protein (SV40 T antigen) and sera diluted 1:400 are incubated with the two proteins. The ratio of binding to the two proteins is calculated and values greater than 1.5 are considered positive.

Sera were obtained from oesophageal cancer patients and the corresponding tumours were examined for immunostaining of p53 protein using a specific polyclonal antibody. Of the sera examined, five were positive in the ELISA with ratios up to 2.6. All patients positive for antibody were positive for p53 protein by immunocytochemistry, suggesting the presence of a p53 mutation in the tumour. However, not all patients with tumours positive by immunostaining had detectable serum antibodies. DNA sequencing of the p53 gene in the various tumours should reveal the specificity and sensitivity of this immunoassay as a non-invasive approach to detecting p53 mutations. It is of interest to establish the temporal development of the antibody response in relation to the natural history of oesophageal cancer as well as cancer of other sites.

2.9.4 Possible role of *ras* gene mutations in human oesophageal carcinogenesis

(C. Galiana, N. Martel and H. Yamasaki; in collaboration with A. Fusco, Naples, Italy; S. Hirohashi, Tokyo, Japan; and T. Nishihira, Sendai, Japan)

Activation by point mutation of the H-, K- and N-*ras* genes is found in many tumours, but no such mutation has yet been found in human oesophageal carcinomas from various parts of the world (Hollstein *et al.*, 1988; Jiang *et al.*, 1989; Galiana *et al.*, 1993). We have confirmed the absence of mutation at codons 12, 13 and 61 of K- and N-*ras* and at codons 12 and 61 of H-*ras* in 25 primary tumours obtained in France. In contrast, among seven human oesophageal carcinoma cell lines with different degrees of tumorigenicity in nude mice, the three highly tumorigenic cell lines showed activation of *ras* oncogenes, two of which were a G^{35} to A^{35} transition at codon 12 of K-*ras* gene and one an H-*ras* G^{35} to T^{35} transversion. Since these cell lines had been established from tumours of Japanese patients, we examined 28 primary oesophageal tumours from Japan, including the primary tumours from which the cell lines had been derived. No *ras* mutation was detected, which suggests that either the *ras* gene mutations found in the cell lines were due to their long-term culture or only a small portion of the original tumours contained such mutations. In order to directly examine the role of *ras* gene mutation, one of the non-tumorigenic cell lines was transfected with a plasmid EJ-ras coding for a mutated H-*ras* gene (G^{35} to T^{35}). Transfected clones expressing high levels of mutated *ras* gene were able to induce tumours in nude mice (Figure 17). Thus, although no primary human oesophageal tumour contained a mutated *ras* gene, our studies do not exclude a significant role of mutated *ras* genes in cell proliferation and malignant transformation of human oesophageal cells.

2.9.5 Prevalence of *ras* gene amplification and p53 gene mutations in human primary oesophageal adenocarcinomas in comparison to squamous cell carcinomas

(C. Galiana, J.-C. Lozano, H. Nakazawa and H. Yamasaki; in collaboration with B. Bancel, Lyon, France)

Apart from the expression of *ras* p21 oncoprotein in some oesophageal squamous cell carcinomas and mutations of K- and H-*ras* genes in oesophageal carcinoma cell lines, mutated *ras* genes have been found to be conspicuously absent from primary tumours of the oesophagus (see above). Mutations of the p53 gene have been found in 30–35% of human oesophageal squamous cell carcinomas (Galiana, 1993). In order to examine possible differences in molecular pathogenesis between squamous cell carcinomas and adenocarcinomas of the oesophagus, we analysed 10 adenocarcinomas for the presence of *ras* gene mutations and amplification and p53 gene mutations. As in squamous cell carcinomas, no *ras* gene mutations were found, but the K-*ras* gene was amplified in four of the samples. No such amplification was observed among 61 squamouss cell carcinomas, 1 pseudo-sarcomatous carcinoma and 8 oesophageal cell lines, nor in 6 adenocarcinomas of the stomach. This K-*ras* amplification in oesophageal tumours did not correlate with any pathological feature of the tumours, with the survival rate of the patients, nor with the presence of other genetic alterations. These findings provide the first evidence for amplification of the K-*ras* gene in human oesophageal cancer, restricted to adenocarcinomas.

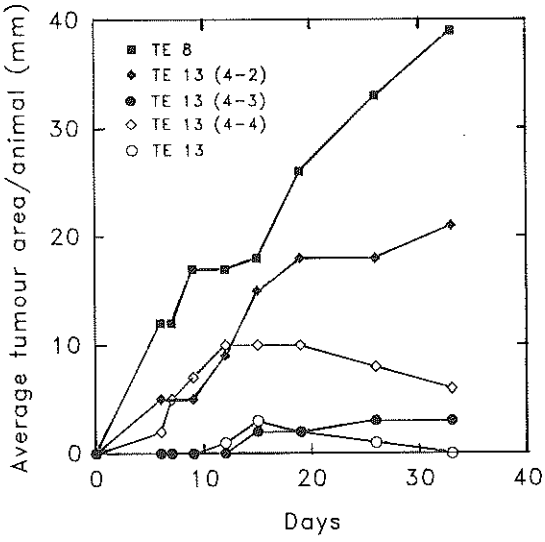

Figure 17. Tumorigenicity of human oesophageal carcinoma cell lines and derived transfected clones (TE 13 (4-2), (4-3), 4-4)) in nude mice. Two million cells were injected per site and per nude mouse. Tumours were measured weekly (length × breadth) and the average from each group is presented.

The prevalence of p53 gene mutations in the adenocarcinomas was very high, SSCP analysis revealing mutations in six out of eight samples (75%).

The higher prevalences of K-*ras* gene amplification and of p53 gene mutations in adenocarcinomas than in squamous cell carcinomas of the oesophagus suggest that different pathways of pathogenesis and/or etiological factors are involved.

2.9.6 Long-term carcinogenicity: effect of temperature on oesophageal carcinogenicity

(H. Yamasaki, J.R.P. Cabral, D. Galendo, M.P. Cross and J. Garcia)

Consumption of very hot drinks is one of the major suspected risk factors for human oesophageal cancer. In the present experiments, the "hot drinking" risk hypothesis is being tested as a possible promoting agent, complete carcinogen or co-carcinogen, using groups of 25 male and 25 female six-week old BDVI rats. The experiment was terminated after 100 weeks, and gross observations indicate a possible co-carcinogenic effect of hot temperature on induction by *N*-nitroso-*N*-methylbenzylamine of oesophageal tumours in the rats. Histological examination is being performed.

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2.10 *Cancer of the stomach*

The causes of most of the cases of stomach cancer, one of the commonest malignancies in the world despite decreasing incidence in recent decades, remain largely unknown. Case–control epidemiological studies are described below and, in respect of dietary factors, in section 2.3.5. Endogenous formation of *N*-nitroso compounds is now strongly suspected of playing a role, and studies of various related aspects are described in sections 2.7.2–2.7.5. Screening for stomach cancer (section 4.4.2) and chemoprevention of precancerous gastric lesions (sections 4.2.2) are both being studied in Venezuela.

2.10.1 Case–control study of stomach cancer in Tachira, Venezuela

(N. Muñoz, S. de Sanjosé, P. Pisani and M. Benz; in collaboration with W. Oliver, J. Vivas and G. Lopez, San Cristobal, Venezuela)

A case–control study to identify the main risk factors and to evaluate the efficacy of a screening programme for gastric cancer in Tachira state, Venezuela, has been initiated (see Section 4.4.2). All new and histologically confirmed cases of stomach cancer diagnosed at the two main hospitals in San Cristobal since January 1991 are being included as well as two controls per case, one from the same hospital and the other from the same neighbourhood as the case and matched by sex and age. Cases and controls must have resided in Tachira state for at least five years. Information on diet is collected by personal interview using a dietary history questionnaire focused on usual diet one year before the disease. Dietary habits when the

study subject was 15–20 years old are also recorded. Information on screening is retrieved from the records of the Cancer Control Centre. Serum samples to measure antibodies to *Helicobacter pylori* and selected micronutrients are being collected in cases and controls; biopsies from tumorous and non-tumorous gastric mucosa are being collected from the cases to look for genetic alterations.

A total of 119 cases, 119 hospital controls and 119 neighbourhood controls have now been interviewed and their biological specimens collected. A preliminary analysis of these 119 triplets will be done during the second half of 1993 to decide on the final sample size required.

2.10.2 Cohort study on intestinal metaplasia in Slovenia

(N. Muñoz, S. Teuchmann and M. Benz; in collaboration with M.I. Filipe, London, UK; and A. Jutersek, I. Matko and V. Pompe-Kirn, Ljubljana, Slovenia)

A cohort of 1525 patients with intestinal metaplasia (IM) diagnosed between 1967 and 1976, subclassified into three types of IM based on mucin staining, were followed up until the end of 1986. The standardized incidence ratio (SIR) for stomach cancer in the whole cohort was 2.23 (95% CI = 1.5–3.1), but it was higher for IM type III (SIR = 3.8; 95% CI = 2.1–6.3) followed by type II (SIR = 2.4; 95% CI = 0.8–5.6). No increased risk was observed for type I (SIR = 1.0; 95% CI = 0.4–2.2).

2.10.3 Alteration of tumour-suppressor genes and microsatellites in stomach tumours

(N. Mironov, Y. Omori, A.-M. Aguelon and H. Yamasaki; in collaboration with O.V. Gorbunov and A.A. Klimenkov, Moscow, Russian Federation)

To investigate the molecular pathogenesis of human gastric cancer, alterations of the p53 gene, connexin 32 gene and APC/MCC genetic loci have been analysed in 22 human gastric tumours from the Russian Federation, a high-incidence area.

Exons 5–8 of the p53 tumour-suppressor gene were analysed for the presence of mutations. Two GC → AT transitions at CpG dinucleotides were found in codons 154 and 175 of exon 5, and a third mutation was an AT → GC transition in codon 234 of exon 7. The AT → GC transition is rarely observed in carcinogen-induced tumours, but could result from an O^4-MeT adduct formed by alkylating nitroso compounds pairing with a G during replication. Two of the three patients with mutated p53 had metastases in regional lymphatic nodules. This suggests that p53 is involved in a late stage of gastric carcinogenesis. All mutations of p53 were found in adenocarcinomas, and none in signet-ring cell carcinomas.

Gap junctional communication capacity is often lost in tumours, suggesting that a gene involved in such communication could be a tumour-suppressor gene. However, we found no mutation in a coding region of the connexin 32 gene, indicating that production of mutated connexin 32 is not involved, or is a rare event, in gastric cancer development. In contrast, one out of seven chemical carcinogen-induced rat liver tumours contained a mutation in the cx32 gene (see Section 2.16.1.6).

The APC gene is responsible for the hereditary syndrome familial adenomatous polyposis. Loss of heterozygosity at APC/MCC genetic loci is frequent in colon cancer, and was found in two of our stomach tumour samples, suggesting the participation of these suppressor genes in the development of some gastric tumours.

In the human genome, there are sequences that contain (CA)n repeats, that are highly polymorphic. We have looked for possible genetic instability in tumours by amplification of these sequences in various chromosomes in normal and tumour tissue. One group of tumours showed no changes in microsatellite DNA, while a second group had a very high frequency of

genetic alteration in such sequences (of the order of 10^4 per genome), mainly loss of heterozygosity and less frequent appearance of new sequences (Figure 18).

Our results suggest that there are different mechanisms of tumour development or progression, one of which includes "explosive" genetic alteration affecting many loci at different chromosomes.

2.10 IARC staff publications

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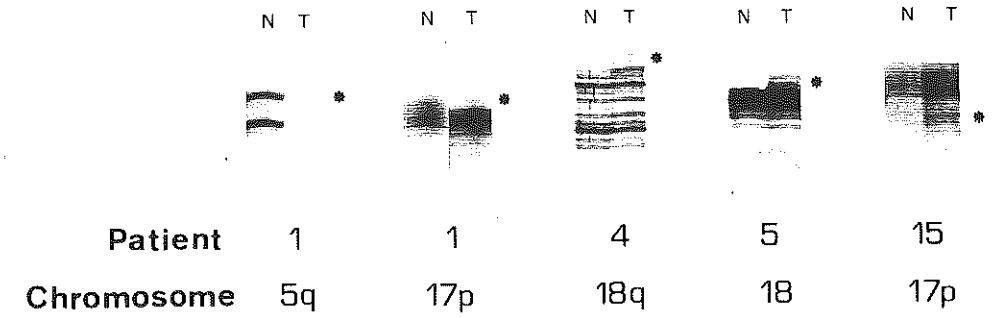

Figure 18. Examples of microsatellite instability in DNA of stomach tumours.

N–normal mucosa; T–tumour. Asterisks show loss at microsatellite sequence (patients 1) or appearances of sequence absent in normal tissue (patients 4, 5 and 15).

2.11 *Cancer of the liver*

Several studies are aimed at understanding the importance and interactions of various etiological factors in human liver carcinogenesis. The work is approached by making precise measurements of individual human exposure and by using both animal models (hepatitis B virus (HBV) transgenic mice, Pekin duck) and field studies to elucidate the mechanism of interaction between HBV and aflatoxin.

Certain other studies are examining gap-junctional intercellular communication in normal and tumorous liver tissue (Section 2.1.6.1), genetic variation in carcinogen-metabolizing liver enzymes (Sections 3.1.2 and 3.1.3) and DNA damage in vinyl chloride-associated liver angiosarcoma (Sections 2.8.3 and 5.1.1). Evaluating liver cancer prevention by prevention of infection with hepatitis B virus is the main aim of the Gambia Hepatitis Intervention Study (Section 4.1).

2.11.1 Cohort study of hepatocellular carcinoma in HBsAg carriers in Thailand

(N. Muñoz, F.X. Bosch, M. Benz and J. Estève; in collaboration with S. Puribahat and P. Srivatanakul, Bangkok, Thailand)

The purpose of this project is to identify liver cancer cases developing within a cohort of subjects identified as carriers of the hepatitis B virus surface antigen (HBsAg) in Bangkok and to describe their exposure to selected risk factors for liver cancer. A set of controls matched to the cases by age, sex and period of recruitment will be identified within the cohort. Active follow-up is being conducted at regular intervals for five years and blood and urine specimens are being collected and stored. Biological markers of exposure to aflatoxin and other environmental carcinogens will be measured in these specimens. Exposure to other risk factors such as tobacco smoking and alcohol is assessed through questionnaires. Individuals with normal liver function are visited yearly and every 3–6 months if any abnormality is detected.

Follow-up of the first subjects recruited into the cohort is now completing its fifth year. Up to December 1992, 2025 subjects had been recruited, of whom 503 have been followed twice, 301 three times, 248 four times and 480 more than five times. 64 subjects have completed the five-year follow-up.

Thirty-five cases of liver cancer and four cases of liver haemangioma have been identified. The data and the urine and serum specimens are regularly transferred to Lyon. A mail-based follow-up system is being implemented to encourage continuous participation.

2.11.2 Follow-up of a cohort of HBsAg-positive blood donors in Catalonia

(F.X. Bosch and M. Benz; in collaboration with V. Moreno, J. Ribes and A. Plasència, Barcelona, Spain)

A cohort of 2515 HBsAg carriers (1772 males, 742 females) was identified among blood donors at five major blood banks in the area of Barcelona in Spain. Passive follow-up has been conducted by record linkage with the mortality files to 1990. For both sexes combined, an excess mortality due to liver cirrhosis has been demonstrated (SMR = 220, 95% C.I. 101–418). In males, one case of liver cancer was found against 0.81 expected. A second follow-up is now taking place using mortality data up to 1992–93.

A new protocol has been prepared to conduct an active follow-up of 555 HBsAg carriers identified by the blood bank of one of the collaborating centres, Ciutat Sanitaria i Universitaria de Bellvitge. Cases of chronic liver disease will be identified and compared with controls in relation to alcohol and tobacco consumption and to the prevalence of selected viral markers.

2.11.3 Epidemiology of cholangiocarcinoma in Thailand

(D.M. Parkin, P. Pisani, H. Ohshima and M. Lang (in collaboration with V. Vatanasapt and S. Sriamporn, Khon Kaen, Thailand)

A cohort study has been started in north-eastern Thailand to clarify the role of diet as a source of carcinogens (aflatoxin, nitrate and nitrosamines) and protective agents (some vitamins and anti-oxidants) and as a vehicle for *Opisthorchis viverrini* (OV) infection in the etiology of cholangiocarcinoma (CCA) and hepatocellular carcinoma (HCC), in a population at very high risk of CCA. Recruitment of the cohort of 10 000 people exploits a screening programme offered to the whole population of the region. Information on betel-nut chewing (Figure 19), usual dietary intake, tobacco and alcohol consumption, together with socio-demographic variables, is being collected through a personal interview. Blood and

Figure 19. Preparation of betel nut in a market in Khon Kaen, Thailand

urine samples will be stored for study of hepatitis B and C infection, intake of aflatoxins and antibodies to OV.

During the first year of recruitment, 1300 subjects were enrolled. A sample of the information obtained through questionnaires has been analysed in order to describe the demographic characteristics and personal habits of the population. This information allows the selection of small groups with particular characteristics (e.g., highly infected with OV vs. non-infected) for cross-sectional studies on markers of endogenous nitrosation, on the expression of some P450 enzymes and on exposure to alkylating agents.

2.11.4 Human exposure to aflatoxin and its association with genetic damage

(C.P. Wild, B. Chapot, R. Montesano, M. Hollstein, S. Chutimataewin and O. Ogunbiyi; in collaboration with A. Abbondandolo, Genoa, Italy; M. Diallo, Conakry, Guinea; A.J. Hall, London, UK; P. Srivatanakul, Bangkok, Thailand; and H. Whittle, Fajara, The Gambia)

Further determination of human exposure to aflatoxin has continued using the aflatoxin–albumin adduct as a marker in new studies in The Gambia, Nigeria, Nepal, Egypt and Guinea and this approach has provided considerable information regarding the exposure of various populations. In The Gambia, measurements of adduct levels in non-vaccinated children from the GHIS study have been made.

The level of genetic damage (micronuclei, chromosome aberrations, sister chromatid exchanges) in peripheral blood cells in 36 Gambian individuals was assessed in relation to exposure to aflatoxin and HBV and a genetic polymorphism in glutathione *S*-transferase (GSTM1). More than 90% of the individuals were exposed to aflatoxins but there was no correlation with any of the markers of genetic damage examined. Analyses of mutation spectra in marker genes (e.g., *hprt*) in peripheral blood lymphocytes from these same individuals could permit a more specific association with aflatoxin exposure to be made.

In a second study in collaboration with the National Cancer Institute, Bangkok, Thailand, aflatoxin–DNA adducts in surgical tissue samples from 15 hepatocellular carcinoma cancer

patients in Thailand were analysed. No adducts were detected, a finding consistent with the low frequency of detection of serum aflatoxin–albumin adducts in Thailand and with the low prevalence of p53 mutations at codon 249 in this set of tumours (Hollstein *et al.*, 1993).

2.11.5 Aflatoxin exposure and metabolism, HBV infection and liver enzymes

(C.P. Wild, B. Chapot and R. Montesano; in collaboration with F. Donato, Brescia, Italy; A. J. Hall, London, UK; H. Whittle and M. Fortuin, Fajara, The Gambia; and C.R. Wolf, Edinburgh, UK)

A group of 117 Gambian children were studied to assess the relationship between (*a*) HBV infection, liver injury and aflatoxin–albumin adducts and (*b*) GSTM1 genotype and aflatoxin–albumin adducts.

All but two children showed detectable aflatoxin–albumin adducts, with levels ranging over two orders of magnitude. HBV carriers showed moderately higher levels of adduct than non-carriers, but the difference was not statistically significant. However, there was a relatively weak but highly significant positive correlation between aflatoxin–albumin adducts and serum transaminases, which was seen both with and without inclusion of HBV carriers in the analysis. This association could be the result of the hepatotoxicity of aflatoxin, but the data are also consistent with the hypothesis that liver damage caused by HBV and/or other factors can alter aflatoxin metabolism resulting in an increased binding to cellular macromolecules including DNA. This hypothesis is being further investigated in appropriate animal models (see below).

The frequency of the GSTM1 gene deletion (17%) was far lower in The Gambia than in European or American populations. In addition, the null genotype was significantly less frequent in some ethnic groups than others within the population. However, no association was observed between aflatoxin–albumin adduct level and the GSTM1 genotype, suggesting that this enzyme is not a major factor in determining the level of adduct formation in this population.

A second genotyping assay for a common mutation (Gough *et al.*, 1990) in CYP2D6 (debrisoquine hydroxylase) was applied to subjects from The Gambia. Among 48 subjects, five had this mutation (three heterozygotes and two homozygotes). It remains to be determined how important this specific mutation is in determining the phenotype in an African population. Studies of other polymorphisms or interindividual variations in expression of enzymes, including CYP3A4, involved in aflatoxin metabolism are being continued.

It is therefore possible to measure aflatoxin and HBV exposure and to determine genotype and/or phenotype for specific metabolizing enzymes in the same individual. This provides an opportunity to examine, in the field, the importance of specific isoenzymes in the metabolism of aflatoxin and the hypothesis that expression of those enzymes is modulated by HBV infection and/or other causes of liver injury.

2.11.6 Aflatoxin-metabolizing enzymes in liver cancer patients

(C.P. Wild, B. Chapot, G. Kirby, R. Montesano and S. Chutimataewin; in collaboration with G.E. Neal, Carshalton, UK; P. Srivatanakul, Bangkok, Thailand; and C.R. Wolf, Edinburgh, UK)

The expression of drug-metabolizing enzymes has been examined in surgically excised liver tissue from the series of Thai liver cancer patients in whom aflatoxin–DNA adduct levels were measured (see above). The levels of cytochrome P450s were lower in tumour tissue than in surrounding normal tissue. The level of CYP3A4 correlated with the *in vitro* activation of

aflatoxin by microsomes from the same samples. The levels of the μ and α forms of glutathione *S*-transferase were generally reduced in the tumours, but the π isoenzyme was increased. The studies will be pursued to identify specific P450s and GSTs involved in aflatoxin metabolism (Kirby *et al.*, 1993).

2.11.7 Experimental studies of aflatoxin metabolism and carcinogenicity

(C.P. Wild, B. Chapot, I. Chemin, G. Kirby, R. Montesano, S. Chutimataewin, M. Lang and L. Barraud)

2.11.7.1 *Hepatitis B transgenic mice*

(in collaboration with F. Chisari, La Jolla, CA, USA)

In parallel with the field studies mentioned above, transgenic mice carrying the human HBV genome are being studied to determine whether the presence of the virus and the associated liver injury are associated with altered metabolism of aflatoxin. Cytochrome P450 Cyp2a-5 was first shown to be important in aflatoxin metabolism in the mouse. The level of this isoenzyme was examined by immunohistochemistry and *in vitro* metabolism of aflatoxin. Cyp2a-5 showed a marked increase in hepatocytes of transgenic mice in parallel to development of liver injury, which is caused by overexpression of the HBV large envelope polypeptide in this lineage. At one month of age, isolated hepatocytes around the central veins were stained strongly with anti-Cyp2a-5 antibody and at 9 and 12 months such cells were widely distributed in the hepatic parenchyma. No alteration in expression of glutathione *S*-transferases was observed in transgenic mice compared with non-transgenic litter mates. These results suggest that liver injury can alter expression of P450 isozymes involved in carcinogen metabolism and work is continuing to examine the specificity of this effect (see also Section 3.1.3).

2.11.7.2 *Pekin duck*

(in collaboration with L. Cova, Lyon, France)

Ducks from two regions of China (Qidong and Shanghai) were examined for the presence of HBV infection, HBV integration into tumours, p53 mutations and the presence of aflatoxin–DNA adducts in the liver. Liver tumours were seen in Qidong in the absence of HBV infection. The presence of aflatoxin–DNA adducts in one of these livers, plus the frequent occurrence of biliary proliferation, suggests a role for aflatoxin in the development of liver cancer in these ducks (Cova *et al.*, 1993). An examination for mutations in exon 7 of the p53 gene, including codon 249, did not reveal the presence of any mutation in the tumours available. It should be noted that the base sequence around the codon 249 hot spot is markedly different in the human and duck p53 genes.

2.11.7.3 *Comparative carcinogenicity*

(in collaboration with N. Ito and R. Hasegawa, Nagoya, Japan)

Three strains of rat and one strain each of mouse, hamster and guinea-pig were treated with aflatoxin daily by gastric intubation for 14 days. Aflatoxin–albumin adducts in serum and aflatoxin–DNA adducts in liver were determined at days 1, 3, 7 and 14. Levels of albumin adducts were dose-related and increased with time in each species and strain. The levels were higher in rats, followed by guinea-pigs, hamsters and mice. Thus, the adduct levels reflected the susceptibility to carcinogenesis between species. However, there were no significant differences between rat strains in adduct levels even though there are reported differences in sensitivity to hepatocarcinogenicity. Aflatoxin–DNA adduct data are still being analysed but

preliminary results indicate a concordance between the albumin and DNA adduct data. The same biomarkers have been measured in human populations and this type of approach may improve attempts at cross-species extrapolations and at quantitative risk assessment.

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2.12 *Cancer of the cervix*

A number of international collaborative efforts in this area have been conducted and/or coordinated by IARC, entailing the linkage of the latest tools of molecular biology for detection of biological agents, especially human papillomaviruses, with the most advanced epidemiological methodology.

2.12.1 Case–control studies of cervical cancer in Spain and Colombia

(N. Muñoz, F.X. Bosch, S. de Sanjosé, D. Magnin and M. Rosato; in collaboration with P. Alonso de Ruiz, Mexico City, Mexico; N. Aristizabal and L. Tafur, Cali, Colombia; N. Ascunce and M. Santamaria, Pamplona, Spain; I. Izarzugaza, Vitoria-Gasteiz, Spain; M. Gili, Seville, Spain; L.C. Gonzalez, Salamanca, Spain; E. Guerrero, Madrid, Spain; I. Lind, Copenhagen, Denmark; C. Navarro and M.J. Tormo, Murcia, Spain; C. Martos and P. Moreo, Zaragoza, Spain; J. Orfila, Amiens, France; K.V. Shah, Baltimore, MD, USA; P. Viladiu, Hospital Sta Catarina, Gerona, Spain; V. Vonka, Prague, Czech Republic; and B. Wahren, Stockholm, Sweden)

Four concurrent case–control studies were organized to evaluate risk factors for cervical cancer in Colombia, a country at high risk for cervical cancer (age-adjusted incidence rate 48 per 100 000) and in Spain, a country at low risk (age-adjusted incidence rates between 5 and 10 per 100 000). Two of the studies included cases of invasive cervical cancer and two included cases of CIN III.

All incident cases of cervical neoplasia occurring in pre-defined populations were identified and invited to participate before treatment. Controls for invasive cancers were a representative sample of the residents of the province or city. Controls for the cases of CIN III were recruited among women participating in screening programmes or providing a cytological specimen for any other reason. For each case one control was selected, matched by age, date of smear-taking and centre, and was eligible if her smear showed no sign of

cervical neoplasia. Husbands of cases and controls were also invited to participate. The total number of subjects interviewed was close to 3000 (918 cases, 912 controls and 1073 husbands of cases and controls).

Exposure to common sexually transmitted agents was assessed by serological assays for herpes simplex virus types 1 and 2 (HSV 1, 2), hepatitis B virus (HBV) and cytomegalovirus (CMV) (Dr B. Wahren, Stockholm), for *Chlamydia trachomatis* (Dr J. Orfila, Amiens) and for syphilis and gonorrhoea (Dr I. Lind, Copenhagen). The presence of human papillomavirus (HPV) was determined by hybridization tests on DNA from exfoliated cells from the cervix and the penis. Three different assays were used, the commercially available ViraPap®, Southern blot and hybridization (dot blot or Southern blot) following HPV DNA amplification using the polymerase chain reaction (PCR) technique.

The statistical analysis of the results is well advanced. The study of invasive cervical cancer has been largely completed (Muñoz *et al.*, 1992; Bosch *et al.*, 1992; Guerrero *et al.*, 1992; Müller *et al.* 1992). The main results of the study of CIN III lesions are being published (Muñoz *et al.*, 1993; Bosch *et al.*, 1993).

The results confirm the central role of HPV in the causation of cervical cancer and CIN III. Table 12 shows the prevalence of HPV DNA in cervical cells, based on PCR analysis, and the risk estimates in both countries.

Using additional tissue specimens from cases, the prevalence of HPV DNA increased from about 70% to 85%. There were no differences in the HPV DNA prevalence between cases of invasive cervical cancer and cases of CIN III lesions or between cases in Spain and cases in Colombia. Among controls, the HPV DNA prevalence was 2–3 times higher in Colombia. HPV 16 was the most prevalent type among cases in both countries and also conveyed the strongest risk. Table 13 shows the ORs for HPV 16, 18, 31, 33, 35 and of unknown type.

Comparison of three different hybridization methods for HPV DNA detection (ViraPap, Southern blot and PCR) confirmed the higher sensitivity of PCR-based technology (Guerrero *et al.*, 1992).

Table 12. Percentage of HPV-positive cases (detected by PCR assay) and controls and odds ratios (OR) for the association between HPV and cervical cancer in Spain and Colombia

Type of lesion	Spain			Colombia		
	HPV-positive (%)		OR	HPV-positive (%)		OR
	Cases	Controls	(95% CI)	Cases	Controls	(95% CI)
Invasive cancer[a]	69.0	4.6	46.2 (18.5–115.1)	72.4	13.3	15.6 (6.9–34.7)
CIN III[b]	70.7	4.7	56.9 (24.8–130.6)	63.2	10.5	15.5 (8.2–29.4)

[a] Odds ratio adjusted for age, study centre, number of sexual partners, age at first birth, education level and Pap smears

[b] Odds ratio adjusted for age, study centre, number of sexual partners, age at first sexual intercourse and number of sexual partners of the husband (in Spain), and antibodies to *C. trachomatis* and smoking (in Colombia)

Table 13. Risk of cervical cancer according to HPV type (detected by PCR assay) in Spain and Colombia

	OR[a] for specific types of HPV			
	16	18	31, 33, 35	Unknown type
Spain				
Invasive cancer	44.8	5/0	13.8	16/0
CIN III	295.5	1/0	28.9	18.7
Colombia				
Invasive cancer	14.9	8.3	31.3	20.6
CIN III	27.1	0/0	23.4	12.0

[a] Odds ratios adjusted as indicated in Table 12

The analyses of risk factors other than HPV for invasive cervical were difficult to interpret because of the recognized underdetection of HPV among cases based on cytological specimens. It was not possible to assess the level of HPV underdetection among controls, since these did not provide biopsies. With this limitation, the results of the study suggest an independent effect for low educational level (invasive cancer only), number of sexual partners, early age at first birth and early age at first intercourse. A protective effect was found for previous screening and for Caesarean section.

To explore risk factors for progression from HPV carrier to CIN III and invasive cancer, some analyses were conducted including only the HPV-positive subjects. Ever use of oral contraceptives was the only exposure related to invasive cervical cancer (OR = 6.5, 95% CI 1.3–31.4) with non-users as reference, and early age at first sexual intercourse was the only independent risk factor for CIN III (ORs = 1.0, 1.3, 6.0 for ages at first intercourse of 20+, 18–19, <18; p for trend = 0.003). There was a strong suggestion that HPV and oral contraceptives interact in the causation of cervical cancer.

The biological specimens collected are being used in the development and testing of new HPV serological methods: an ELISA-based assay with four HPV 16 E6-E7 peptides and a newly developed transcription and translation radioimmunoprecipitation assay (TT-RIPA). Antibodies to these peptides were detected in 50% of sera from women with HPV 16-positive cervical cancer. In contrast, the assay showed very low sensitivity in HPV 16-positive CIN III lesions. The prevalence of antibodies among controls (serum from women with normal cervix or with HPV-negative CIN lesions) was 3% (Müller *et al.*, 1992).

p53 mutations were investigated in a selected sample of biopsies collected among cases of invasive cervical cancer. Unexpectedly a missense mutation of p53 was found in one out of 29 HPV-positive cases, and no p53 mutation in the six HPV-negative cases. The absence of p53 mutations in HPV-negative cases prompted an assessment of tumours for *mdm2* gene amplification. The *mdm2* gene encodes a p53-binding protein and has been found to be amplified in some human tumours lacking p53 mutations. None of 16 tumours (including 4 HPV-negative cases) studied showed amplification of the *mdm2* gene (Kessis *et al.*, 1993).

As an extension of this project, all cases of invasive cervical cancer have been followed up to December 1992 to assess the vital status of the patients and to collect additional clinical information such as stage of the disease at the time of diagnosis, presence of recurrences and some indications on the treatment used. The analysis of this group of over 400 cases will allow

the evaluation of selected HPV markers as prognostic factors for cervical cancer recurrence and survival.

2.12.2 Multicentre case–control study of cancer of the cervix

(N. Muñoz, F.X. Bosch, S. de Sanjosé, M. Rosato and D. Magnin; in collaboration with S. Bayo, Bamako, Mali; N. Chaouki, Rabat, Morocco; S. Chichareon, Hat Yai, Thailand; J. Eluf-Neto, São Paulo, Brazil; C. Ngelangel, Manila, Philippines; P.A. Rolón, Asunción, Paraguay; K.V. Shah, Baltimore, MD, USA; and J. Walboomers and C. Meijer, Amsterdam, The Netherlands)

This multicentre study aims at exploring risk factors for cervical cancer in areas of the world where the incidence of the disease is high and in which very few studies have been completed. Particular attention is devoted to aspects of sexual behaviour and other risk factors for which the epidemiological evidence is limited or contradictory. These include the role of the male as a vector of the relevant sexually transmitted agent(s), the implications of the practice of prostitution by women and the use of prostitutes by men, the use of oral contraceptives and the role of other sexually transmitted agents.

The study in Brazil has been completed (Eluf-Neto *et al.*, 1993), and shows a strong association between HPV DNA and invasive cervical cancer (OR = 37.1, 95% CI 19.6–70.4). HPV 16 was the predominant viral type and showed the strongest relationship with invasive cervical cancer (OR 74.9, 95% CI 32.5–172.7). After taking into account the strong effect of HPV, high parity remained as an additional risk factor and history of previous smears as a protective factor. The results restricted to HPV-positive cases and controls (Table 14) suggest that hormonal factors are risk factors for progression from HPV carrier state to invasive carcinoma.

Table 14. Odds ratios for invasive cervical cancer among women positive for HPV DNA in Brazil

	Cases	Controls	OR (95% CI)
Parity			
0–1	9	5	1.0
2–3	24	9	2.2 (0.4–11.8)
4–5	34	9	2.2 (0.4–12.1)
6–7	24	4	3.8 (0.5–29.2)
8–9	26	3	5.4 (0.8–37.1)
> 10	37	2	12.8 (1.5–109.7)
			p for trend = 0.008
Oral contraceptive use (years)			
None	97	21	1.0
1–4	30	9	1.2 (0.4–4.2)
> 5	27	2	9.0 (1.4–57.4)
			p for trend = 0.02

Serum specimens collected in this study were analysed for HPV antibodies against E6 and E7 of HPV 16 also by the TT-RIPA assay, which has higher sensitivity than assays based on linear epitopes and allows quantitation of the intensity of the reaction. The results suggest that the presence of E6 antibodies is related to the clinical stage and thus could be used as a

prognostic marker. The prevalence of seroreactivity to E7 seems to decrease with age, suggesting that it could be regarded as an insensitive marker of chronic or persistent HPV infection.

In Paraguay, biopsy specimens were collected from 127 women with invasive cervical cancer and exfoliated cells from 118 control women. Preliminary results of hybridization assays indicate that a high proportion of the specimens are inadequate, as 20% of case specimens and 64% of control specimens were negative for β-globin amplification. The HPV prevalence among those with β-globin amplification was 83.3% in cases and 19% in controls, giving a crude OR of 21.3 (95% CI 7.7–60.3). Among cases, HPV 16 was the most common type (68.2%) and among eight HPV-positive controls, three were HPV 16 and five were uncharacterized types.

Data and specimen collection is complete in the Philippines and Thailand and will continue in Mali and Morocco throughout 1993. The total number of women recruited within the six areas will be around 2800 and about 35% are also expected to contribute information and specimens from their husbands. The laboratory work is beginning, using the PCR-based hybridization methods developed in the Free University of The Netherlands.

2.12.3 International prevalence survey of human papillomavirus markers in cervical cancer tissue and sera

(F.X. Bosch, N. Muñoz and D. Magnin; in collaboration with E. Alihonou, Cotonou, Bénin; S. Bayo, Bamako, Mali; N. Chaouki, Rabat, Morocco; H. Cherif Mokhtar, Sétif, Algeria; S. Chichareon, Hat-Yai, Thailand; A. Daudt, Pelotas, Brazil; E. de los Rios, Panama; P. Ghadirian, Montréal, Canada; J.N. Kitinya, Dar es Salaam, Tanzania; M. Koulibaly, Conakry, Guinea; M. Manos, M. Sherman and R. Kurman, Baltimore, MD, USA; C. Ngelangel, Manila, the Philippines; J. Peto, Sutton, UK; Ll. M. Puig Tintoré, Barcelona, Spain; J.L. Rios-Dalenz, La Paz, Bolivia; Sarjadi, Semarang, Indonesia; M. Schiffman, Bethesda, MD, USA; A. Schneider, Ulm, Germany; L. Tafur, Cali, Colombia; A.R. Teyssie, Buenos Aires, Argentina; P.A. Rolón, Asunción, Paraguay; M. Torroella, Habana, Cuba; A. Vila Tapia, Concepción, Chile; H.R. Wabinga, Kampala, Uganda; and W. Zatonski, Warsaw, Poland)

The field part of this study is complete. A total of 1069 subjects are distributed across 22 countries (Table 15). A Steering Committee (comprising representatives of IARC, the UK Cancer Research Campaign, Johns Hopkins University, Baltimore and the US National Cancer Institute) has been formally established to overview the use of the specimens and to advise on the establishment of collaborations with relevant laboratories. The original biopsy slides provided by the collaborators have been reviewed by pathologists with expertise in cervical cancer (M. Sherman and R. Kurman, Johns Hopkins University). Most cases were confirmed as invasive cancer and the histological type and subtype have been recorded. New slides are being prepared for cases in which the material was insufficient to confirm the diagnosis. HPV DNA testing has been initiated at the Johns Hopkins University. Preliminary results indicate that over 85% of the specimens are HPV DNA-positive. HPV 16 was the most common type overall (55%) followed by HPV 18 (14%), HPV 45 (11%) and HPV 31 (5%). The distribution of the various HPV types varied substantially between countries.

Protocols are being prepared for the study of gene mutations in HPV-negative specimens and of HPV integration, and for serological studies.

Table 15. Numbers of subjects and of specimens collected in the the International Biological Study on Cervical Cancer, by country

Country	Questionnaires	Histology slides	Frozen cells	Serum
Algeria	42	20	41	26
Argentina	64	64	61	64
Benin	18	11	13	18
Bolivia	57	56	57	55
Brazil	50	50	50	50
Canada	50	41	51	38
Chile	108	109	85	105
Colombia	56	44	42	56
Cuba	51	51	51	47
Germany	–	18	17	–
Guinea	26	24	22	20
Indonesia	51	50	53	51
Mali	62	63	59	66
Panama	80	80	80	80
Paraguay	109	111	128	110
Philippines	30	30	30	30
Poland	28	25	25	29
Spain	56	56	49	52
Tanzania	51	51	52	51
Thailand	30	30	30	30
Uganda	50	50	48	46
USA	–	13	13	–
Total	1069	1047	1057	1024

2.12.4 Studies of high-risk groups for cervical cancer in Spain and Colombia

(S. de Sanjosé, N. Muñoz and F.X. Bosch; in collaboration with N. Aristizabal and L. Tafur, Cali, Colombia; and V. Palacio and S. Vazquez, Oviedo, Spain)

The relationship between CIN lesions and the prevalence of serological markers of sexually transmitted diseases has been analysed in a population of prostitutes in Spain (Palacio *et al.*, 1992; de Sanjosé *et al.*, 1993). HIV-positive prostitutes were at high risk of CIN as compared to HIV-negative prostitutes (OR = 12.7, 95% CI 3.9–40.9), or as compared to a group of non-prostitutes (OR = 14.2, 95% CI 4.8–42.4). No association was observed between CIN and positivity to serological markers of exposure to *C. trachomatis* and *Treponema pallidum*.

A comparison of the prevalence of CIN lesions in prostitutes and a control group of non-prostitutes in Spain and Colombia has also been completed. Prostitutes in Spain showed an increased risk for CIN lesions (mostly CIN I) (OR = 2.3, 95% CI 1.1–4.5) which was restricted to the women who were HIV-positive. Prostitutes in Cali showed an increased risk for CIN which was not statistically significant (OR = 1.8, 95% CI 0.9–3.5). The prevalence of CIN lesions was similar between non-prostitutes in Spain and in Cali.

2.12 IARC staff publications

Armstrong, B.K., Muñoz, N. & Bosch, F.X. (1992) Epidemiology of cancer of the cervix. In: Coppleson, M., Monaghan, J.M., Morrow, C.P. & Tattersall, M.H.N., eds, *Gynecologic Oncology*, Volume 1, 2nd Edition, Edinburgh, Churchill Livingstone, pp. 11–29

Bosch, F.X., de Sanjosé, S. & Muñoz, N. (1993) Epidémiologie des lésions précurseurs des cancers du col utérin et de la vulve. *Impact Médecin Hebdo* (in press)

Bosch, F.X., Muñoz, N., Sanjosé, S., Izarzugaza, I., Gili, M., Viladiu, P., Tormo, M.J., Moreo, P., Ascunce, N., Gonzalez, L.C., Tafur, L., Kaldor, J.M., Guerrero, E., Aristizabal, N., Santamaria, M., Alonso de Ruiz, P. & Shah, K. (1992) Risk factors for cervical cancer in Colombia and Spain. *Int. J. Cancer*, **52**, 750–758

Bosch, F.X., Muñoz, N., de Sanjosé, S., Navarro, C., Moreo, P., Ascunce, N., Gonzalez, L.C., Tafur, L., Gili, M., Larrañaga, I., Viladiu, P., Daniel, R.W., Alonso de Ruiz, P., Aristizabal, N., Santamaria, M., Guerrero, E., Shah, K.V. (1993a) Human papillomavirus and CIN III: a case-control study in Spain and Colombia. *Cancer Epidemiol. Biomarkers Prev.* (in press)

Bosch, F.X., Muñoz, N., de Sanjosé, S., Viladiu, P., Tormo, J., Puig, F., Ascunce, N., Gonzalez, L.C., Tafur, L., Gili, M., Muniozguren, N., Guerrero, E., Aristizabal, N., Santamaria, M., Alonso de Ruiz, P. & Shah, K.V. (1993b) Le rôle du virus du papillome humain dans l'étiologie du cancer du col utérin: Comparaison de deux pays à incidence élevée ou basse de cancer du col. *Gynécol. Int.*, **2**, 101–106

de Sanjosé, S., Santamaria, M., Alonso de Ruiz, P., Aristizabal, N., Guerrero, E., Castellsagué, X. & Bosch, F.X. (1992) HPV types in normal cervical cytology. In: Muñoz, N., Bosch, F.X., Shah, K.V. & Meheus, A., eds, *The Epidemiology of Cervical Cancer and Human Papillomavirus* (IARC Scientific Publications No. 119), Lyon, IARC, pp. 77–85

de Sanjosé, S., Palacio, V., Tafur, L., Vazquez, S., Espitia, V., Vazquez, F., Roman, G., Muñoz, N. & Bosch, F.X. (1993) Prostitution, HIV and cervical neoplasia: a survey in Spain and Colombia. *Cancer Epidemiol. Biomarkers Prev.* (in press)

Eluf-Neto, J., Booth, M., Muñoz, N., Bosch, F.X., Meijer, C.J.L.M. & Walboomers, J.M.M. (1993) Human papillomavirus and invasive cervical cancer in Brazil. *Br. J. Cancer* (in press)

Guerrero, E., Daniel, R.W., Bosch, F.X., Castellsagué, X., Muñoz, N., Gili, M., Viladiu, P., Navarro, C., Martos, C., Ascunce, N., Gonzalez, L.C., Tafur, L., Izarzugaza, I. & Shah, K.V. (1992) Comparison of Virapap, Southern hybridization and polymerase chain reaction methods for human papillomavirus identification in an epidemiological investigation of cervical cancer. *J. Clin. Microbiol.*, **30**, 2951–2959

Kessis, T.D., Slebos, R.J., Han, S.M., Shah, K., Muñoz, N., Hedrick, L. & Cho, K.R. (1993) P53 gene mutations and MDM2 amplification are uncommon in primary carcinomas of the uterine cervix. *Am. J. Pathol.* (in press)

Müller, M., Viscidi, R.P., Sun, Y., Guerrero, E., Hill, P.M., Shah, F., Bosch, F.X., Muñoz, N., Gissmann, L. & Shah, K.V. (1992) Antibodies to HPV-16 E6 and E7 proteins as markers for HPV-16-associated invasive cervical cancer. *Virology*, **187**, 508–514

Muñoz, N. & Bosch, F.X. (1992) HPV and cervical neoplasia: review of case-control and cohort studies. In: Muñoz, N., Bosch, F.X., Shah, K.V. & Meheus, A., eds, *The Epidemiology of Cervical Cancer and Human Papillomavirus* (IARC Scientific Publications No. 119), Lyon, IARC, pp. 255–266

Muñoz, N., Bosch, F.X., de Sanjosé, S., Tafur, L., Izarzugaza, I. Gili, M., Viladiu, P., Navarro, C., Martos, C., Ascunce, N., Gonzalez, L.C., Kaldor, J.M., Guerrero, E., Lörincz, A., Santamaria, M., Alonso de Ruiz, P., Aristizabal, N. & Shah, K. (1992) The causal link between human papillomavirus and invasive cervical cancer: a population-based case-control study in Colombia and Spain. *Int. J. Cancer*, **52**, 743–749

Muñoz, N., Bosch, F.X., de Sanjosé, S., Vergara, A., del Moral, A., Muñoz, M.T., Tafur, L., Gili, M., Izarzugaza, I., Viladiu, P., Navarro, C., Alonso de Ruiz, P., Aristizabal, N., Santamaria, M., Orfila, J., Daniel, R.W., Guerrero, E. & Shah, K.V. (1993a) Risk factors for CIN III in Spain and Colombia. *Cancer Epidemiol. Biomarkers Prev.* (in press)

Muñoz, N., Bosch, F.X., de Sanjosé, S., Viladiu, P., Tormo, J., Moreo, P., Ascunce, N., Gonzalez, L.C., Tafur, L., Gili, M., Izarzugaza, I., Guerrero, E., Aristizabal, N., Santamaria, M., Alonso de Ruiz, P. & Shah, K.V. (1993b) El virus del papilloma humano en la etiología del cáncer cervicouterino. *PAHO Bull.* (in press)

Muñoz, N., Bosch, F.X., de Sanjosé, S. & Shah, K.V. (1993c) The role of HPV in the etiology of cervical cancer. *Mutat. Res.* (in press)

Palacio V., de Sanjosé, S., Vazquez, S., Puente, M., Bosch, F.X. & Vazquez, F. (1992) Cervical neoplasia and sexually transmitted diseases among prostitutes in Oviedo, Spain (letter). *Int. J. STD AIDS* (in press)

2.13 *Cancer of the breast*

Breast cancer etiology is still an area of much uncertainty. Nutritional factors thought to be involved are being considered in several projects, reported in Section 2.3. Since breast cancer has been observed to have a familial component, the genetic study of the disease has been actively pursued and a gene that appears to be responsible has been localized on chromosome 17 (see Section 3.3.4).

A pilot study of breast cancer screening using physical examination only has been conducted in the Philippines (Section 4.4.1).

2.13.1 Breast cancer and reproductive and endocrine factors in premenopausal Chinese women

(A.J. Sasco, E. Riboli and R. Saracci; in collaboration with M.X. Hu and L. Qing, Guangzhou, China; and S. Stellman, New York, USA)

The aim of this study is to evaluate the relationship between hormonal profiles and breast cancer incidence in women in a low-risk population.

The study is using a case–control approach. Incident cases, all of them pre-menopausal, have been pair-matched to control women on the basis of age and residence. Women taking contraceptive pills or any other hormonal treatment, reserpine or tranquillizers were excluded, as well as women having or having had in the preceding twelve months a pregnancy (whether carried to full term or ending in a spontaneous or induced abortion), women having lactated in the preceding six months and women having documented hormonal disease, gynaecological conditions or chronic debilitating conditions.

A questionnaire administered to cases and controls covered the following items: personal identification data, details of diagnosis, reproductive and contraceptive life history, personal history of diseases, family history of cancer, diet history and other factors. Saliva and blood specimens were collected between days 20 and 24 of the menstrual cycle.

The analysis of the questionnaire data yielded similar results to those obtained in studies conducted in western populations. Breast cancer risk is increased among women with a higher level of education (OR = 3.4 for university education) or with a high-level occupation (OR = 3.6 for professionals). Women born to older mothers are at increased risk (OR = 3.0 for mothers older than 35 years at the subject's birth), but risk is diminished if the woman was breast-fed. A lower risk is also associated with late menarche (OR = 0.35 for menarche above 17 years of age), irregular menstrual cycles and marked periovulatory symptomatology. Risk is increased for late age at birth (OR = 1.8 for above 30 years of age), a history of spontaneous abortions or difficult pregnancies (Sasco & Qing, 1992). Determinations of hormonal levels in the biological samples collected are still to be carried out.

2.13.2 European case–control study of male breast cancer

(A.J. Sasco and R. Saracci)

A protocol has been finalized for an international case–control study to evaluate the role in male breast cancer etiology of reproductive life, personal history of diseases and drug use,

family history of cancer, tobacco and alcohol consumption, nutritional habits, body build and hepatic function. Evaluation of the etiological role of hormones is of particular interest.

The descriptive and analytical epidemiology of male breast cancer has been reviewed. The most noticeable feature is a relatively high incidence of breast cancer among black men, in particular in some African countries, but also in the USA. A meta-analysis of the seven case–control studies of male breast cancer published up to mid-1992 demonstrated an association of male breast cancer risk with marital status, religion, past history of benign breast pathology, gynaecomastia, past history of testicular or liver disease and a positive family history of breast cancer (Sasco *et al.*, 1993).

2.13.3 Survey of breast cancer in the Rhône 'département'

(A.J. Sasco; in collaboration with B. Fontanière, J. Fabry and V. Sciortino, Lyon, France)

No population cancer registry exists for the Rhône 'département'. A comprehensive survey of all treatment institutions, anatomopathological laboratories and social security claims has demonstrated a high incidence of breast cancer in this part of France (80.5 new cases per 100 000 woman-years) (Sasco *et al.*, 1991). A population-based series of ten male breast cancer cases has also been described (Sasco & Fontanière, 1991). A study is now being set up to evaluate the population-based survival of breast cancer patients in the Rhône department.

2.13.4 Case–control study of endometrial cancer following breast cancer

(A.J. Sasco; in collaboration with the French network of cancer registries)

Several case reports (Mignotte *et al.*, 1992), a Swedish case–control study and follow-up results of curative trials of breast cancer with an anti-estrogen, tamoxifen, have indicated a possible increased risk of endometrial cancer among exposed women. As tamoxifen is being proposed as an agent for prevention of breast cancer among high-risk, healthy women, its potential carcinogenic risk urgently needs to be assessed (Sasco, 1991). A pilot study in the Rhône department has led to the proposal to extend the study through the network of French cancer registries and possibly the French Federation of Anti-Cancer Centres and maybe to other countries. This study will compare women who developed endometrial cancer after already having had breast cancer with control women who have only had breast cancer. The exposures of main interest will be treatment with tamoxifen, including consideration of daily dose and duration of treatment.

2.13 IARC staff publications

Coleman, M.P. & Reiter, R.J. (1992) Breast cancer, blindness and melatonin. *Eur. J. Cancer*, **28**, 501–503

Nectoux, J. & Parkin, D.M. (1992) L'épidémiologie du cancer du sein chez l'homme. *Bull. Cancer*, **79**, 991–998

Parkin, D.M. & Nectoux, J. (1991) The changing incidence of breast cancer. *Rev. Endocrine-Related Cancer*, **39**, 21–27

Pisani, P. (1992) Breast cancer: geographic variation and risk factors. *J. Environ. Pathol. Toxicol. Oncol.*, **11**, 313–316

Sasco, A.J. & Fontanière, B. (1991) A population-based series of ten male breast cancer cases (letter to the editor). *Eur. J. Cancer*, **27**, 1713

Sasco, A.J. & Qing, L. (1992) A case–control study of premenopausal breast cancer cases in Guangzhou, People's Republic of China. Abstracts of the XVII Congress of the European Society for Medical Oncology, Lyon. *Ann. Oncol.*, **3**, 60

Sasco, A.J., Fontanière, B., Charbaut-Lagarde, M.O., Kliebsch, U., Hamandjian, P., Cornu-Lugrin, A.E., Schnebelen, J.P., Sciortino, V. & Fabry, J. (1991) A systematic survey of breast cancer incidence in the 'département' of Rhône, France. *Eur. J. Cancer*, **27**, 1696–1701

Sasco, A.J., Lowenfels, A.B. & Pasker-de Jong, P. (1993) Epidemiology of male breast cancer. A meta-analysis of published case-control studies and discussion of selected etiological factors. *Int. J. Cancer*, **53**, 538–549

2.14 *Other specific cancers and risk factors*

2.14.1 Cancers of the pancreas, gallbladder and bile duct

(P. Boyle and R. Saracci; in collaboration with P. Baghurst, Adelaide, Australia; H.B. Bueno de Mesquita, Bilthoven, The Netherlands; P. Ghadirian, Montreal, Canada; G.R. Howe, Toronto, Canada; and W. Zatonski, Warsaw, Poland; A.J. McMichael, A.B. Miller and A.M. Walker continue to participate in this study group)

The principal hypotheses of this study, initiated as part of the SEARCH programme (a network of case–control studies), were that the risk of cancer of the pancreas would increase with increasing intake of dietary fat, alcohol, coffee and artificial sweeteners, and that there would be positive associations with cholecystectomy, pre-existing diabetes, and occupational exposure to certain chemicals. It was postulated that subjects with atopic allergies would have a reduced risk of the disease. Protein intake was evaluated in view of a demonstrated association between high intake and chronic stimulation of pancreatic enzyme production. Finally, associations with specific agents which stimulate the release of gut hormones, and notably cholecystokinin (pancreozymin), were evaluated, since laboratory-based investigations suggested that they may be of etiological importance.

The previously reported elevation in risk associated with cigarette consumption has been confirmed (see papers cited in the 1990–91 IARC Biennial Report; and Bueno de Mesquita *et al.*, 1991; Zatonski *et al.*, 1993; Boyle *et al.*, 1993). There was no association with intake of total fat or protein in the combined analysis (Howe *et al.*, 1992). The strong positive association with total energy intake was almost entirely due to the positive association with carbohydrate intake. In addition, there was a consistent positive association with reported dietary intake of cholesterol, and a consistent inverse association with a number of markers of fruit, vegetable and cereal intake, particularly dietary fibre and vitamin C. All of these associations were relatively strong, with risks between the highest and lowest quintiles of intake varying by a factor of more than two-fold. Studies in the single centres do not support a positive association with lifetime consumption of alcoholic beverages or coffee.

Data accumulating suggest that a history of atopic allergy for which a treatment was specified by the subject may protect against the development of cancer of the pancreas. In the Dutch data, there was no association with a history of cholecystectomy for any condition of the gallbladder after excluding subjects who underwent the procedure during the five-year period before diagnosis in cases and interview in controls (Bueno de Mesquita *et al.*, 1992b). In men, diabetes treated with insulin and diagnosed more than one year previously was positively associated with the risk of pancreatic cancer, but there was no association for diabetes treated with oral anti-diabetics or diet only. The data suggest that in women, early menarche and greater adult stature are positively associated with the risk of the disease (Bueno de Mesquita *et al.*, 1992c).

A remarkable family aggregation has been reported from Montreal (Ghadirian *et al.*, 1991) and the clinical characteristics of cases in Poland have been described (Zatonski *et al.*, 1991).

Analyses in single centres of cancer of the gallbladder (Zatonski *et al.*, 1992) and of the biliary tract (Moerman *et al.*, 1993) have been published.

The advantages of the multicentre approach — the assessment of consistency of associations between centres in which a standard protocol has been used, and where consistency is demonstrated, to combine data to obtain more precise measures of effect, or to combine data to investigate rare types or subtypes of tumour — have been well illustrated by this study.

2.14.2 Brain tumours in children

(J. Little and R. Saracci; in collaboration with M. McCredie, Kings Cross, Australia; N.W. Choi, Winnipeg, Canada; S. Cordier, Paris, France; B. Modan, Tel Hashomer, Israel; G. Filippini, Milan, Italy; R. Gurevicius, Vilnius, Lithuania; R. Peris-Bonet, Valencia, Spain; S. Preston-Martin, Los Angeles, CA, USA; E.A. Holly, San Francisco, CA, USA; B. Mueller, Seattle, WA, USA; and P. Boyle, Milan, Italy)

This study was initiated as part of the SEARCH programme, a network of case–control studies.

Experimental studies have shown that transplacental exposure to a variety of *N*-nitroso compounds in the rat produces neurogenic tumours in the offspring (Magee *et al.*, 1976). Neurogenic tumours have been produced in the mouse and the Syrian golden hamster by administration of *N*-ethyl-*N*-nitrosourea to the dam. These observations generated the primary hypothesis of this study, that brain tumours in children are associated with exposure to preformed *N*-nitroso compounds and their precursors, and that factors influencing the formation and metabolism of these compounds will be of etiological importance. Thus, the exposures under investigation include intake of foods with high concentrations of preformed nitrosamines and sodium nitrites, and of substances inhibiting nitrosation, of the mother during pregnancy and of the index child, and sources of secondary exposure to preformed nitrosamines, notably tobacco smoke, cosmetics and drugs. A second objective is to clarify associations with ionizing radiation and with specific genetic syndromes in the index child. Additional objectives are to test hypotheses related to (*a*) prenatal and postnatal exposure to barbiturates; (*b*) characteristics of the index pregnancy and birth; and (*c*) trauma to the child. Also, associations with a range of other factors with which relationships have been reported in previous studies, e.g. certain parental occupations or parental occupational exposures and family history of cancer, are being investigated.

The study is being carried out in Australia, Canada, France, Israel, Italy, Lithuania, Spain and the USA. Data collection has been completed in six of these countries, and is expected to be completed in all of them by late 1993. To date, in all of the centres combined, a total of just over 1200 cases and more than 1900 control subjects have been investigated.

2.14.3 Brain tumours in adults

(J. Little and R. Saracci; in collaboration with A. Ahlbom, Stockholm, Sweden; P. Boyle, Milan, Italy; N.W. Choi, Winnipeg, Canada; S. Cordier, Paris, France; R. Gurevicius, Vilnius, Lithuania; G. Howe, Toronto, Canada; J. McNeil, Melbourne, Australia; F. Ménégoz, Meylan, France; B. Modan, Tel Hashomer,

Israel; S. Preston-Martin, Los Angeles, CA, USA; P. Ryan, Adelaide, Australia; and J. Wahrendorf, Heidelberg, Germany)

This study was initiated as part of the SEARCH programme, a network of case–control studies.

In experimental studies in a variety of species, the acyl alkyl nitrosamides and related compounds are very powerful carcinogens for the nervous system. Thus, in common with the study of brain tumours in children, experimental data have provided the basis for the main hypothesis, namely that these tumours are associated with exposure to preformed *N*-nitroso compounds and their precursors, and to factors influencing the formation and metabolism of these compounds. In relation to this, diet, occupation, smoking habits, alcoholic beverage consumption, medication use and water source have been investigated. An additional objective is to investigate the association with a range of other factors for which relationships have been reported in previous studies, notably electromagnetic fields, head trauma, medical history and therapeutic drugs, a variety of occupations, exposure to farm animals, noise exposure, tobacco and alcohol consumption, and therapeutic and diagnostic exposure to ionizing radiation. Specific host factors are being considered by reference to genetic syndromes in the index subject and to the history in first-degree relatives of genetic syndromes and cancers of specific types.

Eleven centres in Australia, Canada, France, Germany, Israel, Lithuania, Sweden and the USA are included. Data collection is complete in all but one of the centres, where it is expected to be completed in late 1993. So far, over 2000 cases and 3000 control subjects have been investigated. Centre-specific analyses have been published for Adelaide (Ryan *et al.*, 1992a,b) and Heidelberg (Boeing *et al.*, 1993; Schlehofer *et al.*, 1990, 1992a,b). The role of toxoplasma antibodies was investigated in the centres in Australia (Adelaide and Melbourne) only (Ryan *et al.*, 1993). There was no association between glioma and antibody positivity to *Toxoplasma gondii*. However, a positive association between meningioma was found in the Adelaide study. The Melbourne study did not include cases with meningioma.

2.14.4 Case–control study of plantar melanoma in Paraguay

(D.M. Parkin and P. Pisani; in collaboration with P.A. Rolón, Asunción, Paraguay)

Data collection in this study was completed in 1992. Sixty-two cases and 248 controls have been interviewed. The use of shoes, history of trauma, thermal injury and presence of plantar naevi have been recorded for all subjects, as well as the history of exposure to UV light (important in cutaneous melanoma in European populations). Analysis will start during 1993.

2.14.5 Case–control study of soft tissue sarcoma and non-Hodgkin lymphoma in relation to exposure to herbicides in Viet Nam

(D.M. Parkin and M. Kogevinas; in collaboration with Nguyen Chan Hung and Cung Tuyet Anh, Ho Chi Minh City, Viet Nam; Le Cao Dai, Hanoi, Viet Nam; S. Cordier, Villejuif, France; M. Rafaël, Paris, France; J.M. Rivera-Pomar, Vizcaya, Spain; and S. Stellman, New York, USA)

During the second Indochina war, large quantities of herbicides contaminated with dioxins were sprayed onto the territory of what was, at the time, South Viet Nam. Most of this spraying took place in 1965–71, but because of the relatively long biological half-life of dioxins, human exposure will have been more prolonged. The objective of the study is to investigate whether any excess risk for two cancers — soft tissue sarcoma and non-Hodgkin lymphoma — exists. One hundred and fifty cases of each disease and two hospital controls per case will be

interviewed, and samples of blood and adipose tissue stored. Estimation of exposures is initially based on detailed residential history in relation to the known location, type and quantity of herbicide sprayed by US forces. If positive findings emerge, direct measurements of dioxins in adipose tissue of subjects will be made. A pilot study was completed in early 1993 and the main study has begun.

2.14.6 Genetic alterations in human oral cancer

(M. Hollstein, G. Martel-Planche, M. Laval and N. Lyandrat; in collaboration with S. Thomas, Brisbane, Australia; and D. Sidransky, Baltimore, MD, USA)

Several of the genetic alterations characteristic of oesophageal tumours are also typical of oral cancers, notably abnormalities of the p53 tumour-suppressor gene and amplification of the *erbB*, *int 2* and c-*myc* oncogenes. In Europe, North America and Japan, the major risk factors for both cancer types are tobacco and alcohol consumption. In some areas of the world where oral cancer is the most frequent malignant neoplasm, the predominant risk factor is betel quid chewing. In order to examine whether p53 mutation is important in betel-associated tumours and whether a unique pattern of gene damage occurs, we are examining a series of 40 squamous cell carcinomas of the oral cavity from betel quid chewers residing in Papua, New Guinea for tumour-specific changes of the p53 gene and protein. Results will be compared with those from a histologically similar set of oral carcinomas from North American patients.

2.14 IARC staff publications

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2.15 *Transplacental and multigeneration carcinogenesis*

No new long-term experiments on transplacental and multigeneration carcinogenesis have been undertaken. The molecular analysis of tumour and tissue samples from previously performed long-term animal experiments has been continued to study molecular mechanisms.

2.15.1 Early detection of H-*ras* oncogene activation in morphologically unchanged tissues of mice following transplacental exposure to DMBA

(A. Loktionov, O. Bertrand, H. Yamasaki and L. Tomatis)

We have previously demonstrated that activation of different oncogenes of the *ras* family detectable in tumours induced in mice by transplacental treatment with 7,12-dimethylbenz-[*a*]anthracene (DMBA) depends on the tissue of tumour origin (Loktionov *et al.*, 1990). We decided to test if such tissue-specificity could result from an initial tissue-associated targeting of the carcinogen-induced mutations; alternatively, the initial mutation pattern might be similar in all tissues, with progression-associated expansion of mutated cells occurring only in certain tissues. Preliminary results using an allele-specific PCR-based method show that DMBA-induced activation of H-*ras* can be detected as early as the first day of postnatal development of mice both in the liver (frequent activation of the H-*ras* gene in hepatocellular tumours) and in lung (H-*ras* is never activated in lung adenomas, while K-*ras* activation is common).

2.15.2 Possible role of oncogene/tumour-suppressor gene mutations in germ-line transmission of carcinogenic risk

(O. Bertrand, A. Loktionov, H. Yamasaki and L. Tomatis)

Germ-line mutation of the p53 tumour-suppressor gene may be responsible for an increased risk for cancer in humans (Malkin *et al.*, 1990; Srivastava *et al.*, 1990). In order to examine whether mutagens or carcinogens induce *de novo* germ cell mutations, we analysed the p53 gene in tumour and tissue samples from a recent multigeneration carcinogenesis experiment in mice (Loktionov *et al.*, 1992). No mutation in exons 5, 6, 7 and 8 of the gene was detected using the polymerase chain reaction/single strand conformation polymorphism technique.

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2.16 *Studies on specific mechanisms of carcinogenesis*

2.16.1 The role of cell–cell interaction in carcinogenesis

2.16.1.1 *Decreased gap junctional intercellular communication as a common property of human cancer cell lines*

(M. Mesnil, C. Piccoli and H. Yamasaki; in collaboration with D.J. Fitzgerald, Adelaide, Australia; N. Fusenig, Heidelberg, Germany; N. Marceau, Québec, Canada; C.A. Reznikoff, Madison, WI, USA; and S.H.H. Swierenga, Ottawa, Canada)

Decreased gap junctional intercellular communication (GJIC) has been observed in various transformed animal and human cell types and seems to be necessary for cells to express and/or maintain cancerous behaviour (Mesnil & Yamasaki, 1993). In order to better understand the molecular regulation of GJIC in human cancer cells, the expression and localization of the structural protein of gap junctions, connexin (cx), were estimated in various human cell lines derived from different tumourous tissues such as colon, mesothelium, epidermis, liver and ureter (Fitzgerald *et al.*, 1993; Oyamada *et al.*, 1993; Mesnil *et al.*, 1993a; Linnainmaa *et al.*, 1993). Most of these cell lines completely lost their GJIC (Table 16) and those immortalized by SV 40 showed no detectable transcripts of connexins. However, in some cases, the lack of communication was not due to a lack of synthesis but to aberrant cytoplasmic localization of the connexins (Fitzgerald *et al.*, 1993).

Table 16. Comparison of GJIC (number of communicating cells as tested by dye transfer assay) between tumour cells and their normal counterparts

	Tumour cells	Non-tumoral cells
Mesothelial cells	0–10	18–40
Liver cells	8–10[a]	15–50
Epidermal cells	0–20	30–40

[a] Infected with SV40

2.16.1.2 *Mechanisms of aberrant regulation of gap junctional intercellular communication by carcinogens and in cancer cells*

(M. Mesnil and C. Piccoli; in collaboration with M. Asamoto, Omaha, NE, USA)

Treatment of the highly communicating rat liver epithelial cell line, IAR 20, with 12-*O*-tetradecanoylphorbol 13-acetate (TPA) induced very rapid and complete inhibition of GJIC because of internalization of the connexins into the cytoplasm (Asamoto *et al.*, 1991). This aberrant localization seems to be correlated with the appearance of a phosphorylated isoform (p3), probably as a consequence of activation of protein kinase C by TPA (p3 in Figure 20). The chemically transformed counterparts of IAR 20 cells, IAR 6-1 cells, also show cytoplasmic connexins with a phosphorylation pattern similar to that of the connexins isolated from TPA-treated IAR 20 cells. Thus phosphorylation of connexins appears to be an important regulator of GJIC.

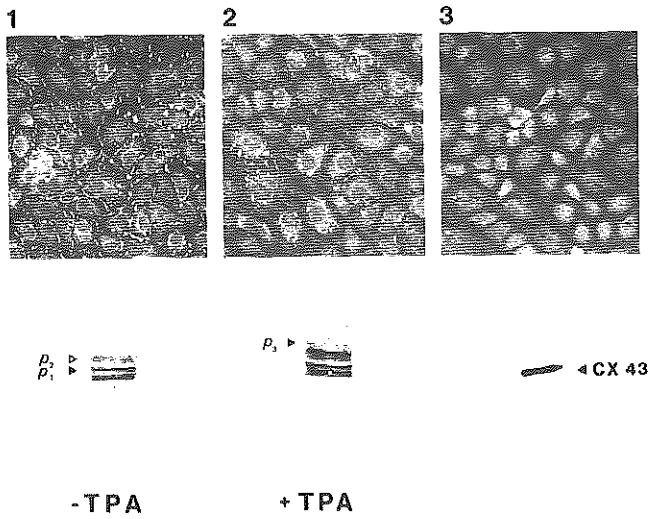

Figure 20. Effect of TPA (100 μg/ml) on connexin 43 localization and phosphorylation in rat liver epithelial cells (IAR 20)

Connexin 43 was detected in cultures by fluorescent immunohistochemistry and the various isoforms of connexin 43 were detected by Western analysis. (1) Untreated cultures showing normal localization of connexin 43 between cells. (2) Cultures treated with TPA for 30 min, showing the cytoplasmic localization of connexin 43 correlated with the appearance of the phosphorylation isoform p3. (3) Preimmune staining and phosphatase-treated sample showing that p1, p2 and p3 are phosphorylated isoforms of connexin 43.

Previous work suggested that GJIC might be also regulated by the expression of cell adhesion molecules such as E-cadherin (a molecule essential for epithelial cell polarity) in mouse epidermal cells (Jongen *et al.*, 1991). Other kinds of cell adhesion molecules seem to be involved in human carcinogenesis, such as DCC, a homologue of the neural cell adhesion molecule (N-CAM), for which the gene has been often found to be deleted in colon carcinomas. However, we found no correlation between the communication capacity of a panel of human colorectal adenocarcinoma cell lines and DCC expression revealed by a reverse transcriptase/polymerase chain reaction method. This lack of correlation suggests that DCC is not a crucial regulator of GJIC (Mesnil *et al.*, 1993a).

GJIC was found to be decreased at confluence in a BALB/c 3T3 cell line which is highly sensitive to carcinogen-induced cell transformation (Yamasaki *et al.*, 1985). We have now demonstrated that such a decrease of GJIC is associated with a lower level of cx 43 mRNA. When these cells were hybridized with others which do not lose GJIC at confluence, all hybrids showed the decreased GJIC phenotype, suggesting that the decrease is genetically a dominant trait (Katoh & Yamasaki, 1991). However, these hybrids were all resistant to chemical induction of cell transformation, suggesting a lack of association between lower GJIC and higher transformability.

Further studies on the relationship between oncogenes and GJIC have confirmed that BALB/c 3T3 cells transformed with membrane or cytoplasmic oncogenes (v-*src*, v-*ras* and polyoma middle T) do not communicate with non-transformed counterparts. On the other hand, those transformed by nuclear oncogenes (v-*myc*, v-*fos* or polyoma large T) communicated with normal cells through GJIC. Cells transformed by nuclear oncogenes, but

not those with membrane or cytoplasmic oncogenes, formed foci on a monolayer of normal cells (Katoh *et al.*, 1993).

2.16.1.3 *Connexin genes as a family of tumour-suppressor genes: transfection into a human cancer cell line*
(M. Mesnil, C. Piccoli, V. Krutovskikh and H. Yamasaki; in collaboration with K. Willecke, Bonn, Germany)

When genes encoding for gap junctional proteins, cx 26, 40 and 43, were transfected into human cervical adenocarcinoma cells (HeLa) which are communication-deficient, all the cells recovered GJIC, as shown by the dye-transfer assay. Although all clones communicated extensively, the cx 26 transfectants almost completely lost (80%) their ability to grow in soft agar. When injected subcutaneously into nude mice, cx 26 transfectants did not produce any tumours within two months, in contrast to the transfectants expressing other types of connexins (Table 17). This lack of tumorigenicity of cx 26 transfectants is probably due to a direct effect on the growth of the cells, as the growth rates of various HeLa clones expressing cx 26 were correlated with the level of cx 26 expression. Thus, the absence of correlation between the dye-transfer ability and growth inhibition seems to suggest another function of cx 26, as a regulator of cell proliferation.

These results are consistent with other reports (Eghbali *et al.*, 1991; Naus *et al.*, 1992; Rose *et al.*, 1993) in which cx 32 and cx 43 have been shown to be tumour-suppressor genes. However, our study is the first to show a complete suppression of tumorigeneicity by cx 26 gene transfection.

Table 17. Phenotypes of connexin-transfected HeLa cells

	GJIC (dye-transfer)	Growth in soft agar (% of colonies/no. of cells seeded)	Tumorigenicity in nude mice (no. of animals bearing a tumour/no. of animals injected subcutaneously)	
			Injected cells	
			10^6	10^5
HeLa	1.0	100%	6/6	5/6
Connexin 40				
clone A	4.5	120%	6/6	2/6
clone B	11.3	70%	6/6	2/6
Connexin 43	18.2	20%	5/6	2/6
Connexin 26	11.8	20%	1/6 (after 3 months)	0/6

2.16.1.4 *Novel interaction of normal and transformed cells: TGF-β1 suppresses the growth of transformed cells with the aid of normal cells*
(P. Silingardi, J.-L. Klein, M. Mesnil and H. Yamasaki)

We have examined the influence of transforming growth factor β1 (TGF-β1) on the growth of H-*ras*-transfected BALB/c 3T3 1-1 cells, both in pure culture and in the presence of their normal counterparts. Considerable cytotoxicity was observed among transformed cells after TGF-β1 treatment, and when a monolayer of normal 3T3 1-1 cells was present, complete and irreversible suppression of H-*ras* foci formation was found (Table 18). These effects appear to differ from those of human placental factors, which suppress the growth of tumorigenic cells effectively in the absence of normal cells (Klein *et al.*, 1991, 1993).

Table 18. Effect of TGF-β1 on the growth of H-ras-transfected BALB/c 3T3 1-1 cells seeded either alone[a] or on a monolayer of confluent 3T3 1-1 cells[b,c]

	No. colonies/plate[a]	No. foci/plate[b]	No. foci/plate[c]
Control[d] (MEM)	24.00 ± 0.66	19.67 ± 0.38	17.68 ± 0.66
TGF-β1[d] (0.5 ng/ml)	17.33 ± 1.71	0	0
TGF-β1[d] (1 ng/ml)	13.00 ± 2.65	0	0

[a] Cells were fixed and stained after 13 days

[b] Cells were kept in culture for 3 weeks

[c] After 3 weeks TGF-β1 treatment was interrupted and cells maintained in culture for 2 more weeks.

[d] Data are reported as mean of three replicates ± SE

The suppression of focus formation in cocultures by TGF-β1 was also observed with v-*src*- and v-MT-transfected 1-1 cells and with cells transformed *in vitro* by either chemical (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine or phorbol 12,13-didecanoate) or physical (UV) agents.

The induction by TGF-β1 of the capacity of normal cells to inhibit the growth of transformed cells was found to not require direct contact between the transformed and normal cells, but instead is probably mediated by a diffusible factor.

2.16.1.5 *GJIC in human normal and tumour cells of surgically removed liver samples*
(V. Krutovskikh, G. Mazzoleni, N. Mironov, Y. Omori, A.-M. Aguelon, M. Mesnil and H. Yamasaki; in collaboration with C. Partensky and F. Berger, Lyon, France)

A newly developed dye-transfer method has been extensively used to measure GJIC directly in freshly removed tissue samples (Krutovskikh *et al.*, 1991).

GJIC in 20 primary human liver tumours with different degrees of malignancy has been studied at the functional and molecular levels. In freshly removed tumour tissue, GJIC capacity was significantly reduced in all samples, regardless of their morphology (Figure 21). In addition, a selective lack of GJIC between tumour and surrounding non-tumorous cells was observed in some cases (Figure 22), probably due to the physical separation between them resulting from encapsulation of tumours.

There was little change in the level of expression of the major liver gap-junction protein, cx 32, in liver tumours, but instead of being localized in the cytoplasmic membrane at intercellular contacts, cx 32 was detected mainly either intracytoplasmically or in plasma membrane free from contact with other cells (Figure 23).

We detected no mutation in the coding sequence of the cx 32 gene from any of the human liver tumours we tested. Thus it is likely that the aberrant localization of cx 32 in tumour cells is due to disturbance of the mechanisms by which this protein is assembled into gap-junction plaques, rather than to any structural abnormality of the cx 32 protein itself. Another member of the connexin family, cx 43, not detectable in non-tumorigenic hepatocytes, was expressed in several tumours, and especially in invasive areas, but was detected in only a few tumour cells and was localized intracytoplasmically, suggesting that this protein is not involved in GJIC in the tumours.

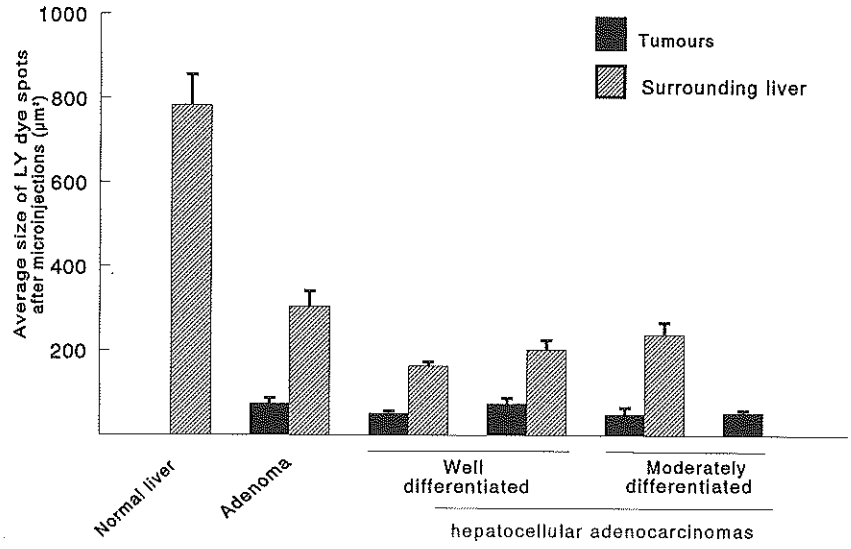

Figure 21. GJIC capacity of various types of human liver tumour. The extent of GJIC was determined by the dye transfer assay performed on fresh surgically removed samples.

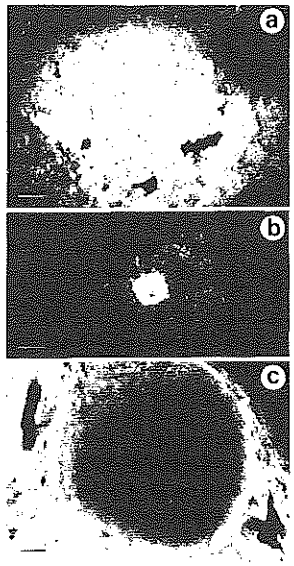

Figure 22. Patterns of Lucifer Yellow dye-transfer between homologous (within tumour or within surrounding tissue) or heterologous (between them) cells.

Microinjection was performed into: (a) surrounding non-tumorous liver (bar 100 µm); (b) hepatocellular carcinoma (bar 100 µm); (c) connective tissue capsula around tumour nodule (bar 150 µm).

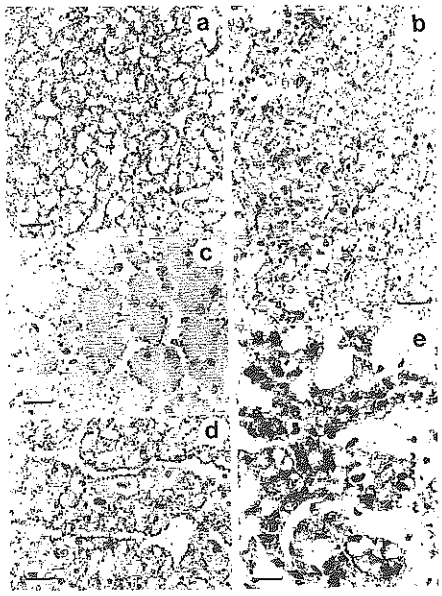

Figure 23. Immunohistochemical localization of cx 32 in various morphological types of human liver tumour.

Cx 32 was visualized by DAB–$NiCl_2$–silver immunostaining, followed by haematoxylin–eosin counterstaining. (*a*) Normal human liver: numerous cx 32-positive gap junctions, revealed as black spots, are present in plasma membranes between individual hepatocytes. Bar, 15 μm. (*b*) Human liver adenoma. Note the irregular cx 32-positive gap junction distribution in tumour cell plasma membrane. Bar 15 μm. (*c*) Acinar type of well differentiated hepatocellular carcinoma (HCC). Atypical concentration of cx 32-positive spots in parts of tumour cell plasma membrane around glandular-like tumour structures, but not between individual cells. Bar 20 μm. (*d*) Trabecular type of well differentiated HCC. Cx 32-positive spots are mostly in parts of the cell plasma membrane around tumour trabecular structures. Bar 20 μm. (e) Moderately differentiated HCC with predominantly solid structure. Intracytoplasmic localization of cx 32 in groups of tumour cells. Bar 15 μm.

2.16.1.6 *Connexin gene mutations in rat liver tumours*
(Y. Omori, N. Mironov, A.-M. Aguelon, V. Krutovskikh and H. Yamasaki; in collaboration with H. Tsuda, Aichi, Japan)

In order to see whether the aberrant GJIC observed in some tumours is caused by mutations of connexin genes, we examined the possible involvement of cx 32 gene modification in rat liver tumours. One out of seven samples tested showed a point mutation, located in the C-terminal cytoplasmic portion of the cx 32 protein, which could explain its aberrant localization (Figure 24). These results suggest that mutation of the cx 32 gene may be a mechanism for the decrease in GJIC in some tumours. However, since we did not detect any cx 32 gene mutation in 20 human liver tumours, such mutations can rarely be responsible for human liver carcinogenesis.

2.16.1.7 *Relationship between cell proliferation, intercellular communication and tumour promotion in rat liver*
(V. Krutovskikh, M. Mesnil and H. Yamasaki; in collaboration with G.M. Ledda-Columbano and A. Columbano, Cagliari, Italy)

Three types of treatment that induce cell proliferation but have different tumour-promoting capacities have been studied in terms of their ability to affect GJIC in rat liver. Compensatory cell proliferation induced by partial hepatectomy (PH), which possesses the strongest tumour-promoting effect, was accompanied by reversible inhibition of GJIC. The decrease in GJIC preceded inhibition of the expression of both liver-specific connexins 32 and 26 (Figure 25).

Proliferation that occurs after carbon tetrachloride (CCl_4) liver poisoning is also considered to be a sort of compensatory regeneration, but unlike partial hepatectomy, it is a

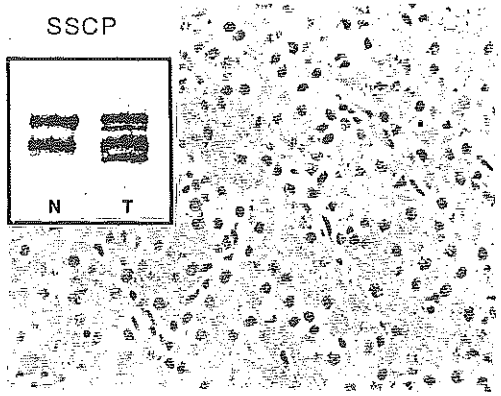

Figure 24. Rat hepatocellular carcinoma with mutated cx 32 gene. Cx 32-positive spots are diffusely scattered in tumour cell cytoplasm. Inset: Single-strand conformation polymorphism pattern for DNA from normal tissue (N) and DNA from the tumour (T)

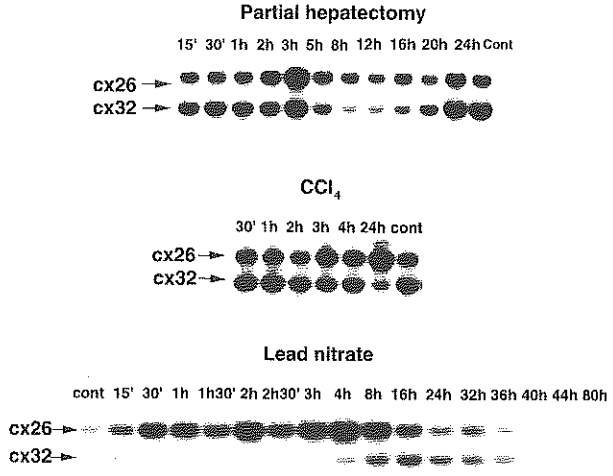

Figure 25. Time-course Northern blot analysis of connexin 26 and 32 gene expression in rat livers after partial hepatectomy, lead nitrate administration and carbon tetrachloride poisoning. Total RNA (20 μg from each sample) was hybridized with radioactively labelled cDNA probes of cx 32 and 26.

local mitogenic event and a less efficient tumour promoter. We found that CCl_4-induced proliferation did not affect GJIC in hepatocytes that are not involved in the local regeneration.

Direct hyperplasia induced in rat liver by lead nitrate administration does not show a tumour-promoting property. Inhibition of GJIC during direct hyperplasia lasted for quite a long time and seemed to be a result of reciprocal disregulation of the expression of two liver-specific connexins (cx 26 and 32). Decreased expression of the principal rat liver connexin, cx 32, was associated with a simultaneous increase in cx 26, which is usually less abundant in rat liver.

The fact that cx 26 expression is specifically increased by this type of cell proliferation stimulus, that does not accelerate hepatocarcinogenesis, is consistent with previous observations suggesting that this particular connexin could act as a tumour suppressor (Lee *et al.*, 1991; and see above).

2.16.2 Evidence for a role of solar infrared (heat) radiation in human skin carcinogenesis

(H. Nakazawa and N. Martel)

While there is no doubt that solar radiation causes skin cancer, especially in white populations, the underlying mechanisms are not well understood (IARC, 1992). The known effects of UV radiation cannot explain all of the events of solar carcinogenesis, and we are therefore studying the possible involvement of the solar infrared (heat) component in human skin carcinogenesis.

Induction of specific programmed cell death (apoptosis) is known to occur after UV irradiation or heat shock in some cell types, and a contribution of the wild-type p53 to the process has been suggested. We have demonstrated that UVB (20 mJ/cm2) or heat (42°C for one hour) efficiently induced apoptosis accompanied by DNA fragmentation in both normal primary proliferating and differentiating keratinocytes *in vitro*. The effect of UVB irradiation on apoptosis lasted only for a short time and was not observed 24 hours after exposure. In contrast, heat shock-induced apoptosis was still detectable up to 24 hours after exposure. Apoptosis occurred less frequently in a cell population exposed to UVB followed by heat shock, perhaps because cells with UV-induced DNA damage (e.g., CC to TT mutation in the p53 gene) could escape from the apoptotic process and survive after heat shock.

Overexpression or cytoplasmic expression of the p53 protein was observed in cell populations exposed to UV and/or heat shock. Aberrant p53 expression is believed to be strongly associated with an early stage of skin carcinogenesis. As demonstrated *in vivo* by Hall *et al.* (1993), we detected UV-induced p53 overexpression by some, though not all, primary keratinocytes, cell lines, both proliferating (low Ca^{++}) and differentiating (high Ca^{++}).

Similarly, in proliferating cells, overexpression of p53 protein was induced by heat shock in certain cell lines, while in differentiating cells it occurred in a different cell line. One possible explanation is that the overexpression of p53 protein induced by UV or heat shock may be accompanied by structural or conformational alteration of the protein. Taken together, our results strongly suggest involvement of the p53 gene in sunlight-associated human skin carcinogenesis through UV- and heat-associated pathways. (This hypothesis is depicted in Figure 26).

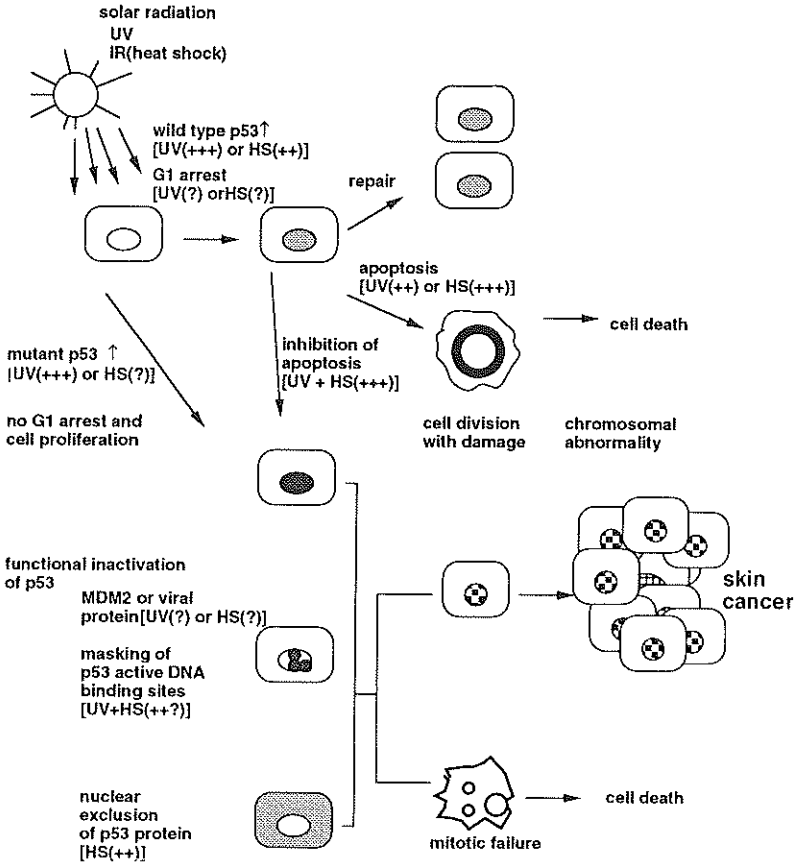

Figure 26. Schematic summary — p53 gene in sunlight-associated carcinogenesis

2.16.3 Sequence specificity of O^6-methylguanine formation in the H-*ras* gene

(N. Mironov, F. Bleicher, G. Martel-Planche, C.P. Wild and R. Montesano; in collaboration with P. Georgiadis and P. Swann, London, UK)

The distribution of *O*^{6}-methylguanine (O^6-MeGua) in the rat H-*ras* gene sequence was studied using PCR by transition of O^6-MeGua to adenine during the reaction. The use of PCR for detection of O^6-MeGua was validated by using oligonucleotides (61 bases) containing one O^6-MeGua residue at a defined site. When rat liver DNA was treated with *N*-methyl-*N*-nitrosourea *in vitro*, a strikingly non-random sequence distribution of O^6-MeGua was observed. 68% of the O^6-methylated guanine bases were found in the middle G of the sequences GGT and GGA in the H-*ras* gene, whereas no methylation was found in the middle G of the sequences AGG, GGG, TGT, TGC, CGA and CGC. No O^6-MeGua was found in the 12th codon (sequence GGA) of the H-*ras* gene, indicating that this site is not a main target for O^6-MeGua formation in this system.

It was further observed that, in the DNA sequence studied, the formation of O^6-MeGua was highest where the G was flanked by pyrimidine–purine or purine–purine sequences on the 5′ side, whereas a purine–purine pair on the 3′ side showed maximal inhibition of O^6-MeGua formation.

2.16 IARC staff publications

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PART 3. STUDY OF HOST FACTORS FOR CANCER AND THEIR INTERACTION WITH ENVIRONMENTAL FACTORS

3.1 *Studies of the cytochrome P450 enzyme system and its role in carcinogenesis*

CYP genes coding for cytochrome P450s are a superfamily of genes regulating the expression of enzymes oxidizing xenobiotics, including those that convert procarcinogens to their ultimate carcinogenic forms. The expression of CYP genes is regulated by environmental and genetic factors and most, if not all, have their individual patterns of regulation. For each isozyme, either genetic polymorphism or wide interindividual variation in expression has been found, leading to great individual variations in capacity to generate carcinogenic metabolites. Our studies focus on the following isozymes: CYP1A1, CYP2A6 (Cyp 2a-5 in mice; previously called IIA3), CYP2E1 and CYP3A4 which appear to be essential in the metabolism of carcinogens such as polycyclic aromatic hydrocarbons, nitrosamines and aflatoxins.

Other studies relating to this topic are described in sections 2.4.2 (genetic susceptibility to lung cancer and environmental tobacco smoke), 2.4.8 (pulmonary carcinogen–DNA adducts, cytochrome P450 enzymes, smoking and occupational exposure in lung cancer patients in Finland), 2.11.3 (liver cancer in Thailand), 2.11.6 (aflatoxin-metabolizing enzymes in liver cancer patients), 2.11.7 (experimental studies of aflatoxin carcinogenicity), and 2.5.4 (Balkan endemic nephropathy).

3.1.1 Individual variability of CYP2A6 and CYP2E1 and its implications in nitrosamine metabolism

(A.-M. Camus, O. Geneste, J.-C. Bérézíat, H. Bartsch and M.A. Lang; in collaboration with P. Honkakoski, Kuopio, Finland; and C.J. Henderson and C.R. Wolf, Edinburgh, UK)

This study has shown that in both mice and humans, individual *N*-nitrosodimethylamine (NDMA) and *N*-nitrosodiethylamine (NDEA) metabolism is highly variable due to variations in the expression of the two enzymes. CYP2A6 seems to be more important in NDEA metabolism and CYP2E1 more important in NDMA metabolism (Camus *et al.*, 1993).

3.1.2 Expression of carcinogen-metabolizing Cyp2a-5 in hepatomas

(M.A. Lang; in collaboration with V. Kobliakov, L. Kulikova and D. Somoilov, Moscow, Russian Federation)

Expression of Cyp2a-5, highly active in the metabolism of NDEA and aflatoxin B_1, was found to be strongly and selectively enhanced in mouse hepatomas (spontaneous and transplanted) compared to normal tissue; other isozymes determined were either not affected

or reduced (Kobliakov *et al.*, 1993). The regulation of expression was at the pretranslational level, possibly through an altered transcription rate. The high level of expression of Cyp2a-5 in tumours or premalignant cells may contribute to a causal process in chemical carcinogenesis.

3.1.3 Increased expression of Cyp2a-5 and related carcinogen metabolism in different types of liver injury

(C.P. Wild, H. Bartsch, R. Montesano, G. Kirby, O. Geneste, J.-C. Béréziat and M.A. Lang; in collaboration with P. Pelkonen, P. Pellinen, P. Honkakoski, M. Pasanen, Kuopio, Finland; and F. Stenbäck, A. Rautio and O. Pelkonen, Oulu, Finland)

Previous studies have shown that Cyp2a-5 activity is selectively increased in liver injury caused by pyrazole and cobalt (Hahnemann *et al.*, 1992). In experiments in mice and hamsters, to find out whether this increase is a general phenomenon related to different types of injury, various types of hepatotoxic chemicals and hepatitis B virus and *Opisthorchis viverrini* liver fluke all selectively increased Cyp2a-5 among several CYP isozymes. This led to increased metabolism of certain nitrosamines and aflatoxin B_1 which are known to be good substrates for Cyp2a-5. Furthermore, in individual cells or sections of liver where the expression of Cyp2a-5 is high, DNA binding of aflatoxin B_1 was elevated. Most of the agents used in this study to cause liver injury are also suspected cocarcinogens, an activity that may be mediated by an ability to increase carcinogen metabolism.

3.1.4 Expression of CYP2A5 and related carcinogen metabolism in nasal epithelium

(J.-C. Béréziat, O. Geneste, H. Bartsch and M.A. Lang)

A high level of expression of CYP2A5 was found in the nasal cavity of rats. The level of CYP2A5 was strongly increased by coumarin, a well known odorant found in several plants. The activity of this enzymes was about 100 times higher in the nasal epithelium than in the liver and was associated with a high level of metabolism of aflatoxin and nitrosamines in nasal tissues. It is therefore possible that the high expression of CYP2A5 in the nasal epithelium could be a risk factor in nasal carcinogenesis and could explain why aflatoxin B_1 and some nitrosamines have previously been found to be nasal carcinogens.

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3.2 *Studies of effects of DNA repair on carcinogenesis*

While the formation of DNA adducts is a critical step in the carcinogenic process, the occurrence of mutations is greatly dependent on the capacity of the cells to repair DNA damage. Thus the capacity of an individual (or cell) to carry out these DNA repair processes could represent an important biomarker for cancer risk. The objective of the studies presented here is to assess the role in cancer induction and development of the repair of some forms of DNA damage induced by UV radiation, alkylating agents and oxygen free radicals.

3.2.1 *In vitro* assay of capacity to repair UV-induced DNA damage: its use in molecular epidemiological studies

(J. Hall, M. Artuso and B.K. Armstrong; in collaboration with D. English, Nedlands, Australia; and L. Grossman, Baltimore, MD, USA)

Individual variation in the capacity of human lymphocytes to repair UV-induced DNA photoproducts has been measured in participants (aged 44 to 68 years) in a population-based case–control study of basal cell carcinoma (BCC) or squamous cell carcinoma (SCC) of the skin in Geraldton, Australia. Blood samples were obtained from 202 (84%) of the 241

subjects invited to participate and informative repair capacity data obtained from 173. It was found that BCC cases had a repair activity 1.16 times higher than the controls (95% CI 1.01–1.11, $p = 0.03$). For the SCC cases, the activity was 1.08 greater than among the controls (95% CI 0.81–1.28, $p = 0.41$). These results contrast with those of Wei *et al.* (1993), who found a lower repair capacity in BCC cases aged less than 40 years than in controls but no such difference in cases over 50 years of age. In order to determine whether similar differences existed in younger subjects among our study population, the analyses were repeated on subjects now aged 45–55 years. As in the overall analysis, the BCC cases had a higher activity than controls, suggesting that if repair declines with age, the major effect in BCC patients occurs before the age of 45. However, the repair activity in our younger SCC cases was lower than in the controls. The lack of association between DNA repair capacity and case/control status found in this study, in contrast to the finding of Wei *et al.*, could in part be explained by the age distribution of the subjects, the higher proportion of the control population having a family history of BCC or SCC, and the high level of sun exposure and consequent risk of skin cancer in Geraldton. Under these conditions, host defences may be overwhelmed, and differences in repair capacity obscured. However, if this were the case, one would expect that other host characteristics such as the skin's reaction to sunlight would have weaker effects in Geraldton than in low-risk areas. This is not the case; the association between ability to tan and risk of either BCC or SCC is at least as strong in these data as has been seen in other studies (Kricker *et al.*, 1991). It remains to be elucidated whether this lack of association is due to induction of repair processes by high exposure to sunlight in all subjects in this population or is a more widespread phenomenon.

3.2.2 Control of expression of enzymes for DNA alkylation damage repair during single and chronic exposures to carcinogens in animal models

(J. Hall, H. Brésil and H. Kang)

The capacity of rodent liver to repair O^6-methylguanine alters after either chronic or single exposure to a variety of carcinogens. In the rat, the level of the mRNA for O^6-alkylguanine-DNA alkyltransferase (AGT) has been shown to increase after treatment with *N*-nitrosodimethylamine (NDMA) (5–20 mg/kg), ionizing radiation (3 Gy) and partial hepatectomy (Figure 27). The time course of this increase parallels the increase in enzyme activity and occurs in both male and female BDIV rats (6–9 weeks old) to similar extents. In contrast, in the Syrian golden hamster, the recovery of AGT to constitutive levels is observed only after exposure to much lower doses of alkylating carcinogen (2.5 mg/kg NDMA), with no increase above the constitutive level of expression being observed and no significant changes in the AGT mRNA. Likewise, hamster (male and female) liver appears to be refractory to the induction of the AGT at either the protein or mRNA level, by ionizing radiation (3–12 Gy) or partial hepatectomy. The molecular events that control the expression of the alkylation-specific DNA repair enzymes and certain other damage-inducible enzymes in both species are under investigation.

The tissue-specific changes in alkylation DNA repair enzymes are being measured following exposure to *N*-nitrosamines having different target organs, to complement studies on adduct formation (Section 2.8.6). AGT activity in the liver of animals was measured 16 hours after exposure to a single dose of *N*-nitrosamine. Inactivation of the constitutively expressed enzyme was found with all compounds although with a markedly different dose response. Exposure to 20 mg/kg NDMA, 20 mg/kg 1,2-dimethylhydrazine, 25 mg/kg *N*-nitrosomethylbenzylamine and 75 mg/kg NNK gave approximately 20% residual AGT activity.

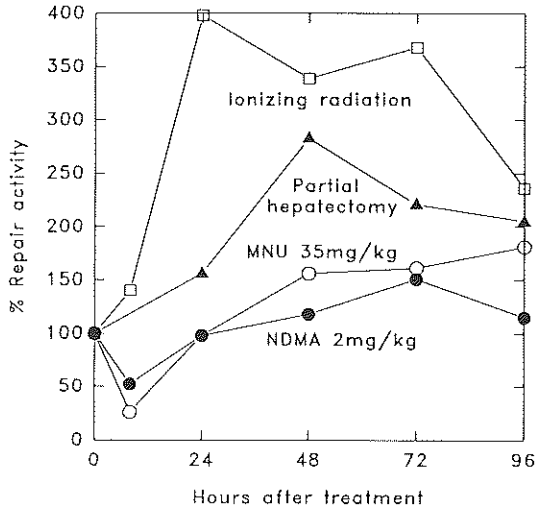

Figure 27. Time course of AGT repair activity in male rats following various treatments

3.2.3 DNA repair modulation by cellular injury

(J. Hall, G. Kirby, C.P. Wild and I. Chemin; in collaboration with F. Chisari, La Jolla, CA, USA)

The modulation of DNA repair by cellular injury resulting from inflammatory processes is being investigated in HBV transgenic mice (see Section 2.11.7). The changes in expression of the AGT in mice aged 1 to 12 months have been followed. Except in the oldest animals, the HBV-positive animals have a higher mean level of AGT than the HBV-negative animals, which is marginally significant in 1- and 6-month-old animals.

3.2.4 Modulation of DNA repair enzymes in human tissues

(J. Hall; in collaboration with R. Butler, Adelaide, Australia; and F. Donato, Brescia, Italy)

The levels of AGT and methylpurine-DNA glycosylase have been measured in protein extracts obtained from 'normal' and colon carcinoma tissue from the same individuals. No differences in the AGT activity were observed, whilst the level of methylpurine-DNA glycosylase activity was significantly higher in the tumour tissue compared with normal tissue (2.94 ± 0.39 versus 4.44 ± 0.45 pmol/mg protein; $p < 0.0005$). Interestingly, the level of this enzyme has also been shown to be significantly higher in the leukocytes of smokers compared to non-smokers, whilst the levels of the AGT were similar (Hall *et al.*, 1993).

3.2 IARC staff publications

Hall, J., Brésil, H., Donato, F., Wild, C.P., Loktionova, N.A., Kazanova, O.I., Komyakov, I.P., Lemekhov, V.G., Likhachev, A.J. & Montesano, R. (1993) Alkylation and oxidative-DNA damage repair activity in blood leukocytes of smokers and non-smokers. *Int. J. Cancer*, **54**, 1–6

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3.3 *Studies on genetic determinants of specific cancers*

In 1985, IARC launched a programme aimed at studying genetic susceptibility to cancer. The general objectives were to evaluate the role and the importance of inherited conditions predisposing to cancer through molecular, familial and population-genetic approaches. Another goal was to establish how molecular genetics can be used to better define the genetic make-up of individuals in epidemiological surveys.

Probably less than 5% of cancers occur in individuals who are strongly predisposed to a particular cancer type. If a genetic marker of risk is identified, these individuals (and their relatives) may benefit from screening and early diagnosis. Molecular investigations may allow the identification of the predisposing genes and elucidation of how they operate. Such information would be of general importance, since the more common non-familial forms of such a cancer may result from somatic mutation of the same gene.

The approach we have chosen in order to study inherited predisposition to cancer is through linkage analysis in high-risk families. In linkage analysis, co-segregation of cancer susceptibility is sought with a specific allele of a polymorphic system which has been mapped to a known chromosomal location.

Four conditions have been studied: three relatively well defined syndromes, X-linked lymphoproliferative syndrome (XLP), familial medullary thyroid cancer (MTC) in the framework of multiple endocrine neoplasia type 2 (MEN 2) and neurofibromatosis type 2 (NF2), and familial breast cancer, now the focus of most of our effort.

3.3.1 X-linked lymphoproliferative syndrome (XLP)

(B.S. Sylla, Q. Wang, J. Lamartine, S. Pauly and G. Lenoir; in collaboration with D. Haber, Charlestown, MA, USA; A. Monaco, Oxford, UK; D.L. Nelson, Houston, TX, USA; G. Romeo, Genoa, Italy; J. Skare, Boston, MA, USA; and S. Tsuji, Niigata, Japan)

The X-linked lymphoproliferative syndrome (XLP) is a rare recessive immunodeficiency characterized by either fatal infectious mononucleosis, hypogammaglobulinaemia or malignant lymphoma, following primary Epstein–Barr virus (EBV) infection. The genetic defect is not known. The XLP gene appears to be involved in the control of EBV infection. XLP disease is an interesting model to study the interaction between an infectious environmental agent (EBV) and a genetic component in the development of malignant lymphoma.

The XLP locus has been mapped to the Xq25-q26 chromosomal region by the genetic linkage approach (Skare *et al.*, 1989; Sylla *et al.*, 1989). A collaborative study with J. Skare has shown that the responsible gene is near DXS42, DXS12 and DXS37 (Figure 28). Efforts have been devoted to establishing the physical map of this region and to identifying the gene involved in the disease. The methodology developed for this project (linkage study, pulse field gel electrophoresis, yeast artificial chromosome (YAC) analysis) has contributed significantly to the progress of our other genetic projects.

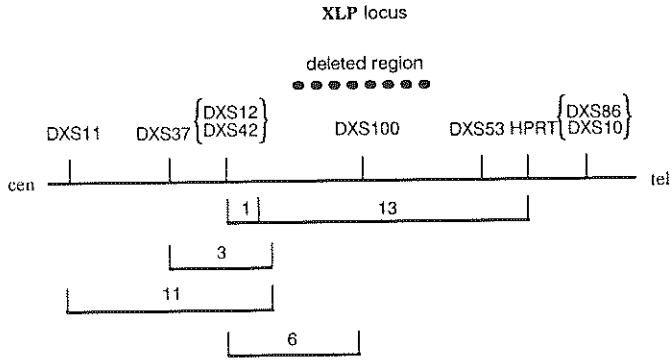

Figure 28. Genetic and physical maps around the XLP locus

3.3.1.1 *Physical mapping of the Xq25-q26 region*

Screening of YAC libraries with probes closely linked to the XLP locus has permitted the isolation of several YAC clones. Mapping of three of them which overlap has indicated that the loci DXS12 and DXS42 are linked within 60 kbp, and both are separated from DXS37 at the genomic level, by at most 3700 kbp (Figure 29) (Wang *et al.*, 1993).

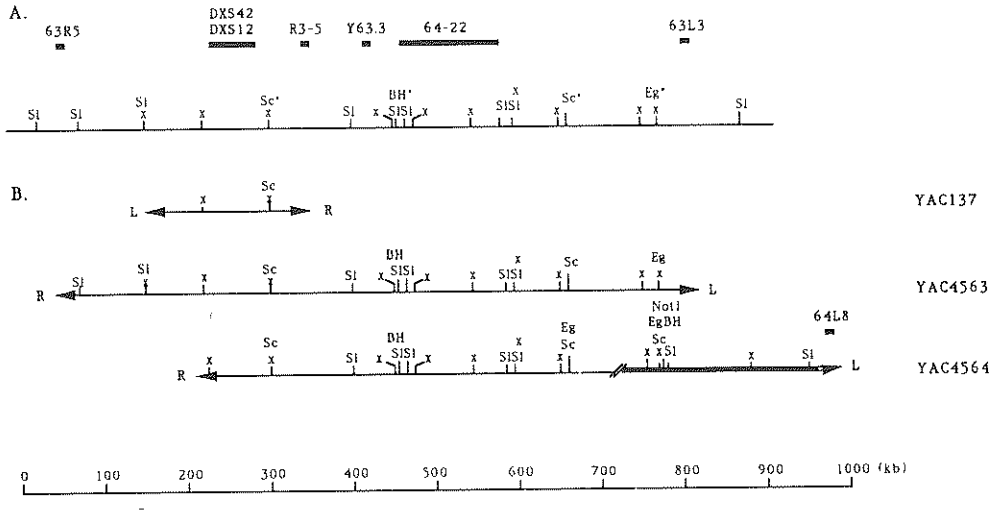

Figure 29. Physical map of overlapping yeast artificial chromosomes (YACs) around DXS12 and DXS42

A Restriction map at the genomic DNA level, illustrating the localization of loci DXS12, DXS42 and the probes generated from the YACs. The methylated restriction sites are indicated by *.

B Three YACs: YAC137 (200 kb), YAC4563 (780 kb) and YAC4564 (800 kb) were mapped and aligned by partial rare-cutter enzyme digestion and PFGE analysis. X = XhoI; SI = SalI, Sc = SacII; BH = BssHII; Eg = AegI. The cocloned region at the left extremity of YAC4564 is indicated by a black box. L = Left end; R = right end

The deleted markers have been used to screen cosmid and YAC libraries and several positive clones have been identified. Analysis of these clones in order to construct a physical map of the deletion and to isolate expressed sequences is in progress.

3.3.1.2 *Search for expressed sequences in the Xq25 region*

Identification of XLP patients with interstitial deletions in the Xq25 region constitutes an important step towards the isolation of the XLP gene. A search for expressed sequences from the deleted region will allow identification of genes that can be tested as candidate genes for the XLP syndrome. Conserved sequences isolated from a phage clone corresponding to the region that is deleted in one of our XLP patients were used to screen a human fetal cDNA library. Analysis of several independent positive cDNA clones indicated that all contained repetitive sequences. cDNAs isolated after screening of placenta and brain cDNA libraries, using CpG-containing cosmid clones mapped on Xq24-q26, were retained in the XLP patient with deletion. Various approaches such as exon amplification and cDNA selection are now being undertaken to isolate genes from YAC and cosmid clones shown to be deleted in our XLP patient.

3.3.2 Multiple endocrine neoplasia type 2 (MEN 2)

(I. Schuffenecker, M.-F. Lavoué and G. Lenoir; in collaboration with the Group for the Study of Calcitonin Tumours: Secretariat, C. Calmettes, Paris, France; E. Modigliani, Paris, France; and S. Narod, Montreal, Canada)

MEN 2A is an autosomal dominant inherited cancer syndrome characterized by medullary carcinoma of the thyroid (MTC), phaeochromocytoma and hyperparathyroidism, accounting for at least 30% of medullary thyroid cancers. Almost all gene carriers will develop the disease (a very high penetrance of the gene), but their identification still relies on a screening test that detects an early stage of the malignancy. Through the Group for the Study of Calcitonin Tumours in France and contacts with various European institutions, over 135 families have been identified, and blood has already been collected from most members.

Recent emphasis has been placed on the study of genetic heterogeneity of MEN 2 and on the development of a screening method using DNA markers. Most of the molecular analysis is now performed in the Genetics Laboratory of the Edouard Herriot Hospital, Lyon, while coordination and analysis of the project remains the responsibility of IARC.

Hereditary MTC appears in three forms: (1) in association with phaeochromocytomas and parathyroid hyperplasia (MEN 2A); (2) with phaeochromocytomas, neuromas of the mucous membranes and a marfanoid appearance (MEN 2B); and (3) without phaeochromocytoma. Despite these differences in presentation, age of onset and clinical severity, limited genetic studies suggest that the three MTC variants may be due to inherited mutations at the same gene locus. By testing 24 families with nine polymorphic markers spanning the centromere of chromosome 10, haplotypes have been constructed, and a segment was found to be shared by seven of these families, suggesting they have a common ancestor (Narod *et al.*, 1992a).

During the last year, DNA screening in families has been developed, with the major focus on the use of highly polymorphic markers (microsatellites). Our group has participated in a consensus statement for early diagnosis of MEN 2 in the framework of a European Community concerted action (Calmettes *et al.*, 1992).

Recently reported germline mutations in the *RET* proto-oncogene (Mulligan *et al.*, 1993; Donis-Keller *et al.*, 1993) will be useful in addressing key questions concerning MTC etiology, such as the role of *RET* in MEN 2B, the correlation between mutation and phenotype in familial MTC, and the involvement of *RET* in sporadic MTC.

3.3.3 Neurofibromatosis type 2

(G. Lenoir and C. Bonnardel; in collaboration with G. Fischer, Lyon, France; S. Narod and G. Rouleau, Montreal, Canada; and G. Thomas, Paris, France)

The neurofibromatoses are autosomal dominant diseases which primarily affect the nervous system. Neurofibromatosis type 1 (NF1), which occurs with an incidence of one in 3000, predisposes mainly to the development of peripheral neurofibromas, cafe au lait macules, optic nerve gliomas and bony abnormalities. Neurofibromatosis type 2 (NF2), which occurs with an incidence of one in 37 000, is mainly associated with the development of schwannomas, and also, to a lesser extent, meningiomas and ependymomas. Linkage studies have shown that, in contrast to NF1 which maps to chromosome 17, NF2 maps to chromosome 22.

Our group has collected a set of 25 cases, 11 with documented familial history, and 14 with bilateral schwannoma without familial history, representing probably new mutations. This panel of families has been used to demonstrate through linkage analysis that NF2 is a genetically homogeneous disease (Narod *et al.*, 1992b) and to demonstrate that pre-sympto- matic diagnosis by linkage analysis using chromosome 22 markers is feasible (Ruttledge *et al.*, 1993). Analysis of a germ-line deletion in one of our NF2 pedigree has indicated that NF2 maps in a 700 kb interval (Sanson *et al.*, 1993) (Figure 30). A new gene encoding a putative membrane-organizing protein was identified in this region by the groups of G. Rouleau and G. Thomas and is the site of germ-line mutations in NF2 patients (Rouleau *et al.*, 1993). It seems to act as a suppressor gene, and is likely to be involved in sporadic schwannomas and meningiomas.

Attempts are being made in our laboratory to correlate the mutations and the phenotypes of the families and to study how the new mutations were generated.

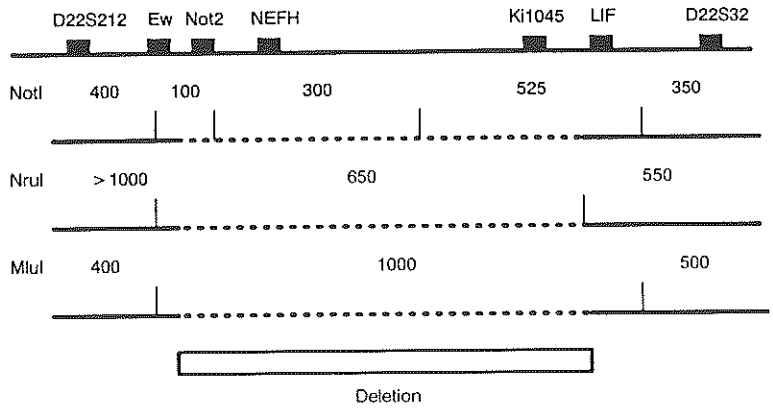

Figure 30. Physical map of the NF2 region

NotI, NruI and MluI restriction map of the NF2 region. The broken line and the open box underneath shows the extent of the deletion seen in family 21. The positions of the markers D22S212, Ew, Not2, NEFH , Ki1045, LIF and D22S32 is shown.

3.3.4 Linkage analysis in familial breast and ovary cancer

(G. Lenoir, B. Sylla, O. Serova, L. Deng, M.-F. Lavoué and C. Bonnardel; in collaboration with J. Feunteun, Villejuif, France; H. Lynch, Omaha, NE, USA; and S. Narod, Montreal, Canada)

The objectives of this project are to locate breast cancer susceptibility genes, to identify them, and to evaluate the biological significance of the hereditary component in breast cancer. It is also intended to evaluate the feasibility of DNA screening in breast cancer families, once genetic markers become available.

Most cases of breast cancer appear to be sporadic. There are, however, some families in which there are several affected individuals, indicating either a chance clustering of sporadic cases or an inherited genetic effect. In other rarer families, there is a clear inherited susceptibility to breast cancer that can be traced through consecutive generations. In these families, breast cancer usually occurs at an early age (before menopause), is often bilateral, and is sometimes associated in the family with other cancer such as ovarian cancer. It has been evaluated that about 4% of breast cancer occurs in such high-risk families, and that cancer risk is transmitted as an autosomal dominant trait.

3.3.4.1 *Linkage studies*

In order to locate any breast cancer susceptibility locus by linkage analysis, we have examined a significant number of breast cancer families, originally identified by Professor H.T. Lynch at Creighton University, Omaha, NE, USA. More than 900 blood samples from over 50 high-risk families were shipped to Lyon between 1989 and 1993.

Following the identification of a breast cancer locus on the long arm of chromosome 17 by linkage analysis (Hall *et al.*, 1990), we demonstrated that this locus corresponds to a breast and ovarian cancer susceptibility locus (Narod *et al.*, 1991). Nineteen North American caucasian families, each with at least four confirmed cases of breast or ovarian cancer, have been studied. Four polymorphic markers (cLB17.1, D17S579, D17S588 and D17S74), which span a region of approximately 15 cM on chromosome 17q21, were typed. Our data confirm the location of a dominant gene conferring susceptibility to breast and ovarian cancer (maximum LOD score = 9.78). Two recombinants in one large family suggest that the breast–ovarian cancer locus lies between D17S588 and D17S579 (Feunteun *et al.*, 1993).

At an international workshop on linkage studies in hereditary breast cancer in December 1991, most of the participants agreed to share their data. As a consequence, a collaborative linkage study involving 214 families, including 57 breast–ovarian cancer families, was performed (analysis performed by D.F. Easton, D.T. Bishop, D. Ford, G.P. Crockford and the Breast Cancer Linkage Consortium) (Easton *et al.*, 1993). The results suggest that a gene (or genes) on chromosome 17q accounts for the majority of families in which both early-onset breast cancer and ovarian cancer occur. In contrast, there was significant evidence for genetic heterogeneity among families without ovarian cancer, with an estimated 45% being linked. This indicates that other genes predisposing to breast cancer exist and should be looked for by linkage using the panel of 17q-unlinked breast cancer families.

Further linkage mapping by our laboratory indicates that this breast cancer locus now termed BRCA1 is located in a 2.8 cM interval between the insulin-like growth factor binding protein 4 (IGFBP4) gene (centromeric) and the polymorphic marker D17S579 (telomeric) (Tonin *et al.*, 1993).

3.3.4.2 *Attempt to identify the breast and ovarian cancer susceptibility gene*

A search for constitutional rearrangements at the 17q breast cancer (BRCA1) locus has been performed using cytogenetic and molecular approaches on over 50 individual families. No deletions or rearrangements have been yet observed.

Candidate genes in this region include EDH17B2, which encodes estradiol 17β-hydroxysteroid dehydrogenase II (17β-HSDII), and RARA, the gene for retinoic acid receptor α. We have typed 22 breast and breast–ovarian cancer families with eight polymorphisms from the chromosome 17q12-21 region, including two in the EDH17B2 gene. Genetic recombination with the breast cancer trait excludes RARA from further consideration as a candidate gene for BRCA1. Both BRCA1 and EDH17B2 map to a 6 cM interval (between THRA1 and D17S579) and no recombination was observed between the

two genes. However, direct sequencing of overlapping DNA amplification products containing the entire EDH17B2 gene in four unrelated affected women did not reveal any sequence variation, other than previously described polymorphisms. Mutations in the EDH17B2 gene, therefore do not appear to be responsible for the hereditary breast–ovarian cancer syndrome (Simard *et al.*, 1993).

A major effort to generate a physical map of the region is continuing, with the use of yeast artificial chromosomes and cosmids. Identification of putative genes located in this region through screening of a cDNA library is also in progress.

3.3.4.3 *DNA screening in the families based on linked DNA markers*

In a large family containing 23 women with breast or ovarian cancer, susceptibility is linked to a gene on chromosome 17 (maximum two-point LOD score 4.20 at 5 cM distance from the D17S250 locus). Because linkage in this family is not in doubt, DNA probes can be used to identify probable gene carriers from birth. Using five linked DNA markers, and by incorporating clinical data, 34 probable gene carriers have been identified. By age 70 the cumulative risk for breast cancer among carriers is estimated to be 73% and the risk for ovarian cancer is 83%, but the risk is not evenly distributed among generations of women (Figure 31). The incidence rate of breast cancer among women born after 1930 was five times greater than that among those born before 1930 ($p = 0.044$). The increase cannot be explained by improved methods of detection or by changing patterns of fertility and is more likely to be the effect of a changing environment (Narod *et al.*, 1993).

In the same family, high-risk members of the kindred were offered counselling based on 17q markers. Family members responding positively received one-to-one genetic counselling in a structured setting. Predisclosure education was conducted, and post-disclosure assessment of the immediate impact of this information was made (Lynch *et al.*, 1993).

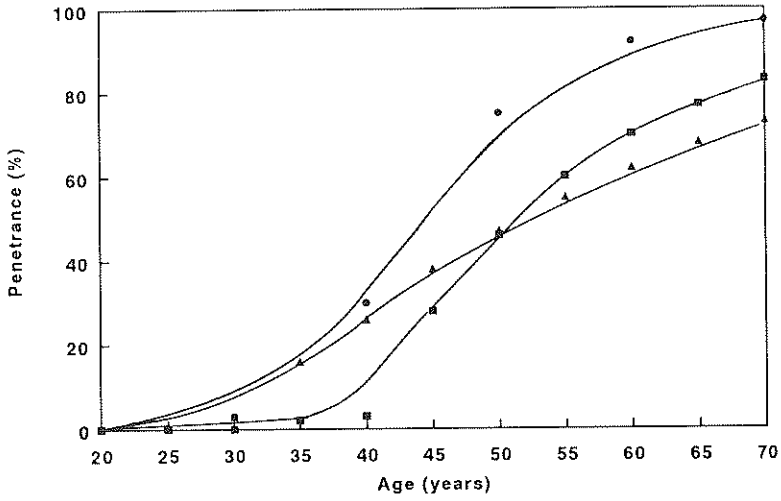

Figure 31. The cumulative indidence of cancer of the breast and of the ovary among 34 gene carriers identified in Family 1816

•, Breast or ovary cancer; ▲, Breast cancer; ■, Ovary cancer

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PART 4. RESEARCH ON THE PREVENTION AND EARLY DETECTION OF CANCER

4.1 *The Gambia Hepatitis Intervention Study*

(A.D. Jack, M. Fortuin, N. Maine, B.K. Armstrong, F.X. Bosch, D.M. Parkin, R. Montesano, C.P. Wild, N. Charnay and H. Renard; in collaboration with M.O. George, K. Cham, K. Jobe, Y. Lowe and P.E. Crivelli, Banjul, The Gambia; B.M. Greenwood, H.C. Whittle, M. Mendy and E. Bah, Fajara, The Gambia; A. Cali, Naples, Italy; L. Chieco-Bianchi and A. Del Mistro, Padua, Italy; F. Aiuti, Rome, Italy; M. Rizzetto, Turin, Italy; R.L. Robertson, South Hadley, MA, USA; M. Kane, Geneva, Switzerland; A.J. Hall, London, UK; and H. Inskip, Southampton, UK)

This is a long-term intervention study aimed at evaluating the effectiveness of hepatitis B vaccination in the prevention of persistent hepatitis B infection, chronic liver disease and primary hepatocellular carcinoma. A total of 124 577 children have been recruited into the study in two cohorts of vaccinated and unvaccinated groups. The effect of vaccination will be evaluated in these children over a period of 30 to 40 years.

The study is being conducted in collaboration with the Government of The Gambia and the Medical Research Council of the United Kingdom, with funding from the Direzione Generale per la Cooperazione allo Sviluppo of the Ministry of Foreign Affairs, Italy, and a contribution from the Medical Research Council of Sweden.

4.1.1 Monitoring the effect of HBV vaccination

A group of 1041 children (Group 1) who have been vaccinated with hepatitis B vaccine are being followed up in order to monitor the levels of antibody and the duration of protection against persistent infection. These children were selected from the first four centres where the use of the vaccine was introduced.

The fifth-year follow-up of these children was concluded in July 1992 with 71% of all recruited children traced and bled. While the majority of the children still showed protective levels of antibody, the rate of antibody decay now appears to be more marked than in previous years (Table 19). Despite this trend, 95% of children were still free of infection at age 5. No follow-up for serology was scheduled for year 6. Instead, the children in this group were visited towards the end of 1992 to check on their state of health and to assist with treatment where necessary. Serological testing will be resumed in August 1993.

A second group of children (Group 2), consisting of randomly selected children aged between 3 and 4 years who had not received hepatitis B vaccine, were screened for evidence of infection. The aim was to describe the occurrence of hepatitis B infection among the unvaccinated members of the GHIS cohort and thereby allow the estimation of vaccine

Table 19. Hepatitis B status of children in Group 1 at each of the first five years of follow-up[a]

	HBsAb HBcAb-	HBsAb- HBcAb-	HBsAb+ HBcAb+	HBsAb- HBcAb+	Total
1st year	716 (94)*	11 (1)	33 (4)	4 (0.5)**	764
2nd year	664 (95)	27 (4)	8 (1)*	4 (0.6)**	703
3rd year	655 (93)	30 (4)	13 (2)*	6 (0.9)****	704
4th year	659 (91)	27 (4)	29 (4)**	4 (0.6)**	721
5th year	622 (84)	80 (11)	27 (4)	11 (1.5)****	740

[a] The number of asterisks indicates the number of children positive for HBsAg.

efficacy in the short-term to be made. 824 children were recruited, of whom 29% showed evidence of past exposure to hepatitis B infection, with 13% also positive for HBsAg. Of this latter group, 86% were still found to be chronic carriers a year later, a status which is considered a major risk factor for later development of the malignant state.

By comparing children in groups 1 and 2, it has been possible to estimate vaccine efficacy in protecting against infection and chronic carriage at 84% and 94% respectively (Table 20).

Table 20. Estimates of vaccine efficacy by zone in children aged 3 to 4 years

Zone	Efficacy against infection (95% CI)	Efficacy against chronic carriage (95% CI)
1	91% (80-96%)	100% (86-100%)
2	75% (45-89%)	89% (17-99%)
3	76% (51-88%)	100% (72-100%)
4	86% (74-92%)	86% (54-96%)
Total	84% (78-89%)	94% (84-98%)

4.1.2 Cancer registration

A population-based cancer registry was launched at the beginning of the study, with the primary aim of monitoring the occurrence of primary liver cancer in The Gambia, so as to provide the basis for future evaluation of the effect of vaccination on the incidence of this tumour in the Gambian population. Since then its role has been expanded to include all the other types of cancer which are diagnosed within the health service.

Six years of data on the incidence of different forms of malignancy are now available through the cancer registry. Liver cancer continues to be the most commonly reported malignancy in males and is a close second to cervical cancer in females. Attempts continue to be made to strengthen the reporting system through regular visits to all units, the recruitment of additional staff for the registry, training of a Gambian pathologist and the provision of equipment for local histopathological examination. A two-week introductory course in ultrasonography was held in October 1992, aimed at improving the diagnostic capabilities of the reporting staff.

4.1.3 Ancillary studies

Several such studies are either in progress or have been concluded during 1992/1993.

Interest in the occurrence of breakthrough infections with hepatitis B virus in the face of apparently protective levels of antibody has led to the investigation of possible viral variants. There is evidence to suggest that one such infection was from a mutant virus which was not neutralized by antibodies induced by hepatitis B vaccination.

The fieldwork for a study to evaluate environmental and genetic predispositions to HBe antigenaemia has been concluded and serological assays and HLA typing are in progress. This study should help identify factors which may enhance the aggregation of such a highly infectious state within families.

The concern that nation-wide hepatitis B vaccination may, in the long run, alter the pattern of clinical presentations of hepatitis B infection, in terms of both frequency and severity, has led to the launching of a surveillance system to monitor the occurrence of jaundice. Three sentinel areas have been identified and a reporting system introduced using traditional healers to whom the majority of patients with jaundice present for treatment. The reports from one such herbalist have been validated in 73% of cases, implying that the traditional sector is an important source of information on jaundice. An attempt is now being made to encourage the referral of patients to enable serological tests to be performed.

Mathematical models to predict post-vaccination antibody decline and the impact of vaccination on the dynamics of infection are being developed in collaboration with the University of Cambridge and Imperial College (London) respectively.

A number of studies have shown that aflatoxin exposure in The Gambia is high and widespread (see Section 2.11). Methods are being explored by which it might be feasible to reduce this exposure, initially in small controlled intervention trials with accurate monitoring of exposure levels.

4.1 IARC staff publications

Allen, S.J., Wild, C.P., Wheeler, J.G., Riley, E.M., Montesano, R., Bennett, S., Whittle, H.C., Hall, A.J. & Greenwood, B.M. (1992) Aflatoxin exposure, malaria and hepatitis B infection in rural Gambian children. *Trans. R. Soc. Trop. Med. Hyg.*, **86**, 426–430

Chotard, J., Inskip, H.M., Hall, A.J., Loik, F., Mendy, M., Whittle, H., George, M.O. & Lowe, Y. (1992) The Gambia Hepatitis Intervention Study: follow-up of a cohort of children vaccinated against hepatitis B. *J. Infect. Dis.*, **166**, 764–768

Del Mistro, A., Chotard, J., Hall, A.J., Whittle, H., De Rossi, A. & Chieco-Bianchi, L. (1992) HIV-1 and HIV-2 seroprevalence rates in mother-child pairs living in The Gambia (West Africa). *J. Acq. Immune Defic. Syndr.*, **5**, 19–24

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Hall, A.J. and The Gambia Hepatitis Study Group (1991) The Gambian Hepatitis B Control Programme. In: Hollinger, F.B., Lemon, S.B. & Margolis, H.S., eds, *Proceedings of The 1990 International Symposium on Viral Hepatitis and Liver Disease: Contemporary Issues and Future Prospects* (Houston, 4–5 April 1990), Baltimore, Williams & Wilkins, pp. 712–716

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Inskip, H.M., Hall, A.J., Chotard, J., Loik, F. & Whittle, H. (1991) Hepatitis B vaccine in the Gambian Expanded Programme on Immunization: factors influencing antibody response. *Int. J. Epidemiol.*, **20**, 764–769

Inskip, H.M., Hall, A.J., Temple, I.K., Loik, F., Herbage, E., Chotard, J. & Whittle, H. (1991) Response to hepatitis B vaccine in relation to the hepatitis B status of family members. *Int. J. Epidemiol.*, **20**, 770–773

Robertson, R.L., Hall, A.J., Crivelli, P.E., Lowe, Y., Inskip, H.M. & Snow, S.K. (1992) Cost-effectiveness of immunizations: the Gambia revisited. *Health Pol. Plan.*, **7**, 111–122

Temple, K., Greenwood, B.M., Inskip, H.M., Hall, A.J., Koskela, M. & Leinonen, M. (1991) The antibody response to pneumococcal capsular polysaccharide vaccine in Gambian children. *Pediatr. Infect. Dis.*, **10**, 386–390

Vall Mayans, M., Inskip, H.M. & Hall, A.J. (1991) Accuracy of answers obtained using a one-year recall period compared to a series of fortnightly recalls. *Int. J. Epidemiol.*, **20**, 774–776

Wild, C.P., Hudson, G.J., Sabbioni, G., Chapot, B., Hall, A.J., Wogan, G.N., Whittle, H., Montesano, R. & Groopman, J.D. (1992) Dietary intake of aflatoxins and the level of albumin bound aflatoxin in peripheral blood in The Gambia, West Africa. *Cancer Epidemiol. Biomarkers Prev.*, **1**, 229–234

4.2 *Other studies of primary prevention of cancer*

4.2.1 Review of results of chemoprevention trials for cancer

(D.M. Parkin and N. Muñoz; in collaboration with E. Buiatti, Florence, Italy; and M. Hakama, Tampere, Finland)

Chemoprevention studies have been extensively used in recent years to assess the contribution of dietary constituents — including micronutrients and oligoelements — to cancer etiology, and their potential for preventive interventions. Most such studies measure, as intermediate endpoints, cellular changes that have been associated with cancer (with varying degrees of certainty). Few such studies reported final results before 1991, but by the end of 1993 a substantial number will be complete. Work began in late 1992 to create an inventory of these studies, and a meeting will be held in early 1994 to review the results of completed studies.

4.2.2 Chemoprevention trial on precancerous lesions of the stomach in Venezuela

(N. Muñoz and S. de Sanjosé; in collaboration with N. Alvarez, O. Andrade, E. Cano, D. Castro, G. Lopez, W.E. Oliver, S. Peraza, V. Sanchez and J. Vivas, San Cristobal, Venezuela; E. Buiatti, Florence, Italy; P. Correa, New Orleans, LA, USA; and G. Sobala, Bradford, UK)

An intervention study is being set up in Tachira state, taking advantage of the infrastructure created for the screening programme for stomach cancer (see Section 4.4.2). The principal aim of this double-blind randomized trial is to determine whether treatment for *Helicobacter pylori* infection followed by treatment with certain anti-oxidants (β-carotene, vitamins C and E) can interrupt the gastric carcinogenic process by blocking the progression from chronic gastritis and intestinal metaplasia to dysplasia and cancer. The protocol requires recruitment of 3000 subjects 35–64 years of age. At recruitment a dietary questionnaire is completed, a gastroscopy performed taking five biopsies and blood and urine specimens are collected from each participant. These procedures will be repeated at the end of the treatment phase, three years later. Annual physical examination and collection of biological specimens will be performed on all participants and gastroscopy on a subsample.

Pilot studies have shown a high prevalence (90%) of *H. pylori* resistant to metronidazole and that treatment with bismuth sub-citrate and amoxycillin for two weeks eradicated *H. pylori* in only 6.5% as compared to 2.0% in those receiving a placebo. This contrasts with

eradication rates of 36–60% reported in trials using the same treatment regimen in Europe and North America (Muñoz *et al.*, 1992). In view of these disappointing results, anti-*H. pylori* treatment phase has been deleted and the main trial commenced in May 1992 randomizing the subjects to treatment with anti-oxidant vitamins (vitamins C, E and β-carotene) as three capsules a day each containing 250 mg of vitamin C, 200 mg of vitamin E and 6 mg of β-carotene, or with a placebo. Treatment is distributed every 1–2 months for three years (Muñoz *et al.*, 1993). A total of 1032 subjects have been recruited and randomized to the two groups up to June 1993. Compliance with the treatment has been satisfactory, with 84.6% of subjects leaving less than 10% of the capsules. Only 6.4% of the subjects have withdrawn from the trial and two presented allergic reactions.

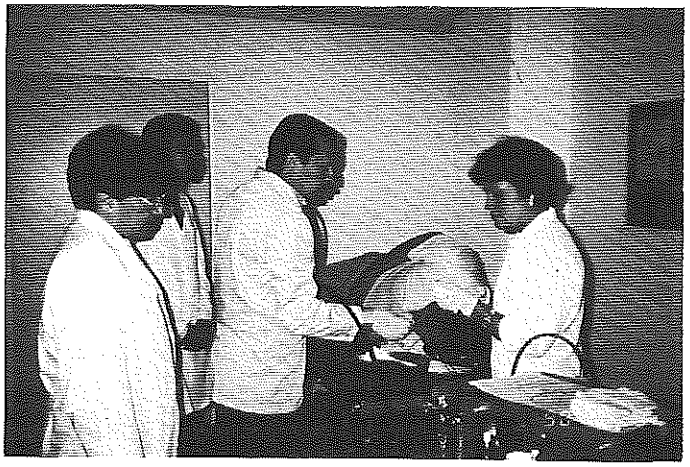

Figure 32. Endoscopic examination of a participant in the Venezuela chemoprevention trial

Consultant pathologists (Dr I. Filipe from Guy's Hospital and Dr J. Torrado from San Sebastian, Spain) have visited the study centre to collaborate with the local pathologists in establishing the immunohistochemical techniques for mucins and Lewis antigen alterations, to be used as end-points in addition to histological diagnosis.

Among the first 1000 subjects recruited, only four had normal gastric mucosa. Superficial gastritis was present in 34, chronic gastritis in 500, chronic atrophic gastritis without intestinal metaplasia (IM) in 125, IM in 293 and dysplasia in 44. Of the cases with IM, 151 (51.5%) were Type I, 56 (19.1%) Type II, 72 (24.5%) Type III and 14 (4.9%) unknown type.

966 of the 1000 subjects were found positive for *H. pylori* in the gastric biopsies using Giemsa stain.

Patients with IM type III are being examined every year including gastroscopy and five gastric biopsies. So far, 46 of these subjects have undergone the second examination.

Recruitment of study subjects has been slower than expected because participation in the screening programmme has considerably fallen during the past year due to problems with the X-ray mobile units used for screening, and to the deteriorating socio-economic situation of the country.

A small sub-trial involving 80 subjects is planned, in order to compare the effect of Sydney triple therapy (bismuth, amoxycillin and metronidazole) with omeprazole plus clarithromycin. The best treatment will be continued until *H. pylori* eradication has been achieved in

100 subjects. Since eradication of *H. pylori* may influence the efficacy of the anti-oxidant treatment, and specifically the effect of ascorbic acid, the information derived from this sub-trial will be used in the interpretation of the results from the main trial.

It is now expected that recruitment will take at least two years and therefore reduction of the sample size or extension of the intervention study to another high-risk area for stomach cancer in Costa Rica is being considered.

4.2 IARC staff publications

Muñoz, N., Buiatti, E., Vivas, J., Oliver, W., Cano, E., Peraza, S., Castro, D., Sanchez, V., Andrade, O. & Benz, M. (1992) Difficulty in eradicating *Helicobacter pylori* in a high-risk population for stomach cancer in Venezuela (Abstract). *Irish J. Med. Sci.*, **161** (Suppl. 10), 6

Muñoz, N., Vivas, J., Buiatti, E., Peraza, S., de Sanjosé, S., Cano, E., Castro, D., Sanchez, V., Andrade, O., Benz, M. & Oliver, W. (1993) Chemoprevention trial of precancerous lesions of the stomach in Venezuela (Abstract). *Eur. J. Cancer Prev.*, **2** (Suppl. 1), 5

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Tuyns, A.J. & Sasco, A.J. (1993) Prevention, knowledge and action (editorial). *Eur. J. Cancer Prev.*, **2**, 91–93

4.3 *Safe handling of carcinogens and destruction of carcinogenic wastes*

4.3.1 Validation and publication of methods for the safe destruction of carcinogenic wastes

(M. Castegnaro; in collaboration with M. De Méo and G. Dumenil, Marseille, France; G. Lunn and E.B. Sansone, Frederick, MD, USA; S. Hansel, M. Milhavet, M. Siméon and M.H. Sportouch, Montpellier, France; J. Barek, Prague, Czech Republic; J. Gallelli, Bethesda, MD, USA; and L.G. Israels, Winnipeg, Canada. Supported in part by the French Ministry of the Environment)

The volumes concerning the methods for the degradation of mycotoxins and polycyclic heterocyclic hydrocarbons have been published.

In view of mutagenic activity of Mn^{2+}, a method was worked out to eliminate Mn^{2+} after treatment of samples with acidic potassium permanganate by precipitation under alkaline conditions, followed by filtration or centrifugation. This procedure leaves no mutagenic activity in the aqueous residues.

A new programme to investigate methods for the degradation of cytostatic drugs has been initiated. The efficiency of three methods for degradation of the most commonly used drugs (see Table 21) is being evaluated.

Detailed protocols for three degradation methods using oxidation by 5% NaOCl or 12.5% in case of failure, oxidation by 30% H_2O_2, and oxidation by 30% H_2O in the presence of ferrous chloride have been agreed upon by the collaborators. Residues will be tested for mutagenic activity in *Salmonella typhimurium* strains TA98, TA100, TA102 and TA97, with and without metabolic activation, and for genotoxic activity, by the single cell gel assay technique (De Méo *et al.*, 1991).

Table 21. List of compounds being considered for degradation techniques

Azathioprine	Ifosfamide	Streptozotocin	Etoposide
Fluorouracil	Bleomycin	Thiotepa	Dacarbazine
Cyclophosphamide	Daunorubicin	Pentostatin	Fludarabine
Cytarabine	Vindesine	Idarubicin	6-Mercaptopurine
Doxorubicin	Plicamycin	Dactinomycin	Carboplatin
Melphalan	Asparaginase	Vinorelbine	Carmustine
Methotrexate	Mitoxantrone	Rubidazone	Teniposide
Cisplatin	Amsacrine	Epirubicin	Mitomycin
Vincristine	Mechlorethamine	Pirarubicin	Aclacynomycin
Vinblastine	Floxuridine	Lomustine	

4.3.2 Safe handling of genotoxic substances

(M. Castegnaro and W. Davis, in collaboration with A. Nageotte, Lyon France; X. Rousselin and J. Dayan, Paris, France; and A. Picot, C. Pleven and F. Zajdela, Orsay, France)

A course was organized on 24 and 25 February 1992, on the safe handling of cytostatic drugs for health workers, which was followed, for those who wished, by a training session at Edouard Herriot Hospital (Lyon, France).

A document has been prepared for publication by the 'Institut national de recherche et sécurité' (Paris, France), dealing with good laboratory practices for handling of genotoxic substances and methods for decontamination of chemical carcinogens.

4.3 IARC staff publications

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Castegnaro, M., Barek, J., Jacob, J., Kirso, U., Lafontaine, M., Sansone, E.B., Telling, G.M. & Vu Duc, T., eds (1991) *Laboratory Decontamination and Destruction of Carcinogens in Laboratory Wastes: Some Polycyclic Heterocyclic Hydrocarbons* (IARC Scientific Publications No. 114), Lyon, IARC

Castegnaro, M. (1993) Disposal of carcinogens from research facilities and hospitals. In: Purchase, R., ed., *The Laboratory Environment*, London, Royal Society of Chemistry (in press)

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Castegnaro, M., de Méo, M., Laget, M. & Duménil, G. (1993) Methods for degradation of polycyclic heterocyclic compounds in laboratory wastes. In: Garrigues, P. & Lamotte, M., eds, *Polycyclic Aromatic Compounds, Synthesis, Properties, Measurements, Occurrence and Biological Effects*, New York, Londonm Gordon & Breach, pp. 135–141

De Méo, M., Laget, M., Castegnaro, M. & Duménil, G. (1991) Genotoxic activity of potassium permanganate in acidic solutions. *Mutat. Res.*, **260**, 295–306

Fouillet, B., Chambon, P., Chambon, R., Castegnaro, M. & Weill, N. (1991) Effects of ozonation on mutagenic activity of polycyclic aromatic hydrocarbons. *Bull. Environ. Contam. Toxicol.*, **47**, 1–7

Fouillet, B., Chambon, P., Chambon, R., Castegnaro, M. & Weill, N. (1991) Influence of ozonation on mutagenic activity of benzidine in water. *Bull. Environ. Contam. Toxicol.*, **47**, 738–742

Rousselin, X., Dayan, J., Pleven, C., Castegnaro, M., Picot, A. & Zajdela, F., eds (1993) *Prévention et sécurité lors de la manipulation des substances génotoxiques utilisées au laboratoire*, Paris, Publications de l'INRS (in press)

4.4 *Studies of screening for cancer*

4.4.1 Screening for cancer of the breast in the Philippines

(D.M. Parkin and P. Pisani; in collaboration with A.V. Laudico, C. Ngelangel, E. Robles, M.-L. Munson and M.G. Reyes, Manila, Philippines)

Screening for breast cancer by mammography, with or without physical examination of the breast, can reduce mortality from breast cancer in women over 50 years of age. However, since the procedure is expensive, such programmes are inappropriate for developing countries, even where breast cancer incidence is moderately elevated. The Manila area of the Philippines is one such area, and a protocol has been developed for a randomized controlled trial of screening for breast cancer in 330 000 women aged 35–64 years, using physical examination by trained nurses as the sole screening modality. A pilot study of 14 000 women in the age range 35–64 was undertaken in 1991–92 to investigate various aspects of feasibility and compliance, and to estimate predictive value of physical examination in this population. In the two districts chosen, 96.5% of women agreed to be examined, and 169 (1.1%) were judged to have breast lumps requiring hospital referral for confirmation and investigation. Compliance with hospital referral was poor (42% only), and special follow-up was required. About half of those finally examined in hospital were confirmed to have a mass requiring biopsy, and one in eight of those were cancers (six cases in total). Financial support for implementation of the randomized trial is now being sought.

4.4.2 Screening for cancer of the stomach in Latin America

(D.M. Parkin and P. Pisani; in collaboration with W. Oliver, San Cristobal, Venezuela; and R. Sierra, San José, Costa Rica)

The case–control study to evaluate the efficacy of the on-going screening programme for gastric cancer by photofluoroscopy, followed by endoscopy, in Tachira province, Venezuela, has been completed. It showed that the population coverage is too low for a mass survey, and that the people who accepted to undergo the examination were self-selected for being at high risk of gastric cancer. A pilot study has been started to test different procedures aimed at improving compliance.

Examination of the feasibility of conducting a randomized trial indicated that the facilities available to the programme are insufficient for the required sample size. Similar considerations apply to a planned screening programme in Costa Rica. The feasibility of pre-selecting a high-risk population in Costa Rica by means of serum determination of pepsinogen levels is under investigation. This would also allow the evaluation, in a prospective design, of the long-term risk of stomach cancer predicted by this serum parameter.

4.4.3 Screening for cancer of the cervix in developing countries

(D.M. Parkin and R. Sankaranarayanan; in collaboration with C. Ngelangel and D. Esteban, Manila, the Philippines; K. Jayant, Barshi, India; and M. Krishnan Nair, N. Sreedevi and B. Mathew, Trivandrum, India)

Screening for cancer of the cervix by means of cytological examination of Pap smears is a well established preventive measure, but relatively few studies have reported on the effectiveness of such programmes in developing countries.

A limited screening programme has been in existence in certain municipalities of the greater Manila area for 15 years. As part of a case–control study of possible etiological factors

(see Section 2.12.2), information is being collected on previous screening history. At the same time, the subjects' knowledge of and attitudes to screening and preventive health care are assessed, so that the magnitude of any selection bias related to these variables can be estimated.

Detection of early invasive cancers by simple visual inspection of the cervix in asymptomatic women is being promoted by the WHO as a less resource-intensive alternative to cytological screening. The sensitivity and specificity of this procedure is being tested in Barshi Tehsil, Sholapur district, Maharashtra and in Kazhakuttam panchayath, Trivandrum, Kerala. In a population-based survey in Barshi, more than 2000 women aged $\geqslant$ 35 years have been subjected to unaided visual inspection by speculum examination to score the clinical appearance of the cervix and Pap smear, in an on-going programme. A similar trial involves approximately 6000 women, in Kazhakuttam panchayath, Kerala. A controlled trial involving more than 70 000 women aged $\geqslant$35 years in Kerala, India is being planned to evaluate the relative effectiveness of Pap smear versus aided visual inspection or unaided visual inspection in detecting invasive cancer at early stage and in preventing (*a*) late-stage (III and IV) disease, (*b*) invasive cancer of the cervix, and (*c*) death from cervix cancer.

4.4.4 Evaluation of screening for neuroblastoma

(J. Estève and P. Roy, in collaboration with the Association pour le Dépistage du Neuroblastome and the Study Group for the Evaluation of Neuroblastoma Screening in Europe (SENSE); F. Berthold and F. Herrmann, Cologne, Germany; V. Combaret, J. Greffe, P. Mathieu and T. Philip, Lyon; A. Craft, Newcastle, UK; R. Erttmann, Hamburg, Germany; J. Jenker, Rome, Italy; J. Mann, Birmingham, UK; F. Schilling, Stuttgart, Germany; and I. Storm-Mathisen, Oslo, Norway)

Several feasibility studies for neuroblastoma screening have been set up in Europe leading to a protocol for the evaluation of screening strategies for this childhood disease. The Agency provided epidemiological and statistical input in the preparation of this protocol. A consensus meeting was organized at the Agency with members of the SENSE Group and epidemiologists with expertise in the evaluation of screening (S. Duffy, Cambridge, UK; C. Hill and H. Sancho-Garnier, Villejuif, France). The Group concluded that screening at six months was not advisable given the heavy overdiagnosis observed in Japan, where a programme has been in operation for several years, and in several feasibility studies. In addition, it was clear that even under an optimistic hypothesis on sojourn time (the length of time spent in the detectable pre-clinical phase), only a large international study would have enough power to evaluate correctly screening for neuroblastoma at either 12 or 18 months or both.

4.4 IARC staff publications

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Sasco, A.J. (1991) Screening for nasopharyngeal carcinoma. In: Miller, A.B., Chamberlain, J., Day, N.E., Hakama, M. & Prorok, P.C., eds, *Cancer Screening*, Cambridge, Cambridge University Press, pp. 375–386

Sasco, A.J. (1991) Validity of case–control studies and randomized controlled trials of screening (letter to the editor). *Int. J. Epidemiol.*, **20**, 1143–1144

Sasco, A.J. (1993) Specific aspects of screening for laryngeal cancer. In: Proceedings of the international colloquium "Perspectives on secondary prevention of laryngeal cancer". *Excerpta Medica* (in press)

Sasco, A.J., Segnan, N., Steiner, W., Succo, G., Terracini, B., Valente, G. & Vershulst, J. (1993) Report of an international workshop on perspectives on secondary prevention of laryngeal cancer. *Eur. J. Cancer* (in press)

Walter, S.D., Kubik, A., Parkin, D.M., Reissigova, J., Adamec, M. & Khlat, M. (1992) The natural history of lung cancer estimated from the results of a randomized trial of screening. *Cancer Causes Control*, **3**, 115–123

PART 5. DEVELOPMENT OF METHODS FOR CANCER RESEARCH

5.1 *Methods for measuring and monitoring exposure to particular carcinogens*

5.1.1 Development of methods for biological monitoring of exposure to chemical carcinogens that yield DNA etheno adducts

(A. Barbin, J. Nair, F. El-Ghissassi, Y. Guichard and H. Bartsch; in collaboration with M.-J. Marion, O. Froment, C. Trépo and J.-C. Contassot, Lyon, France; J. Swenberg, Chapel Hill, NC, USA; M.F. Rajewsky, Essen, Germany; P. Brandt-Rauf, New York, USA; J. Miller, Madison, USA; H.V. Gelboin and S.S. Park, Bethesda, MD, USA. Supported by a contract with the Groupe de Recherche sur les Hépatites, Cirrhoses et Cancers de Foie (INSERM, Lyon) and Elf-Atochem (Paris) and by grants from the Weisbrem-Benenson Foundation (Fondation de France, Paris), the Commission of the European Communities (STEP-CT91-0145) and the University of North Carolina)

Etheno-bridged nucleobases such as 1,N^6-ethenoadenine (ϵA), 3,N^4-ethenocytosine (ϵC) and N^2,3-ethenoguanine (ϵG) are formed by a variety of mutagens and carcinogens such as vinyl halides, urethane, acrylonitrile and mucochloric acid (Bartsch *et al.*, 1993). Etheno adducts in DNA exhibit promutagenic properties and they do not seem to be repaired in liver from preweanling rats exposed to vinyl chloride (VC) (Swenberg *et al.*, 1992). These data suggest that etheno adducts could play a critical role in chemical carcinogenesis and provide a strong rationale for examining the utility of such adducts as biomarkers of exposure.

Mutation in *ras* genes from tumours of humans and rodents exposed to VC have been studied, as described in Section 2.8.3.

5.1.1.1 *Analysis of DNA etheno adducts formed* in vivo

A new, ultrasensitive method based on immunoaffinity purification and ^{32}P-postlabelling has been developed to measure 1,N^6-ethenodeoxyadenosine 3′-monophosphate (3′-ϵdAMP) and 3,N^4-ethenodeoxycytidine 3′-monophosphate (3′-ϵdCMP) in DNA (Guichard & Barbin, 1992; Guichard *et al.*, 1993; Figure 33). This method requires 50 μg of DNA and has a detection limit of 20 attomole which is equivalent to ~4 adducts per 10^{10} parent bases.

Analysis of liver samples from unexposed humans and rodents revealed the presence of background levels of ϵA and ϵC in DNA. This background appears to be highly variable, the molar ratios of adduct to parent base ranging from $< 4 \times 10^{-10}$ up to 1×10^{-7}. The origin of this background is being investigated (see below).

3′-ϵdAMP and 3′ϵdCMP were detected for the first time in liver and lung DNA of adult and newborn mice treated with urethane, vinyl carbamate or vinyl carbamate epoxide by i.p. injection.

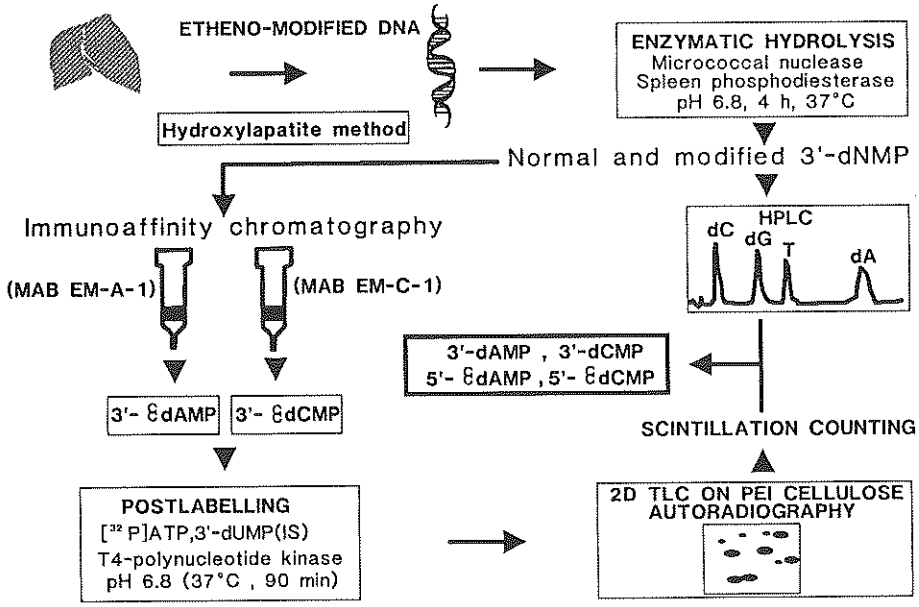


Figure 33. Procedure for ultrasensitive quantification of DNA etheno adducts

The accumulation and persistence of 3′-εdAMP and 3′-εdCMP is being investigated in several organs of rats exposed to 500 ppm VC (4 h/day, 5 days/week) for 1 to 8 weeks, in order to determine whether εA and εC are repaired in adult animals. 3′-εdAMP and 3′-εdCMP are also being analysed in liver DNA from adult rats exposed to various concentrations of vinyl fluoride (25, 250 or 2500 ppm in air, 6 h/day, 5 days/week for 2 or 52 weeks).

5.1.1.2 *Formation of etheno adducts* in vitro

A sensitive assay has been developed to investigate the metabolic or chemical pathways that lead to the formation of etheno adducts (Barbin *et al.*, 1993). cAMP is incubated with the chemical to be tested and an activating system. The formation of 1,N^6-etheno-cAMP is measured by HPLC/fluorimetry, following immunoaffinity purification. This assay has been used to measure the kinetics of activation of VC by rat liver microsomes. In addition, immuno-inhibition assays using monoclonal antibodies directed against specific P450 isozymes have been carried out. The data point to three P450 isozymes involved in VC activation in rat liver: 2B1, 2E1 and an unidentified high-affinity isozyme which activates VC at low concentrations.

Incubation of rat liver microsomes with 25 mM cAMP, deoxyadenosine, deoxycytidine, 3′-dAMP or 3′-dCMP and cofactors for lipid peroxidation ($FeSO_4$ or cumene hydroperoxide) (Figure 34) led to formation of etheno moieties (Barbin *et al.*, 1993). 1,N^6-Etheno cAMP was also produced when cAMP was incubated with arachidonic acid and ferrous sulfate. These data suggest that the background levels of εA and εC observed in DNA *in vivo* (see above)

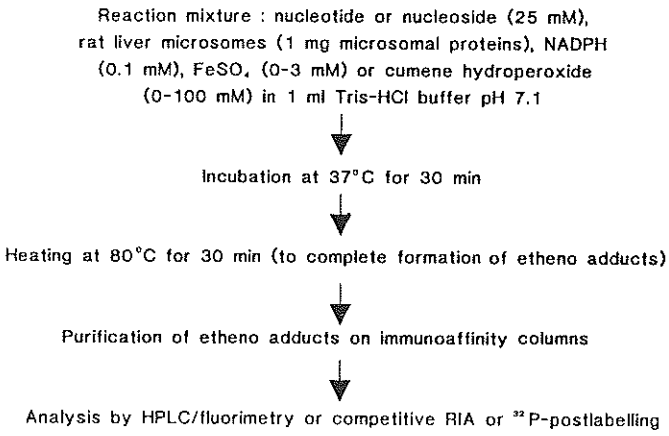


Figure 34. *In vitro* assays to detect etheno (ϵ) adducts formed from nucleotides (nucleosides) and lipid peroxidation products

could result from lipid peroxidation. This hypothesis is now being assessed by analysing human and animal tissues in which lipid peroxidation is increased.

5.1.1.3 *Long-term biomarkers of exposure to vinyl chloride*

A cohort of workers exposed to VC is being followed for the appearance of liver angiosarcoma and of specific tumour markers in blood. The cohort includes workers currently exposed to low levels (< 1 ppm) of VC and retired workers who were heavily exposed to VC before 1975. A bank of sera and lymphocytes from these workers has been established.

An increased level of von Willebrand factor has been observed in VC-exposed workers, as compared to controls (Froment *et al.*, 1992). The serum level of this factor was markedly elevated in three patients with hepatic angiosarcoma and was relatively high in 17 VC-exposed workers with no sign of hepatic disease nor characterized angiopathy. Another tumour marker, the mutant p21 protein produced by the K-*ras* oncogene activated in human liver angiosarcoma, is being measured in the serum of VC-exposed workers. The preliminary data again show elevated levels of mutant p21 in the serum of angiosarcoma patients and of some exposed workers and indicate that this protein could be an earlier marker for liver angiosarcoma.

5.1.2 Biomonitoring of human exposure to alkylating carcinogens by non-invasive means

(D.E.G. Shuker and V. Prevost)

The use and relevance of excreted DNA adducts as markers of exposure to alkylating carcinogens has been reviewed (Shuker & Farmer, 1992). Analytical methods for urinary 3-alkyladenines (3-alkAde), based on immunoaffinity clean-up using a widely cross-reactive monoclonal antibody followed by separation and quantification by gas chromatography–mass spectrometry (Prevost *et al.*, 1993), have been developed and are now finding use in many studies.

5.1.2.1 *Effect of smoking on excretion of 3-alkyladenines*

Three volunteers collected consecutive 24-h urines during a 10-day period during which consumption of preformed 3-MeAde from foods was strictly controlled. On days when the subjects smoked, consistent increases in urinary 3-MeAde were observed which rapidly returned to background levels on cessation of smoking (Prevost *et al.*, 1992; Shuker *et al.*, 1993). An increase in excretion of 3-EtAde was also seen. Since background levels of 3-EtAde are very low and stable (about 1 nmol/person/24 h), excretion of this marker of ethylation was studied in two cigarette smokers on free-choice diets. A very good correlation of 3-EtAde excretion with nicotine intake was seen ($r = 0.93$) (Prevost & Shuker 1992). Recent results have shown that tobacco smoke contains a directly acting ethylating agent that reacts with DNA *in vitro* to give 3-EtAde. The identity and significance of this activity is currently under investigation.

5.1.2.2 *Excreted adducts derived from alkylating chemotherapeutic drugs*
(in collaboration with A.J. Likhachev, St Petersburg, Russian Federation; and M. Clavel, Lyon, France)

In patients receiving combination chemotherapies that included methylnitrosourea, for various malignancies, a dose-dependent excretion of 3-MeAde was seen. Wide variations in the excreted levels of 3-MeAde for a given dose were noted, but the significance of this was not clear, especially due to the rather advanced illness of the patients. Some indication of a correlation between 3-MeAde excretion and levels of 7-methylguanine and O^6-methylguanine in lymphocyte DNA was observed. These rather exceptional studies have nonetheless proved encouraging for the application of a non-invasive approach to the determination of exposure to alkylating agents.

Studies on alkylating chemotherapeutic drugs have been extended to chloroethylnitrosoureas, in particular, fotemustine. Fotemustine is mutagenic in a number of test systems and its activity profile is similar to that of carcinogenic nitrosoureas such as 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (MeCCNU) (Ashby *et al.*, 1993). The major urinary 3-alkyladenine arising from fotemustine and bischloroethylnitrosourea (BCNU) both *in vitro* and *in vivo* (rats and humans) appears to be 3-vinyladenine.

5.1.3 Validation of a new fluorometric assay for benzo[*a*]pyrene diol epoxide adducts in human white blood cell DNA

(M. Rojas, K. Alexandrov and H. Bartsch; in collaboration with E. Kriek, Amsterdam, The Netherlands)

A new fluorometric assay has been validated for quantitation of benzo[*a*]pyrene diol-epoxide (BPDE) adducts in DNA of human white blood cells (WBC) (Alexandrov *et al.*, 1992). This assay can measure 1 BPDE adduct per 10^8 unmodified nucleotides in WBC DNA. The quantity of DNA required depends on the level of modification and can vary from 5 to 500 μg. High levels of BPDE–DNA adducts were found in WBC from seven lung cancer patients (range 62–533 adducts/10^8 nucleotides). In 13 samples from healthy subjects (smokers and non-smokers), the presence of BPDE–DNA adducts was detected in only one smoker, and at a lower level than in the lung cancer patients. BPDE–DNA adduct levels measured by the fluorometric assay were compared with results from ELISA and ^{32}P-postlabelling assays done in another laboratory. A highly significant correlation and

proportionality was found between the levels of BPDE–DNA adduct measured by fluorescence and those from the ^{32}P-postlabelling assay, but the correlation with the ELISA data was poor, the latter assay grossly overestimating BPDE–DNA adduct levels. This highly sensitive and specific fluorescence assay is suitable for measuring BPDE–DNA adducts not only in tissues but also in human WBC from occupationally exposed subjects and smokers.

5.1.4 Biomonitoring of exposure to the food-borne carcinogen PhIP

(M. Friesen, L. Garren, C. Malaveille, J.-C. Béréziat, J. Hall and H. Bartsch; in collaboration with F.F. Kadlubar, D. Lin and K. Kaderlik, Jefferson, AR, USA; H.A.J. Schut, Toledo, OH, USA; and M. Vanderlaan, Livermore, CA, USA)

Sensitive and specific methods involving gas chromatography/negative ion chemical ionization mass spectrometry (Lin *et al.*, 1993) have been developed to measure human exposure to 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), a compound formed during the cooking of meat which has been shown to cause colon cancer in rats. The methods measure levels of unmetabolized PhIP in the urine or faeces (Friesen *et al.*, 1993) or, after alkaline hydrolysis of PhIP from DNA, in exposed tissues (Kadlubar *et al.*, 1993). The method for PhIP–DNA adducts, which has been tested in bacteria, rats and beagle dogs at levels down to one adduct per 10^8 nucleotides (for 200 μg DNA), is currently being validated by comparison with ^{32}P-postlabelling. Work is also in progress to extend the method to the measurement of PhIP–DNA adducts in lymphocytes from humans exposed to PhIP through the diet and to study the effect on adduct levels of acetylation and oxidation phenotype.

5.1.5 Postlabelling methodology study

(M. Castegnaro; in collaboration with D.H. Phillips, Sutton, UK)

A collaborative study to evaluate interlaboratory variability in the use of ^{32}P-postlabelling techniques for detection of DNA adducts, with or without adduct enrichment techniques, has been initiated. Four samples have been circulated to 15 laboratories (sample 1: DNA isolated from epidermis of untreated male Parker mice; sample 2: DNA isolated from epidermis of benzo[*a*]pyrene-treated male Parker mice; sample 3: DNA isolated from human peripheral lung tissue of tobacco smokers; sample 4: DNA isolated from liver of mice treated intraperitoneally with 2-acetylaminofluorene). Participants were deliberately not asked to adhere to a provided protocol, but rather to use the experimental procedures and conditions that have evolved in their laboratories. Thus the primary aim of the trial was to determine the extent of reproducibility or variability occurring with ^{32}P-postlabelling as it is currently performed. The results are presented in Table 22.

There was a good qualitative and reasonable quantitative agreement between the participating laboratories. There is, however, some indication that adduct levels, at least for samples 2 and 4, were somewhat underestimated.

Although different investigators used a variety of conditions for DNA digestion, labelling and chromatography, no trends were noted that indicated specific sources of variability.

Table 22. Statistical parameters from the interlaboratory study on postlabelling

Sample	Method[a]	No. of results	Median	Mean adducts/10^8 nucleotides (S.D.)		Coefficient of variation (%)	Outliers
2 BP-DNA	Standard	11	208.0	205.1	(141.7)	69.1	1
	Nuclease P1	15	105.5	121.9	66.5)	54.5	1
	Butanol	9	93.0	113.5	(80.6)	71.0	1
3 Human	Nuclease P1	15	16.1	17.6	(9.7)	55.4	1
lung	Butanol	9	15.7	27.0	(26.4)	97.6	0
4 AAF-DNA	Standard	11	310.0	271.0	(151.7)	56.0	0
	Nuclease P1	15	15.1	6.8	(13.3)	79.5	0
	Butanol	9	245.0	265.7	(173.3)	65.2	1

[a] Without adduct enrichment (standard method), or using the nuclease P1 or butanol enrichment procedures

BP, benzo[*a*]pyrene; AAF, 2-acetylaminofluorene

5.1.6 Validation of method for fumonisin analysis

(M. Castegnaro)

In an evaluation of analytical methodology for fumonisins B_1 and B_2 (coordinated by the Community Bureau of Reference of the CEC, Brussels), the method most commonly used by the 24 laboratories that sent back results was the HPLC method of Shephard *et al.* (1990). Generally good results were obtained. A further study using contaminated maize as a matrix for analysis is planned.

5.1.7 International Mycotoxin Check Sample Programme

(M. Friesen, E. Bayle and L. Garren. Supported by the Joint FAO/WHO Food Contamination Monitoring Programme and the IUPAC Commission on Food Chemistry)

Since 1979, the IARC has provided laboratories around the world with an annual service of analytical quality assurance for the analysis of mycotoxins in foods. Participants analyse identical portions of a homogeneous food sample for mycotoxins using methods of their choice. They are then provided with the distribution of the results from all participants, with which they can compare their own results. In a study completed in 1992, 209 laboratories in 57 countries participated in the analysis of aflatoxins in maize and peanuts, 125 laboratories in 43 countries in the analysis of aflatoxin M_1 in milk and 126 laboratories in 27 countries in the analysis of aflatoxins in cotton seed meal.

5.1.8 Development of general procedures for the detection of 7-alkylguanines

(D.E.G. Shuker and M.-J. Durand)

5.1.8.1 *Immunoaffinity purification of 7-alkylguanines*

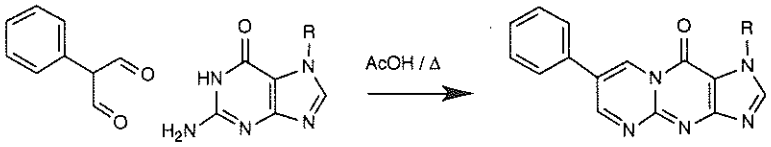
An antiserum that cross-reacts with a number of 7-alkylguanines has been prepared using an antigen based on 7-(2-carboxyethyl)guanine. Immunoaffinity columns made using the IgG

fraction of this serum were found to retain 7-methyl-, 7-ethyl-, 7-hydroxyethyl- and 7-(2,3-dihydroxypropyl)guanine. Samples of human gastric juice spiked with methyl- and ethylurea were treated with nitrite at pH 2 and then incubated with calf thymus DNA. The recovered DNA was analysed for 7-alkylguanines using immunoaffinity purification and HPLC–fluorescence and for 3-alkyladenines by immunoaffinity purification and gas chromatography–mass spectrometry as described in Section 5.1.2. Readily detectable levels of methylated and ethylated bases were seen (Durand, 1993). This method is being developed as the basis of a procedure to identify alkylating agents responsible for the mutagenic and potentially carcinogenic activity of certain gastric juices.

5.1.8.2 *Fluorescent postlabelling of 7-alkylguanines using phenylmalondialdehyde* (in collaboration with D. Molko, Grenoble, France)

7-Alkylguanines react with phenylmalondialdehyde to produce highly fluorescent derivatives. As part of a project to develop methods for the identification of genotoxic substances in biological fluids (such as gastric juice) or foods, this reaction is being used to determine 7-alkylguanines in DNA which has been incubated with samples of interest.

Reaction of a series of 7-alkylguanines including 7-methyl-, 7-ethyl- and 7-(2,3-dihydroxypropyl)-guanine with phenylmalondialdehyde gives fluorescent products (Figure 35), that show similar spectral properties.



R = H-, CH_3-, CH_3CH_2-, $HOCH_2CH_2$-, $HOOCCH_2$-, $HOOCCH_2CH_2$-

Figure 35. Formation of fluorescent derivatives of 7-alkylguanines by reaction with phenylmalondialdehyde

The pure compounds have been characterized by mass spectrometry and nuclear magnetic resonance spectroscopy (Shuker *et al.*, 1993). The analysis of a standard mixture by fluorescence detection following reverse-phase HPLC separation showed a detection limit of less than 1 pmol of each compound. In order to improve the sample clean-up before analysis, an antiserum has been prepared against the fluorescent derivative of 7-(2-carboxyethyl)guanine bound to carrier protein via the carboxyl group. The antiserum cross-reacted with a number of phenylmalondialdehyde derivatives (Durand, 1993).

5.1.9 HPLC photohydrolysis colorimetric method for analysis of *N*-nitroso compounds in human body fluids and foods

(B. Pignatelli, P. Thuillier and H. Bartsch)

Known *N*-nitroso compounds (NOC) constitute only a minor fraction of total NOC that are formed or occur in body fluids and in human diets. A new selective and sensitive method for separating and detecting NOC of unknown structure has been developed and validated with mixtures of 28 reference NOC that include nitrosamides, bulky alkylnitrosamines and NOC carrying hydroxyl and carboxylic groups (Pignatelli *et al.*, 1993). NOC are first separated

by reverse-phase HPLC (column ODS C_{18}) and then photolytically cleaved by UV irradiation. The resulting nitric oxide is oxidized and hydrolysed to nitrite ion which is detected spectrometrically by formation of an azodye with Griess reagent. As only compounds liberating nitric oxide after UV irradiation can be detected, the method has a high selectivity. Sensitivity was 1 to 10 ng (0.006–0.05 nmol) depending on the individual NOC.

Application of this method to the analysis of NOC in a dichloromethane extract of nitrosated gastric juice revealed the presence of three unknown NOC which were different from eight reference NOC separated under the same conditions. Similarly, in addition to nitrosopyrrolidine, six NOC of unknown structure were separated by HPLC analysis of an extract of a genotoxic nitrosated fish sauce sample. These two examples illustrate the usefulness of the method to separate, detect and characterize unknown NOC.

5.1.10 Separation of exfoliated colonic cells

(I.K. O'Neill, A. Ellul and A. Loktionov; in collaboration with J. Cummings and S.A. Bingham, Cambridge, UK. Supported by the US National Institutes of Health (CA-39417))

Methods for isolation of human exfoliated cells (Albaugh *et al.*, 1992) and detection of p53 mutations (Ma *et al.*, 1993) are being incorporated into a procedure for short-term screening for chemopreventive agents. F344 rats are administered i.p. 50 mg/kg dimethylhydrazine in a regimen known to yield colon tumours within 30 weeks, and exfoliated cells are periodically collected and assayed for K-*ras* mutations at the codon 12/13 hot-spot. The aims are to (i) establish whether these mutations can be detected early enough for incorporation in volunteer chemopreventive trials and (ii) distinguish whether mutation occurred in stem cells or in proliferating crypt cells, with the latter potentially reversible by dietary manipulation.

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5.2 *Methods for detecting or measuring particular kinds of carcinogenic activity*

5.2.1 Non-genotoxic carcinogenic activity

5.2.1.1 *Inhibition of gap junctional intercellular communication as a detection assay for liver tumour promoters in long-term cultures of rat hepatocytes*
(M. Mesnil, C. Piccoli and H. Yamasaki)

Among various cocultures of hepatocytes with other cell types, we found that mouse embryonal cells (BALB/c 3T3 cells) were the most effective in maintaining the differentiation of rat hepatocytes *in vitro*. Because most human cancers have an epithelial origin and since tumour promoters generally inhibit gap junctional intercellular communication (GJIC) (Swierenga & Yamasaki, 1992), we are examining the use of such a hepatocyte culture system for the detection of tumour-promoting agents by measurement of GJIC after exposure to the test compound. A single application of the strong rat liver tumour-promoter phenobarbital drastically inhibited GJIC between hepatocytes in a coculture for several hours, while treatment for three weeks provoked a constant decrease of GJIC (50%) during the whole period of treatment (Mesnil *et al.*, 1993).

5.2.1.2 *Quantitative estimation of the effect of liver tumour-promoting agents on gap junctional communication and connexin expression in rat liver* in vivo
(V. Krutovskikh, G. Mazzoleni, M. Mesnil and H. Yamasaki; in collaboration with L. Wärngard and U. Ahlborg, Stockholm, Sweden)

Gap junctional intercellular communication (GJIC) was inhibited in liver tissue from rats treated with liver-specific tumour-promoting agents such as phenobarbital, dichlorodiphenyltrichloroethane (DDT), polychlorobiphenyls and clofibrate, as assayed by the direct dye-transfer assay. To obtain more quantitative data, tissue samples from the same experiment were stained with antibodies specific to the major liver gap junction protein, connexin 32 (cx 32). Quantitative measurement of the immunostaining revealed significant

reductions in both the number of gap junctions per hepatocyte and the size of individual gap junctions in all samples from treated animals (see Figure 36). This finding, together with our observation of unchanged levels of cx 32 gene expression (estimated by Northern analysis) in treated rat livers, suggests a direct effect of tumour promoters on gap junctions.

Average number of gap junctions per hepatocytes, average size of individual gap junctions (spots) and percentage of area occupied by cx 32-positive structures were measured with a Leica Quantimeter 570 Image Processing and Analysis System from ten random fields in each sample.

5.2.1.3 *Effect of anti-tumour-promoting treatment on GJIC in rat liver*

(V. Krutovskikh; in collaboration with P. Martel, Jouy-en-Josas, France; and M. Suschetet, Dijon, France)

It is believed that one of the principal pathways of tumour-promoter action is the inhibition of GJIC in target tissues. Since flavonoids have been found to exert anti-tumour-promoting effects on experimental liver carcinogenesis, we have studied the effects of diverse flavonoid compounds on GJIC in rat liver at different stages of medium-term hepatocarcinogenesis experiments. The rats were treated with the test compounds at the 'promotion' stage of the experiment. The functional dye-transfer assay revealed a significant difference between flavonoids in terms of their capacity to affect GJIC in rat liver. Thus, tangeretin strongly decreased GJIC at 72 days of the experiment and the inhibitory effect was even more evident at 132 days. Tangeretin appears to be a stronger inhibitor of GJIC than phenobarbital, which was used in this study as a positive control that acts as a strong liver tumour promoter. In contrast, flavanone and quercetin treatment led to increased GJIC in rat liver, with their effects most evident at the late point of the experiment (132 days). Flavone did not induce any change of GJIC in rat liver. The effects on GJIC were, to a certain extent, correlated with yields of preneoplastic foci in the treated rats.

5.2.1.4 *Preliminary studies for the establishment of a human epithelial cell transformation system*

(P. Silingardi, H. Yamasaki and M. Mesnil; in collaboration with A. Yilmaz and N. Odartchenko, Epalinges, Switzerland)

In an attempt to improve *in vitro* assay systems for carcinogen detection, we have studied a human epithelial cell transformation model, using ductal luminal cells desquamated in human milk during the first days of lactation, that were immortalized by microinjection of wild-type SV40 DNA. From this non-clonogenic cell line, further cell lines, both clonogenic and tumorigenic, were isolated. Our initial aim was to detect biological markers, varying during mammary carcinogenesis, that could be useful to identify transformed epithelial cells.

GJIC studies revealed very low communication capacities for all the mammary cell lines tested and no significant correlation with the different steps of transformation. A lack of expression of the major connexins (26, 32 and 43) and of E-cadherin, a calcium-dependent cell adhesion molecule, was also observed. Study of the involvement of SV40T large antigen in the development of the tumorigenic phenotype suggested an association of this onco-protein with immortalization but not with the acquisition of tumorigenic capacities.

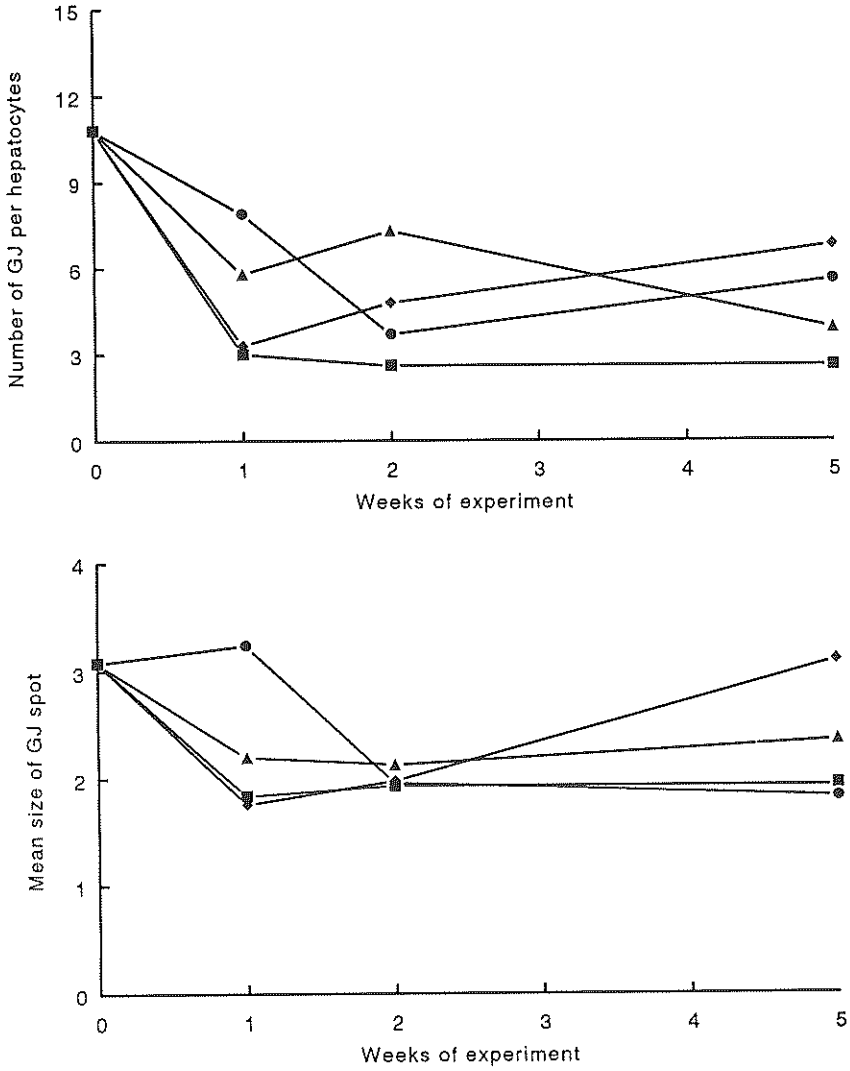

Figure 36. Quantitative analysis of connexin 32 expression in rat livers treated with different liver tumour-promoting agents: phenobarbital (●), polychlorinated biphenyls (■), dichlorodiphenyltrichloroethane (▲) and clofibrate (◆), revealed by immunostaining.

Average number of gap junctions per hepatocyte, average size of individual gap junctions (spots) and percentage of area occuped by cx 32-positive structures were measured with a Leica Quantimeter 570 Image Processing and Analysis System from ten random fields in each sample.

Among the cellular oncogenes known or suspected to be involved in mammary tumorigenesis, we found a slight but significant amplification of c-*myc* in the two tumorigenic cell lines. We are trying to transform the initial non-clonogenic cell line with known

carcinogens to evaluate if chemical induction of transformation is associated with both the growth capacity in soft agar and the biological markers we have studied.

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Swierenga, S.H.H. & Yamasaki, H. (1992) Performance of tests for cell transformation and gap-junction intercellular communication for detecting nongenotoxic carcinogenic activity. In: Vainio, H., Magee, P.N., McGregor, D.B. & McMichael, A.J., eds, *Mechanisms of Carcinogenesis in Risk Identification* (IARC Scientific Publications No. 116), Lyon, IARC, pp. 165–193

Zeng, Y., Ohshima, H., Bouvier, G., Roy, P., Jianming, Z., Li, B., Brouet, I., de Thé, G. & Bartsch, H. (1993) Urinary excretion of nitrosamino acids and nitrate by inhabitants of high- and low-risk areas for nasopharyngeal carcinoma in southern China. *Cancer Epidemiol. Biomarkers Prev.*, **2**, 195–200

PART 6. DISSEMINATION OF INFORMATION, AND EDUCATION AND TRAINING IN CANCER RESEARCH

6.1 *Publication of information directories on cancer research*

6.1.1 *Directory of On-Going Research in Cancer Epidemiology*

(M.P. Coleman, E. Démaret, S. Whelan and R. Sankaranarayanan; in collaboration with H.-J. Baur and J. Wahrendorf, Heidelberg, Germany. Partially supported by the Europe Against Cancer Programme of the Commission of the European Communities)

The Directory is a compilation of abstracts of current, unpublished research in cancer epidemiology, produced in collaboration with the German Cancer Research Centre in Heidelberg. The Directory has been published annually since 1976, but as from 1992 the cycle became biennial. The 1992 Directory contains information on 1197 projects carried out in 70 countries, as well as information on 350 biological materials banks and an address list of all population-based cancer registries. Eight indexes (by investigator, key-word, cancer site, study type, chemical, occupation, country and cancer registry) facilitate access to the information.

To simplify complex searching, for example using key-words from several indexes, a diskette-based index to the Directory has been provided with recent editions. The entire Directory content will be included in a CD-ROM publication now in preparation (Section 6.1.3).

A user survey carried out in 1992 to find out how the Directory is being used and how it could be improved yielded responses from about 50% of the investigators mailed.

Development of EPIBASE, a database management system to run the project entirely at IARC on a microcomputer, is now complete.

The mailing cycle for the 1994 Directory started in January 1993 and most of the material for this issue has been edited, key-worded and entered into the computer.

6.1.2 *Directory of Agents Being Tested for Carcinogenicity*

(M.J. Ghess, J.D. Wilbourn and H. Vainio)

The *Directory of Agents Being Tested for Carcinogenicity* (formerly Information Bulletin on the Survey of Chemicals Being Tested for Carcinogenicity) was initiated in 1973 in collaboration with the US National Cancer Institute. For Directory No. 15, letters were sent out in September 1991 to all collaborating laboratories as well as newly identified institutes asking for updated and new information. Each entry gives the name, Chemical Abstracts Registry Number and synonyms for the chemical, use category, purity, species, strain, sex and number of animals, route of administration and dose levels, duration, starting date, stage of experiments and principal investigator.

The Directory of Agents No. 15, published in June 1992, gives information on 796 chemicals or agents being tested in 82 institutes in 22 countries; a total of 262 published reports on 168 chemicals or agents are listed.

Analysis of the data reported in Directories Nos 13, 14 and 15 shows that the number of carcinogenicity studies being undertaken in each two-year period appears to be diminishing.

6.1.3 Electronic publications

(H. Vainio, J. Cheney, G. Zizka, E. Démaret, J. Wilbourn and M.-J. Ghess)

A range of IARC information resources have been prepared for electronic publication on CD-ROM (compact disk, read-only memory). These comprise the full series of *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* (Vols. 1–55), the *Directory of On-going Research in Cancer Epidemiology*, the *Directory of Agents Being Tested for Carcinogenicity*, the IARC/EPA Genetic Activity Profile data-base and cancer incidence and mortality data. In addition, structured data-bases containing sub-sets of the information in the IARC Monographs will be included to allow more rapid and efficient searching than can be achieved by free-text searching. Data-base building from the electronic resources provided by IARC and from OCR-scanned volumes of the Monographs that were not available in electronic form, as well as development of powerful search software, are being carried out by I.S. Grupe, Inc. (Lombard, Illinois, USA).

The CD-ROM will be published, with full user documentation, by Silver Platter Information Inc., in late 1993.

6.1 IARC staff publications

Coleman, M., Wahrendorf, J. & Démaret, E., eds (1992) *Directory of On-Going Research in Cancer Epidemiology 1992* (IARC Scientific Publications No. 117), Lyon, IARC

Ghess, M.-J., Wilbourn, J.D. & Vainio, H., eds (1992) *Directory of Agents Being Tested for Carcinogenicity*, No. 15, Lyon, IARC

6.2 *Other scientific publications*

(J. Cheney, E. El Akroud, M. Mainaud, A. Romanoff and J. Thévenoux)

All proposals for IARC publications are critically reviewed by the Advisory Committee on Publications to ensure scientific quality and compatibility with the Agency's overall programme. A reduced emphasis has been placed on proceedings of conferences and symposia in view of the often rather ephemeral nature of such material.

The major publication of the biennium was Volume VI of *Cancer Incidence in Five Continents*, the largest volume yet produced by IARC (see Section 1.1.1). The printed volume is accompanied by diskettes carrying the data contained in both volumes V and VI. An important new reference publication in experimental tumour pathology is the multi-fascicle *International Classification of Rodent Tumours*, produced in collaboration with the Institute of Experimental Pathology of the Hannover Medical School. Three of these fascicles on rat tumours have appeared and several further ones are in press. A subsequent set on mouse tumours is in preparation.

New publications that have appeared during the period under review are the following:

Atlas of Cancer Incidence in the Former German Democratic Republic (IARC Scientific Publications No. 106)

Atlas of Cancer Mortality in the European Economic Community (IARC Scientific Publications No. 107)

Environmental Carcinogens: Methods of Analysis and Exposure Measurement, Vol. 11, *Polychlorinated Dioxins and Dibenzofurans* (IARC Scientific Publications No. 108)

Environmental Carcinogens: Methods of Analysis and Exposure Measurement, Vol. 12, *Indoor Air* (IARC Scientific Publications No. 109)

Laboratory Decontamination and Destruction of Carcinogens in Laboratory Wastes: Some Mycotoxins (IARC Scientific Publications No. 113)

Laboratory Decontamination and Destruction of Carcinogens in Laboratory Wastes: Some Polycyclic Heterocyclic Hydrocarbons (IARC Scientific Publications No. 114)

Mycotoxins, Endemic Nephropathy and Urinary Tract Tumours (IARC Scientific Publications No. 115)

Mechanisms of Carcinogenesis in Risk Identification (IARC Scientific Publications No. 116)

Directory of On-going Research in Cancer Epidemiology 1992 (IARC Scientific Publications No. 117)

Cadmium in the Human Environment: Toxicity and Carcinogenicity (IARC Scientific Publications No. 118)

The Epidemiology of Cervical Cancer and Human Papillomavirus (IARC Scientific Publications No. 119)

Cancer Incidence in Five Continents, Vol. VI (IARC Scientific Publications No. 120)

International Classification of Rodent Tumours (IARC Scientific Publications No. 122) (Fascicles 1–3)

Cancer in Italian Migrant Populations (IARC Scientific Publications No. 123)

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 53. *Occupational Exposures in Insecticide Application, and Some Pesticides*

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 54. *Occupational Exposures to Mists and Vapours from Strong Inorganic Acids; and other Industrial Chemicals*

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 55. *Solar and Ultraviolet Radiation*

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 56. *Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins*

SEARCH: A Computer Package to Assist the Statistical Analysis of Case–Control Studies (IARC Technical Reports No. 2)

Comparative Study of Anti-Smoking Legislation in Countries of the European Economic Community (IARC Technical Reports No. 8)

Epidémiologie du cancer dans les pays de langue latine (IARC Technical Reports No. 9)

Nitroso Compounds: Biological Mechanisms, Exposures and Cancer Etiology (IARC Technical Reports No. 11)

Epidémiologie du cancer dans les pays de langue latine (IARC Technical Reports No. 12)

Health, Solar UV Radiation and Environmental Change (IARC Technical Reports No. 13)

Epidémiologie du cancer dans les pays de langue latine (IARC Technical Reports No. 14)

Directory of Agents Being Tested for Carcinogenicity, No. 15

6.3 *Organization of scientific meetings*

6.3.1 **Workshop on Repair of DNA Alkylation Damage**, 22–27 September 1991, Courmayeur, Italy

This meeting was organized by T. Lindahl (ICRF) and R. Montesano (IARC), with financial support from the EEC Concerted Action on DNA Repair, the Italian Cancer Society and the "Assessorato della Sanità e Assistenza Sociale (Région Autonome Vallée d'Aoste). It was attended by some 65 participants, mainly from Europe. The topics of discussion covered studies on enzymology, molecular biology of various enzymes responsible for the repair of DNA alkylation damage and their role in mutagenesis and carcinogenesis and the efficacy of chemotherapy of cancer patients with alkylating agents.

6.3.2 Cadmium in the Human Environment – Toxicity and Carcinogenicity, 25–27 September 1991, Gargnano, Italy

In response to an initiative of the French Ministère de l'environnement, the Agency organized this symposium in collaboration with the Institute of Occupational Health of the University of Brescia (Professors L. Alessio and P. Apostoli) which provided the local organization of the meeting, jointly organized with the International Union of Pure and Applied Chemistry (IUPAC) and the International Programme on Chemical Safety (IPCS), with the sponsorship of some fifteen national and international organizations. Professors Gunnar Nordberg (University of Umeå, Sweden), Robert Herber (IUPAC) and Lorenzo Alessio (Institute of Occupational Health, Brescia) coordinated the programme and edited the proceedings (Nordberg *et al.*, 1992). 159 participants from 18 different countries attended the symposium.

6.3.3 Meetings on (a) Biomonitoring and Susceptibility Markers in Human Cancer: Applications in Molecular Epidemiology and Risk Assessment; (b) Nitroso Compounds: Biological Mechanisms, Exposures and Cancer Etiology, 27 October–2 November 1991, Kona, Hawaii, USA

These meetings were held in Kona, Hawaii, USA, from 27 October to 2 November 1991 with a total attendance of 210 scientists. The first meeting discussed a wide range of types of biomarkers, exposures and sites at risk (Bartsch *et al.*, 1992) and the second discussed nitroso compound research (Bartsch & O'Neill, 1992).

6.3.4 Workshop on Human Papillomavirus and Cervical Cancer, 25–28 November 1991, Brussels, Belgium

The second international workshop on the epidemiology of cervical cancer and human papillomavirus concluded that the evidence was compelling in considering the association between cervical cancer and HPV infection as causal (Bosch *et al.*, 1992; Muñoz *et al.*, 1992).

6.3.5 Symposium on Postlabelling Methods for the Detection of DNA Adducts, 24-27 June 1992, Lyon, France

This meeting critically reviewed the present state of methodology for the detection of carcinogen–DNA adducts and other forms of induced DNA damage using postlabelling methods (Phillips *et al.*, 1993). It was organized in collaboration with Dr D.H. Phillips (Institute for Cancer Research, Sutton, UK) and held at IARC on 24–27 June 1992.

A unique aspect of this meeting was an international effort by 15 different laboratories to quantify DNA–carcinogen adduct levels in standard DNA and human tissue samples (see Section 5.1.5). While the results were generally similar between most laboratories, an unexpected finding was the apparent under-estimation of total adduct levels by this method, implying that DNA adduct levels in human tissues may actually be higher than is currently estimated.

A particularly significant finding reported at the meeting was that paraffin-embedded tissues, if fixed for no more than 48 hours in formalin, are quite suitable for ^{32}P-postlabelling analyses.

6.3.6 Symposium on Biopersistence of Respirable Fibres and Minerals, 7–9 September 1992, Lyon, France

This workshop was organized in collaboration with the French Institut national de la santé et de la recherche médicale (INSERM) and Centre national de la recherche scientifique (CNRS) and with the support of the Commission of European Communities, the Ministère de la recherche et de la technologie and the Ministère du travail, de l'emploi et de la formation professionnelle, France and the National Institute of Environmental Health Sciences, USA, together with several eminent industrial firms and associations all over the world. Professor J. Bignon from INSERM, Dr J.C. Touray from CNRS and Dr R. Saracci from IARC co-edited the proceedings of the workshop. 110 participants from 21 different countries were present.

6.3.7 Symposium on DNA Adducts of Carcinogenic and Mutagenic Agents: Chemistry, Identification and Biological Significance, 18–21 November 1992, Huddinge, Sweden

This meeting, organized in collaboration with K. Hemminki, reviewed the present state of knowledge on physicochemical properties, methods for synthesis and detection, and the biological relevance of several hundred known DNA adducts that are produced by almost 20 classes of carcinogens and mutagens. Chapters that comprehensively cover reactive intermediates and DNA-bound products formed by carcinogenic agents *in vitro* and *in vivo*, based on articles published over the last 10 years, were prepared and reviewed for publication in the IARC Scientific Publications series (Hemminki *et al.*, 1993).

6.3.8 Meeting on Molecular Epidemiology of Cancer, 7-11 December 1992, Courmayeur, Italy

The meeting was organized in collaboration with D. Forman, London, with the financial support of the European Science Foundation, the Commission of the European Communities, the Région Autonome Vallée d'Aoste and the Italian Cancer Society. It was attended by some 90 participants, mainly from Europe, and notable successes were the balance between laboratory-based scientists and epidemiologists (approximately 60:40) and the bringing together of individuals with direct experience of molecular epidemiology and others keen to learn.

The conference covered a wide range of topics, from the highly technical (new techniques for identifying rare somatic mutations; strategies for the development of a vaccine against human papillomavirus) to broader-based reviews (currently available biomarkers for assessing carcinogen exposure, aflatoxin and liver cancer, radon and lung cancer) to the philosophical (how do epidemiologists define a causal relationship). However, the general theme adhered to by all speakers was how to understand the carcinogenic process as it occurs in humans and in terms that can lead to disease prevention.

6.3.9 Ole Møller Jensen Memorial Symposium on Nutrition and Cancer, 15–17 March 1993, Lyon, France

To honour the important contributions of Dr Ole Møller Jensen to cancer epidemiology, particularly in the field of diet and cancer, a symposium was held at IARC, where Dr Jensen had been a staff member for many years. The symposium, which was organized in collaboration with Dr John Cummings on behalf of the International Union of Nutritional Sciences with the support of the CEC Programme "Europe Against Cancer" and the EUROFOODS-ENFANT Concerted Action, was attended by 110 scientists from 21 countries.

6.3 IARC staff publications

Bartsch, H., O'Neill, I.K. & Kadlubar, F., eds (1992) Biomarkers in human cancer: predisposition and use in risk assessment. *Environ. Health Persp.*, Vols 98 and 99

Bartsch, H. & O'Neill, I.K., eds (1992) *Nitroso Compounds: Biological Mechanisms, Exposure and Cancer Etiology* (IARC Technical Reports No. 12), Lyon, IARC

Bosch, F.X., Muñoz, N., Shah, K.V. & Meheus, A. (1992) Second International Workshop on the Epidemiology of Cervical Cancer and Human Papillomavirus. *Int. J. Cancer*, **52**, 171–173

Hemminki, K., Dipple, A., Shuker, D., Kadlubar, F.F., Segerbäck, D. & Bartsch, H., eds (1993) *DNA Adducts: Identification and Biological Significance* (IARC Scientific Publications No. 125), Lyon, IARC (in press)

Muñoz, N., Bosch, F.X., Shah, K.V. & Meheus, A., eds (1992) *The Epidemiology of Cervical Cancer and Human Papillomavirus* (IARC Scientific Publications No. 119), Lyon, IARC

Nordberg, G.F., Herber, R.F.M. & Alessio, L., eds (1992) *Cadmium in the Human Environment: Toxicity and Carcinogenicity* (IARC Scientific Publications No. 118), Lyon, IARC

Phillips, D.H., Castegnaro, M. & Bartsch, H., eds (1993) *Postlabelling Methods for the Detection of DNA Damage* (IARC Scientific Publications No. 124), Lyon, IARC

6.4 *Cancer research awards*

6.4.1 Research training fellowships

(R. Montesano, M. Davis and E. El Akroud)

The Fellowships Selection Committee met twice in Lyon over the period to review applications; the members of the Committee were:

Dr V.N. Anisimov (1992)	Laboratory of Experimental Tumours, N.N. Petrov Research Institute of Oncology, St Petersburg, Russian Federation
Dr A. Brøgger (1992–1993)	Department of Genetics, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway
Dr J. Cairns (1992–1993)	Clinical Trial Service Unit, Radcliffe Infirmary, Oxford, UK
Dr H. Esumi (1993)	National Cancer Center Research Institute, Tokyo
Dr J. Gordon McVie (1992)	Scientific Department, Cancer Research Campaign, London
Dr B. Mansourian (1992–1993)	Office of Research Promotion and Development, WHO, Geneva, Switzerland
Dr N. Odartchenko (1993)	UICC Representative, Swiss Institute for Experimental Cancer Research, Epalinges s/Lausanne, Switzerland
Dr B. Terracini (1992–1993)	Department of Biomedical Science and Human Oncology, University of Turin, Turin, Italy
Dr S. Watanabe (1992)	National Cancer Center Research Institute, Division of Epidemiology, Tokyo, Japan
Dr D.G. Zaridze (1993)	Institute of Carcinogenesis, Cancer Research Center, Academy of Medical Sciences, Moscow, Russian Federation

The Agency representatives were Dr R. Montesano, Dr N. Muñoz (1992), Dr E. Riboli (1993) and Dr H. Vainio (1992–1993).

In 1992, a total of 9 fellowships were awarded out of 50 applications; in 1993, 13 out of 54 eligible candidates received fellowships. In 1992, five fellowships were tenable at the IARC and in 1993 three. The distribution of fellowships awarded by discipline is given in Table 23; the list of Fellows is given in Table 24.

The Italian Association for Cancer Research generously provided US$100 000 in 1992–1993 in support of the Fellowships Programme.

6.4.2 Visiting Scientist awards

In 1992, this Award was given to Dr N. Pearce (Department of Medicine, Wellington School of Medicine, Wellington, New Zealand) who spent a period of one year in the Unit of Analytical Epidemiology and in 1993 to Dr P.C. Gupta (Tata Institute of Fundamental Research, Bombay, India), who will spend one year in the Unit of Descriptive Epidemiology.

Table 23. Distribution of research training fellowships awarded by discipline

Scientific discipline	No. of fellowships		
	1992	1993	1986-93
Epidemiology and biostatistics	2	2	10
Chemical carcinogenesis	3	3	30
Viral carcinogenesis	0	1	18
Cell biology, cell differentiation and cell genetics	2	4	54
Biochemistry and molecular biology	2	2	65
Others	0	1	153
Total	9	13	410

Table 24. Fellowships awarded in 1992 and 1993

Name	Institute of origin	Host institute
1992		
DEBANT, A.B.	INSERM U 210 Immunologie Cellulaire et Moléculaire Faculté de Médecine Pasteur Nice, France	Dana Farber Cancer Institute Division of Tumour Immunology Harvard Medical School Boston, MA, USA
DEMERS, P.	University of Washington Department of Environmental Health Seattle, WA, USA	IARC Unit of Analytical Epidemiology Lyon, France
DENG, L.	Hunan Medical University Cancer Research Institute Changsha, Hunan, China	IARC Unit of Mechanisms of Carcinogenesis Programme on Viral & Hereditary Factors in Carcinogenesis Lyon, France
IMYANITOV, E.N.	N.N. Petrov Research Institute of Oncology St Petersburg, Russian Federation	Max Planck Institute of Biochemistry Department of Molecular Biology Martinsried bei München, Germany
KANG, H.-I.	Institute of Medical Science University of Tokyo Department of Cancer Cell Research Tokyo, Japan	IARC Unit of Mechanisms of Carcinogenesis Lyon, France
LIANG, Y.Y.	Cancer Institute Department of Chemical Etiology and Carcinogenesis Beijing, China	IARC Unit of Mechanisms of Carcinogenesis Lyon, France
OMORI, Y.	Research Institute for Tuberculosis and Cancer Department of Cell Biology Sendai, Japan	IARC Unit of Multistage Carcinogenesis Lyon, France
SARDET, C.C.	Centre de Biochimie - CNRS Nice, France	Whitehead Institute Cambridge, MA, USA
VARGHESE, C.	Regional Cancer Centre Division of Cancer Epidemiology and Clinical Research Trivandrum, India	Cambridge University Institute of Public Health Department of Community Medicine Cambridge, UK
1993		
ELJAAFARI-CORBIN, A.	INSERM CJF 9015 Hôpital Robert-Debré Paris, France	ARIAD Pharmaceuticals Inc. Cambridge, MA, USA
FU HUA	Shanghai Medical University Department of Preventive Medicine Shanghai, China	IARC Unit of Analytical Epidemiology Lyon, France
GOLDBERG, G.S.	Cancer Research Center of Hawaii Honolulu, Hawaii, USA	University of Western Ontario Department of Anatomy London, Ont., Canada

Name	Institute of origin	Host institute
LANDESMAN, Y.	Weizmann Institute of Science Department of Molecular Genetics & Virology Rehovot, Israel	Beth Israel Hospital Harvard Medical School Department of Microbiology and Molecular Genetics Boston, MA, USA
MOISEENKO, V.V.	Medical Radiological Research Center, Obninsk, Russian Federation,	National Radiological Protection Board Cytogenetics Laboratory Didcot, UK
OHUCHI, T.	Research Institute for Microbial Diseases Osaka University Department of Oncogene Research Osaka, Japan	Rockefeller University Laboratory of Molecular Oncology New York, NY, USA
PALKAMA, T.	Department of Bacteriology & Immunology University of Helsinki Helsinki, Finland	Department of Pathology Tufts University School of Medicine Boston, MA, USA
PERINI, G.	Istituto di Genetica Biochimica ed Evoluzionistica del Consiglio Nazionale delle Ricerche Pavia, Italy	University of Massachusetts Medical Center Program in Molecular Medicine Worcester, MA, USA
SOUCEK, P.	Center of Occupational Diseases National Institute of Public Health Prague, Czech Republic	Center in Molecular Toxicology Vanderbilt University, College of Medicine Nashville, TN, USA
SUNG, N.S.	Lineberger Comprehensive Cancer Center University of North Carolina Chapel Hill, NC, USA	Institute of Virology Chinese Academy of Preventive Medicine Beijing, China
TAKAHASHI, S.	Nagoya City University Medical School First Department of Pathology Nagoya, Japan	IARC Unit of Mechanisms of Carcinogenesis Lyon, France
TAO, X.-G.	Shanghai Medical University School of Public Health Department of Environmental Health Shanghai, China	The Johns Hopkins University School of Hygiene and Public Health Department of Epidemiology Baltimore, MD, USA
YIN FEN	School of Public Health Shanghai Medical University Shanghai, China	Unit of Mechanisms of Carcinogenesis IARC Lyon, France

6.5 *Conduct of training courses*

(W. Davis (until August 1992), M. Davis (since September 1992), C. Déchaux)

Ten courses were held during the period under review.

6.5.1 Advanced statistical methods in cancer epidemiology, 15–19 July 1991, Lyon, France

This course was the seventh in a very successful series held at the Agency. The programme was coordinated by Dr Jacques Estève (IARC).

There were 53 participants coming from 18 countries.

6.5.2 Detection of health hazards in human populations exposed to chemical mutagens and carcinogens, 9–20 September 1991, Harare, Zimbabwe

Following similar courses in Nairobi, Bombay and Mexico, Professor Clever Nyathi of the University of Zimbabwe offered to host this course organized in collaboration with the United Nations Environment Programme (UNEP), the International Programme on Chemical Safety (IPCS) and with the support of the Institute of Occupational Health, Helsinki, the International Labour Office and FINNIDA. Drs Harri Vainio and Chris Wild from IARC coordinated the scientific programme of the course. There was a total of 24 participants (12 from Zimbabwe and 12 from outside, 11 of whom were funded by UNEP).

6.5.3 Safe handling of cytostatic drugs for health workers, 24–25 February 1992, Lyon, France

As a follow-up to the first successful course of March 1990, the Agency organized, in collaboration with the French Institut national de recherche et de sécurité (INRS) a further two-day course for the training of nurses and public health workers. Dr Xavier Rousselin (INRS) and Dr Marcel Castegnaro (IARC) coordinated the programme. 43 French participants attended this session.

6.5.4 Pathology of cancer for non-pathologists, 15–19 June 1992, Oslo, Norway

This new topic for an IARC course was introduced at the suggestion of Professor Olav Iversen (Institute of Pathology, University of Oslo), with the support of the Norwegian Cancer Society and the Norwegian Medical Association Cancer Committee. Professor Iversen coordinated the scientific programme of the course, which was attended by 34 participants coming from 26 different countries.

6.5.5 European Educational Programme in Epidemiology – Fifth and Sixth Residential Summer Courses, 22 June–10 July 1992, Florence and 21 June–9 July 1993, Montecatini Terme, Italy

At this annual summer course, 79 participants from 23 countries were present in 1992 and 62 participants from 19 countries in 1993. Dr Rodolfo Saracci (IARC) was the Programme Director.

6.5.6 Cancer epidemiology with emphasis on occupational cancer and cancer registration, 9–20 November 1992, Ahmedabad, India

This course, directed by Drs Paolo Boffetta and Manolis Kogevinas from IARC, and supported by UNEP and IPCS, was held at the National Institute of Occupational Health, Ahmedabad (Director: Dr S.K. Kashyap) and was attended by 23 participants coming from 11 countries.

6.5.7 Cancer epidemiology (in French), 23 November–4 December 1992, IARC, Lyon, France

Dr Jacques Estève (IARC) and Denis Hémon (INSERM) assumed the task of scientific programme directors of the third course in this series conducted in collaboration with the French Institut national de la santé et de la recherche médicale. 39 participants from 10 different countries attended the course.

6.5.8 Epidemiology of nutrition and cancer, 1–12 March 1993, Lyon, France

Dr Elio Riboli, Head of the IARC Nutrition Programme, directed this first course on the epidemiology of nutrition and cancer. 29 different nationalities were represented in a total of 38 participants.

6.5.9 Cancer epidemiology, 17–28 May 1993, Ostrava, Czech Republic

In collaboration with the University Hospital and Cancer Registry of Northern Moravia, Ostrava (Dr F. Beska) and the Cancer Research Institute of the Slovak Academy of Sciences, Bratislava (Dr I. Plesko), the Agency organized this course with the help of Dr Elsebeth Lynge from the Danish Cancer Registry, Copenhagen, as programme director. There were 31 participants from 9 different countries.

PART 7. SCIENTIFIC SUPPORT ACTIVITIES

7.1 *Computing support service*

(M. Smans, B. Charnay, P. Damiecki, X. Nguyen-Dinh, H. Renard and B. Kajo)

Following the last biennium's major re-design of the Agency's central computing facilities, considerable effort has been devoted to the expansion of the local area network, to better integrate central and personal computing. Management of the central facilities has now become an almost routine task, so that more time is available to assist users.

Transition to the new version of the centralized word-processing system is now complete, but it is clear that this older style of office automation is reaching the limit of its capabilities, and different ways of meeting the Agency's needs are being kept under review.

The Computing Services Group spends most of its time helping the growing number of users to identify and apply appropriate equipment and software from among an ever-expanding range of powerful tools, while constantly investigating new developments to keep the service to the agency at its best possible level.

7.2 *Library and information services*

(H. Miido, M. Coudert and L. Ossetian)

The library receives 200 journal titles and holds approximately 10 500 bound volumes of journals. The journal collection is managed with the Sydney Library System, an automated system for processing books and journals. The total number of library books is over 7000, including WHO publications and annual reports. Electronic book ordering from a US agent has become operational.

During the period under review, 1100 literature searches and 239 monthly updates were performed using in-house CD-ROMs, and 464 online searches of external databases were made. The library uses mostly Medline and Cancerlit databases on CD-ROM.

The on-line ordering of 5722 photocopies of articles not available in-house from other libraries constituted an increase of 23% over the previous two-year period.

Current Contents on diskette is accessed by staff directly on personal computers linked to the Agency network. The system enables users both to identify new literature and to automatically request reprints from authors. During the period July 1991–June 1993, a total of 2660 reprints were requested through this centralized system.

A database of reprints held in personal collections in-house now indexes a total of 36 705 articles.

An on-line computerized catalogue of the books received in the Library since 1989 has been set up. When the retrospective cataloguing is complete by the end of 1993, IARC staff will be able to use any of the VAX computer terminals in the Agency to identify the locations of books in the library or in Unit holdings. In addition, the library has established a direct link

with the on-line public access catalogue to the WHO HQ library in Geneva to allow direct searching for documents held there.

Requests from external libraries were fulfilled for photocopies of 1628 articles from journals in the IARC library.

7.2 IARC staff publications

Miido, H. (1992) Use of medical library systems: geographic analysis. *Elec. Libr.*, **10**, 27–32

Miido, H. (1993) CD-ROM use and file interaction: An analysis of a sample of medical libraries. *J. Information Sci.*, **19**, 235–238

Miido, H. & Armstrong, B.K. (1992) From search results to interlibrary loan and reprint requets through automation. In: Raitt, D.I., ed., *Online Information 1992* (16th International Online Meeting Proceedings, London 8–10 December 1992), Oxford, Learned Information, pp. 479–485

7.3 *Banks of biological specimens*

Various banks of biological samples are available at IARC as a result of past or on-going collaborative studies. Such samples are made accessible to scientists not involved in the original studies, under appropriate conditions. These banks include over 70 000 sera mainly from a study of the role of Epstein–Barr virus in the etiology of Burkitt's lymphoma and nasopharyngeal carcinoma, and 124 Burkitt's lymphoma cell lines from this and other studies (see Section 2.6.1). More recently, a collection has been assembled of viable leukocytes and lymphoblastoid cell lines from members of cancer families studied within the framework of genetic projects. One part of this is a unique bank of samples from French families participating in the medullary thyroid cancer project (Section 3.3.2) and the other is related to the familial breast cancer project, and will be maintained only for the duration of this project.

A very large collection of blood samples is being made in the context of the European Prospective Investigation into Cancer and Nutrition (Section 2.3.1). Some four million "straws" containing aliquots of plasma, serum, buffy coat and red blood cells will be shipped to Lyon from seven countries by the end of 1996, and are being stored in liquid nitrogen. Blood samples collected as part of the Gambia Hepatitis Intervention Study (Section 4.1) to assess the efficiency of the vaccination are also used in ancillary studies of other related scientific questions.

The ethical issues related to the use of biological specimens are examined on a study-by-study basis by a Biological Specimen Committee that interacts closely with the Agency's Ethical Review Committee.

7.4 *Common Laboratory Services*

These services include animal breeding and maintenance of the animal house, the histology laboratory and the glass-washing service. The Agency's scientists use animals bred in-house for the majority of their work, since they now have considerable detailed knowledge of the spontaneous tumour rates in the strains used — BDIV and BDVI rats, C57BL/6 and CD1 mice. Facilities for the maintenance of nude mice are also available. The histology laboratory processes all the histological material from experimental animals in the Agency as well as biopsy material sent by Agency researchers doing field work abroad. The glass-washing facility is a unified service for the experimental work carried out in chemistry, biochemistry and cell culture.

7.5 *Publications and presentations support service*

(J. Cheney, J. Déchaux, G. Mollon and J. Thévenoux)

Advice and assistance are routinely provided to scientific staff on all aspects of document preparation and publication.

An advanced desktop publishing system is used for the typographic make-up of publications and other documents.

Illustrations for IARC publications and for journal articles, lectures and poster presentations by the scientific staff, as well as for other purposes are prepared by a draughtsman and a photographer. A computerized graphics system with a variety of software is used to produce both slides and printed illustrations. Photomicroscopy and the photographic recording of experimental results such as chromatograms, electrophoretograms and autoradiograms constitute an integral part of the laboratory research activity of the Agency.

Annex 1

PARTICIPATING STATES AND REPRESENTATIVES AT THE THIRTY-THIRD SESSION OF THE IARC GOVERNING COUNCIL

29–30 April 1992

Finland

Professor J.K. Huttunen (*Chairman*)
Director-General
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Norway

Dr Berit Mørland (*Vice-Chairman*)
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Belgium

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Secrétaire Général
Ministère de la Santé publique et de l'Environnement
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Ms. Jette Mersing*
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Dr Diane McLaren
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World Health Organization

Dr M. Abdelmoumène
Deputy Director-General

Mr C.G. Sandström
Chief, Budget

Dr J. Stjernswärd
Chief, Cancer and Palliative Care

Mr T. Topping
Office of the Legal Counsel

Mr E.E. Uhde
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Professor L.G. Israels
Incoming Chairman, Scientific Council

Professor A.J. McMichael
Outgoing Chairman, Scientific Council

Professor P.G. Smith
Scientific Council member

Mr A.J. Turnbull
Executive Director, UICC

PARTICIPATING STATES AND REPRESENTATIVES AT THE THIRTY-FOURTH SESSION OF THE IARC GOVERNING COUNCIL

28–30 April 1993

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Graf G. von Westphalen
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Federal Ministry of Finance
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Annex 2

MEMBERS OF THE IARC SCIENTIFIC COUNCIL AT ITS TWENTY-EIGHTH SESSION

3–6 February 1992

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Professor of Occupational and Environmental Health
Department of Community Medicine
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MEMBERS OF THE IARC SCIENTIFIC COUNCIL AT ITS TWENTY-NINTH SESSION

11–14 January 1993

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Professor G.R. Mohn
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Dr V. Koroltchouk
Cancer and Palliative Care

UICC

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Annex 3

STAFF AT IARC*

1 July 1991–30 June 1993

Office of the Director	
Director, IARC	Dr L. TOMATIS
Deputy Director	Dr B.K. ARMSTRONG
Special Adviser on Biostatistics	Dr J. ESTEVE
Statistician	Dr A. ROGATKO (until 30.6.92)
Scientist	Dr E. CARDIS
Technical Assistant	Mr K. ZAID (until 8.5.92)
Administrative Assistants	Mr C. AUGROS
	Mrs M. DAVIS
	Mrs A. GESER
	Mrs E. RIVIERE
Secretaries	Mrs B. ANDRIEUX (half-time)
	Mrs C. DECHAUX
	Miss A. DUFOURNET
	Mrs W. FEVRE-HLAHOLUK
	Mrs A. RIVOIRE
Computing Service Group	
Head/Computer Systems Manager	Mr M. SMANS
Computer Analyst/System Manager	Mr P. DAMIECKI
Scientific Software Manager	Ms B. CHARNAY
Programme Analyst	Mr X. NGUYEN-DINH
Assistant (Computing)	Miss H. RENARD
Computer Operator	Mrs B. KAJO
Secretary	Mrs J. NORTH (from 6.1.92 until 31.7.92)
Gambia Hepatitis Intervention Study	
Project Leaders/Epidemiologists	Dr H. INSKIP (until 19.10.91)
	Dr A. JACK (from 1.9.91)

* The list shows the position of staff and established units as at 30 June 1993

Medical Officer	Dr M. FORTUIN (until 31.1.93)
Statistician/Programmer	Mr N. MAINE (from 5.4.92)
Secretary	Miss S. COTTERELL
Editorial and Publications Services	
Head, Editorial & Publications Services/Editor	Dr J. CHENEY
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Education and Training	
Responsible Officer, Fellowships	Dr R. MONTESANO
Administrative Assistant	Mrs M. DAVIS
Secretaries	Mrs C. DECHAUX
	Mrs E. EL AKROUD
Library	
Librarian	Mrs H. MIIDO
Technical Assistant (Search Analyst)	Mrs M. COUDERT
Assistant (Library)	Mrs L. OSSETIAN
Division of Scientific Activities	
Unit of Analytical Epidemiology	
Chief	Dr R. SARACCI
Scientists	Dr P. BOFFETTA
	Dr P. BOYLE, Head, SEARCH Programme (until 18.10.91)
	Mr R. KAAKS (from 1.1.93)
	Dr E. KOGEVINAS
	Dr J. LITTLE
	Dr E. RIBOLI (Head, Programme of Nutrition and Cancer)
	Dr A.J. SASCO (from INSERM, on special assignment to IARC)
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	Mr B. HEMON
	Mr P. MAISONNEUVE (31.10.91)
	Miss R. WINKELMANN
	Mrs A. ARSLAN
Secretaries	Mrs A. HANSS-COUSSEAU

	Miss S. HAVER
	Miss A. SHANNON
	Mrs S. SOMERVILLE
	Mrs S. STALLARD
Unit of Field and Intervention Studies	
Chief	Dr N. MUÑOZ
Scientist	Dr F.X. BOSCH
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	Miss D. MAGNIN
	Mr M. ROSATO (from 1.9.91)
Secretary	Mrs H. LORENZEN-BIEHE
Unit of Descriptive Epidemiology	
Chief	Dr D.M. PARKIN
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	Dr R. SANKARANARAYANAN (from 29.3.93)
	Dr P. PISANI (from 14.7.91)
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	Dr M. CASTEGNARO
	Dr M. FRIESEN
	Dr M. LANG
	Dr C. MALAVEILLE
	Dr I.K. O'NEILL
	Dr H. OHSHIMA
	Dr B. PIGNATELLI
	Dr D. SHUKER
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	Mrs G. BRUN

	Miss A.-M. CAMUS
	Mrs L. GARREN
Laboratory Technicians	Mrs I. BROUET
	Mrs A. ELLUL
	Mrs A. HAUTEFEUILLE
	Miss J. MICHELON
	Mr. A. SCHOUFT
	Mr P. THUILLIER
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	Mrs Z. SCHNEIDER (half-time)
	Mrs M. WRISEZ
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	Dr M. HOLLSTEIN
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Laboratory Research Assistants	Miss H. BRESIL
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	Mr J. GARCIA
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	Miss A. MUNNIA (until 31.12.91)
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	Mrs A. TROCHARD
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	Mr R. DRAY

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	Dr A. LOKTIONOV (until 28.2.93)
	Dr N. MIRONOV
	Dr H. NAKAZAWA
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	Miss N. MARTEL
	Mrs C. PICCOLI
Laboratory Aides	Mrs M. ESSERTEL
	Mrs N. GRANDCLAUDE
	Miss M. MARANHAO
	Mrs S. VEYRE
Secretary	Mrs C. FUCHEZ
Unit of Carcinogen Identification and Evaluation	
Chief	Dr H. VAINIO
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	Dr H. MØLLER (from 19.1.92)
	Mrs I. PETERSCHMITT (half-time)
	Mr J. WILBOURN
Technical Editor	Mrs C. PARTENSKY
Technical Assistants	Mrs J. CAZEAUX
	Mrs M.-J. GHESS
	Mrs D. MIETTON
Secretary	Miss S. REYNAUD
Clerk	Mrs M. LEZERE
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Director	Mr H.R. CROCKETT
Administrative Assistant	Mrs J. MARTINEZ
Translation	
Translators	Mrs L. EYDOUX (until 20.8.91)
	Dr N. GAUDIN (from 24.10.92)
Secretary	Mrs A.-C. MORET
Personnel	
Personnel Officer	Mrs A. ESCOFFIER
Clerk	Mrs C. MOGENET
Budget and Finance	
Budget and Finance Officer	Mr M.P. JOHNSON

Finance Officer	Mr S. SAPRA
Assistant (Accounting)	Mrs M. HERIN
Assistant (Payments)	Mrs F. ROMAGNAN
Secretary	Mrs D. MARCOU-HANSSON
Clerk (Cashier)	Mr D. HORNEZ
Clerk (Accounts)	Mrs D. LOMBARDO
Clerks (Finance)	Mrs F. FLORENTIN (half-time)
	Miss A. MILONE (half-time)
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Administrative Services Officer	Mr G. GUILLERMINET
Administrative Assistant	Mrs R. SEXTIER
Clerk	Mrs M. LEPETIT
Switchboard Operator	Mrs R. KIBRISLIYAN
Driver	Mr J.-F. DURAND-GRATIAN
Usher (Messenger)	Mr D. LAGARDE
Maintenance Technicians	Mr M. BARBIEUX
	Mr M. BAZIN
	Mr J.-P. BONNEFOND
	Mr G. THOLLY
Assistant (Registry)	Mrs M. GREENLAND
Clerk (Registry)	Mrs L. VIGIER
Assistant (Supplies)	Mrs J. POPOFF
Clerks (Supplies)	Mrs M. FILIPPI
	Mrs L. GRAVIER (half-time)
	Mr M. PRAT
Equipment Operators (Reproduction)	Mr D. GRAIZELY
	Mr M. JAVIN
Documents and Stenographic Pool	
Assistant	Mrs M.-H. CHARRIER
Clerk-stenographers	Mrs M. CAMPBELL (until 31.7.92)
	Miss B. GEOFFRE
	Miss C. HUGHES (until 31.7.92)
	Miss G. RAWLING
Clerk	Mrs M.-B. D'ARCY (until 27.4.92)

SHORT-TERM STAFF
(CONSULTANTS AND TEMPORARY STAFF)
1 July 1991–30 June 1993

Office of the Director	
Consultant	Professor R. SOHIER†
Assistant (Statistics)	Mrs C. LAVÉ*
Editorial and Publications Service	
Consultant	Mr G.E.A. ZIZKA*
Clerk–stenographer	Mrs E. BRUSSIEUX
Education and Training	
Consultant	Dr W. DAVIS
Computing Services Group	
Secretary	Mrs J. NORTH
Division of Scientific Activities	
Unit of Analytical Epidemiology	
Medical Officer	Dr P. ROY
Scientists	Dr T. PARTANEN* Dr P. TONIOLO
Technical Officers	Mr R. KAAKS Ms N. SLIMANI*
Clerks (Statistics)	Miss C. CASAGRANDE* Mr D. COLIN*
Unit of Field and Intervention Studies	
Medical Officer	Dr I. KATO*
Clerk	Miss M. BENZ
Unit of Descriptive Epidemiology	
Scientist	Ms A. KRICKER*

*Still on short-term employment on 30 June 1993

† Deceased 22 December 1991

Unit of Environmental Carcinogens and Host Factors	
Scientists	Dr O. TAYLOR
	Dr A. LOKTIONOV*
Laboratory Research Assistant	Mrs A. KOJO
Laboratory Technician	Miss F. EL GHISSASSI*
Unit of Mechanisms of Carcinogenesis	
Laboratory Technicians	Miss B. CHAMBE
	Mrs L. FOURNIER
	Miss F. VEYRAT-DUREBEX
Laboratory Aides	Mr J. DELZOPPO
	Mr R. GRILLET
	Mr C. MARIOTTO
	Mr R. REVEL
	Mr S. SEBAOUI
Unit of Carcinogen Identification and Evaluation	
Scientist	Dr E. MATOS
Secretary	Mrs J. ATHERTON* (half-time)
Clerks	Mr J. CEREDA (part-time)
	Miss S. RUIZ*

Division of Administration and Finance

Personnel	
Social Adviser	Mrs M.A. VIOT-COSTER* (part-time)
Documents and Stenographic Pool	
Clerk-stenographers	Miss C. HUGHES
	Miss S. LILLE
Registry	
Clerk	Mrs A.M. BEH

*Still on short-term employment on 30 June 1993

Annex 4

VISITING SCIENTISTS AND TRAINEES

Scientists

Dr A. Ahnoux, Unit of Descriptive Epidemiology (3–14 August 1992)

Dr F. Akhtar, Unit of Descriptive Epidemiology (17–23 April 1993)

Dr K. Alexandrov, Unit of Environmental Carcinogens and Host Factors

Dr M.T. Alvarez, Unit of Mechanisms of Carcinogenesis, ICRETT Fellowship (7–11 October 1991)

Ms M. Aoun, Unit of Descriptive Epidemiology, WHO Fellowship (30 November–11 December 1992)

Dr M. Artuso, Unit of Mechanisms of Carcinogenesis, Special Training Award (from 11 May 1992)

Dr P. Arvela, Unit of Environmental Carcinogens and Host Factors (23–27 November 1992)

Dr T. Bandaletova, Unit of Mechanisms of Carcinogenesis, Special Training Award (until 3 February 1992)

Mr L. Barraud, Unit of Mechanisms of Carcinogenesis, Special Training Award (1 February–15 August 1992)

Dr T. Bellander, Unit of Analytical Epidemiology (16–20 September 1991)

Dr F. Bianchini, Unit of Mechanisms of Carcinogenesis, Fellowship from the Commission of the European Communities (until 30 November 1991), Special Training Award (1 December 1991–30 March 1992), Fellowship from the European Environmental Research Organization (from 1 April 1992)

Dr M. Billaud, Programme on Viral and Hereditary Factors in Carcinogenesis, Unit of Mechanisms of Carcinogenesis, Special Training Award from Ligue National contre le Cancer and Fellowship from Fondation pour la Recherche Médicale (from 7 December 1992)

Ms K. Blachnik, Unit of Analytical Epidemiology (from 1 June 1993)

Dr F. Bleicher, Unit of Mechanisms of Carcinogenesis, Special Training Award (until 14 September 1991)

Mr G. Bouvier, Unit of Environmental Carcinogens and Host Factors (until 31 October 1992)

Dr P. Boyle, Unit of Analytical Epidemiology (1–5 June 1992)

Dr P. Brandt-Rauf, Unit of Carcinogen Identification and Evaluation (7 June–15 August 1991)

Professor R. Burton, Unit of Descriptive Epidemiology (from September 1992)

Dr R.N. Butler, Unit of Mechanisms of Carcinogenesis, ICRETT Fellowship (1–28 November 1991)

Dr A. Calender, Programme on Viral and Hereditary Factors in Carcinogenesis, Unit of Mechanisms of Carcinogenesis, Special Training Award (until 30 April 1992)

Professor A. Cali, The Gambia Hepatitis Intervention Study (5–18 February 1992)

Dr X. Castellsagué, Unit of Field and Intervention Studies, IARC Research Training Fellowship (17 June 1991–17 July 1992)

Dr P. Chattopadhyay, Unit of Analytical Epidemiology (1 April–31 May 1992)

Dr I. Chemin, Unit of Mechanisms of Carcinogenesis, Special Training Award (from 1 September 1992)

Dr E. Chokonunga, Unit of Descriptive Epidemiology (1–15 May 1993)

Miss S. Chutimataewin, Unit of Mechanisms of Carcinogenesis, Special Training Award (until 26 May 1992)

Dr D. Consonni, Unit of Analytical Epidemiology (1 May–16 June 1993)

Dr S. Cordier, Unit of Analytical Epidemiology (1–5 June 1992 and 5 October 1992–28 May 1993)

Dr T.A. Cung, Unit of Descriptive Epidemiology (31 August–28 September 1992)

Dr M. D'Errico, Unit of Mechanisms of Carcinogenesis (21 June–2 July 1993)

Dr P. Demers, Unit of Analytical Epidemiology (from 12 October 1992)

Dr L. Deng, Programme on Viral and Hereditary Factors in Carcinogenesis, Unit of Mechanisms of Carcinogenesis, IARC Research Training fellowship (from 10 December 1992)

Dr E. de Stefani, Unit of Field and Intervention Studies (2–15 May 1992)

Dr M.S. Diallo, Unit of Mechanisms of Carcinogenesis, ICRETT Fellowship (15 March–23 April 1993)

Dr F. Donato, Unit of Mechanisms of Carcinogenesis, Fellowship from the Brescia (Italy) Regional Association of Cancer Research (16 March–15 September 1992, 24 November–4 December 1992, 19 April–23 April 1993), Unit of Analytical Epidemiology (11–17 March 1993)

Dr A. Esteve, Unit of Mechanisms of Carcinogenesis, Special Training Award (18 March 1992–17 March 1993), Fellowship from the Commission of the European Communities (from 18 March 1993)

Dr A.C. Fassa, Unit of Analytical Epidemiology (11 January–5 March 1993)

Dr C.M. Friedenreich, Programme of Nutrition and Cancer, Unit of Analytical Epidemiology, IARC Research Training Fellowship (until 30 September 1991)

Dr C. Galassi, Unit of Analytical Epidemiology (26 January–26 March 1992)

Dr J. Galceran, Unit of Descriptive Epidemiology (31 May–6 June 1992)

Professor L. Gandolfi, The Gambia Hepatitis Intervention Study (23 November–12 December 1992)

Dr M. Goldberg, Unit of Environmental Carcinogens and Host Factors, ICRETT Fellowship (19 October–24 November 1992)

Dr H. Greenfield, Programme of Nutrition and Cancer, Unit of Analytical Epidemiology, Fellowship from the Australian Department of Industry, Trade and Commerce (20 July 1991–15 July 1992)

Dr R.C. Gupta, Unit of Environmental Carcinogens and Host Factors (11 June–3 July 1992)

Mr M. Haba, Unit of Descriptive Epidemiology, French Government Fellow (21–26 October 1991)

Dr A.J. Hall, The Gambia Hepatitis Intervention Study (14–28 February 1993)

Dr M. Hergenhahn, Unit of Environmental Carcinogens and Host Factors (until 30 April 1993)

Professor G. Howe, Unit of Analytical Epidemiology (1–5 June 1992)

Dr H.M. Inskip, The Gambia Hepatitis Intervention Study (12–21 May 1992)

Dr J. Iscovich, Unit of Descriptive Epidemiology (11–23 October 1992 and 29 March–23 April 1993)

Dr Liu Long-Jian, Unit of Analytical Epidemiology, MRC Fellowship, UK (28 June–4 July 1993)

Dr H.-I. Kang, Unit of Mechanisms of Carcinogenesis, IARC Research Training Fellowship (from 9 November 1992)

Mrs S. Kaplan, Unit of Analytical Epidemiology (1–5 June 1992 and 19–23 October 1992)

Dr T. Kauppinen, Unit of Analytical Epidemiology (11–15 May 1992)

Dr G.M. Kirby, Unit of Environmental Carcinogens and Host Factors and Unit of Mechanisms of Carcinogenesis, IARC Research Training Fellowship (14 October 1991–13 October 1992)

Dr M. Konstandi, Unit of Environmental Carcinogens and Host Factors, Fellowship, University of Ioannina, Greece (from 3 September 1992)

Dr M. Koulibaly, Unit of Descriptive Epidemiology, French Government Fellow (16–22 December 1991)

Ms A. Kricker, Unit of Descriptive Epidemiology (10 June 1991–20 January 1992)

Miss I. Kusters, Unit of Mechanisms of Carcinogenesis, Special Training Award (18 November 1991–30 June 1992)

Miss H. Labidi, Unit of Analytical Epidemiology (1 June–10 July 1992)

Dr P. Lejeune, Unit of Environmental Carcinogens and Host Factors (29 June–10 July 1992)

Dr Y.-Y. Liang, Unit of Mechanisms of Carcinogenesis, IARC Research Training Fellowship (from 12 January 1993)

Dr S.-H. Lu, Unit of Mechanisms of Carcinogenesis (17–26 June 1992 and 10–22 May 1993)

Mr P. Maisonneuve, Unit of Analytical Epidemiology (1–5 June 1992)

Miss V. Marle, Unit of Analytical Epidemiology (19 June–19 December 1992)

Ms T. Mathiesen, Unit of Descriptive Epidemiology (17–25 June 1992 and 28 October–3 November 1992)

Professor B. Modan, Unit of Analytical Epidemiology (1–5 June 1992)

Dr L. Muriale, Unit of Analytical Epidemiology (4–22 May 1992)

Dr J. Nair, Unit of Environmental Carcinogens and Host Factors (from 23 March 1992)

Miss S. Nambooze, Unit of Descriptive Epidemiology (16–26 August 1992)

Dr S.A. Narod, Programme on Viral and Hereditary Factors in Carcinogenesis, Unit of Mechanisms of Carcinogenesis (1–5 July 1991 and 20–25 March 1992)

Dr F. Ogunbiyi, Unit of Mechanisms of Carcinogenesis, IARC Research Training Fellowship (6 January–23 December 1992)

Dr N. Onier, Unit of Environmental Carcinogens and Host Factors (29 June–10 July 1992)

Dr V. Oumansky, Unit of Environmental Carcinogens and Host Factors (29 June–10 July 1992)

Dr T. T. Partanen, Unit of Analytical Epidemiology (from 1 May 1993)

Dr N. Pearce, Unit of Analytical Epidemiology (from 12 October 1992)

Miss P. Pelkonen, Unit of Environmental Carcinogens and Host Factors, Young Scientist Fellowship, University of Kuopio (20 September–6 November 1992 and 2–29 March 1993)

Dr A. Pham Hoang, Unit of Descriptive Epidemiology (3–10 August 1992)

Dr M. Plummer, Programme of Nutrition and Cancer, Unit of Analytical Epidemiology (20–25 January 1992)

Dr N. Popova, Unit of Environmental Carcinogens and Host Factors, European Science Foundation Fellowship (27 October–27 December 1992)

Professor S. Preston-Martin, Unit of Analytical Epidemiology (1–5 June 1992)

Dr D. Quong, Unit of Environmental Carcinogens and Host Factors (14 March–6 April 1993)

Dr R. Ramirez, Unit of Field and Intervention Studies, Fellowship from Fondo de Investigaciones Sanitarias (9 September 1991–31 July 1992)

Dr L. Reyes, Unit of Descriptive Epidemiology, Fellowship from the International Cancer Research Technology Transfer Programme (9 June–9 July 1992)

Dr M.G. Reyes, Unit of Descriptive Epidemiology, Fellowship from the International Cancer Research Technology Transfer Programme (21 September–21 October 1992)

Ms L. Rezzig, Unit of Descriptive Epidemiology, WHO Fellowship (30 November–11 December 1992)

Dr M. Rojas-Moreno, Unit of Environmental Carcinogens and Host Factors

Dr P. Roy, Unit of Analytical Epidemiology (4–15 January 1993)

Dr P. Ryan, Unit of Analytical Epidemiology (1–5 June 1992)

Ms E. Sala, Unit of Descriptive Epidemiology, WHO Fellow (13 July–4 August 1992)

Dr S. de Sanjosé Llongueras, Unit of Field and Intervention Studies, Special Training Award

Dr S. Satarug, Unit of Environmental Carcinogens and Host Factors, Australian National Health and Medical Research Council Fellowship (from 23 March 1993)

Dr H. Schut, Unit of Environmental Carcinogens and Host Factors, NCI Fogarty Fellowship (from 23 February 1993)

Dr O. Serova, Programme on Viral and Hereditary Factors in Carcinogenesis; Unit of Mechanisms of Carcinogenesis, IARC Research Training Fellowship and Fondation Mérieux fellowship (from 2 December 1991)

Dr K.V. Shah, Unit of Field and Intervention Studies (23 March–14 April 1992)

Dr S. Shankar, Programme of Nutrition and Cancer, Unit of Analytical Epidemiology, NCI Fellowship (27 May–28 August 1992)

Dr M. Siddiqi, Unit of Mechanisms of Carcinogenesis, Fellowship from the Commission of the European Communities (until 15 December 1991)

Dr L. Simonato, Unit of Analytical Epidemiology (27 July–7 August 1992)

Ms T. Sørlie, Unit of Mechanisms of Carcinogenesis, Special Training Award (from 3 May 1993)

Ms S. Sriamporn, Unit of Descriptive Epidemiology (7 September–5 October 1991, 9–15 February, 18–25 June 1992 and 3–15 May 1993)

Dr H. Sriplung, Unit of Descriptive Epidemiology (10–15 May 1993)

Dr S. Stellman, Unit of Descriptive Epidemiology (19 May–14 August 1992 and 4 February–30 April 1993)

Mr C.A. Stiller, Unit of Descriptive Epidemiology (22–28 March 1992)

Dr P. Stolba, Programme on Viral and Hereditary Factors in Carcinogenesis, Unit of Mechanisms of Carcinogenesis, fellowship from the Commission of the European Communities (17 May–17 August 1993)

Dr H.H. Storm, Unit of Descriptive Epidemiology (31 May–5 June 1992)

Dr W. Tarkowski, Unit of Descriptive Epidemiology, Fellowship from the International Cancer Research Technology Transfer Programme (6 November–6 December 1991 and 2–16 December 1992)

Dr P. Toniolo, Programme of Nutrition and Cancer, Unit of Analytical Epidemiology (15 April–30 May 1993)

Dr J. Trédaniel, Unit of Analytical Epidemiology (from 1 October 1992)

Dr H. Tulinius, Unit of Descriptive Epidemiology (13–19 December 1992)

Dr V. Vatanasapt, Unit of Descriptive Epidemiology (10–15 May 1993)

Dr P. Vizcaino, Unit of Descriptive Epidemiology (1–7 March 1992) and Unit of Field and Intervention Studies, Fellowship from Fundación para el Fomento en Asturias de la Investigación Cientifica y la Tecnologia

Dr H.R. Wabinga, Unit of Descriptive Epidemiology (17–27 June 1992)

Dr Q. Wang, Unit of Descriptive Epidemiology, Visiting Scientist Award (5 November 1991–30 October 1992)

Dr T.H. Way, Unit of Descriptive Epidemiology, SEARO Visiting Scientist Grant (25 November–13 December 1991)

Dr V.B. Yermilov, Unit of Environmental Carcinogens and Host Factors, ICRETT Fellowship (22 May–21 June 1993)

Dr A. Zarba, Unit of Mechanisms of Carcinogenesis, ICRETT fellowship (16 November–11 December 1992)

Trainees

Miss E. Albujár, Unit of Descriptive Epidemiology, Special Training Award (21 October 1991–19 September 1992)

Mr R. Artiges, Unit of Mechanisms of Carcinogenesis (1 June–25 July 1992)

Dr T. Bandaletova, Unit of Environmental Carcinogens and Host Factors, Special Training Award (17 February 1992–30 June 1993)

Ms V. Benhaïm, Unit of Analytical Epidemiology, Special Training Award

Miss M. Benz, Unit of Field and Intervention Studies, Special Training Award (until 18 September 1991)

Mr O. Bertrand, Unit of Multistage Carcinogenesis, Fellowship from the Association pour la Recherche sur le Cancer (from 1 January 1992)

Mr G. Bonzon, Unit of Mechanisms of Carcinogenesis (29 March–30 April 1993)

Miss K. Bori, Unit of Mechanisms of Carcinogenesis (25 January–26 February 1993)

Ms S. Chappuis, Programme on Viral and Hereditary Factors in Carcinogenesis, Unit of Mechanisms of Carcinogenesis, MRT fellowship (from 2 September 1991)

Mr P. Chatard, Unit of Analytical Epidemiology, Special Training Award (8 March–31 July 1993)

Mr O. Chatard, Unit of Analytical Epidemiology, Special Training Award (from 1 June 1993)

Ms M. Cordier, Programme on Viral and Hereditary Factors in Carcinogenesis, Unit of Mechanisms of Carcinogenesis, IARC Special Training Award and supported by La Fondation Mérieux (until 30 September 1991)

Mr J. David-Ameline, Programme on Viral and Hereditary Factors in Carcinogenesis, Unit of Mechanisms of Carcinogenesis (2 September 1991–31 August 1992)

Ms K. de Bruin, Unit of Analytical Epidemiology, Special Training Award (until 31 July 1991)

Dr C. Chiodino, Unit of Multistage Carcinogenesis, Fellowship from the Commission of the European Communities (until 31 December 1991)

Miss B. Donvito, Unit of Environmental Carcinogens and Host Factors, Special Training Award (from 5 November 1992)

Mr F. Duchêne, Unit of Analytical Epidemiology (2–20 November 1992)

Miss M.-J. Durand, Unit of Environmental Carcinogens and Host Factors, Special Training Award (1 July 1992–28 May 1993)

Mr L. Espinosa, Unit of Mechanisms of Carcinogenesis (19 April–7 May 1993)

Miss V. Gaborieau, Unit of Analytical Epidemiology, Special Training Award (5 April–24 June 1993)

Miss C. Gaillard, Unit of Mechanisms of Carcinogenesis (1–5 February 1993)

Mrs C. Galiana, Unit of Multistage Carcinogenesis, Fellowship from the Association pour la Recherche sur le Cancer (until 31 December 1992) and Special Training Award

Ms H. Garcia-Giannoli, Unit of Analytical Epidemiology, Special Training Award (from 18 May 1993)

Miss B. Garnier, Unit of Mechanisms of Carcinogenesis (1–12 July 1991)

Miss S. Gazzo, Unit of Mechanisms of Carcinogenesis (3 June–12 July 1991)

Mr O. Geneste, Unit of Environmental Carcinogens and Host Factors, Special Training Award

Ms C. Gros, Unit of Analytical Epidemiology, Special Training Award (13 April–3 July 1992)

Mr Y. Guichard, Unit of Environmental Carcinogens and Host Factors, Special Training Award

Miss M. Holzmann, Unit of Field and Intervention Studies, Special Training Award (1 June–24 July 1992)

Dr M. Hours, Unit of Analytical Epidemiology, Special Training Award (1 February–31 May 1993)

Mr M. Jones, Unit of Descriptive Epidemiology, Special Training Award (from 2 March 1992)

Mr J.L. Klein, Unit of Multistage Carcinogenesis, Special Training Award

Miss M. Koppe, Unit of Mechanisms of Carcinogenesis (22 June–2 July 1993)

Mr J. Lamartine, Programme on Viral and Hereditary Factors in Carcinogenesis, Unit of Mechanisms of Carcinogenesis

Dr V. Ledda-Columbano, Unit of Multistage Carcinogenesis, ICRETT Fellowship (1–9 May 1992)

Miss S. Leijabok, Unit of Descriptive Epidemiology, Special Training Award (from 12 October 1992)

Mr J.C. Lozano, Unit of Multistage Carcinogenesis, Special Training Award

Ms P. Médina, Unit of Analytical Epidemiology, Special Training Award (1 September–31 December 1991)

Mr L. Navarro, Unit of Multistage Carcinogenesis (27 January–28 February 1992)

Ms M.P. Paperin, Programme on Viral and Hereditary Factors in Carcinogenesis, Unit of Mechanisms of Carcinogenesis (29 March–30 April 1993)

Ms O. Patria, Unit of Analytical Epidemiology, Special Training Award (5 April–19 June 1993)

Dr S. Pinloche, Programme on Viral and Hereditary Factors in Carcinogenesis, Unit of Mechanisms of Carcinogenesis (4 November 1991–30 April 1992)

Dr D. Pobel, Unit of Analytical Epidemiology, Grant from the French Ministry of Research and Technology (until 30 April 1993)

Miss F. Raffalli, Unit of Environmental Carcinogens and Host Factors (from 18 January 1993)

Ms S. Royer, Programme on Viral and Hereditary Factors in Carcinogenesis, Unit of Mechanisms of Carcinogenesis (30 March 1992–30 April 1992)

Ms I. Schuffenecker, Programme on Viral and Hereditary Factors in Carcinogenesis, Unit of Mechanisms of Carcinogenesis (4 November 1991–30 April 1992)

Mr A. Souabni, Unit of Mechanisms of Carcinogenesis (from 7 June 1993)

Miss C. Sunyach, Unit of Environmental Carcinogens and Host Factors, Special Training Award (from 2 November 1992)

Mr A. Sylla, Programme on Viral and Hereditary Factors in Carcinogenesis, Unit of Mechanisms of Carcinogenesis (1 October–20 December 1991)

Ms M.T. Valdivieso, Unit of Descriptive Epidemiology, Special Training Award (from 18 March 1993)

Miss B. Vozar, Unit of Mechanisms of Carcinogenesis (30 March–4 May 1992)

Mr S. Wagner, Programme on Viral and Hereditary Factors in Carcinogenesis, Unit of Mechanisms of Carcinogenesis (4 January–28 February 1992)

Dr Q. Wang, Programme on Viral and Hereditary Factors in Carcinogenesis, Unit of Mechanisms of Carcinogenesis, Special Training Award (until 15 February 1993)

Mr J. Watanabe, Unit of Multistage Carcinogenesis (26 April–15 May 1992)

Dr L. Wärngård, Unit of Multistage Carcinogenesis, Fellowship from INSERM (1–30 September 1991 and 16 March–12 April 1992)

Dr E.C. Weatherhead, Office of the Director, Special Training Award (from 10 May 1993)

Miss M. Zucchini, Unit of Mechanisms of Carcinogenesis (19–23 April 1993)

Annex 5

RESEARCH AGREEMENTS IN OPERATION BETWEEN IARC AND VARIOUS INSTITUTIONS

1 July 1991–30 June 1993

Cancer registries

DEB/73/16	International Association of Cancer Registries (Provision of a secretariat and other supporting services)
DEP/87/01	Hanoi Cancer Institute, Hanoi, Viet Nam (Cancer Registry of Hanoi)
DEP/87/02	National Institute of Public Health, Bamako, Mali (Cancer Registry of Mali)
DEP/87/09	La Paz Cancer Registry, Oncological Society of Bolivia, La Paz, Bolivia (La Paz Cancer Registry)
DEP/88/05	Cancer Registry of Tanzania, Pathology Department, Muhimbili Medical Centre, University of Dar-es-Salaam (Cancer Registry of Tanzania)
DEP/89/10	Department of Pathology, National University of Trujillo, Trujillo, Peru (Cancer Registry of Trujillo)
DEP/89/11	Department of Epidemiology and Preventive Medicine, University Hospital, Sétif, Algeria (Cancer Registry of Sétif)
DEP/90/02	Faculty of Medicine, Prince of Songkla University, Hat-Yai, Songkla, Thailand (Population-based cancer registry of Songkla Province)
BRI/91/01	Danish Cancer Registry, Danish Cancer Society, Copenhagen, Denmark (European Network of Cancer Registries)
DEP/91/02	South Pacific Commission, Noumea, New Caledonia (Cancer registration in the Pacific area)
DEP/91/04	National Centre of Anatomo-Pathology, Faculty of Medicine, University of Conakry, Conakry, Guinea (Cancer Registry of Conakry)
DEP/91/05	Department of Pathology, Makerere University Medical School, Kampala, Uganda (Kampala Cancer Registry)
DEP/92/01	Oncology Center of Ho Chi Minh City, Republic of Vietnam (Population-based cancer registry for the city of Ho Chi Minh)
DEP/92/02	Argentinian Association on Education and Prevention of Cancer, Bahia Blanca, Argentina (Cancer registry of Southern Buenos Aires Province)

DEP/92/05	National University of Rwanda, Butaré, Rwanda (Cancer Registry of Butaré)
DEP/92/08	Zimbabwe Cancer Registry, Harare, Zimbabwe (Zimbabwe Cancer Registry)
DEP/92/10	Faculty of Medical Sciences, University of Niamey, Niamey, Niger (Cancer Registry of Niger)
DEP/92/15	National Cancer Institute, Bangkok, Thailand (IARC support for a monograph on "Cancer in Thailand" and a workshop on "The Cancer Control Program : Putting Science into Practice")
DEP/92/16	Barshi Rural Cancer Registry, Ashwini Rural Cancer Research and Relief Society, Barshi, India (Barshi Rural Cancer Registry)
DEP/93/01	National Cancer Registry of Uruguay, National Institute of Oncology, Montevideo, Uruguay (National Cancer Registry of Uruguay)

Incidence studies

DEP/91/01	Israel Center for Registration of Cancer and Allied Diseases, Jerusalem, Israel (Cancer risk in second-generation migrants to Israel: Phase II)
DEP/92/03	Lithuanian Oncological Centre, Vilnius, Lithuania (European childhood leukaemia/lymphoma incidence study (ECLIS))
DEP/92/04	Estonian Cancer Registry, Institute of Experimental & Clinical Medicine, Tallinn, Estonia (European childhood leukaemia/lymphoma incidence study (ECLIS))
DEP/92/12	Institute of Hematology, Minsk, Belarus (European childhood leukaemia/lymphoma incidence study (ECLIS))
DEP/92/13	Petrov Research Institute of Oncology, St Petersburg, Russian Federation (European childhood leukaemia/lymphoma incidence study (ECLIS))
DEP/92/14	Research Institute of Pediatric Hematology, Moscow, Russian Federation (European childhood leukaemia/lymphoma incidence study (ECLIS))
AEP/93/01	Israel Center for Registration of Cancer and Allied Diseases, Jerusalem, Israel (Cancer risk in a cohort of immigrants from the former Soviet Union to Israel)

Second cancers and DNA damage following chemotherapy

BRI/89/07	TNO Medical Biological Laboratory, Rijswijk, The Netherlands (Study of the relationship between cis-platinum adduct levels and therapeutic efficacy in testicular cancer patients)
BRI/89/09	Biological Research Center, National Hellenic Research Foundation, Athens, Greece (Detection of methylation adducts in Hodgkin's disease patients)
BRI/89/10	Department of Radiation Genetics and Chemical Mutagenesis, Sylvius Laboratories, State University of Leiden, Leiden, The Netherlands (Measurements of micronuclei in lymphocytes as an indication of DNA damage following chemotherapy in Hodgkin's disease patients)
BRI/89/11	Gustave Roussy Institute, Villejuif, France (Pilot study for the detection of methylation adducts in lymphoma patients)

BRI/91/02	Lymphoma Clinic, University of Athens School of Medicine, Athens, Greece (Pilot study for the detection of methylation adducts, oncogene mutation, micronuclei and DNA repair in Hodgkin's disease patients treated with MOPP/ABV chemotherapy)
AEP/92/02	Unit of Hematology, Hospital Lyon-Sud, Pierre-Bénite, France (DNA damage following MOPP/ABV chemotherapy in Hodgkin's disease patients)
AEP/92/03	Edouart Herriot Hospital, Lyon, France (DNA damage following MOPP/ABV chemotherapy in Hodgkin's disease patients)

Studies on breast cancer

DEB/86/10	Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, NY, USA (Breast cancer and hormonal profile in Chinese and Chinese-American women)
DEB/86/14	Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, NY, USA (Biochemical analyses for studies of (*a*) urinary levels of oestrogens and progesterone in relation to passive smoking in nonsmoking women, and (*b*) breast cancer and hormonal profile in males)
DEP/91/03	Clinical Epidemiology Unit, College of Medicine, University of the Philippines, Manila, Philippines (Pilot study into the feasibility of screening for breast cancer in the population of metropolitan Manila)
DEP/92/07	Rizal Cancer Registry, Rizal Medical Centre, Manila, Philippines (Feasibility of screening for breast cancer in the population of Metropolitan Manila)

Studies on cervical cancer

DEB/85/17	Foundation for Higher Education, Cali, Colombia (Case-control study on risk factors for cervical cancer)
FIS/88/01	Department of Immunology and Infectious Diseases, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, MD, USA (Human papilloma virus (HPV) and cervical cancer: analysis of specimens for HPV-DNA)
FIS/90/05	WHO Collaborating Centre for the Community Control of Hereditary Diseases, Department of Human Genetics and Teratology, National Institute of Hygiene, Budapest, Hungary (Cytogenetic studies)
FIS/90/08	Faculty of Medicine, Prince of Songkla University, Hat-Yai, Thailand (Multicentric case-control study on cervical cancer)
FIS/90/09	National Institute of Public Health Research, Bamako, Mali (Multicentric case-control study on cervical cancer)
FIS/90/10	Clinical Epidemiology Unit, College of Medicine, University of the Philippines, Manila, Philippines (Multicentric case-control study on cervical cancer)
FIS/90/11	Department of Pathology, Ministry of Health, Asunción, Paraguay (Multicentric case-control study on cervical cancer)

FIS/90/13	National Institute of Oncology, Rabat, Morocco (Multicentric case–control study on cervical cancer)
FIS/91/01	Maria Sklodowska Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland (International biological study on cervical cancer)
FIS/91/02	Department of Pathology, Diponegoro University Medical Faculty, Semarang, Indonesia (International biological study on cervical cancer)
FIS/92/01	Department of Immunology and Infectious Diseases, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland, USA (International biological study on cervical cancer: polymerase chain reaction (PCR) testing of specimens with histology confirming the presence of malignancy)
FIS/92/02	Department of Experimental Virology, Institute of Hematology and Blood Transfusion, Prague, Czech Republic (Role of HPV in the development of cervical cancer)
FIS/93/01	Department of Pathology, Free University Hospital, Amsterdam, The Netherlands (Multicentric case control study on cervical cancer)
FIS/93/03	Laboratory of Microbiology and Immunology, Institute of Virology, Tours, France (Role of HPV DNA in the development of cervical cancer)

Studies on liver cancer

DIR/86/01	Medical Research Council, London, UK (Gambia Hepatitis Intervention Study)
FIS/87/01	National Cancer Institute, Bangkok, Thailand (Cohort study of HBsAg carriers in Bangkok)
FIS/88/02	Institute of Oncology, University of Padua, Italy (Natural history of human retrovirus infections in The Gambia)
FIS/88/03	Division of Gastroenterology, Hospital San Giovanni Battista, Turin, Italy (Causes of non-response to hepatitis B vaccine)
FIS/88/04	Department of Clinical Immunology, University of Rome, Italy (Causes of non-response to hepatitis B vaccine)
FIS/88/05	Department of Social Medicine and Public Health, University of Singapore, Singapore (Cohort study on hepatitis B carriers and liver cancer)
FIS/89/09	Mount Holyoke College, South Hadley, MA, USA (Cost effectiveness of addition of hepatitis B virus vaccination to expanded programme on immunization in the Gambia)
DEP/92/09	Cancer Unit, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand (Prospective study on the etiology of liver cancer in Northeastern Thailand)
MCA/93/01	Research Division, National Cancer Institute, Bangkok, Thailand (Investigation of expression of hepatic carcinogen metabolising enzymes in the Thai population)

Studies on malignant melanoma

DEP/87/08	Cancer Registry, Department of Pathology, National University, Asunción, Paraguay (Case–control study of etiological factors of plantar melanoma in Paraguay)

Studies on nutrition and on cancer of the gastrointestinal tract

DEB/84/01	Singapore Cancer Registry, Department of Pathology, University of Singapore, Singapore (Development of methodology for the conduct of diet-directed case–control studies in Singapore)
FIS/87/05	Cancer Registry, Department of Pathology, National University, Asunción, Paraguay (Case–control study on oesophageal cancer in Paraguay)
AEP/88/02	Department of Epidemiology and Statistics, Hospital San Jaume i Santa Magdalena, Mataro, Spain (Case–control study on stomach cancer and diet)
AEP/89/01	Rowett Research Institute, Aberdeen, UK (Nutritional assessment component of EEC breast and colorectal cancer study)
AEP/89/02	Department of Epidemiology and Statistics, Hospital San Jaume i Santa Magdalena, Mataro, Spain (Planning phase of a project on prospective studies on diet and cancer)
AEP/89/03	Unit of Epidemiology, National Institute for the Study and Treatment of Cancer, Milan, Italy (Planning phase of a project on prospective studies on diet and cancer)
AEP/89/04	Gustave Roussy Institute, Villejuif, France (Planning phase of a project on prospective studies on diet and cancer)
AEP/89/05	Institute of Anatomy, University of Turin, Italy (Planning phase of a project on prospective studies on diet and cancer)
DEP/89/05	Cancer Control Centre, San Cristobal, Venezuela (Case–control study to investigate the effect of screening by X–ray examination in preventing death from gastric cancer)
AEP/89/07	School of Public Health, Granada, Spain (Nutritional assessment component of EEC breast and colorectal cancer study)
AEP/89/08	St Vincent Hospital, Dublin, Ireland (Nutritional assessment component of EEC breast and colorectal cancer study)
AEP/89/09	National Institute of Public Health and Environmental Hygiene, Bilthoven, The Netherlands (Nutritional assessment component of EEC breast and colorectal cancer study)
AEP/89/10	Unit of Epidemiology, Oncological Centre, Aviano, Italy (Nutritional assessment component of EEC breast and colorectal cancer study)
AEP/89/11	Department of Hygiene and Epidemiology, University of Athens Medical School, Athens, Greece (Nutritional assessment component of EEC breast and colorectal cancer study)
FIS/89/11	Institute of Oncology, Ljubljana, Slovenia (Precancerous lesions of the stomach in Slovenia)
AEP/89/13	Department of Biochemistry, University of Glasgow, UK (Nutritional assessment component of EEC breast and colorectal cancer study)
FIS/89/13	Unit of Epidemiology, Cancer Registry of Majorca, Spain (Family studies on diet and colorectal cancer: pilot study)

AEP/89/22	University Institute of Social and Preventive Medicine, Lausanne, Switzerland (Nutritional assessment component of EEC breast and colorectal cancer study)
AEP/89/23	Unit of Cancerology and Haematology, University Hospital, Luxembourg (Nutritional assessment component of EEC breast and colorectal cancer study)
AEP/89/24	Italian League against Cancer, Ragusa, Italy (Planning phase of a project ón prospective studies on diet and cancer)
AEP/90/01	Institute of Epidemiology and Biometry, German Cancer Research Centre, Heidelberg, Germany (Planning phase of a project on prospective studies on diet and cancer)
AEP/90/02	National Institute of Public Health and Environmental Hygiene, Bilthoven, The Netherlands (Planning phase of a project on prospective studies on diet and cancer)
AEP/90/03	Department of Nutrition and Biochemistry, School of Public Health, Athens, Greece (Planning phase of a project on prospective studies on diet and cancer)
AEP/90/05	"Preventicon", Utrecht, The Netherlands (Planning phase of a project on prospective studies on diet and cancer)
FIS/90/12	Cancer Control Center, San Cristobal, Venezuela (Etiology and prevention of stomach cancer in Venezuela)
AEP/91/05	Dunn Clinical Nutrition Centre, Cambridge, UK (Laboratory analyses of nitrogen in urine samples collected in the methodological phase of the European Prospective Study on Nutrition, Cancer and Health)
AEP/91/07	National Institute of Nutrition, Rome, Italy (Biochemical analyses of plasma samples collected in the methodological phase of the European Prospective Study on Nutrition, Cancer and Health)
AEP/91/08	Naylor Dana Institute for Disease Prevention, American Health Foundation, New York, USA (Laboratory analyses of urine samples collected in the pilot phase of the European Prospective Study on Nutrition, Cancer and Health)
AEP/91/09	Laboratory of Biochemistry, Micro-Nutrients and Free Radicals, University of Sciences, Pharmacology and Biology, Grenoble, France (Biochemical analyses of plasma samples collected in the methodological phase of the European Prospective Study on Nutrition, Cancer and Health)
AEP/93/02	Institute of Epidemiological and Clinical Research, Mataró, Spain (European prospective investigation into cancer and nutrition (EPIC))
AEP/93/03	Department of Health, Planning and Order, Government of Navarra, Pamplona, Spain (European prospective investigation into cancer and nutrition (EPIC))
AEP/93/04	Health Administration of Guipuzcoa, San Sebastian, Spain (European prospective investigation into cancer and nutrition (EPIC))
AEP/93/05	Andalusian School of Public Health, Granada, Spain (European prospective investigation into cancer and nutrition (EPIC))
AEP/93/06	Department of Epidemiology, Health Council, Murcia, Spain (European prospective investigation into cancer and nutrition (EPIC))

AEP/93/07	Department of Health Planning, Regional Administration of Public Health, Oviedo, Spain (European prospective investigation into cancer and nutrition (EPIC))
AEP/93/08	Medical Research Council Biostatistics Unit, University of Cambridge, UK (European prospective investigation into cancer and nutrition (EPIC))
AEP/93/09	Imperial Cancer Research Fund, Cancer Epidemiology Unit, Radcliffe Infirmary, Oxford, United Kingdom (European prospective investigation into cancer and nutrition (EPIC))
AEP/93/10	German Research Cancer Centre, Division of Epidemiology, Heidelberg, Germany (European prospective investigation into cancer and nutrition (EPIC))
AEP/93/11	Department of Nutrition and Biochemistry, Athens School of Public Health, Athens, Greece (European prospective investigation into cancer and nutrition (EPIC))
AEP/93/12	Department of Epidemiology, University of Utrecht, The Netherlands (European prospective investigation into cancer and nutrition (EPIC))
AEP/93/13	Department of Epidemiology, National Institute of Public Health and Environmental Protection (RIVM), Bilthoven, The Netherlands (European prospective investigation into cancer and nutrition (EPIC))
AEP/93/14	Cancer Epidemiology Research Unit (U.351), National Institute for Health and Medical Research (INSERM), Gustave Roussy Institute, Villejuif, France (European prospective investigation into cancer and nutrition (EPIC))
AEP/93/15	Department of Epidemiology, National Institute for Research and Treatment of Cancer, Milan, Italy (European prospective investigation into cancer and nutrition (EPIC))
AEP/93/16	Ragusa Cancer Registry, Italian League Against Cancer, Ragusa, Sicily, Italy (European prospective investigation into cancer and nutrition (EPIC))
AEP/93/17	Department of Biomedical Sciences and Human Oncology, University of Turin, Turin, Italy (European prospective investigation into cancer and nutrition (EPIC))
AEP/93/20	Unit of Epidemiology, Centre for Preventive Oncology (CSPO), Florence, Italy (European prospective investigation into cancer and nutrition (EPIC))
FIS/93/02	Institute of Health Investigations, San José, Costa Rica (Aetiology and prevention of stomach cancer in Costa Rica)

Studies on occupational cancer

DIR/87/02	Department of Biomedical Science and Human Oncology, University of Turin, Italy (Study on early lesions produced by low environmental exposures [passive smoking and pollution] and by low levels of occupational exposures)
AEP/89/14	Netherlands Cancer Institute, Antoni van Leeuwenhoek Huis, Amsterdam, The Netherlands (Feasibility phase of an international study of cancer risk in biology research laboratory workers)
AEP/89/16	National Institute of Health and Medical Research (INSERM), Villejuif, France (Feasibility phase of an international study of cancer risk in biology research laboratory workers)

AEP/89/17	Institute of Occupational Health, Helsinki, Finland (Feasibility phase of an international study of cancer risk in biology research laboratory workers)
AEP/89/18	College of Physicians and Surgeons of Columbia University, New York, USA (Feasibility phase of an international study of cancer risk in biology research laboratory workers)
AEP/89/19	National Institute of Agronomical Research (INRA), Paris, France (Feasibility phase of an international study of cancer risk in biology research laboratory workers)
AEP/89/20	TEAGASC, Agricultural and Food Development Authority, Dublin, Ireland (Feasibility phase of an international study of cancer risk in biology research laboratory workers)
AEP/89/21	National Institute of Public Health, Rome, Italy (Feasibility phase of an international study of cancer risk in biology research laboratory workers)
AEP/89/25	Institute of Anatomy, University of Turin, Italy (Non-occupational exposure to asbestos and mesothelioma)
AEP/89/26	Norwegian Cancer Registry, Norwegian Radium Hospital, Oslo, Norway (Multicentric study of workers exposed to styrene)
AEP/90/04	National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands (IARC international register of workers exposed to phenoxy acid herbicides and their contaminants)
AEP/90/06	Department of Epidemiology, London School of Hygiene and Tropical Medicine, London, UK (Lung cancer mortality among iron and steel workers in the steel valley of South-East Brazil)
AEP/90/07	National Centre for Scientific Research (CNRS), Paris, France (International study of cancer risk in biology research laboratory workers in Europe)
AEP/90/08	National Institute for Research and Security (INRS), Vandoeuvre, France (International study of cancer risk in biology research laboratory workers in Europe)
AEP/90/09	German Cancer Research Centre, Division of Epidemiology, Heidelberg, Germany (International study of cancer risk in biology research laboratory workers in Europe)
AEP/90/10	United Kingdom Co-ordinating Committee on Cancer Research, London, UK (International study of cancer risk in biology research laboratory workers in Europe)
AEP/90/12	Institute of Pathology, University of Oslo, Oslo, Norway (International study of cancer risk in biology research laboratory workers in Europe)
AEP/90/13	Department of Epidemiology, Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden (International study of cancer risk in biology research laboratory workers in Europe)

AEP/90/14	TEAGASC, Agriculture and Food Development Authority, Dublin, Ireland (International study of cancer risk in biology research laboratory workers in Europe)
AEP/90/15	National Institute of Public Health, Rome, Italy (International study of cancer risk in biology research laboratory workers in Europe)
AEP/90/16	National Institute of Health and Medical Research (INSERM), Villejuif, France (International study of cancer risk in biology research laboratory workers in Europe)
AEP/90/17	National Institute of Agronomical Research (INRA), Paris, France (International study of cancer risk in biology research laboratory workers in Europe)
AEP/90/18	Netherlands Cancer Institute, Antoni van Leeuwenhoek, Amsterdam, The Netherlands (International study of cancer risk in biology research laboratory workers in Europe)
AEP/91/01	Atomic Energy Agency, Paris, France (International study of cancer risk in biology research laboratory workers in Europe)
AEP/91/02	Institute of Occupational Health, Helsinki, Finland (International study of cancer risk in biology research laboratory workers in Europe)
AEP/91/03	National Institute of Health and Medical Research, Le Vesinet, France (International study of cancer risk in biology research laboratory workers in Europe)
AEP/92/01	Danish Cancer Registry, Danish Cancer Society, Copenhagen, Denmark (IARC International Register of Workers exposed to Phenoxy Acid Herbicides and their Contaminants)
AEP/92/04	Department of Health Services Administration and Community Medicine, Faculty of Medicine, University of Alberta, Edmonton, Canada (International cancer study among pulp and paper industry workers)
AEP/92/05	German Cancer Research Centre, Division of Epidemiology, Heidelberg, Germany (Man-made mineral fibres (MMMF) historical cohort study)
AEP/92/06	National Institute of Oncology, Montevideo, Uruguay (Case–control study to evaluate the importance of exposures in the occupational environment for the occurrence of cancer in Uruguay)
AEP/92/07	Department of Social Medicine, Faculty of Medicine, Federal University of Pelotas, Pelotas, Brazil (International cancer study among pulp and paper industry workers)
AEP/92/08	Directorate of Labour Inspection, Oslo, Norway (Multicentric study of workers exposed to styrene)
AEP/92/09	Department of Epidemiology, National Institute for Research and Treatment of Cancer, Milan, Italy (International study of cancer risk in biology research laboratory workers)
AEP/92/10	National Institute of Occupational Health, Solna, Sweden (Pilot phase of the case–control study on lung cancer in the rock/slag wool sub-cohort of the man-made mineral fibres (MMMF) historical cohort study)

AEP/92/11 Danish Cancer Registry, Danish Cancer Society, Copenhagen, Denmark
(Pilot phase of the case-control study on lung cancer in the rock/slag wool sub-cohort of the man-made mineral fibres (MMMF) historical cohort study)

AEP/92/12 Norwegian Cancer Registry, Norwegian Radium Hospital, Oslo, Norway
(Pilot phase of the case-control study on lung cancer in the rock/slag wool sub-cohort of the man-made mineral fibres (MMMF) historical cohort study)

AEP/92/13 Municipal Institute of Medical Research, Barcelona, Spain
(International cancer study among pulp and paper industry workers)

AEP/92/14 Institute of Occupational Medicine, WHO Collaborating Centre for Occupational Health, Lodz, Poland
(International cancer study among pulp and paper industry workers)

AEP/93/18 Institute of Occupational Health, Helsinki, Finland
(International cancer study among pulp and paper industry workers)

AEP/93/19 Department of Occupational Medicine, Sahlgren Hospital, University of Gothenburg, Sweden
(Multicentric study on European mercury workers)

Studies on the effects of active and passive smoking

AEP/87/02 Department of Epidemiology and Statistics, Hospital San Jaime i Santa Magdalena, Mataro, Spain
(International collaborative study on lung cancer in non-smokers)

AEP/87/03 Department of Hygiene and Epidemiology, School of Medicine, University of Athens, Athens, Greece
(International collaborative study on lung cancer in non-smokers)

AEP/87/04 Maria Sklodowska-Curie Memorial Centre, Institute of Oncology, Warsaw, Poland
(International collaborative study on lung cancer in non-smokers)

DEP/89/12 Tata Institute of Fundamental Research, Bombay, India
(Prospective study on tobacco-related cancers and other diseases in the city of Bombay)

AEP/89/15 Department of Chest Diseases, Postgraduate Institute of Medical Education and Research, Chandigarh, India
(International collaborative study on lung cancer in non-smokers)

AEP/91/06 Unit of Respiratory Diseases, Saint Louis Hospital, Paris, France
(Passive smoking in Europe)

Studies on chemical carcinogenesis

DEC/81/35 National Institute of Hygiene, Budapest, Hungary
(Long-term carcinogenicity testing of environmental chemicals)

DEC/83/11 Institute of Oncology, Medical Academy, Sofia, Bulgaria
(Mycotoxins and individual oxidative susceptibility in relation to endemic nephropathy and tumours of the urinary system)

DEC/84/01 Research Department, National Board of Occupational Safety and Health, Solna, Sweden
(Long-term carcinogenicity testing of environmental chemicals)

DEC/86/07 Laboratory of Carcinogenic Substances, Oncological Research Centre, Moscow, Russian Federation
(Role of prezygotic events in increasing cancer risk in subsequent generations)

ECH/87/06	Laboratory of Microbiology, Faculty of Pharmacy, Marseille, France (Studies of methods for degradation of chemical carcinogens)
MCA/89/07	Department of Biochemistry, University of Kashmir, Srinagar, India (Detection of DNA alkylation adducts and oncogene activation in human tissues)
ECH/89/08	Medical Research Council, London, UK (Mass spectrometry of nucleotide modifications and adducts in recoverable microcapsules)
MCA/91/01	University of Western Australia, Nedlands, Australia (*In vitro* repair assay for UV damage: its use in molecular epidemiological studies for exposure assessment)
CIE/92/01	Lithuanian Oncological Centre, Vilnius, Lithuania (Carcinogenicity testing of sulfuric acid mists in rats)
ECH/92/02	Cancer Unit, Faculty of Medicine, Srinagarind Hospital, Khon Kaen, Thailand (Study on effect of *Opisthorchis viverrini* infestation on induction of P450's and DNA repair enzymes, and on endogenous nitrosation)
ECH/92/03	Department of Clinical Engineering, McGill University, Montreal, Canada (Microencapsulation of DNA and polynucleotides for the *in vivo* entrapment of carcinogenic agents)
ECH/92/24	Cancer Research Centre, Moscow, Russian Federation (Effect of treatment with β-carotene, vitamins C and E and/or anti-*Helicobacter pylori* drugs on chronic gastritis)
MCA/92/01	Institute of Carcinogenesis, Cancer Research Centre, Moscow, Russian Federation (Perinatal exposure to aflatoxins)
MSC/92/01	Agrotechnological Research Institute, Wageningen, The Netherlands (The role of biotransformation of chemicals in nongenotoxic carcinogenesis)
MSC/92/02	Brunel University, Human Cancer Genetics Unit, Uxbridge, UK (Induction of the infinite self-renewal (ISR) phenotype as an objective test endpoint for the detection of nongenotoxic carcinogens)
MSC/92/03	Institut du Cancer et Immunogénétique, Laboratoire de Pharmacologie cellulaire et moléculaire, Villejuif, France (The relationship between ornithine decarboxylase (ODC) activity and carcinogenic process)
MSC/92/04	Imperial Chemical Industries, Central Toxicology Laboratory, Macclesfield, UK (Study on the mechanisms by which nongenotoxic chemicals produce cancer in the rodent liver)
MSC/92/05	Institute for Cancer Research, Laboratory for Environmental and Occupational Cancer, Oslo, Norway (The importance of gap junctional intercellular communication and growth factors in nongenotoxic mechanisms of carcinogenesis)
MSC/92/06	Institute of Toxicology, University of Würzburg, Würzburg, Germany (The mechanisms of cell transformation using the SHE cell system as a model)
MSC/92/07	National Institute of Cancer Research, Laboratory of Chemical Carcinogenesis, Genoa, Italy (The mechanistic role of changes in pattern and morphological organization of proteins of the nuclear matrix and other physicochemical parameters related to chromatin conformation)

MSC/92/08	Istituto Superiore di Sanità, Laboratorio di Tossicologia Applicata, Rome, Italy (The possible effects of nongenotoxic carcinogens on cellular DNA replication)
DEP/92/06	Oncology Center of Ho Chi Minh, Republic of Vietnam (Pilot study to investigate the feasibility of case-control studies of soft tissue sarcoma and non-Hodgkin's lymphoma in the south of Vietnam)
DEP/93/02	Oncology Center of Ho Chi Minh, Republic of Vietnam (Case-control studies of soft tissue sarcoma and non-Hodgkin's lymphoma in the south of Vietnam)

Annex 6

MEETINGS AND WORKSHOPS ORGANIZED BY IARC

1 July 1991–30 June 1993

Meeting on the occupational analysis of the IARC larynx cancer study	Lyon, 15 July 1991
International course on advanced statistical methods in cancer epidemiology	Lyon, 15–19 July 1991
Meeting of industrial hygienists to rank case–controls concerning exposure to phenoxy herbicides and contaminants	Lyon, 2–6 September 1991
International course on the detection of health hazards in human populations exposed to chemical mutagens and carcinogens	Harare, Zimbabwe 9–20 September 1991
Working group on the LANGUAL food coding system and food composition data–bases	Lyon, 11–12 September 1991
Meeting of EUROFOODS Project Management Group	Lyon, 12–13 September 1991
Workshop to review dosimetric information provided by participating centres in the international collaborative study of nuclear industry workers	Lyon, 16–18 September 1991
Meeting on repair of DNA alkylation damage	Courmayeur, Italy 22–27 September 1991
International symposium on the toxicity and carcinogenicity of cadmium in the human environment	Gargnano, Italy 25–27 September 1991
Workshop on cancer registries in Latin America and Annual Meeting of International Association of Cancer Registries	Quito, Ecuador 2–5 October 1991
SEARCH working group on childhood brain tumours	Lyon, 8–11 October 1991
IARC working group on occupational exposures to mists and vapours from strong inorganic acids; and other industrial chemicals (Volume 54)	Lyon, 15–22 October 1991
International meeting on (1) Biomonitoring and susceptibility markers in human cancer: applications in molecular epidemiology and risk assessment (2) Nitroso compounds: biological mechanisms, exposures and cancer etiology	Hawaii, USA, 27 October– 2 November 1991
Ad hoc working group on the Agency's planning mechanisms	Lyon, 6 November 1991
Scientific Council Peer Review Committee	Lyon, 7–8 November 1991

Groupe de travail Rhône–Alpes sur l'utilisation des matériels DIGITAL	Lyon, 13 November 1991
Meeting of the working group of the IARC multicentric study on lung cancer in non-smokers	Lyon, 19–21 November 1991
Third meeting of collaborators in the CEC-BRIDGE Programme	Lyon, 22–23 November 1991
Workshop on HPV and cervical cancer	Brussels, 25–28 November 1991
Meeting of the coordinating committee of the EPIC project	Cambridge, 27–29 November 1991
Meeting of the study group of the international collaborative study of cancer risk among nuclear industry workers	Lyon, 2–4 December 1991
Workshop on linkage studies in hereditary breast cancer	Lyon, 5–6 December 1991
IARC/ECETOC meeting of industry associations representatives	Lyon, 12 December 1991
Meeting of Council of Europe Study Group on Epidemiology. Special Committee of the SIEAD/ OPA/Chernobyl Programme	Lyon, 18–19 December 1991
Working group meeting on tamoxifen and cancer of the endometrium	Lyon, 19 December 1991
Working group meeting on breast cancer in the Rhône 'Department'	Lyon, 7 January 1992
Meeting of the working group for IARC historical prospective study of workers employed in the MMMF industry	Lyon, 20–22 January 1992
Working group meeting on smoking in the Rhône 'Department'	Lyon, 21 January 1992
Working group meeting on tamoxifen and cancer of the endometrium	Lyon, 23 January 1992
Working group meeting on survival and breast cancer in the Rhône 'Department'	Lyon, 31 January 1992
IARC Scientific Council	Lyon, 3–6 February 1992
International monitoring of the incidence of skin cancer and related biological phenomena in relation to environmental change	Lyon, 10 February 1992
IARC Working Group on ultraviolet and solar radiation (Volume 55)	Lyon, 11–18 February 1992
The Gambia Hepatitis Intervention Study, 7th Steering Committee meeting	Lyon, 19–20 February 1992
Meeting of the collaborators of the study on cervical cancer in Spain and Colombia	Lyon, 12–14 February 1992
Information meeting on biological samples banks	Lyon, 20 February 1992
Course on safe handling of cytostatic drugs	Lyon, 24–25 February 1992

Liaison committee meeting of the IARC historical prospective study of workers employed in the MMMF industry	Lyon, 26 February 1992
US/European workshop on epidemiologic studies of man-made vitreous fibre production workers	Boston, USA, 16–18 March 1992
Meeting of the coordinating committee of the EPIC project	Lyon, 7–10 April 1992
Meeting of principal investigators of EPIC study from France, Italy, Spain and UK	Lyon, 26–27 February 1992
IARC Fellowships Selection Committee	Lyon, 23–24 April 1992
Planning meeting of the IARC working group on quantitative risk assessment	Lyon, 27–28 April 1992
IARC Governing Council	Lyon, 29–30 April 1992
Editorial board meeting for the workshop on bio-persistence of respirable synthetic fibres and minerals	Lyon, 4 May 1992
Training course for interviewers of the pilot phase of the case-control study on lung cancer in the IARC study on cancer risk among European man-made mineral fibre workers	Lyon, 5–7 May 1992
Meeting of industrial hygienists for the international study in the pulp and paper industry	Lyon, 13–15 May 1992
Editorial board meeting for Volume VI of *Cancer Incidence in Five Continents*	Lyon, 25–26 May 1992
EUROCIM/Network meeting	Lyon, 1–3 June 1992
SEARCH collaborative studies meeting on brain tumours in children	Lyon, 1–3 June 1992
SEARCH collaborative studies meeting on brain tumours in adults	Lyon, 3–5 June 1992
IARC working group on some naturally occurring substances: some food items and constituents, spices, pyrolysis products and mycotoxins (Volume 56)	Lyon, 9–16 June 1992
Meeting of industrial hygienists of the IARC multi-centric study on workers exposed to styrene	Copenhagen, Denmark, 10–12 June 1992
International course on pathology of cancer for non-pathologists	Oslo, 15–19 June 1992
Meeting to discuss IARC classification of certain man-made vitreous fibres	Lyon, 17 June 1992
Man-made mineral fibres liaison committee	Lyon, 17 June 1992
European Educational Programme in Epidemiology – annual residential summer course	Florence, Italy, 22 June–10 July 1992
Meeting on ^{32}P-postlabelling methods for the detection of DNA adducts	Lyon, 24–27 June 1992
International Association of Cancer Registries (IACR) annual meeting	Ottawa, Canada, 28–30 June 1992

Meeting on the composition of southern European food	Lyon, 6–8 July 1992
Working group on standardization of 24-hour diet recall methods in the EPIC project	Lyon, 8–10 July 1992
Workshop on respirable synthetic fibres and minerals	Lyon, 7–9 September 1992
Meeting of the Liaison Committee for the IARC historical cohort study on workers in the European MMMF industry	Lyon, 10 September 1992
Meeting on the industrial hygienists for the IARC historical cohort study on workers in the European MMMF industry	Lyon, 10–11 September 1992
Meeting of the steering committee of the European Network of Cancer Registries	Lyon, 22–23 September 1992
Meeting on the combined analysis of case–control studies of cutaneous melanoma	Lyon, 28–29 September 1992
Scientific Council Peer Review Committee meeting	Lyon, 30 September–1 October 1992
IARC working group on occupational exposures of hairdressers and barbers and personal use of hair colouring materials: some hair dyes, cosmetic colourings, industrial dyestuffs and aromatic amines (Volume 57)	Lyon, 6–13 October 1992
Meeting on the international monitoring of the incidence of skin cancer and related biological phenomena in relation to environmental change	Lyon, 19–21 October 1992
Working group on 24–hour diet recall in the EPIC study	Lyon, 2–4 December 1992
Meeting on molecular epidemiology of cancer	Courmayeur, Italy, 7–11 December 1992
Meeting of the EPIC project steering committee	Lyon, 15–17 December 1992
Working group meeting for the multicentric cohort study on mercury workers in Europe	Lyon, 17–18 December 1992
IARC Scientific Council	Lyon, 11–14 January 1993
Preparatory meeting for the IARC working group on the evaluation of carcinogenic risks to humans: some metals and metal compounds, and occupational exposures in the glass industry	Lyon, 18–22 January 1993
IARC working group on beryllium, cadmium, mercury and exposures in the glass industry (Volume 58)	Lyon, 9–16 February 1993
Editorial meeting on quality and quality control for cancer registries	Lyon, 12 February 1993
Working group on the standardization of 24-hour diet recall in the EPIC project	Lyon, 17–19 February 1993
Meeting on cancer risks among nuclear industry workers	Lyon, 22–23 February 1993
International course on the epidemiology of nutrition and cancer	Lyon, 1–12 March 1993

Meeting of the Liaison Committee for the feasibility study on cancer risk in European asphalt workers	Breukelen, The Netherlands, 12 March 1993
Ole Møller Jensen Symposium on nutrition and cancer	Lyon, 15–17 March 1993
Meeting of the epidemiology group for the IARC multicentric cohort study of workers exposed to styrene	Lyon, 25–26 March 1993
Steering Committee of the EPIC project	Lyon, 30–31 March 1993
Group meeting of participating laboratories in the EC Environment Programme	Lyon, 2 April 1993
Meeting of the Scientific Council of the Students' Association Against Cancer	Lyon, 2 April 1993
Neuroblastoma study group meeting	Lyon, 5–6 April 1993
Working group for the IARC historical cohort study on workers in the European MMMF industry	Lyon, 5–7 April 1993
IARC Fellowships Selection Committee	Lyon, 15–16 April 1993
IARC Governing Council	Lyon, 28–30 April 1993
Steering committee meeting of the European Network of Cancer Registries	Lyon, 3–4 May 1993
Cancer epidemiology course	Ostrava, Czech Republic, 17–28 May 1993
Preparatory meeting to the workshop on quantitative estimation and prediction of cancer risks to humans	Lyon, 24–28 May 1993
Liaison committee meeting for the feasibility study on cancer risk in European asphalt workers	Lyon, 7 June 1993
IARC working group on hepatitis viruses (Volume 59)	Lyon, 8–15 June 1993
European Educational Programme in Epidemiology Annual Residential Summer Course	Montecatini Terme, Italy, 21 June–9 July 1993
Dosimetry and Epidemiology Subcommittee Meeting of the international collaborative study of cancer risk among nuclear industry workers	Lyon, 24–25 June 1993
Meeting of the working group on the LANGUAL food coding system and food composition data-bases	Lyon, 24–26 June 1993

Annex 7

VISITORS TO IARC

1 July 1991–30 June 1993

A total of 1412 persons from 56 countries visited the Agency during the period under review. The following gave lectures:

Dr M. Ahotupa, University of Turku, Finland
Pro-oxidant states: physiological and pathophysiological implications

Dr B. Ames, University of California at Berkeley, CA, USA
Understanding the causes of ageing and cancer
Animal cancer tests — what are they telling us?

Dr G. Ames, University of California at Berkeley, CA, USA
Bacterial permeases as model systems for multidrug resistance and cystic fibrosis

Dr W. Anwar, Ain Shams University, Cairo
Cytogenetic studies on high-risk populations in Egypt

Dr V.E. Avvedimento, Faculty of Medicine and Surgery, Catanzaro, Italy
Ras oncogene and cAMP-dependent protein kinase: molecular mechanisms of control of growth and differentiation in thyroid neoplastic cells

Professor J. Bernard, President, National Ethics Council for Health and Life Sciences, Paris
Hématologie géographique
Naissance et évolution de la bio-éthique

Professor G. Berry, University of Sydney, Australia
Predictions of future mortality in former workers at the Wittenoom crocidolite asbestos mine and mill in Western Australia

Dr H. Blum, Medical Clinic, Zurich, Switzerland
Hepatitis B virus mutants: molecular and clinical implications

Professor G. Bornkamm, Institute for Clinical Molecular Biology and Tumour Genetics, Munich, Germany
A rapid quantitative test system for activators of protein kinase C including tumour promoters and other EBV-inducing agents

Dr C. Bréchot, Necker Hospital Faculty of Medicine, Paris
Hepatitis B and C viruses-related liver carcinogenesis

Professor R. Burton, University of Newcastle, NSW, Australia
Natural cytotoxic cells and tumour surveillance

Dr C. Calleman, School of Public Health and Community Medicine, University of Washington, Seattle, WA, USA
Acrylamide — toxicology, dosimetry and mathematical modelling

Dr I. Chernozemski, National Oncological Centre, Sofia
Epidemiology of Balkan endemic nephropathy and urinary tract tumours in Bulgaria

Dr N. Christie, New York University Medical Centre, Tuxedo, NY, USA
Nickel carcinogenesis and the possible role of oxidative damage

Dr M.G. Deo, Cancer Research Institute, Tata Memorial Centre, Bombay, India
Enhancing factor — a novel growth modulator

Dr J.C. Drapier, Institut Curie, Paris
Nitric oxide, a cytokine-induced gas that targets iron

Dr H. Esumi, National Cancer Center Research Institute, Tokyo
Carcinogenicity of food-borne mutagens, heterocyclic amines

Dr N. Francey, Barrister, Australian Federation of Consumer Organizations, and
Dr S. Woodward, Director, Action on Smoking and Health, Australia
Federal Court of Australia determines that passive smoking causes lung cancer

Dr A. Fusco, Faculty of Medicine and Surgery, Naples, Italy
Molecular biology of human thyroid papillary carcinomas

Dr A.S. Gleiberman, Cancer Research Centre, Moscow
Cell interaction in alphafetoprotein regulation in adult mouse liver

Dr M. Hamaguti, National Cancer Center Research Institute, Tokyo
Highly sensitive exon-trapping systems (SETS)

Dr D. Harber, MGH Cancer Center, Charlestown, MA, USA
Functional analysis of mutations in the Wilms' tumour gene

Professor N. Ito, Nagoya City University, Japan
Medium-term liver bioassay for rapid detection of carcinogens and chemopreventive agents

Dr A.D. Jack, Gambia Hepatitis Intervention Study, IARC, Banjul, The Gambia
The Gambia Hepatitis Intervention Study: an update

Dr M.K. Jacobson, University of North Texas, TX, USA
Human niacin nutrition: implications for cancer prevention

Dr G. Kissilev, Russian Embassy, Paris
Le rôle de l'aluminium dans la cancérogenèse

Dr T. Kuroki, Institute of Medical Science, University of Tokyo, Japan
Predominant expression of a Ca^{2+}-independent protein kinase C, nPKCeta in epithelial tissues

Dr J. Laitinen, University of Helsinki, Finland
Chromatin structure in normal and *ras* oncogene-transformed fibroblasts

Dr S. Lang, University of Kuopio, Finland
Biological effects of nuclear fuel particles

Professor J.A.H. Lee, University of Washington, Seattle, WA, USA
Temporal and geographical changes in the precursor to cutaneous melanoma

Professor L. Le Marchand, University of Hawaii at Manoa, Honolulu, HI, USA
Protective effect of fruits and vegetables on cancer risk

Dr F.P. Li, Dana Farber Cancer Institute, Boston, MA, USA
From cancer families to tumour-suppressor genes

Dr J.B. Little, Harvard School of Public Health, Boston, MA, USA
Molecular mechanisms for radiation mutagenesis: implications for carcinogenesis

Professor P.H.M. Lohman, Sylvius Laboratories, University of Leiden, The Netherlands
An IPCPEMC method for comparing and combining short-term genotoxicity data

Professor A.B. Lowenfels, Westchester County Medical Center, Valhalla, NY, USA
Pancreatitis and the risk of pancreatic cancer

Dr W.K. Lutz, Institute of Toxicology, Swiss Federal Institute of Technology, Schwerzenbach, Switzerland
From carcinogen exposure to cancer risk

Dr C. Magnani, University of Turin, Italy
Health effects of environmental exposure to asbestos in Casale Monferrato, Italy

Mrs M.J. Marion, INSERM U.151, Lyon, France
Mécanisme de la transformation néoplasique induite par le chlorure de vinyle — application à la définition de marqueurs tumoraux

Dr S. Narod, Montreal General Hospital, McGill University, Montreal, Canada
Congenital malformations and childhood cancer in England and Wales

Dr M. Negishi, LRDT, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA
Structure-activity relationships of cytochrome P450 isozymes: changing the substrate preference with single amino-acid substitutions

Professor K. Nose, Showa University, Tokyo
Active oxygen and cell growth control

Professor G. Orth, Pasteur Institute, Paris
(1st Annual Conference in memory of Professeur Roger Sohier)
Papillomavirus et cancer humain

Dr M. Ozturk, Massachusetts General Hospital, Charleston, MA, USA
Hot spot p53 mutation in primary liver cancer

Mr R. Peto, Imperial Cancer Research Fund, Oxford, UK
(General Motors Foundation Visiting Professorship)
How to live forever by preventing both cardiovascular disease and cancer

Dr D.H. Phillips, Royal Marsden Hospital, Surrey, UK
DNA adduct formation in animals and humans exposed to complex mixtures of carcinogens

Professor L. Pylev, Institute of Carcinogenesis, Cancer Research Centre, Moscow
Factors modifying asbestos carcinogenesis

Dr R. Riley, Richard Russell Agricultural Research Center, Athens, GA, USA
Recent studies on the mechanism of action of fumonisins

Professor U. Rovigatti, Institute of Cellular and Molecular Oncology, Bobigny, France
A new human virus, MFV, isolated from neuroblastoma cluster cases can induce N-*myc* DNA amplification

Dr T. Sekiya, National Cancer Center Research Institute, Tokyo
Detection of DNA aberrations in human cancers by single-strand conformation polymorphism analysis of polymerase chain reaction products

Dr P. Shields, National Cancer Institute, Bethesda, MD, USA
Genetic and acquired predispositions to lung cancer

Dr L. Simonato, Venice Tumour Registry, Padua, Italy
The Venice Tumour Registry feasibility project

Dr B. Singer, Donner Laboratory, University of California, Berkeley, CA, USA
What is new in alkylation mutation and repair?

Dr G. Sobala, General Infirmary, Leeds, UK
Ascorbic acid in the human stomach

Dr S. Stellman, American Health Foundation, New York, NY, USA
Agent Orange, phenoxy herbicides, and dioxins health effects on American Armed Forces in Vietnam

Mr C.A. Stiller, Childhood Cancer Research Group, University of Oxford, UK
The geography of childhood leukaemia

Dr D. Stuehr, The Cleveland Clinic Foundation, Cleveland, OH, USA
Structure and function of brain and macrophage nitric oxide synthases

Dr J. Sunyer, University of Barcelona, Spain
Excess cancer risk in a community environmentally exposed to hexachlorobenzene and PCBs

Dr H. Tomasson, University of Iceland, Reykjavik
Breast cancer, genetic susceptibility and oral contraceptives — an Icelandic case-control study

Dr P. Toniolo, New York University Medical Center, NY, USA
Organochlorine residues in blood and risk of breast cancer. The New York University Prospective Study

Dr S. Troyanosky, German Cancer Research Centre, Heidelberg, Germany
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Dr V. Vonka, Institute of Hematology and Blood Transfusion, Prague
Serological studies on human papillomaviruses

Dr R. Wainstok de Calmanovici, University of Buenos Aires, Argentina
Influence of hepatic and mammary tumours on HCB-induced porphyria in rats

Professor R. Wilson, Harvard University, Cambridge, MA, USA
A statistical study of the results of carcinogenicity testing by the National Toxicology Program

Annex 8

INTERNAL REPORTS

IARC Internal Report 91/001	Report of an Ad-hoc IARC Monographs Advisory Group on Viruses and other Biological Agents such as Parasites
IARC Internal Report 91/002	Mechanisms of Carcinogenesis in Risk Identification. A Consensus Report of an IARC Monographs Working Group, 11–18 June 1991
IARC Internal Report 92/001	International Collaborative Study of Cancer Risk Among Nuclear Industry Workers. II – Protocol (prepared by E. Cardis and J. Estève)
IARC Internal Report 92/002	Cancer Mortality in an International Cohort of Workers Exposed to Chlorophenoxy Herbicides, Chlorophenols and Contaminants (M. Kogevinas *et al.*)
IARC Internal Report 92/003	Guidelines on Confidentiality in the Cancer Registry
IARC Internal Report 92/004	CANREG – Cancer Registration Software for Microcomputers (S. Olivier and D.M. Parkin)
IARC Internal Report 93/001	Cancer Incidence and Mortality in an International Cohort of Workers Exposed to Styrene (M. Kogevinas)

Annex 9

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PUBLICATIONS OF THE INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

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