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WORLD HEALTH ORGANIZATION

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

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1980

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CONTENTS

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INTRODUCTION

As in previous years, the first part of the Annual Report presents a brief general review of the programmes and objectives of the Agency. More detailed discussion follows in the reports of the individual Divisions.

General comments

In addition to the classical descriptive and analytical epidemiological studies on carcinogenic stimuli, epidemiology can contribute to an understanding of carcinogenic mechanisms. Thus, putative biological mechanisms may be deduced from field studies, which if consistent with laboratory data may suggest the biological background for some human cancer patterns, and nowadays, more sophisticated techniques are becoming available, which may be applied to large-scale epidemiological investigations in man to test etiological hypotheses.

The concept of a discrete carcinogenic stimulus, chemical or physical, as a direct cause of cancer is well comprehended, and has been extensively investigated. Nevertheless, not all carcinogenic agents are initiators of the carcinogenic process nor are they all genotoxic, and neither is initiation always of exogenous origin nor complete. The view that carcinogenesis in man is multistage and that numerous exogenous or endogenous factors modulate neoplastic development is now becoming generally accepted. Such modulating agents include factors influencing metabolism of carcinogens, both their activation and inactivation, DNA repair, promotion and inhibition, and immunological host status. All these are areas in which there have been a marked increase in basic research in the last decade. Many of these factors involve interplay between environmental and genetic influences. Thus, the etiology of cancer should be considered not just in terms of a single substance acting by a single mechanism, but rather as a series of events involving several factors, of which only a few have been identified and studied.

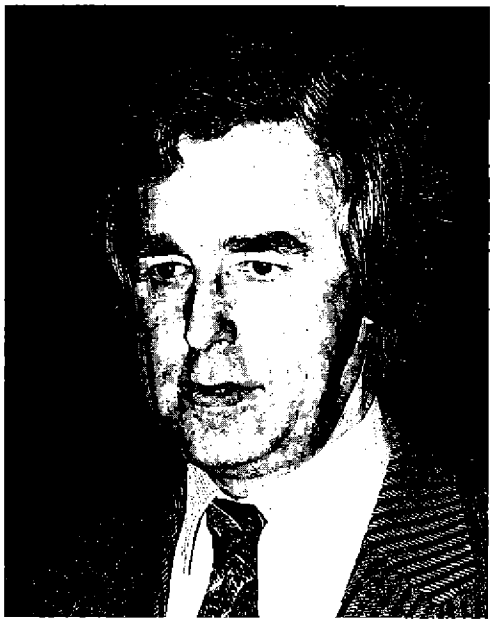
The complexity of the carcinogenic process has significant implications for cancer control and for research strategies. It underlines the inherent biological difficulties in quantitative extrapolation of carcinogenic risk from animals or *in vitro* models to man, when so many variables have not been measured and where the level of target cell exposure is unknown.

This is especially true for carcinogenicity studies of low dose exposures where cancer development or inhibition might be predominantly dependent on modulating factors, both exogenous and endogenous.

It has been suggested that the role of diet and behavioural patterns on the development of human cancer might be partly explicable in relation to their effects for example on endocrine status or intestinal flora. Furthermore, some aspects of individual susceptibility almost certainly relate to individual differences in the metabolic activation of carcinogens, e.g., environmental or hereditary control of enzyme induction as well as modifications in late stage carcinogenesis. It might thus be possible to prevent cancer through interference in other carcinogenic mechanisms than the removal of a defined initiator. In man, such an approach would imply modifications of aspects of life-style,

Fig. 1 New members of the Scientific Council

Professor G. Della Porta
1980–1983



Professor A. Georgii
1980–1983

Professor B. Gustafsson
1980–1983

and chemoprevention. There is evidence that certain contraceptives inhibit endometrial and ovarian cancer. The Agency has begun some programmes and workshops directed to elucidating some of these biological problems involved in defining more objectively 'life-style'.

In epidemiological studies it has not been usual, in evaluating the carcinogenicity of discrete chemicals, to distinguish between promoters and initiators, nor has this been attempted in the *IARC Monographs* series on the evaluation of the carcinogenic risk of chemicals to humans. This is largely due to the problems associated with studying the mechanisms by which certain factors are associated with an increased risk of cancer in humans. Another reason for not attempting a distinction has been the public health implications. The control of promoters may require very different public health strategies from the control of initiators, since promoters may include a very wide variety of agents, acting through widely differing mechanisms. However, the necessity to examine further such distinctions is well illustrated by the recent controversy on the mechanisms involved in the carcinogenic activity attributed to hormones and to artificial sweeteners.

Cancer as a socio-economic problem

Over 40 years ago the association between cancer patterns and social gradient was recognized, and there is now renewed interest in this observation. The importance of the social gradient effect is far greater than implied is simple differences in exposure to discrete carcinogens. It is now clear that social gradient is significantly associated with many aspects of life-style and related cultural habits, and not only with generally recognized occupational exposures. These data imply significant biological and metabolic variations between different social groups. Such variations would almost certainly be of importance in explaining some of the differences in cancer patterns between different socio-economic groups which in other respects may not be very different. These data offer an immense intellectual challenge to the oncologist and epidemiologist for both scientific and sociological reasons. They also emphasize the great difficulties inherent in preventive strategy from the public health viewpoint. They suggest however that a healthy life-style designed to reduce cancer risk would also be healthy in terms of other diseases.

The possibility of life-style changes

It was assumed at one time that the general population would not be prepared to adopt markedly changed life-styles. However, there is growing evidence that the public will modify life-style habits if convinced that such change will be of value to health. This is demonstrated by the very considerable changes in cigarette consumption in certain population groups in many countries, and by a 50% fall since 1963 in saturated fat intake for example that has been reported from the United States. This reduction in consumption of saturated fats was largely due to developments in research on cardiovascular disease and the consequent dietary recommendations of the cardiologists.

Unfortunately, oncologists have been much more conservative in their research efforts in this area. The situation in the study of malignant disease, however, is very complex and no simple solutions are possible at present. Research requiring extensive population studies has been handicapped frequently by inadequate technological methods, but today, it is possible not only to measure very low levels of carcinogens and their metabolites within the body, but also in the diet and ambient environment. Techniques have also been developed that permit variations in carcinogen metabolism and chemicals in tissues to be studied in man and in animals under different conditions, and certain mutagenic screening tests may be valuable in this context. In this way, the

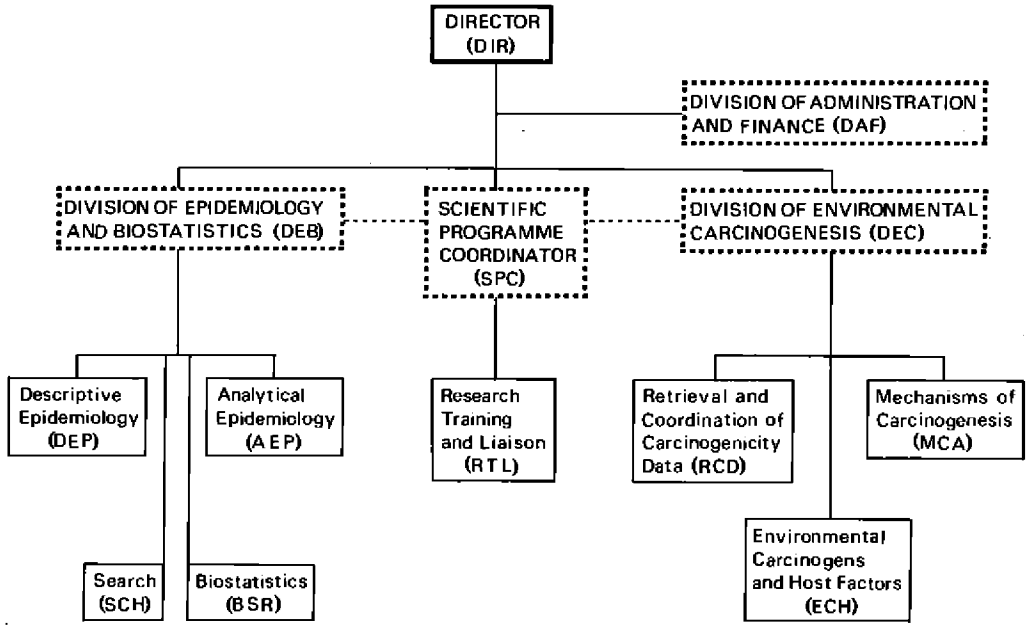


Fig. 2 The structure of the Agency

study of life-style factors which formerly was only considered a theoretical possibility, may now prove to be measurable in biochemical terms.

Several hypotheses have already been formulated and could be tested through intensified, if sometimes tedious, systematic programmes in different environmental settings. Such studies should not only permit better understanding of mechanisms of risk extrapolation from *in vivo* or *in vitro* models, but also an expression of life-style factors in terms of human physiology. With this in mind, a combined epidemiological and laboratory programme is being developed by the Agency to provide a more systematic approach to the study of such factors, for which the Agency appears unusually suited.

Organization

The Agency, since its inception, has directed its resources largely to multidisciplinary studies in environmental carcinogenesis, with both laboratory and field components. The integration of the various disciplines into the research programmes, and involvement of external institutions and laboratories proceeded pragmatically as the programmes developed. Gradually, the Agency's scientific programme took shape with three major aspects:

1. The development and carrying out of specific research programmes.
2. Scientific evaluation of available data.
3. Education and information.

These three aspects meet the requirements of the Agency both to provide a service and to be an international research resource. Further, they have permitted multidisciplinary investigations in environmental carcinogenesis. Projects have not only been directed to the nature of cancer causation in man *per se*, but also to practical preventive problems, as for example, through the

evaluation of pesticides and industrial and pharmaceutical products, which are of concern to both industrialized and developing countries.

It has been decided in the interests of programme efficiency to make certain modifications in the former 'unit' structures which have now been regrouped as follows:

- those related to epidemiology and biostatistics
- those related to the laboratory back-up programmes which serve to complement and balance the epidemiological programmes, and
- general research, education and information.

This restructuring does not represent any fundamental change in the Agency's activities, but is purely an administrative action to facilitate the carrying out and coordination of multidisciplinary programmes. The new organigram is shown in Figure 2.

Funding

During 1980 the income of the Agency totalled US$ 9 177 000. Of this US$ 7 104 000 represented contributions from the Participating States, the remaining US$ 2 073 000 coming from grants and contracts. The details are given in Table 1.

Table 1. Income and expenditure for 1980[a]

	Amount (US $)	Percentage of total
Income		
Statutory budget	7 104 000	77.41
Extra-budgetary	2 073 000	22.59
	9 177 000	100.—
Expenditure		
Intramural		
Headquarters scientific staff	3 137 000	34.18
Administrative staff and office services	1 322 000	14.41
Building management	785 000	8.55
Laboratory research (supplies and staff salaries)	1 141 000	12.43
Publication and information programme	598 000	6.52
Other (data processing, library, organizational and scientific meetings)	518 000	5.64
Total	7 501 000	81.73
Extramural		
Contractual and collaborative research	1 147 000	12.50
Fellowships	356 000	3.88
Duty travel	173 000	1.89
Total	1 676 000	18.27
Grand total	9 177 000	100.—

[a] All figures are estimates. Statutory budget income and expenditure details are those contained in the approved budget (GC/18/3) as amended, whereas the extra-budgetary figure is based on information available in September.

Personnel

In June 1980, the Agency's staff of 150 consisted of 38 scientists, 54 technicians and 58 administrative and secretarial staff. Thirteen visiting scientists, consultants and fellows contributed to the research programmes of the Agency during the year.

There have been several changes in the scientific staff in the last twelve months. Dr Laima Griciute, Chief of the Unit of Environmental Carcinogens, left the Agency after six years. Three epidemiologists have left: Drs O. M. Jensen, F. Repetto and M. Stukonis. From the Division of Environmental Carcinogens there have been several departures: Dr R. Althouse, Mrs C. Desgranges-Blanc, Mrs L. Saint Vincent. Mr G. Toussaint and Mr E. A. Walker.

Mr W. A. Prichard, Director of Administration and Finance has retired. His place has been taken by Mr Keiji Saita, formerly Director of Support Programme, at the WHO Regional Office for Africa.

DIVISION OF EPIDEMIOLOGY AND BIOSTATISTICS

Dr C. A. LINSELL (Director)

1. INTRODUCTION

Following the organization of the Agency into two major Divisions, the programmes in epidemiology and biostatistics were grouped into four areas: Descriptive Epidemiology, Analytical Epidemiology, Surveillance of Environmental Aspects Related to Cancer in Humans (SEARCH) and Biostatistics. While it may be difficult to justify a clear-cut division between the practice of different aspects of modern epidemiology and biostatistics, the designation of programme areas is necessary for the day-to-day management of the Division. The overall objectives of the regrouping of the Agency's research resources were to ensure programme integration within disciplines and to expand interdisciplinary exchange between field and laboratory.

Scientists involved in epidemiological research are drawn from a number of disciplines: from clinical medicine, public health administration, pathology and biostatistics, and all have a part to play in successful field programmes. The Division is fortunate in having staff trained in a variety of basic disciplines, and the present reorganization should allow full advantage to be taken of their expertise in mounting interdisciplinary field programmes, with access to the Agency laboratory programmes in Lyon. When the laboratory investigations required by field programmes cannot be met in-house, these will be carried out in collaboration with suitable national laboratories.

Recently, the epidemiologists of the Agency have been studying the concept which has become known as the SEARCH programme. In essence, this programme is designed to promote the systematic correlation of the resources of the Agency's programme in descriptive epidemiology with collections of environmental data and further to promote case-control studies on an international scale, with an emphasis on factors of life style that can be associated with cancer risk. To facilitate the correlation of data on cancer occurrence and environmental and life style factors, an expanded programme of descriptive epidemiology has been prepared. This was discussed by the 16th Scientific Council of the Agency. Staff positions and resources have been made available and active collaboration has been established with the Dissemination of Statistical Information Unit of WHO. Current analytical epidemiology programmes of the Agency will be brought into the SEARCH programme when appropriate, and a series of multi-centred case-control studies on selected cancers, as discussed below under the SEARCH programme, is being prepared. This programme will not compromise the current and future projects of the analytical epidemiology programme, as it is anticipated that the major funding will be found from financial resources other than the regular budget of the Agency.

The Agency's *Monograph* series, which has been concerned with specific chemical carcinogens, is now also reviewing the hazards associated with individual industries or industrial processes. This will require increased liaison between the Division's programme on occupational cancer and the monograph programme of the Division of Environmental Carcinogenesis.

The programme on occupational hazards in the man-made mineral fibre industry is entering its final phase, and discussions on further Agency activities in relation to this industry must be held in the near future. This programme, which has studied the hazards in a number of countries rather than in a single factory or country, has demonstrated that the Agency can gain ready acceptance and credibility when dealing with a sensitive health and economic issue.

The method of financing the IARC Research Centres has been changed, and financing will in future be confined to support for specific programmes. Some major analytical epidemiology programmes of the Agency are drawing to a close, specifically those in Iran on oesophageal cancer and in Africa on Burkitt's lymphoma and liver cancer. Facets of these programmes are being continued in other geographical areas, such as the Far East.

2. DESCRIPTIVE EPIDEMIOLOGY (Dr C. S. Muir)

The aim of the programme of descriptive epidemiology is to map the occurrence of cancer throughout the world and improve the comparability of incidence data. The existence of differences in cancer risk in different populations facilitates the formulation and testing of etiological hypotheses by the Agency and other bodies[1, 2].

2.1. *Expanded programme*

As indicated above, an expanded programme of descriptive epidemiology has been prepared and submitted to detailed examination within the Division and by the 16th Scientific Council. This programme covers all available resources of morbidity and mortality data. Emphasis is placed on the acquisition of data at a regional or provincial level to allow correlations or cohort studies to be mounted using defined populations of up to one million. A number of cancer registries have been in existence for some years, and mortality data have been collected for many decades. It should therefore be possible to study time trends and to establish cohort studies in the selected populations.

2.2 *Cancer registries*

(*a*) *International Association of Cancer Registries (IACR)* (Dr C. S. Muir and Miss S. Whelan)

The Agency continued to act as the secretariat for the Association's activities (RA/73/016). The membership increased to 110 in 1980 with the admission of three new voting members, Louisiana, USA (Dr E. T. Krementz); Utah, USA (Dr J. L. Lyon), and Hawaii, USA (Dr W. Rellahan); and the affiliation of three non-voting registries, Arkansas, USA (Dr Ruth Steinkamp); Atlanta, USA (Dr Margaret Child) and Columbia-Presbyterian Medical Centre, New

[1] Muir, C. S. & Nectoux, J. (1980) In: Fraumeni, J. F. & Schottenfeld, P., eds, *Cancer Epidemiology and Prevention*, New York, Plenum (in press).

[2] Day, N. E. (1980) In: Symington, T. & Carter, R. L., eds, *Scientific Foundations of Oncology, Supplement*, London, Heinemann Medical Books (in press).

York, USA (Dr Mary McCrea Curnen); and of three non-voting members interested in cancer registration, Professor Bruce Armstrong, National Health and Medical Research Council, Unit of Epidemiology and Preventive Medicine, Perth, Western Australia; Dr A. Vloemans, Netherlands Ministry of Health; and Dr T. Watts, University Teaching Hospital, Lusaka, Zambia.

A two-day scientific meeting of the Association was held in Oslo, Norway, in August 1980, on the theme 'Computation of Rates for Defined Groups—Matching of Numerator and Denominator'. The problems posed by urban and rural differences, small geographical areas, occupation, social class, ethnic and religious groups and migrants were examined. It is planned to hold a further association meeting on cancer registration and patient data systems prior to the 13th UICC Congress to be held in Seattle in 1982.

The IACR and the Agency are collaborating in the production of Volume IV of *Cancer Incidence in Five Continents* (see section 2.3), and on a revision of the monograph *Cancer Registration and its Techniques*[3]. A collaborative descriptive study on malignant melanoma is planned.

Elections of officers to the Association were held in November 1979. Professor P. Correa, Louisiana, USA, is now President; Professor E. Saxén of the Finnish Cancer Registry is General Secretary; and Professor Ed. 'B. Attah, Nigeria; Dr Joyce Ford, Australia; Dr I. Fujimoto, Japan; Professor G. Riotton, Switzerland; Dr J. Staszewski, Poland; Dr E. A. Clarke, Canada; Dr J. Young, USA; and Dr A. P. Mirra, Brazil are regional representatives.

Registries supported by Agency programmes

(*b*) *Caspian Cancer Registry* (see section 3.4 (*d*))

(*c*) *Singapore Cancer Registry* (RA/67/009) (Professor K. Shanmugaratnam, Mrs A. Arslan and Dr N. E. Day)

The Singapore Cancer Registry, inaugurated in 1968, has continued its activities through 1980. Cancer incidence data on the three major ethnic groups—Chinese, Malays and Indians—for the period 1973–1977 have been submitted for inclusion in *Cancer Incidence in Five Continents*, Vol. IV. A monograph is being prepared describing the results of the first 10 years of registration, 1968–1977. The analyses have emphasized differences by Chinese dialect group and by birthplace, and examined trends in incidence seen over this period.

(*d*) *Cancer registration in 'Latin-speaking' countries* (Dr A. J. Tuyns and Mrs J. Nectoux)

The group studying the epidemiology and registration of cancer in 'Latin-speaking' countries held its fifth meeting on 15–16 May 1980 in Viana do Castelo (Portugal), where Dr J. M. de Carvalho runs a Cancer Registry covering the northern part of Portugal.

The inaugural lecture, 'Cancer Registries as seen by a Public Health Man', was given by Dr J. J. Viñes, Sub-director General, Preventive Medicine and Community Health, National Institute of

[3] MacLennan, R., Muir, C. S., Steinitz, R. & Winkler, A. (1977) *Cancer Registration and its Techniques (IARC Scientific Publications No. 25)*, Lyon, International Agency for Research on Cancer.

Health, Madrid, Spain. A total of 22 papers were presented, on the following topics: skin cancer (coordinator: Dr A. Zubiri, Zaragoza Cancer Registry, Spain), stomach and colo-rectal cancers (Dr D. Serrao, Cancer Registry of Porto, Portugal), childhood cancers (Dr G. Pastore, Cancer Registry of Turin, Italy) and nutritional factors in digestive tract cancers (Dr J. Faivre, Cancer Registry of Dijon, France).

(*e*) *Advice to cancer registries and other bodies*

The publication of the monograph *Cancer Registration and its Techniques* (see section 2.2(*a*)) has enabled many queries about cancer registration to be answered more expeditiously. However, many enquirers, notably from developing countries, have not given sufficient thought to the aims of their projected registries.

Algeria: Advice was given on the analysis of relative frequency data for nasopharyngeal cancer collected in West Algeria by Mme Fouzia Medjahed, Oran.

The material from Algeria collected by Prof. A. Yaker and Dr N. Dekkar has now been published *in toto*[4].

France: Close contacts have been maintained with the cancer registries operating in the departments of Bas-Rhin, Calvados, Côte-d'Or, Doubs and Isère.

A new 'Service', dealing with the measurement of cancer mortality and morbidity in France, has been created at the National Institute of Health and Medical Research (INSERM). The 'Comité de Surveillance' is chaired by Dr J. F. Duplan, and Dr A. J. Tuyns is a member of the Committee.

Dr Muir attended the meeting of the Governing Council for the Registre Bourguignon des Tumeurs Digestives, and Dr Tuyns that of the Registre des Tumeurs de l'Isère. Dr Tuyns continues to act as adviser to a Digestive Tract Cancer Registry established in Caen (Calvados).

Dr Goldschmidt, Strasbourg, was provided with information on Grawitz tumours and other urinary-tract cancers.

Liberia: Dr A. Sobo visited the Agency on 4–8 February and presented results from the Liberian Cancer Registry. Arrangements were made to send the Registrar, Mrs E. V. Benjamin, to the Tayside Regional Registry, Scotland (Dr L. Cameron) for further training.

United Kingdom: Information was given to Dr L. G. Morgan, Clydach, Swansea, on the availability of mortality data in the Bordeaux area in relation to the use of arsenic-contaminated copper sulfate sprays in vineyards.

Switzerland: The Association of Cancer Registries in Switzerland has now assembled data from five registries for the years 1974–1976, and Dr A. J. Tuyns assisted in analysing the first results. On this basis, the Scientific Advisory Group to the Association will now prepare a paper summarizing the major findings.

(*f*) *Relative frequency*

Since its inception, the Agency has encouraged pathologists and others who collect relative frequency data to exploit this material, by offering them advice on analytical methods and the

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[4] Yaker, A. & Dekkar, N. (1980) *Profil de la morbidité cancéreuse en Algérie 1966–1975*, Alger Editions, S.N.G.D., pp. 1–136.

presentation of data for publication. As part of an expanded descriptive epidemiology programme, it is now proposed systematically to seek such information from those parts of the world in which neither cancer incidence nor mortality data are likely to be available for some time. It is proposed to store the information received in standard form at the Agency; and, when sufficient material has accumulated, to publish a monograph comprising the basic information with appropriate comment. Dr E. P. van der Esch of The Netherlands Cancer Institute, Amsterdam, has been acting as an advisor to this project.

(*g*) *Multiple tumours* (Mrs J. Nectoux)

The coding of multiple tumours, synchronous or metachronous, poses many problems to cancer registries. With increased survival and the possibility of developing further neoplasms following treatment with chemotherapeutic agents with carcinogenic potential, this problem is becoming of greater importance. As a first step, a small working party has been established to define the problem and to examine existing coding schemes (Dr P. Schaffer, Registre des Tumeurs du Bas-Rhin, Strasbourg; Dr L. Raymond, Registre Genevois des Tumeurs, Geneva).

(*h*) *Time trends in cancer*

Several Agency staff members were asked to participate or to act as session chairmen in a symposium on time trends in cancer organized by the Norwegian Cancer Society and held in early August 1980 in Oslo. The contributions comprised a general assessment of the role of time trends in the determination of cancer etiology[5], an overview of time trends in malignant melanoma of the skin[6], a comparative analysis of time trends in larynx and lung cancer[7], and time trends, cohort effects and ageing as influences on cancer incidence[8].

(*i*) *Reviews*

Reviews on the geographical pathology of digestive-tract cancers and on the epidemiology of breast cancer have now been published[9, 10]. A general review of cancer time trends in France has been published[11].

(*j*) *Cancer of the kidney*

A review was prepared on the geographical distribution and etiological factors of cancer of the kidney[12]. It is likely that over half of tumours of the renal parenchyma and pelvis in males are associated with tobacco smoking. The extraordinarily high incidence of renal pelvic tumours in

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[5] Muir, C. S. In: Magnus, K., ed., *Time Trends in Cancer*, New York, Hemisphere (in press).
[6] Muir, C. S. & Nectoux, J. In: Magnus, K., ed., *Time Trends in Cancer*, New York, Hemisphere (in press).
[7] Tuyns, A. J. In: Magnus, K., ed., *Time Trends in Cancer*, New York, Hemisphere (in press).
[8] Day, N. E. & Charnay, B. In: Magnus, K., ed., *Time Trends in Cancer*, New York, Hemisphere (in press).
[9] Tuyns, A. J. & Repetto, F. (1979) *Rév. Epidemiol Santé publ.*, 27, 465–477.
[10] Tuyns, A. J. & Repetto, F. (1979) *Sénologia*, 4, 241–249.
[11] Tuyns, A. J. & Repetto, F. (1980) *Rév. Practicien*, 30, 187–195.
[12] Muir, C. S. & Nectoux, J. (1980) In: Sufrin, G. & Beckley, S. A., eds, *Renal Adenocarcinoma (UICC Technical Report Series Vol. 49)*, Geneva, pp. 133–155.

localized areas of Bulgaria and Yugoslavia that are frequently associated with the so-called Balkan nephropathy remains unexplained, although there is experimental evidence of a possible role of silica in water and of ochratoxin contamination of foodstuffs.

(*k*) *Malignant melanoma*

During preparation of a review on time trends in malignant melanoma of the skin, it became apparent that there was a scarcity of data on incidence by sub-site and histological type by sex for a variety of climates and latitudes. A study to obtain such information in collaboration with members of the International Association of Cancer Registries is planned. At the same time, information will be collected on non-cutaneous sites of malignant melanoma. Dr E. P. van der Esch, pathologist at The Netherlands Cancer Institute, Amsterdam, who has been associated with a large clinical trial on this tumour, is acting as a consultant.

2.3 Cancer Incidence in Five Continents, *Volume IV* (Dr C. S. Muir and Miss S. Whelan; in collaboration with Dr J. A. H. Waterhouse and Miss J. Powell, Birmingham Cancer Registry, UK, RA/78/019)

Data have now been received from 87 registries, covering 120 ethnic groups, for Volume IV of this series, which is scheduled to appear in 1981. These are now being processed at the Birmingham and West Midlands Regional Cancer Registry, UK, where computer programmes have been designed to translate the raw material received into the age-specific and age-standardized incidence rate tables which are given for each population by site in the volume. The data are subjected to stringent consistency checks by the computer and are then examined in detail by the editorial board to ensure that the quality criteria laid down for inclusion in this series have been met.

The importance of reliability of registration is emphasized throughout the volume. It will also include tables on the proportions of registrations with histological verification of diagnosis, the percentage of registrations based only on a death certificate and correlations with available mortality data by broad age-groups for all sites.

In the main summary tables, cumulative rates for age-spans 0–64 and 0–74 are presented for the first time, in addition to a rate standardized to the world population and a truncated standardized rate covering the age-span 35–64 which appeared in previous volumes. Incidence rates standardized for age to a European and African population structure will not be included in this volume.

While cancer registries use the International Classification of Diseases (ICD), many have their 'house rules' for coding of selected neoplasms, generally those of uncertain behaviour. The results of a comprehensive survey of coding practices will constitute one of the chapters in Volume IV. Dr L. Sobin of the WHO Cancer Unit is collaborating in this work.

Data for urban and rural areas have been provided separately for 41 populations, and a synoptic presentation of that material will be given in a chapter devoted to the topic. Urban and rural age-standardized and cumulative rates will also be included in the main summary tables.

Professor K. Shanmugaratnam (Singapore Cancer Registry), who represents the International Association of Cancer Registries on the editorial board, is coordinating a study on the distribution of specific histological types of cancer of the urinary bladder, thyroid and Hodgkin's disease, in which 61 of the contributing registries have indicated they will participate.

2.4 *Commentary on* Cancer Incidence in Five Continents (Dr M. K. Stukonis and Mr S. Sabai)

Work continues on a monograph that will present incidence data from the three previous volumes of *Cancer Incidence in Five Continents,* emphasizing changes over time, by a series of diagrams and charts, supported by a brief explanatory text. This presentation is designed for use by medical administrators, non-specialist scientists and informed laymen.

The diagrams for the commentary are being produced by the Mathematics Department of the University of Namur, Namur, Belgium (Professor E. Schifflers, RA/78/025).

Selected material from the commentary, which will be particularly useful for studying time trends and geographical differences, has been published[13].

2.5 *Clearing-house for on-going research in cancer epidemiology* (Dr C. S. Muir, Mrs A. Nagy-Tiborcz and Mrs E. Démaret; in collaboration with Professor G. Wagner, Dr C. O. Köhler and Mr K. Schlaefer, German Cancer Research Centre, Heidelberg, Federal Republic of Germany)

The clearing-house for on-going research in cancer epidemiology was created in 1974 by the Agency and the German Cancer Research Centre, Heidelberg, Federal Republic of Germany (RA/74/003) and operates with the support of the International Cancer Research Data Bank of the National Cancer Institute (Bethesda, MD, USA).

The goal of the clearing-house is to publish annually information on on-going studies in cancer epidemiology in the *Directory of On-going Research in Cancer Epidemiology* and to provide an *ad hoc* search service. 'Epidemiology' has hitherto been interpreted rather broadly in this series, although the clearing-house does not solicit information on clinical trials, diagnosis or mass-screening programmes, unless these include an epidemiological evaluation.

The first, second and third directories contained 622, 908 and 1025 projects, respectively. The 1979 directory[14] contained 1092 projects; copies were distributed to all principal investigators who submitted entries, ministries of health, medical journals, cancer research centres, cancer registries, industries, libraries, medical research boards, research workers and selected individuals.

Analysis shows that the United States and the United Kingdom were by far the largest contributors, followed by Japan, Canada and Australia. While Europe as a whole had an increased contribution over that of previous years, the contributions from Africa, South America and the Middle East remained about the same.

As in previous years, the cancer sites most frequently studied were lung, breast, gastrointestinal tract, urinary bladder, liver and cervix, and few investigations were being carried out on cancers of the pancreas or prostate or on malignant melanoma, despite their importance in many parts of the world.

The clearing-house service is now well established and widely known and used by epidemiologists. The information collected is available through the directories and is also available

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[13] International Agency for Research on Cancer (1979) *IARC intern. tech. Rep., No. 79/004.*

[14] Muir, C. S. & Wagner, G., eds (1979) *Directory of On-going Research in Cancer Epidemiology (IARC Scientific Publications No. 28),* Lyon, International Agency for Research on Cancer.

through the CANCERPROJ data base (a subfile of the popular CANCERLINE) and RPROJ (a subfile of TOXLINE). The increasing number of the directories sold by the Agency shows a growing interest in the clearing-house and its service.

Mailing for the 1980 directory began in September 1979, and 2926 letters of invitation to participate were sent to research workers in 103 countries. The 1980 directory contains 1261 projects reported from 69 countries.

3. ANALYTICAL EPIDEMIOLOGY

3.1 *Studies on alcohol and cancer (Dr A. J. Tuyns, Dr F. Repetto and Dr J. Estève)*

For several years, the Agency has undertaken an extensive research programme on the role of alcoholic beverages in cancer, supported by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) (Rockville, MD, USA). The experience acquired by the Agency in this particular field is well recognized, and the results obtained by Agency studies as well as others have been reviewed on various occasions[15-17]. The programme comprises several epidemiological studies on oesophageal and other cancers of the digestive tract and laryngeal cancer as well as laboratory investigations.

(*a*) *Oesophageal and other cancers in Normandy* (Dr A. J. Tuyns; in collaboration with Dr G. Péquignot, Nutrition Section of the French National Institute of Health and Medical Research (INSERM), Le Vésinet, France) (RA/75/015)

The collection of data has now been terminated, and complete dietary data, including alcohol consumption, smoking histories and other data, have been obtained for 744 cases of oesophageal cancer and 1976 population controls. The latter represent approximately 74% of the target population. The data are being computerized.

The oesophageal cancer cases are first being evaluated and checked against mortality data. A preliminary analysis indicates that the standardized mortality rates per 100 000 were 31.7 (males) and 1.5 (females) in Calvados, and 30.7 (males) and 1.8 (females) in the adjacent department of Orne, while the corresponding figures for France were 14.0 (males) and 1.0 (females). In other European countries the mortality rates for males range from 3 to 7 per 100 000; in non-European countries for which mortality data are available, the highest rates were observed in Uruguay (16.2), Hong Kong (11.5), Chile (9.5) and Singapore (9.3).

Various laboratory studies have beeen undertaken, simultaneously at the François Baclesse Centre and the Agency, and the results have been published[18]. Samples of the spectrum of alcoholic drinks consumed in the region, notably apple cider and its distillates, were collected from retail

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[15] Tuyns, A. J. (1979) *Fermented Food Beverages in Nutrition*, New York, Academic Press, pp. 427-437.
[16] Tuyns, A. J. (1979) *Cancer Res.*, 39, 2840–2843.
[17] Tuyns, A. J. (1980) *Cancer Epidemiology and Prevention*, Philadelphia, Saunders (in press).
[18] Tuyns, A. J., Castegnaro, M., Toussaint, G., Walker, E. A., Griciute, L. L., Le Talaer, J. Y., Loquet, C., Guerain, J. & Drilleau, J. F. (1980) *Bull. Cancer*, 67, 15–28.

outlets and from farms; several distillates produced under experimental conditions in the laboratory were also examined. When these samples were analysed for the presence of selected nitrosamines, small amounts were found, mostly in beer, which is not widely drunk in Normandy or Brittany.

Mutagenicity tests on the apple cider-based drinks and on other commercially available alcoholic beverages (beer excepted) have shown a weak response. This can be attributed neither to nitrosamines nor to polycyclic aromatic hydrocarbons but to some other as yet unidentified compounds.

The results of these studies contrast with the overwhelming evidence that the consumption of alcoholic beverages as such entails a considerably increased risk for cancer of the upper aero-digestive tract and liver.

(*b*) *Alcohol in relation to other diseases* (Dr A. J. Tuyns)

The risk of ascitic cirrhosis has previously been shown to be a function of daily intake of alcohol in grams [19]. An investigation is now being carried out to determine whether the various alcoholic beverages are equally cirrhogenic; preliminary results seem to indicate that they are not.

An investigation in Rouen on a possible relationship between diet, alcohol and tobacco consumption and acute myocardial infaction continues. This study is also being carried out in collaboration with Dr G. Péquignot (RA/77/028).

(*c*) *Cancer of the gastrointestinal tract in Belgium* (Dr A. J. Tuyns)

This study is based on the observation that gastric cancer and rectal cancer are far more common in the Flemish provinces than in the Walloon provinces of Belgium. There are minor differences in the dietary patterns of these two sub-population groups, and these are now being investigated in a retrospective case-control study in the provinces of Oost-Vlaanderen and Liège. Coordination is ensured by the Laboratory of Epidemiology at the School of Public Health in Brussels (Mrs L. Ravet-Ramioul) (RA/78/014).

(*d*) *Cancer of the larynx in southern Europe* (Dr A. J. Tuyns, Dr F. Repetto and Dr J. Estève; in collaboration with Dr B. Terracini, Institute of Pathology, University of Turin, Italy (RA/78/017); Dr F. Berrino, National Cancer Institute, Milan, Italy (RA/78/018); Dr A. Zubiri, Cancer Registry of Zaragoza, Zaragoza, Spain (RA/78/015); and Dr A. del Moral Aldaz, Health Department of Navarra, Pamplona, Spain (RA/78/016))

Cancer of the larynx is associated with tobacco smoking and alcohol consumption. The role of the latter is believed to be important in heavy smokers only and is uncertain in light smokers. This assumption is based, however, on studies that were carried out in populations with an overall heavy tobacco consumption and light alcohol consumption. The second part of the premise needs to be confirmed, i.e., the absence of an effect of alcohol among non-or light smokers. This is now being studied in populations known for their relatively moderate smoking habits and for their high

[19] Péquignot, G., Tuyns, A. J. & Berta, J. L. (1978) *Int. J. Cancer*, 7, 113–120.

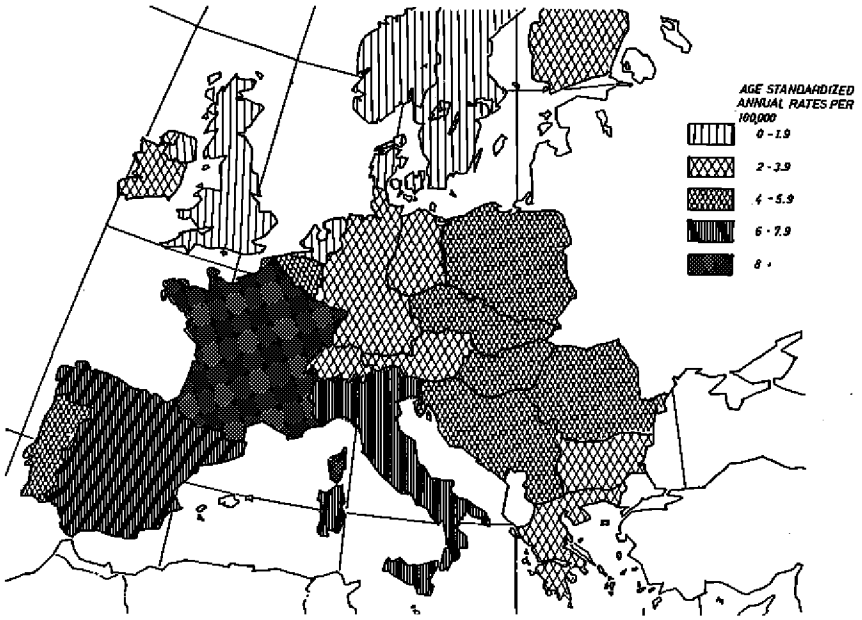

Fig. 1 Mortality rates for cancer of the larynx in males in Europe, 1975–1976

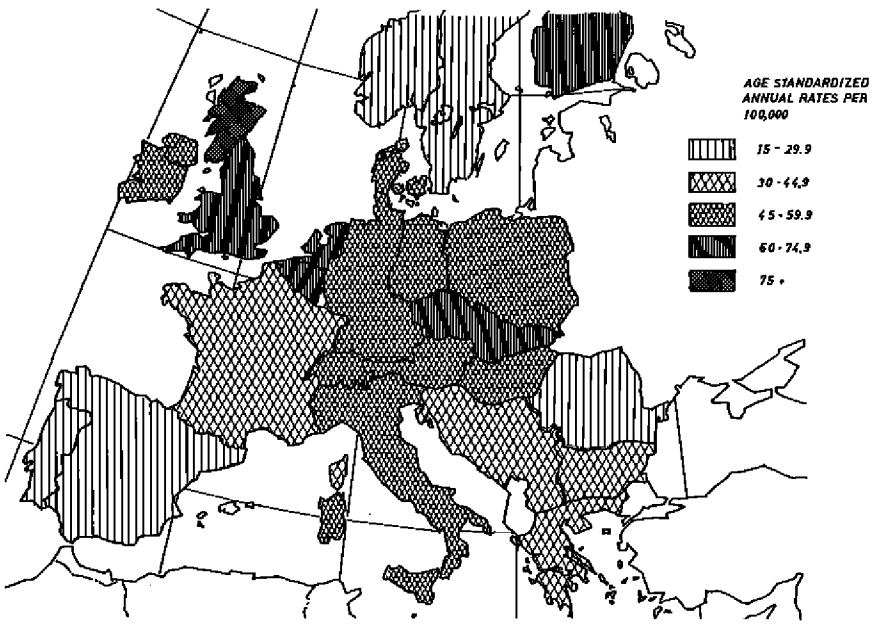

Fig. 2 Mortality rates for cancer of the lung in males in Europe, 1975–1976

consumption of alcohol, namely in France, Spain and Italy. These three countries rank first in Europe for laryngeal cancer mortality rates in males (Fig. 1), further contrasted with moderate rates for lung cancer (Fig. 2).

The aim of the study is to clarify the respective roles of tobacco and alcohol in terms of quality and quantity consumed. It also includes an assessment of diet, using a version of the standard questionnaire previously used in the studies on oesophageal cancer. Occupational histories are also recorded, to verify the impact of some suspect occupational exposures. Dr W. Lehmann, of the Department of Otorhinolaryngology, Cantonal Hospital of Geneva, designed the clinical questionnaire.

Six centres are participating in the study: Turin and Varese (Italy), Zaragoza and Pamplona (Spain), Geneva (Switzerland) and Caen (France). Dr H. Sancho-Garnier and Dr H. Benhamou of the Gustave Roussy Institute (Villejuif, France) are contributing to the testing of the clinical questionnaire used to obtain the precise location of the laryngeal tumour.

Data are now being collected from patients and population control groups in all centres. In Caen, where a study on oesophageal cancer was already in operation (see section 3.2 (*a*)), the initial phase of the study (interviewing patients and population controls) has been terminated.

The problems presented by a study involving several regions differing in cultural patterns and languages required close contact among the principal investigators, and meetings were held in Geneva (17–18 October 1979), Milan (7–8 February 1980) and Porto (12–14 May 1980). In addition, a refresher seminar for the interviewers/dieticians involved in the field work took place at the Agency from 14–18 April 1980.

Time trends for laryngeal cancer mortality and morbidity have been reviewed: there is a general tendency towards an increase of this cancer in most countries, which parallels that of lung cancer, although the slope is less marked for cancer of the larynx. A cohort analysis showed that the increase in laryngeal cancer occurred in later generations than did lung cancer and in females rather than males. The features that characterize the trends are consistent with the preponderant role of smoking and drinking, but are insufficient to demonstrate the mode of action; this is expected to be clarified by the retrospective case-control study described above.

(*e*) *Primary liver cancer in Geneva, Switzerland* (Dr A. J. Tuyns)

An epidemiological investigation aimed at clarifying the unusually high incidence of primary liver cancer in Geneva is being carried out in collaboration with Mr L. Raymond, Geneva Cancer Registry (RA/76/012).

A preliminary study carried out by Dr M. Voirol and Dr F. Infante on the alcohol consumption of the first 22 cases of primary liver cancer, showed an average consumption of 85 g per day for patients with primary liver cancer with cirrhosis, and 45 g for the controls; for patients with primary liver cancer without cirrhosis, the figure was 87 g and for controls, 44 g.

3.2 *Prenatal events and childhood cancer* (Dr N. Muñoz; in collaboration with Dr N. Wald, Department of the Regius Professor of Medicine, Radcliffe Infirmary, Oxford, UK; and Dr A. Pasca, Institute of Microbiology, University Medical School, Pécs, Hungary)

Two or more prenatal serum samples have been collected from over 30 000 pregnant women in Oxford, UK and Pécs, Hungary. Congenital malformations and cancers occurring in the

offspring of these mothers are being identified through record linkage. This study will link serologically confirmed viral infections with the occurrence of congenital malformations and childhood cancers.

3.3 *Liver cancer*

(*a*) *Swaziland* (Dr F. G. Peers and Dr N. Muñoz)

The primary objective of this study is to assess the impact of measures to decrease aflatoxin contamination of food on liver cancer in Swaziland. The natural history of hepatitis B virus in this population will also be determined.

A programme under the Ministry of Agriculture of Swaziland to decrease aflatoxin contamination of crops has been underway for some years. Following the 1968 Agency study on liver cancer and aflatoxin in Swaziland, post-harvest improvement schemes were initiated in 1972. It is estimated that this programme has covered 45% of the homesteads of Swaziland. A stratified random sampling of crops in Swaziland has been completed in collaboration with the Grain Sampling Unit of the Ministry of Agriculture. In spite of difficulties with recruitment of Swazi laboratory staff, analysis of these samples for aflatoxin contamination is currently underway. The Swaziland Government has constructed laboratory and office facilities specifically for the Agency project, and the laboratories have been equipped under the IARC/United Nations Environment Programme agreement. When the specimens collected in the present survey have been analysed, an estimate can be made of the impact of the measures taken during the last decade on aflatoxin contamination.

Cancer registration has been re-established in Swaziland, and over 100 cases were registered during a trial period between July and December 1979. Although this registration has been limited mostly to clinically diagnosed cancer cases, special efforts are being made to provide a histopathological service for the country, which should not only improve the validity of the present study but provide a permanent essential medical service for Swaziland. α-Fetoprotein tests, pathognomonic for liver cancer, have been established at the Agency laboratory. A preliminary analysis of the trial period of registration suggests that liver cancer occurrence has not markedly changed since it was assessed between 1964 and 1968.

A prevalence survey of hepatitis B markers was carried out in 1973, using blood specimens submitted to the Central Public Health Laboratory and Blood Bank at Manzini. A recent analysis of the 669 sera showed the prevalence of hepatitis B surface antigen carriers to be 14.5% of males and 9.4% of females. No significant differences were observed in prevalence between the four geographical areas of Swaziland in which marked variation of liver cancer occurrence has previously been recorded. To enable this analysis to be undertaken and to assist in an accurate geographical allocation of cancer cases for these four areas, a gazetteer of homesteads in Swaziland has been prepared. Further studies to define the natural history of hepatitis infection in Swaziland include a prevalence survey of hepatitis B markers, linked to the United States Agency for International Development survey of water-borne diseases, in the general population of the four topographical areas of Swaziland for comparison with that of 1973. Five hundred pregnant women will be the subjects of a study on the perinatal transmission of hepatitis B virus infection, and the children of antigen-positive mothers will be followed for a period of one year to detect any evidence of hepatitis.

(*b*) *Cohort study on hepatitis B virus and liver cancer* (Dr N. Muñoz; in collaboration with Professor Phoon Wai On and Miss Jong Lee Chen, Department of Social Medicine and Public Health, University of Singapore, Singapore; Dr Ong Yong Wan, Director of Blood Bank, Singapore; Dr Oon Chong Jin, Department of Internal Medicine, University of Singapore, Singapore (RA/79/021))

The aim of this study is to determine the risk of developing primary liver cancer among Chinese carriers of hepatitis B surface antigen by a prospective follow-up. Cohort members for this study are being identified from the following sources:

(i) *The blood bank*

A total of 2704 Chinese males over 38 years of age have been identified from the master computer list of blood donors. Of these, 39 (1.4%) have been suspended as donors because they are hepatitis B surface antigen-positive by counterimmunoelectrophoresis. The remainder will be invited by letter to give a further donation and their sera will be tested for the antigen using reverse passive haemagglutination. In addition, blood specimens from 403 Chinese males over 38 years of age have been collected prospectively and tested for hepatitis B surface antigen by reverse passive haemagglutination. Liver function tests have been performed in all cases of antigenaemia.

(ii) *Singapore Anti-Tuberculosis Association (SATA)*

Blood specimens are taken for routine laboratory tests from tubercular or suspected tubercular patients. Aliquots of 167 sera have been provided since May 1980 for hepatitis B virus testing.

(iii) *Old peoples' homes*

Thirty government homes, with 2500 residents, have been visited; however, it is estimated that very few residents will fulfill the criteria for inclusion in this study since most of them are over 60 years of age.

(iv) *Singapore Action Group for the Elderly (SAGE)*

This group has about 2000 members. Screening for hepatitis B surface antigen has been offered by the Department of Social Medicine, and in the first month 29 subjects were added to the study.

(v) *General practitioners*

About 20 general practitioners attending male Chinese patients in Singapore have agreed to collaborate and to provide blood specimens for screening of hepatitis B surface antigen. The Department of Social Medicine will offer liver function tests to those patients found positive for these antigens.

(*c*) *Perinatal studies on hepatitis B virus and liver cancer* (Dr N. Muñoz; in collaboration with Dr E. Domingo and Dr A. Lingao, Department of Internal Medicine, Philippines General Hospital, Manila)

(i) *Perinatal transmission of hepatitis B virus*

The object of this study is to assess the prevalence and predisposing factors of perinatal transmission of hepatitis B virus among Filipino children born to carrier mothers, as compared

with non-carrier mothers. A total of 500 mothers will be included in the study. Blood specimens will be obtained from the mother at the time of delivery and from the umbilical cord. It is estimated that about 10% of the mothers will be carriers of hepatitis B surface antigen. Follow-up of the children will be organized, and blood specimens will be taken at 1, 3, 6, 9 and 12 months of age.

(ii) *Case-control study of parents of patients with liver-cell cancer and of control patients*

The objective of this study is to compare the hepatitis B viral serological profile of parents and, if possible, of siblings of patients with hepatocellular carcinoma with that of parents and siblings of control patients. Parents and siblings of 50 patients with liver-cell cancer and of 50 control patients will be included in the study. Two years will be needed to collect this number of subjects from the general hospitals in Manila, Cebu City and Roxas City.

(*d*) *Hepatitis B markers in liver cancer*

(i) *Serological study* (Dr N. Muñoz and Dr P. Sizaret; in collaboration with Professor D. Trichopoulos, Department of Hygiene and Epidemiology, University of Athens Medical School, Athens)

The study on the association of hepatitis B markers and α-fetoprotein in liver cancer in Greece has been completed[20]. Professor Trichopoulos acted as consultant for the Agency in Egypt, where existing information indicated that a low frequency of hepatocellular carcinoma was associated with low levels of aflatoxin contamination of foodstuffs but with a high prevalence of hepatitis B surface antigen. He confirmed that relative frequency data suggest that hepatocellular carcinoma is indeed a rare disease in Egypt. Several serological studies indicate that the prevalence of hepatitis B surface antigen is probably below 5% among adults and below 10% among children. The relatively high frequency in children appears to be a recent phenomenon. It was confirmed that contamination of food by aflatoxin is low in Egypt, and so the situation in that country does not appear to contradict the association of hepatitis B virus and hepatocellular carcinoma.

(ii) *Studies on fixed liver tissue* (Dr N. Muñoz; in collaboration with Dr N. C. Nayak, All-India Institute of Medical Sciences, New Delhi; Dr C. Quenum, Dakar University, Senegal; Dr C. Cuello, Universidad del Valle, Cali, Colombia; and Dr A.-M. Mandard, François Baclesse Regional Centre, Caen, France) (RA/77/020)

Liver specimens from 904 patients on whom autopsy had been performed were included in this study. Basic demographic data, information on drinking habits, clinical and histopathological data and information on the number of paraffin blocks were recorded using a standard form. Liver sections were stained for hepatitis B surface antigen using the same batch of orcein, in a reference laboratory or at the local pathology laboratories, followed by a reexamination of all positive slides and a random sample of the negative slides by the reference laboratory.

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[20] Trichopoulos, D., Sizaret, P., Tabor, E., Gerety, R. J., Martel, N., Muñoz, N. & Theodoropoulos, G. (1980) *Cancer*, **46**, 736–740.

Table 1. Proportion of orcein-positive liver diseases in countries with high, intermediate and low risks for hepatocellular carcinoma

Risk area	Hepatocellular carcinoma		Cirrhosis								Miscellaneous		Total
			Alcohol		Non-alcohol		Non-specific		Total				
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.
High-risk	91	71.4	5	0	17	58.8	17	52.9	39	48.7	42	4.8	172
Intermediate-risk	69	79.7	7	0	8	62.5	66	66.6	81	60.5	71	2.8	221
Low-risk	117	30.8	141	2.8	37	21.6	46	21.7	224	9.8	170	0.6	511
Total	277	56.3	153	2.6	62	37.1	129	48.8	344	26.2	283		904

Three main groups of liver diseases were studied: 1) hepatocellular carcinoma, 2) cirrhosis, and 3) miscellaneous liver diseases such as parasitic diseases, metastatic liver cancer and metabolic diseases.

The specimens came from countries classified into three groups according to their risk for hepatocellular carcinoma; high-risk group: Senegal, Nigeria, Singapore (Chinese), Philippines; intermediate-risk group: Japan, India, Greece, Spain; low-risk group: USA, Mexico, Jamaica, Colombia, Brazil, UK, France, Australia. Table 1 gives a summary of the results. The association of hepatitis B virus with hepatocellular carcinoma, which has been demonstrated in several sero-epidemiological studies, is confirmed in these tissue studies in all geographical areas. A positive association between this virus and the non-alcoholic type of cirrhosis is observed in the three risk groups, but no association was observed for the alcoholic type of cirrhosis. The prevalence of hepatitis B surface antigen in hepatocellular carcinoma and non-alcoholic cirrhosis was higher in high-risk and intermediate-risk areas than in the low-risk areas.

3.4 *Oesophageal cancer* (Dr N. Muñoz and Dr N. Day)

(*a*) *Collaborative study on precancerous lesions of the oesophagus* (in collaboration with Dr R. H. Caselletto and Dr R. Drut, National University of La Plata, Argentina; Dr A.-M. Mandard, François Baclesse Regional Centre, Caen, France)

Collection of the oesophagi of patients who had died from diseases other than cancer of the oesophagus continues in areas of varying incidence. As there are few opportunities to obtain material sufficiently soon after death, collection is slow, but this criterion must be met, as histological evaluation is not possible in the presence of autolytic change.

Post-mortem specimens from 210 subjects have been received, but only 127 were suitable for inclusion in our study. The latter have been circulated among the participating pathologists, and histopathological evaluation of the lesions using a standard protocol will soon be complete.

(*b*) *Collaborative study on screening for precancerous lesions of the oesophagus in the high-risk area of Iran* (in collaboration with Professor M. Crespi and Dr A. Grassi, Regina Elena Institute, Rome; Dr A. Nadim and Dr B. Aramesh, Institute of Public Health Research, Teheran; and Dr A. Mojtabai and Dr C. Amiri, Cancer Institute, Teheran)

The endoscopical and histological evaluation of precancerous lesions of the oesophagus in 430 subjects from a high-risk population in Iran has been completed[21]. Plans for a similar survey in a low-risk area are underway.

These studies suggest that the earliest lesion in the development of oesophageal cancer is chronic oesophagitis with atrophic and dystrophic changes in the epithelium, followed by dysplasia, *in situ* cancer and finally invasive carcinoma. Chronic oesophagitis appears early in life and increases in severity with age.

[21] Crespi, M., Muñoz, N., Grassi, A., Aramesh, B., Amiri, G., Mojtabai, A. & Casale, V. (1979) *Lancet, ii*, 217–221.

(*c*) *Collaborative study on precancerous lesions of the oesophagus in a high-risk population of the Peoples' Republic of China* (in collaboration with Dr Li Ping Wu, Dr Chu Chuan Yen, Dr Wang Kao Ching, Dr Li Jun Yao, Dr Su Fang Zheng, Dr Cai Hai Ying and Dr Liu Fu Sheng, Beijing Cancer Institute, Beijing; Dr Yang Wen Hsien, Professor Shen Chum, Dr Qiu Song Lang, Dr Si Je Qiao, Dr Yang Kwan Re and Dr Huang Ha, Honan Medical College and Honan Cancer Institute, Honan, Peoples' Republic of China; and Professor M. Crespi and Dr A. Grassi, Regina Elena Institute, Rome)

A total of 523 individuals from the Cheng Guan commune of Lin County were included in this study. One-third of them had been examined by the Chinese balloon technique five years before, and dysplasia was diagnosed cytologically. A second third were age- and sex-matched controls of the first group and had had a normal oesophageal cytology in the same survey. The remaining third had not been included in the cytological survey five years before. For each subject a questionnaire containing basic demographic data, information on smoking, alcohol, dietary habits and symptomatology was completed, followed by a physical examination and an endoscopic examination with guided cytology and biopsies. Specimens of hair were collected from all individuals for zinc analysis, and blood was collected in a selected sample of individuals. Chronic oesophagitis was observed in 90% of the subjects (mild oesophagitis, 56%; moderate, 28%; and severe, 6%). The endoscopic and histological characteristics of the chronic oesophagitis were similar to those observed in Iran. This lesion increases in severity with age, and moderate and severe degrees were more frequent in males than in females. Incontinent cardias was relatively infrequent (13%), as in Iran, and oesophageal varices were found in 7.6% of the subjects. Dysplasia was diagnosed in 39 patients (7.5%), oesophageal cancer in 10 (2%), and adenocarcinoma of the gastric fundus in three patients. Only one other gastric cancer was detected and that was in the antrum. Of the 10 oesophageal cancer cases, six were located at the oesophageal-gastric junction. The cytological and histological evaluation of these lesions is still in progress, and their possible relationship with specific nutritional deficiencies (vitamin A, riboflavin and zinc) is being explored. If positive associations are found it will be possible to carry out intervention studies in this population.

A similar epidemiologic and endoscopic survey is being proposed in a low-incidence area for oesophageal cancer and in other high-risk areas with completely different environments in the Peoples' Republic of China.

(*d*) *Oesophageal cancer in Iran*

(i) *Caspian Cancer Register* (Mr P. Ghadirian, University of Teheran, Teheran)

The cancer register has been less active than previously, but registration continues. The data for the 10 years July 1968–June 1978 are being put into a uniform format and duplicates removed. A complete analysis of this material is planned.

(ii) *Field studies* (Dr B. Aramesh, Mr P. Ghadirian and Dr G. Stein)

The field studies which began in June 1978, but which were prematurely terminated in November 1978, had involved collection of biological samples, and these were stored at the University of Teheran until early 1980. The samples were finally shipped to the Agency in the first months of 1980 and are currently being studied for:

1) *Mutagenicity testing of urine* (Miss A. Camus and Mrs A. Hautefeuille, Division of Environmental Carcinogenesis)

In a pilot study, samples of urine collected for mutagenicity testing were assayed for the presence of mutagenic metabolites using a method described by Yamasaki & Ames[22].

2) *Morphine metabolites in the urine* (Dr C. Gorodetzky, National Institute on Drug Abuse, Lexington, KY, USA)

Urine samples have been sent to the US National Institute on Drug Abuse for both qualitative and quantitative assays of morphine metabolites. These assays may demonstrate regional variations of opium use.

3) *Antipyrine half-life determinations* (Professor M. Roberfroid, Free University of Brussels, Louvain, Belgium; Dr A. H. Conney, Hoffmann La Roche Inc., Nutley, NJ, USA)

Initial samples of saliva specimens from 50 individuals aged 6–19 years have been sent to Professor Roberfroid, who has been supplied with labelled antibody by Dr A. Conney. The results are being analysed. These assays assess indirectly the ability of the liver to metabolize chemicals.

3.5 *Gastric cancer and nitrosamines* (Dr N. Muñoz; in collaboration with Mr E. Walker, Mr M. Castegnaro and Dr H. Ohshima, Division of Environmental Carcinogenesis; Professor M. Crespi, Regina Elena Institute, Rome (RA/79/016); and Dr C. Walters, British Food Manufacturing Industry Research Association, Kent, UK)

The aim of the study is to compare the potential for formation *in vivo* of *N*–nitroso compounds in the gastric mucosa of subjects with chronic atrophic gastritis and of subjects with a normal gastric mucosa or with superficial gastritis. The pilot study to determine the most suitable conditions in which specimens of gastric juice, blood and urine should be collected, stored and tested is continuing. Specimens from 47 patients have been collected, 37 with chronic atrophic gastritis and 15 with a normal mucosa or superficial gastritis. Preliminary results indicate that there are no consistent differences among the three specimens which are being collected from each patient (fasting, one hour and three hours after standard meal). The level of volatile nitrosamines is higher in patients with chronic atrophic gastritis than in the control patients, but there is a large variation within each group. To evaluate the degree of individual variation, specimens are being collected during five consecutive days from five patients. Analyses for total *N*–nitroso compunds will be done in the next stage. It is estimated that specimens from at least 30 more subjects will have to be analysed before evaluation and decision as to whether this study should be extended to areas with high and low risk for gastric cancer.

[22] Yamasaki, E. & Ames, B. N. (1977) *Proc. natl Acad. Sci. USA*, **74**, 3555–3559.

3.6 *Long-term effects of pesticides on human health in Colombia* (Dr N. Muñoz and Dr N. Day; in collaboration with Dr L. Tomatis, Division of Environmental Carcinogenesis; Dr E. Guerrero and Dr M. Restrepo, National Institute of Health, Bogota (RA/79/012); Dr J. Ospina, National Cancer Institute, Bogota; Dr J. Davies, Department of Epidemiology and Public Health, University of Miami, Miami, USA; and Dr J. Litvak, WHO Regional Office for the Americas, Washington DC)

A number of pesticides are teratogenic or carcinogenic in experimental animals, but their long-term effects on human health are almost unknown. Colombia is one of the largest users of pesticides in the world, and these substances have been in extensive use there for at least 25 years. Several groups with high occupational exposure have been identified, including people exposed in plants where pesticides are formulated, those loading them into aircraft and those using pesticides in agricultural settings, sanitary campaigns and in floriculture. The total population of these groups is estimated at 25 000 persons. Two main studies have been proposed: the possible teratogenic effects of pesticides will be explored using a case-control study within a retrospective cohort study; the carcinogenic effects of pesticides will be explored in a retrospective cohort study using three main sources—pesticide formulating plants, aerial spray companies and sanitary campaigns.

A feasibility study has demonstrated that 5000 subjects 15–35 years of age can be included in the teratogenic study. The expected number of children with major congenital malformations in this cohort is 100. As it is expected that the job turnover in this cohort will be high, prospective rather than retrospective follow-up must be considered. For the carcinogenic study it has been found that a cohort of 2000 males aged 20–25 can be studied. In this population the expected number of cancers—liver, soft tissue, lymphomas and brain tumours—for which there is some evidence, clinical or experimental, of association with pesticide exposure, is small; however, an association will be detected if the relative risk is very high. In both studies, pesticide exposure will be assessed by interview and by determination of pesticides, or their metabolites, in blood, urine and air samples. Interview by questionnaire will be validated by a review of industrial and medical records. The feasibility study has shown that records of pesticide exposure by type of pesticide and length of exposure are available at each of the industrial companies, and that medical records can be traced for 70% of the patients reporting for medical care. Questionnaires to be used in both studies have been pre-tested in a random sub-sample of 100 individuals from each occupational group.

The Agency will supervise the epidemiology in the definitive studies, for which a proposal to funding agencies has been prepared in consultation with the WHO Regional Office for the Americas.

3.7 *Case-control study of lung cancer in Cubans* (Dr O. Joly and Dr N. Muñoz; in collaboration with Dr M. Caraballoso, Institute of Oncology and Radiobiology, Havana) (RA/77/016)

This study has been extended to examine the risk of developing lung cancer by histological type and by type of tobacco used by both male and female Cuban smokers. It has accessed all new female and male cases of lung cancer which occurred in the city of Havana during a two-year period, and includes two matched controls—a hospital control and a neighbourhood control. All cytological or histological slides are reviewed by a committee of pathologists.

In all, 596 women suspected of having lung cancer were interviewed; 166 had lung cancer confirmed by cytology or histology, and in a further 156 the diagnosis was confirmed only by radiology. A total of 982 males suspected of having lung cancer were interviewed; in 382 patients the diagnosis was confirmed by cytology or histology, and in a further 250 only by radiology.

The collation of questionnaires of both cases and controls will soon be complete, and analysis should commence at the end of this year.

Chemical analysis of Cuban cigarettes showed no significant differences in the tar content between Cuban cigarettes and reference cigarettes. However, the Cuban cigarettes produce higher levels of carbon monoxide and carbon dioxide but a lower content of nicotine than the reference cigarettes.

3.8 *Etiological studies of Burkitt's lymphoma* (Dr A. Geser)

(*a*) *Prospective study of Burkitt's lymphoma in the West Nile District Uganda Virus Research Institute, Entebbe*

Principal Investigator: Dr S. Etyono

(i) *Burkitt's lymphoma (BL) case detection*

BL case detection was not carried out during most of 1979, and it is therefore not possible to say whether the decline in incidence which was reported in the previous *Annual Report*[23] has continued. This is clearly an important matter to pursue, and it is hoped that as soon as local conditions permit, the Uganda Virus Research Institute will resume field activities in the West Nile, including BL detection and registration of cases. The Virus Institute will also resume the malaria surveys in the area to find out whether the decline in malaria parasitaemia, which was indicated in our survey results from 1972–1977, continues.

The occurrence of space-time clustering of BL has been invoked as an argument in favour of an environmental or even infectious etiology of BL, and at the end of the prospective study in the West Nile District of Uganda, the opportunity was taken to review observations made in both Uganda and Tanzania[24]. It appears that time-space clustering is not a constant epidemiological characteristic of BL, indicating that the previous observation may have been an artefact due to changes in diagnostic procedures.

Dr G. Olwit, the Ugandan epidemiologist who worked on the BL project in the West Nile, is presently following a training course in epidemiology at the London School of Hygiene and Tropical Medicine, and he is expected to return to work with the Uganda Virus Research Institute from October 1980. He is performing a detailed analysis of the study of child mortality which was carried out in connection with the prospective BL study in the West Nile. The results of this study seem to confirm that the infant mortality rate was indeed unexpectedly low in the West Nile children whom we followed from 1972–1978.

(ii) *Epstein Barr virus (EBV) and Burkitt's lymphoma (BL)*

As mentioned in the previous *Annual Report*[25], 3360 sera collected in the study in the West Nile District were tested for EBV antibody activities, i.e., against viral capsid antigen, early antigen

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[23] International Agency for Research on Cancer (1979) *Annual Report, 1979*, Lyon, p. 77.
[24] Siemiatycki, J., Brubaker, G. & Geser, A. (1980) *Int. J. Cancer*, **25**, 197–203.
[25] International Agency for Research on Cancer (1979) *Annual Report 1979*, Lyon, p. 79.

and nuclear antigen. The results are now being analysed in cooperation with the Programme of Biostatistics, with the main objective being to see whether EBV infection varies by age and sex, from place to place, and over time, in a manner commensurate with the hypothesis that the virus plays an etiological role in BL.

Sera from two further pre-bled BL cases which were detected in the West Nile District after 1977, together with the control sera, have been tested for anti-EBV antibodies at the Agency, and parallel testing will be undertaken at Dr Henle's laboratory in Philadelphia.

A special study was made of EBV antibody levels measured at high and low altitudes in the West Nile and North Mara[26]. The prevalence of EBV antibodies and the level of the antibody titres are very similar in the lowlands and on the high plateaux in East Africa, even though the incidence of BL varies so drastically between the two areas. This finding does not support the idea that EBV infection is related causally to BL.

(iii) *Malaria suppression trial and Burkitt's lymphoma (BL) incidence, Mara Region, Tanzania*
Tanzania Shirati Mission Hospital, Musoma, Tanzania (RA/71/007) (RA/77/008)
Principal Investigator: Dr G. Brubaker

This project is a joint effort between the Tanzanian Government, the Malaria Division, WHO and the Agency, and it is envisaged that it will continue to the end of 1982. The project continues actively to detect BL cases, both in North and South Mara. At the same time, a malaria prophylaxis trial is being carried out in North Mara. The goal is to reduce the prevalence of malaria parasitaemia from its previous level of around 40% to below 10%, to see whether this reduction in malaria will result in a decreasing incidence of BL in the area.

1) *BL detection*

Case detection is carried out in North Mara by a field supervisor from Shirati Hospital, assisted by the eight drug distributors of the malaria project who continuously visit all villages in the area. In 1979, a total of five BL cases, confirmed by histology, were detected in North Mara. The numbers of BL cases and the incidence per 100 000 population between 1964 and 1979 are shown in Table 2; it can be seen that the decline in BL incidence, which became noticeable from 1974 onwards, continues. The decline is statistically significant, but it is evidently not due to our efforts with malaria suppression in the area, since they have not yet had the desired effect (see below).

In South Mara, BL detection is carried out by one scout who systematically visits all health centres in the area to detect BL suspects and bring them in for diagnosis and treatment at Shirati Hospital. Four suspects were found in South Mara in 1979, but specimens for histological confirmation were not obtained and the final number of confirmed cases is thus unknown.

2) *Malaria suppression*

As reported last year[27], chloroquine distribution in North Mara failed to reduce the malaria parasitaemia there. A meeting of Agency personnel involved in the project and malaria experts, held at the Agency in October 1979, considered the reasons for this failure. It was concluded that it could be due either to an inadequate consumption of chloroquine by the recipients or to an

[26] Geser, A., Brubaker, G. R. & Olwit, G. (1980) *Rev. Epidemiol. Santé publ.* (in press).
[27] International Agency for Research on Cancer (1979) *Annual Report, 1979*, Lyon, p. 79.

Table 2. Numbers of patients with onset of Burkitt's lymphoma (BL) from 1964–1979 and annual incidences per 100 000 population in North Mara, Tanzania

Year	Number of cases in North Mara	Population in North Mara lowlands[a]	BL incidence per 100 000 in North Mara
1964	6	132 510	4.5
1965	7	136 090	5.1
1966	9	139 770	6.4
1967	4	143 590	2.8
1968	8	147 420	5.4
1969	6	151 400	4.0
1970	4	155 490	2.6
1971	11	159 680	6.9
1972	7	164 000	4.3
1973	7	168 420	4.2
1974	5	172 970	2.9
1975	5	177 640	2.8
1976	6	182 440	3.3
1977	2	187 360	1.1
1978	2	192 430	1.0
1979	5	197 400	2.5
All	95	159 680[b]	3.8

[a] Calculated on the basis of the 1967 census in Tanzania, assuming a yearly population increase of 2.7 %
[b] Mid-year 1971 population

increased chloroquine resistance in local malaria parasites. To test the first possibility it was decided to double the chloroquine dosage from approximately 5 mg/kg twice monthly to approximately 10 mg/kg twice monthly; this was introduced from December 1979.

To test the second possibility—increased resistance to chloroquine in North Mara—Dr Draper, London School of Hygiene and Tropical Medicine, conducted a chloroquine sensitivity survey in children in North Mara during November/December 1979. The results of the *in vitro* testing, using Dr Draper's micro method, showed that the sensitivity to chloroquine was no lower in malaria parasites collected from a random sample of 0–9-year-old children in North Mara than elsewhere. An *in vivo* test carried out by Dr Draper in 90 children in North Mara with parasitaemia showed that about 80% of them cleared their parasites in the seven days following consumption of the chloroquine tablets (10 mg/kg). It is therefore not lack of chloroquine sensitivity that is responsible for the failure to suppress malaria in our study population.

The continued monitoring of malaria parasitaemia in both North and South Mara indicates that from the beginning of 1980 a moderate impact may be being made on malaria in North Mara. The results of the malaria surveys are shown in Table 3. Since February, the prevalence of parasitaemia has been about 30% less in North Mara than in South Mara, which may indicate that the doubling of the chloroquine dosage in North Mara may now be having an effect. There is, of course, also the possibility that these differences reflect variation in rainfall in North and South Mara, but reports from Dr Brubaker show that so far this year, the rainfall has been similar in the two areas.

In view of the absence of widespread chloroquine resistance in local malaria parasites, it appears likely that the children in the trial are not getting their chloroquine tablets as regularly as they should. We therefore made a detailed enquiry in a sample of the villages in North Mara to find out how many of the children are regularly participating in the chloroquine distribution. On the basis of the answers given by the children, and/or their mothers, it appeared that nearly 85% of the

Table 3. Prevalence of malaria parasitaemia in North and South Mara, Tanzania, January-May 1980

Month	North Mara			South Mara		
	Total no. examined	No. with parasitaemia	%	Total no. examined	No. with parasitaemia	%
Jan.	427	117	27	69	11	16
Feb.	444	136	31	74	36	49
Mar.	414	64	15	76	16	21
Apr.	337	82	24	49	22	45
May	348	106	31	24	13	54
Total	1970	505	26	296	98	33

0–9-year-olds participate in all drug distribution sessions held by their tenhouse chairman *(balosi)*. However, by checking the books which the *balosis* maintain to record chloroquine distribution, it appeared that they are somewhat irregular in arranging the distributions and that over the last year they have, on average, omitted about one-third of all the scheduled distributions. It could well be, therefore, that this irregularity reduces the amount of chloroquine consumed, although it is evident that the children and their parents are willing and indeed keen to participate in the scheme.

An attempt is now being made by Dr Brubaker and his staff to motivate the *balosis* to adhere strictly to their distribution schedule. The continued monitoring of malaria parasitaemia in the survey area will reveal whether or not this measure proves effective.

3.9 *Large-bowel cancer* (Dr O. M. Jensen and Dr D. G. Zaridze)

The programme is investigating the relationship between dietary factors, 'precursor' lesions, and the occurrence of large-bowel cancer. It is being implemented through analytical studies focused on: (*a*) biochemical aspects of diet and intestinal content; and (*b*) histological aspects of 'precursor' lesions, in particular, polyps.

An important element of the two types of studies is the development of standardized techniques of measurement, such as the measurement of dietary components, and assessment of histological lesions.

(*a*) *International study of diet and faecal characteristics in relation to colorectal and other cancers* (Dr O. M. Jensen and Dr D. C. Zaridze; in collaboration with Dr P. Helms, University of Aarhus, Aarhus, Denmark; Dr R. Seppänen, Social Insurance Institute, Helsinki; and Dr R. Williams, MRC Dunn Nutrition Unit, Cambridge, UK)

Large-bowel cancer is associated with factors prevalent in affluent societies and correlates internationally with the consumption of fat or meat. It has been suggested that long-chain fatty acids of animal dietary fat or endogenous bile acids are converted by bowel flora into promoters of bowel carcinogenesis. It has also been proposed that dietary fibre exerts a protective effect, by diluting the intestinal contents and, possibly, by modifying bacterial or other types of metabolism in the intestine.

The Agency has been able to bring together groups of investigators in national laboratories to test some aspects of these hypotheses in populations with greater contrasts in incidence than are observed nationally; the Agency has supervised study design and organized the collection of data and biological material in a standard manner in each population[28].

In earlier investigations, when rural Finnish and urban Danish male populations were compared, the results suggested a possible protective role of dietary fibre[29]. This study has now been repeated, including collection of further information on dietary intake and on the faecal excretion of suspect bile acids, in urban and rural populations in both Denmark (Copenhagen, Them) and Finland (Helsinki, Parrikalen) under the supervision of Dr P. Helms (RA/77/027) and Dr R. Seppänen (RA/77/030), respectively.

The results of preliminary analysis are as follows: (1) No trends paralleling the colon cancer incidence emerged with regard to average daily fat intake. (2) A significant difference was found between the areas with regard to total dietary fibre intake, which was inversely correlated with the incidence of large-bowel cancer. (3) Bile acid concentration in the stool correlated positively with the incidence of large-bowel cancer, in contrast to results of the first study. Further parameters such as faecal bacteriology, faecal mutagenicity and urinary phenols showed no association with the trends in colon cancer incidence among these four populations. This study of four Scandinavian populations with a little more than three-fold difference in colon cancer incidence supports the hypothesis that, given a high level of fat intake, dietary fibre exerts a risk-modifying effect.

(*b*) *Risk of large-bowel cancer in spouses* (Dr O. M. Jensen; in collaboration with Dr J. Ericsson, Swedish Cancer Registry, Stockholm; and Dr A. M. Bollander, Swedish Death Registry, Stockholm)

Spouses may be assumed to share diet and dietary habits. In collaboration with the Swedish Cancer and Death Registries, a study was carried out to determine the risk of large-bowel cancer among spouses of patients who died from colon and rectal cancer in 1961. The risk of colorectal cancer was not increased among spouses of persons with large-bowel cancer; nor was there an increased risk of diseases which have been suggested to be etiologically related to cancer of the large bowel, except for a marginal increase in heart diseases among women[30].

There was no proof that spouses in this study did indeed share the same diet throughout married life. Further investigations are needed to determine whether uniform exposure can indeed be assumed in such studies. The results of this study do not exclude the possibility that premarital (childhood or adolescent) diet may be involved in the etiology of cancer of the colon.

(*c*) *Colo-rectal polyps in Lyon* (Dr O. M. Jensen and Dr D. G. Zaridze; in collaboration with Professor R. Lambert, Division of Epidemiology, National Centre for Scientific Research, Lyon, France)

Adenomatous polyps of the colon and rectum are regarded as precancerous lesions, appearing on average approximately 10 years earlier than invasive neoplasms and probably sharing common

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[28] IARC Intestinal Micro-ecology Group (1977) *Lancet, ii*, 207.

[29] International Agency for Research on Cancer (1977) *Annual Report, 1977*, Lyon, pp. 37–38.

[30] Jensen, O. M., Sigtryggsson, P., Nguyen-Dinh, X., Bollander, A.-M., Vercelli, M. & MacLennan, R. (1980) *Lancet, i*, 1161.

etiological factors. In this study, dietary habits of persons with endoscopically diagnosed and histologically verified large-bowel polyps will be compared with those of controls.

(*d*) *Large-bowel pathology in autopsy series* (Dr O. M. Jensen, Dr D. G. Zaridze and Dr J. Estève; in collaboration with Dr N. M. Gibbs, St Luke's Hospital, Guildford, UK)

This collaborative study seeks to correlate variation in possible precursor or associated pathology with variation in incidence of cancers of the colon and rectum, in particular between colorectal polyps and cancer. Autopsy material has been collected, using standardized protocols, in the following areas, with descending levels of large-bowel cancer incidence: Aberdeen, Scotland (Dr J. Simpson, Dr S. Ewen and Dr J. Clark); Tromsö, Norway (Dr H. Stalsberg and Dr J. Eide), and Kuopio, Finland (Dr G. Koskela). 'Blind' histological evaluation of slides of a random sample has been completed, and statistical analysis is underway.

3.10 *Studies in the industrial environment* (Dr R. Saracci)

This programme was established to provide new information on cancers due to occupational and related environmental exposures. It is currently being implemented by:

(i) testing of hypotheses on occupational exposures, by *ad hoc* case-control and cohort studies; these are conducted at the international level to increase the size of the populations studied and hence the chance of detecting an effect;

(ii) exploration of the possible roles of occupational and related environmental factors in the etiology of specific cancers;

(iii) exploration of time and space variations, within and between countries, of specific cancers in relation to indicators of occupation and social class.

The following projects are in progress:

(*a*) *Health risks from mineral fibres*

(i) *Man-made mineral fibre production* (Dr R. Saracci, Dr L. Simonato, Dr A. Geser and Dr J. Estève; in collaboration with Professor E. D. Acheson, School of Medicine, Southampton, UK (RA/78/021); Dr O.M. Jensen, Danish Cancer Registry, Copenhagen (RA/78/020); Dr P. Westerholm, Landsorganisation i Sverige, Stockholm; Dr S. Krantz, National Board of Occupational Safety and Health, Stockholm (RA/79/001); Dr K. Magnus, Norwegian Cancer Registry, Oslo (RA/78/022); and Dr P. A. Bertazzi, Work Clinic, Milan, Italy (RA/80/002)

Man-made mineral fibres, in particular glass wool and rock wool, are used increasingly as reinforcement for plastics and, more importantly, for thermal and acoustic insulation, where they represent a substitute for asbestos. It is thus important to determine whether man-made mineral fibres, which are carcinogenic when instilled into the pleural cavity of experimental animals, cause cancer in man.

A historical cohort study of the health risks associated with mineral fibres is underway in 13 production facilities located in seven western European countries (Denmark, Federal Republic of Germany, Finland, Italy, Norway, Sweden, United Kingdom). Collection of data started in the second half of 1978, using a common protocol with local adaptations. First a nominal roll and an

employment history of all workers who had ever been employed at each plant was established. Every worker with more than one year of employment was then followed up to ascertain the cause of death stated on the death certificate. For workers at most plants, cancer occurrence is also being ascertained through cancer registry records.

The concentrations of airborne fibres and their size distribution were estimated at individual work stations in 12 of the 13 plants (one has been closed) by the Institute of Occupational Medicine, Edinburgh, Scotland (Mr J. Dodgson and Dr A. Seaton). Table 4 provides a summary of some key features of this international study. The data collection phase is nearing completion, and the data are being sent, with a series of controls at the national level, to the Agency for a central analysis.

Table 4. Man-made mineral fibre historical cohort study

	Rock wool	Glass wool	Continuous filament	Total
No. of plants	7	4	2	13
For mortality	7	4	2	13
For cancer incidence	6	3	-	9
For environmental survey	6	4	2	12
Man-years	45 900	64 400	18 000	128 300
Man-years (≥ 20 years)	6 270	5 900	1 580	13 750

Mortality and cancer occurrence will be compared in workers exposed to mineral fibres in the production facilities and in the general population of the areas where the factories are located. Groups of workers with different degrees and lengths of exposure will also be compared. Analysis will take place during the last months of 1980 and in 1981, and the results will be available by the end of 1981.

(ii) *Man-made mineral fibre users* (Dr R. Saracci, Dr A. Geser, Dr N. Day and Dr L. Simonato; in collaboration with Dr A. Englund and Mr G. Engholm, 'Bygghälsan', The Swedish Foundation for Occupational Safety and Health in the Construction Industry, Stockholm)

This case-control study is being supported financially by both 'Bygghälsan' and the Swedish Working Environment Fund. Its purpose is to assess the risk for workers engaged in occupations such as the building, construction and demolition industries, which are known to expose them to higher concentrations of fibres than occur in the man-made mineral fibre industry.

A preliminary analysis of mortality among workers in these industries up to 1978 was undertaken at the Agency in early 1980. It is now considered necessary to re-examine the cancer registry files for this population in order to assess the accuracy of death certification, and especially to examine in detail cases of mesothelioma and all other deaths in which a diagnosis of mesothelioma could be entertained.

(iii) *Mesothelioma in central Turkey* (Dr R. Saracci and Dr L. Simonato; in collaboration with Dr Y. I. Baris, Department of Chest Diseases, Hacettepe University, Ankara) (RA/78/012)

The endemicity of mesothelioma in certain areas of rural Central Anatolia, Turkey, was reported in 1978 by investigators from the Department of Chest Diseases, Hacettepe University, Ankara [31]. Investigation of this outbreak, in which the reported risk appears to be far higher than that in any occupational asbestos exposure, is of great scientific and public health interest. As neither industrial processes nor obvious exposure to asbestos are involved, the presence of a local mineral fibre appeared the most reasonable hypothesis. A survey was carried out in collaboration with Dr Baris and the Medical Research Council Pneumoconiosis Unit, Penarth, UK. Two villages in the Urgüp district (province of Nevsehir) were investigated: Karain (population 575), where a very high incidence of pleural mesothelioma has been reported [31], and Karlik (population 479), the nearest village to Karain about 3 km away by road, where the disease is unknown (Fig. 3).

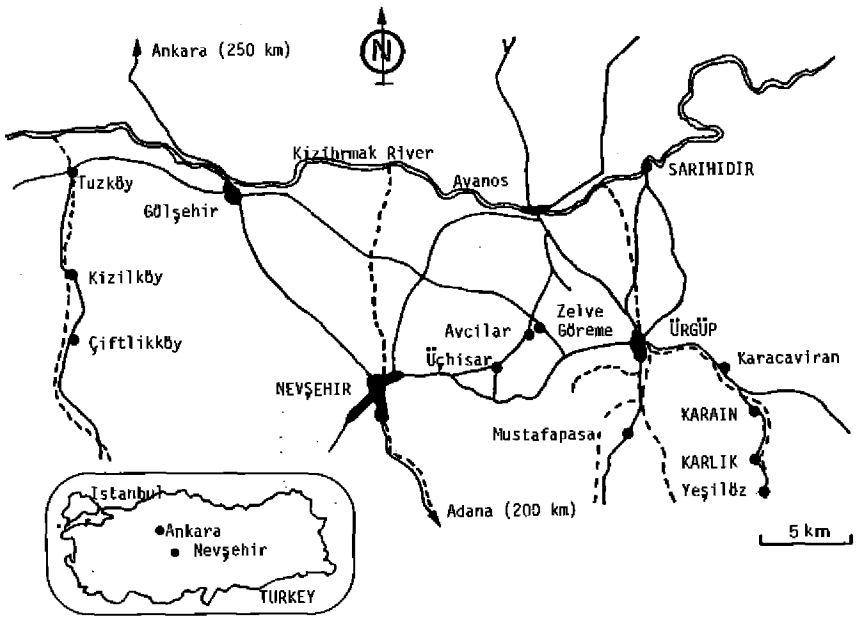

Fig. 3 Villages in Turkey where the occurrence of pleural mesothelioma has been investigated

Demographic data on population structure, births and deaths were abstracted from available local registries (1970–1978). Adults aged 20 and over were administered a questionnaire (including selected items of family, residential and occupational history), and a chest X-ray was taken. The films were read 'blindly' by a panel of four readers, using the UICC/ILO radiological classification of pneumoconiosis. Second readings of a subset of the films were performed. As this is one of the rare occasions in which the UICC/ILO classification has been employed in a general rather than in an occupational population, a detailed assessment of this method of reading X-ray films, including

[31] Baris, Y. I., Sahin, A. A., Ozesmi, M., Kerse, I., Ozen, E., Kolacan, B., Altinörs, M. & Göktepeli, A. (1978) *Thorax, 33*, 181–192.

evaluation of intra- and inter-observer variability, is underway. During the field study, environmental samples of air and water were collected for fibre counting, size determination and elemental chemical identification. Preliminary analysis of the collected data indicates no major differences in the concentration of airborne mineral fibre in the two villages.

Further environmental samples will be collected during 1980, and an environmental and population survey will be done in a third village, Sarihidir, where rocks relatively rich in fibres of the zeolite family have been identified. Assessment of these investigations should make it possible to establish whether a simple 'fibre hypothesis' can reasonably explain the mesothelioma endemic in the villages examined, or whether a more complex etiology should be sought.

(*b*) *Laboratory studies relevant to lung cancer* (see section 4.6 (*a*) of the report of the Division of Environmental Carcinogenesis)

(i) In conjunction with Dr C. Giuntini of the Italian National Research Council (University of Pisa), the Agency is evaluating lung function tests capable of early recognition of the effects of inhaled particles. A detailed analysis of a series of 18 such tests, which were performed on 60 asymptomatic smokers and 60 non-smokers, to identify the tests which best discriminate between the two groups, has been made, and a paper presenting the results and methodology of this study is in preparation.

(ii) It is proposed to use the available data on lung function tests to compare lung function impairment and the activity of pulmonary aryl hydrocarbons hydroxylase (AHH) in surgical lung specimens.

(iii) Determination of the pulmonary microsomal mono-oxygenase metabolism of 105 cancer patients has been completed.

(*c*) IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans (Dr R. Saracci and Dr L. Simonato)

Dr Saracci and Dr Simonato act as the secretariat for the epidemiological component of this programme (see p. 57), which in 1980 produced Volumes 22–25. They have been involved in the evaluation of risks from exposure to a complex chemical environment, such as occurs in occupational situations, which will now form the basis of a new series of *Monographs*. One of the major deficiencies of data for the *Monograph* series is the lack of information on human exposures. The importance of a systematic approach in the collection and evaluation of data from epidemiological studies on individual carcinogens and occupational exposures is again emphasized. Some aspects of the SEARCH Programme (see below) are relevant to this problem.

(*d*) *Cancer mortality in relation to occupation and social class* (Dr W. P. D. Logan and Dr C. S. Muir)

Work continues on this historical review of trends and the relation between cancer, occupation and social class based on the *Decennial Occupational Cancer Mortality Supplements* of the Registrar General of England and Wales[32].

[32] Muir, C. S. & Wagner, G., eds (1980) *Directory of On-going Research in Cancer Epidemiology (IARC Scientific Publications No. 35)*, Lyon, International Agency for Research on Cancer.

A series of international comparisons of socio-economic relationships has now been completed, and the consistency of the associations between occupation and social class can now be tested. Thus, internationally, oesophageal cancer risk tends to be lower in higher socio-economic groups and *vice versa*; stomach cancer shows a trend in the same direction. On the other hand, cancer of the colon shows no consistent pattern. For lung cancer, almost without exception, mortality or incidence was lower in higher socio-economic groups; the same pattern was reflected, although not so consistently, in women.

Since 1931, there has been a systematic gradient of risk by social class for cancer as a whole in England and Wales, the higher standard mortality ratios (SMRs) being seen in the poor: 75 in social class I and 131 in social class V in 1971. For married women, this gradient did not appear until 1961, and was less steep; whereas for single women no such gradient has been observed.

To illustrate the type of information which the monograph presents, stomach cancer may be cited as one of the types most frequently found in persons of a less affluent social class. This has been a consistent finding in England and Wales since 1921 in males, and since 1931, when such data first became available, for married women. The trend was similar in single women. The virtually universal decrease of stomach cancer is now well established. It is interesting to note that in England and Wales during the period 1931–1971 cumulative mortality rates for these three groups fell, the proportionate reduction being about the same in each. In males (Fig. 4), the reduction was seen in all social classes, again in much the same proportion.

Consideration of each cancer site has now been completed, and publication in 1981 is anticipated.

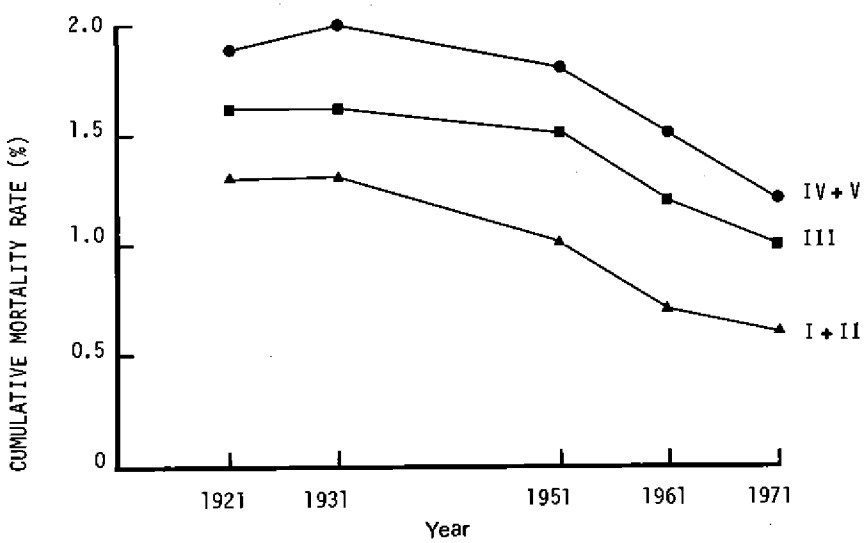

Fig. 4 Cumulative mortality rates for cancer in men in England and Wales during 1931–1971, by social class

(*e*) *Occupational cancer epidemiology for industrial medical officers and hygienists: courses held at the Agency* (Dr W. Davis and Dr R. Saracci)

The epidemiological approach to occupational disease, including cancer, is becoming increasingly important both as a research instrument and as a tool for health surveillance in the workplace. Two courses were held at the Agency in 1980 in order to introduce industrial physicians and hygienists to the key concepts in this field (see p. 148).

4. SURVEILLANCE OF ENVIRONMENTAL ASPECTS RELATED TO CANCER IN HUMANS (SEARCH) (Dr C. Agthe)

The reorganization of the Agency involved the creation of a new programme on the Surveillance of Environmental Aspects Related to Cancer in Humans (SEARCH).

4.1 *Background*

A programme for an International Cancer Surveillance network was first discussed in the 1976 *Annual Report* of the Agency[33]. The Director emphasized that 'the present situation demands a long-term commitment to the collection of cancer data in man relative to his environment'. The importance of the epidemiological study of lifestyle and the value of comparing industrialized with non-industrialized countries were also stressed.

A meeting attended by the directors of a number of cancer registries was held in November 1977 in Lyon during which suggestions were made for feasibility studies. During 1978, feasibility studies were undertaken to evaluate the types and availability of environmental data in three areas: Birmingham (UK), Singapore and Bombay. It was concluded that many such data are available but they are often difficult to locate. Nonetheless, these surveys supported the general impression that existing environmental data could form a basis for a number of correlation studies, as described below under the Extensive Programme.

Early in 1979, Dr C. Agthe was appointed by the Agency to develop the programme further; numerous experts from various disciplines were consulted during that year, and their suggestions are incorporated in the current design of the programme.

4.2 *The programme*

The programme on the Surveillance of Environmental Aspects Related to Cancer in Humans (SEARCH) is intended to be a step towards the 'creation of a mechanism for the systematic identification of the risks associated with specific environmental hazards and environments' (IARC Scientific Council, 1977, SC/13/WP/3). The programme incorporates two basic components:

(*a*) the Extensive component, which consists of correlation studies based on data on cancer occurrence and environmental factors; and

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[33] International Agency for Research on Cancer (1976) *Annual Report, 1976*, Lyon, pp. 24–26.

(*b*) the Intensive component, which consists of a series of case-control studies for investigation of a defined number of factors in cancer.

(*a*) *The Extensive SEARCH programme*

The Extensive component will utilize available data on cancer occurrence to carry out correlation studies and, when possible, selected environmental data over time to establish trends. These studies will be carried out systematically in selected areas or countries to ascertain the consistency and strength of any association which may be demonstrated.

When data on cancer incidence or mortality are available from national sub-areas, efforts will be made to obtain environmental data from the same sub-areas. The advantage of using sub-areas is that they may more readily show differences in cancer occurrence and environmental parameters, which will help to identify risk factors.

(*b*) *The Intensive SEARCH programme*

The Intensive component will consist of a series of coordinated and standardized case-control studies, to be undertaken in a few areas of about 0.7–2.0 million inhabitants. Emphasis will be placed on life style factors in different environmental settings. The programme will involve the simultaneous study of several cancers and many factors in life style and the ambient environment. The questionnaires used in the case-control studies will be revised following analysis of each study, to refine the hypotheses to be tested in the next series of studies.

The studies will investigate factors judged to be causally related to human cancer, in order to assess the relative risk of these factors and to analyse their possible influence as confounders. In addition, the studies will evaluate the possible role of factors suspected on the basis of experimental evidence and/or human observation. Finally, the studies will assess unevaluated factors, namely those which might rationally be viewed as possibly related to increased or decreased cancer risk but for which there is very little or no evidence at the present time.

(*c*) *Interrelationship of the two programme components*

The correlation studies are especially useful for investigating ambient environmental factors, whereas the case-control studies lend themselves better to investigations on life style. It is highly desirable, but not essential, that correlation studies also be undertaken in areas where the series of case-control studies are being carried out, to gain a better understanding of the possible influence of changes in the ambient environment. The two programmes will thus complement each other; and greater confidence can be placed in a hypothesis confirmed by both methods and in chosen environmental settings.

4.3 *Relationship of the SEARCH programme to other Agency programmes*

The Agency's current activities in descriptive epidemiology will provide the data on cancer occurrence that are essential to the Extensive SEARCH programme. These data will also be analysed with regard to trends, which will be of importance for the correlation studies.

The Intensive SEARCH programme will maintain close liaison with the Agency programme in analytical epidemiology. The statisticians at the Agency will play an important role in this programme in view of the complexity of the data analysis which results from the size and the multifactorial nature of the study.

4.4 *Implementation*

With the reorganization of the Agency, it was possible from January 1980 to appoint Dr A. Geser, an epidemiologist previously with the Unit of Biological Carcinogenesis, to the SEARCH programme.

The programme as outlined above was submitted in February 1980 to the Scientific Council, which debated the relative merits of designing epidemiological studies that test specific etiologic hypotheses as opposed to approaches that depend upon detecting unexpected associations. The Council endorsed the SEARCH programme, suggesting that it should complement and not overshadow the Analytical Epidemiology Programme, nor disturb the overall balance of the Agency's efforts.

The Governing Council, which met in May 1980, allotted a further sum to maintain the present core programme for the remainder of 1980 and 1981, but noted that further additional funds from sources outside the regular budget will be sought to cover the expenses once the programme has become fully operational.

During 1980, visits were made to various countries in order to assess suitable areas for inclusion in the programme. At the same time, environmental data are being collected from international sources so that they can be correlated with data on cancer mortality available at the national level. More refined correlation studies, involving national sub-areas, as well as the Intensive programme, will be started when enough areas have expressed their willingness to participate.

5. BIOSTATISTICS (Dr N. E. Day)

After 11 months as a consultant to the Agency, Professor N. E. Breslow returned to his post at the Department of Biostatistics, University of Washington, Seattle, WA, USA. Dr N. E. Day returned to the Agency in September 1979 from his sabbatical leave at the National Cancer Institute, Bethesda, MD, USA.

The arrival of Dr J. Wahrendorf in April 1980 was a further step in the development of the Biostatistics Programme. Dr Wahrendorf is responsible for developing statistical methods for analysing carcinogenesis experiments, for consultation to the Division of Environmental Carcinogenesis for the *IARC Monograph* series, and for current and future carcinogenicity experiments. He is also involved in the analysis of the large-bowel cancer studies (see section 3.9).

After several alternatives for computer management for the Agency were studied, a VAX 11/780 computer from the Digital Equipment Corporation was purchased in January 1980. The necessary conversion of programmes and transfer of data have been completed. Implementation of software and relevant procedures will be completed by the end of the third quarter of 1980. This reorganization of the computing facilities should provide a more flexible approach to biostatistical consultation without increasing computer costs.

5.1 *Evaluation of cervical cancer screening programmes* (Dr N. E. Day, Mr X. Nguyen-Dinh and Mrs A. Arslan)

In collaboration with the Cancer Unit, WHO, Geneva, and the WHO Regional Office for Europe, a meeting was held in Copenhagen on 3–5 December 1979. Participants included representatives of cervical cancer screening programmes and cancer registries from Canada, Finland, Iceland, Norway, Sweden, Switzerland, UK and USA.

A considerable number of screening programmes have now been in operation for more than 15 years, and it was felt that a coordinated assessment of the results of these programmes might assist in developing a unified evaluation of the Papanicolaou (Pap) test itself, and of different screening strategies.

The two basic questions on which attention might be focused are:

1. What protection does a woman receive against the development of clinically apparent, invasive cancer of the cervix by having regular Pap tests?
2. What are the consequences of a given strategy of screening in a population, in terms of subsequent morbidity and mortality in that population? That is, who should be screened, what age and how often?

The purpose of the meeting was to ascertain whether a joint approach was feasible and, if so, to define the initial steps to be taken.

It was felt that, as a first step, a collaborative effort should concentrate on the directly observable, operational features of a screening programme. At a later stage, an investigation could be made of whether possible differences between the definitions of different precancerous and preclinical lesions, as used in each screening programme, might account for any differences in performance that might be observed.

The following programmes agreed to attempt to put their data into a standard form: Aberdeen, UK (Dr E. Macgregor), Finland (Dr M. Hakama), Iceland (Dr G. Johannesson), Ostfold County, Norway (Professor P. Kolstad and Miss A. Hougen), Stockholm (Dr F. Pettersson) and Toronto, Canada (Dr A. Clarke).

The analysis of the results from Iceland has been updated[34].

5.2 *International radiation study of cervical cancer* (Dr J. Estève and Miss D. Magnin)

An International Radiation Study of Cervical Cancer was set up 20 years ago to investigate the risk of radiation-induced leukaemia among women who had been treated with radium and/or X-rays for cancer of the uterine cervix. The study involved clinical follow-up of over 30 000 patients treated at 30 radiotherapy centres in nine countries[35].

At two meetings held at the Agency, on 4–5 October 1979 and 14–15 May 1980, it was agreed to reactivate the study and to enlarge it to include population cancer registries. The purpose of reactivating the study is firstly to evaluate the carcinogenic risk for malignancies other than leukaemia, particularly in the non-pelvic regions of the body, and secondly to re-evaluate the

[34] Johannesson, G., Geirsson, G., Day, N. & Tulinius, H. (1980) *Acta pathol. scand.* (in press).
[35] Boice, J. D. & Hutchison, G. B. (1980) *J. natl Cancer Inst.*, **65**, 115–129.

negative finding with regard to leukaemia by extending the study to areas that have more systematic follow-up facilities and by increasing the length of follow-up.

Initial studies proposed at these meetings are currently underway and include:

(*a*) *Dosimetry studies* (Dr M. Stovall, M. D. Anderson Hospital and Tumor Institute, Houston, TX, USA)

The purpose of these studies is to investigate the radiation dose received by different organs in the body during different treatment regimens and the accuracy with which those doses can be estimated.

(*b*) *Cohort studies in cancer registries*

The cancer registries participating at present are those of Denmark, Finland, Norway, Slovenia (Yugoslavia), South Thames (United Kingdom), New Brunswick (Canada), Connecticut (USA) and Sweden. Additional registries in Canada, the United Kingdom and the USA are being approached. These studies should be completed by December 1980.

(*c*) *Case-control studies within the cancer registry cohort*

The cohort studies that are underway will not provide precise information on dose. To achieve a more accurate estimation of the relationship between risk and dose, it is planned to investigate the treatment received by each woman who has developed a second tumour and by a control series of women who have not. This will lead to an accurate estimate of organ dose, on the basis of the results of the dosimetry studies. A case-control study of leukaemia is to be conducted first.

(*d*) Follow-up of women included in the initial clinical series until the end of 1980, in those clinics where initial feasibility studies showed this to be possible.

A meeting to review progress is planned for December 1980.

5.3 *Immunogenetics of nasopharyngeal carcinoma (NPC)* (Professor S. H. Chan, University of Singapore, Singapore (RA/70/017))

The collection and screening of maternal antisera for HLA A, B, C and DR locus antigens continue.

Dr Chan has now typed 313 newly-diagnosed cases of NPC in the three major Chinese dialect groups, and 497 normal individuals including 167 with nasopharyngeal symptoms found negative on biopsy. The relationship between NPC risk and locus A and B antigens can now be described: the three antigens associated with increased risk are A2, B17 and BW46 (formerly SIN2).

Thirty-four families of NPC cases have been typed sufficiently to assign haplotypes to the case. In addition, data from spouses and children of renal transplant patients, and of individuals with a range of disorders, demonstrated 423 non-NPC haplotypes.

The association between A2 and BW46, known to exist in the general Chinese population, is considerably stronger among cases with NPC, suggesting that it is the haplotype which is important.

A2 appears to be a risk factor only in the presence of BW46, and BW46 a risk factor only in the presence of A2. The relative risk for the haplotype A2 BW46 is 2.79.

The effect of phenotype and of Epstein-Barr virus antibody titres on survival, for which preliminary work suggests an association, is being analysed at the Agency, as is the interaction of age with the HLA-associated risk.

5.4 *Scandi-Afro-Swiss-Immuno-Breast (SASIB) breast cancer study* (Dr L. Muenz, Biometry Branch, National Cancer Institute, Bethesda, MD, USA; Miss D. Magnin; in collaboration with Dr J. Stjernsward, Berne)

The Biostatistics Programme is still handling data processing and statistical aspects of this international collaborative trial of radiation therapy for stage II breast cancer. A meeting was held in Berne in February 1980 between investigators and representatives of the clinics involved in the study. It was decided that each of the 455 patients will be followed for five years from the date of their entry into the study, which means that the study will continue for approximately another three years.

5.5 *Breast and ovarian cancer in Iceland* (Dr M. Gonzalez)

Collaboration with the Icelandic Cancer Registry (Dr H. Tulinius) continues. The basic results of the study on familiality in breast cancer[36] are given in Table 5, where a gradient of risk with degree of familial relationship can be seen clearly. There is no significant excess risk among those related by marriage to the proband. A study of ovarian cancer and reproductive factors is underway.

Table 5. Familiality of breast cancer in Iceland: observed and expected numbers of relatives of breast cancer cases who themselves developed breast cancer

	Observed	Expected	O/E	P value[a]
Mothers	30	15.13	1.98	< 0.001
Sisters	50	16.90	2.96	< 0.001
Daughters	3	1.74	1.72	NS
Grandmothers, maternal	5	7.37	0.68	NS
Grandmothers, paternal	11	6.12	1.80	NS
Aunts, maternal	38	26.27	1.45	< 0.02
Aunts, paternal	41	26.49	1.55	< 0.01
Cousins	46	36.58	1.26	NS
Women related by marriage	53	51.36	1.03	NS

[a] NS – not significant

[36] Tulinius, H., Day, N. E., Sigvaldason, H., Bjarnason, O., Johannesson, G., Gonzalez, M., Grimsdottir, K. & Bjarnadottir, G. (1980) In: Gelboin, H. V., MacMahon, B., Matsushima, T., Sugimura, T., Takayama, S. & Takebe, H., eds, *Genetic and Environmental Factors in Experimental and Human Cancer*, Tokyo, Japan Scientific Societies Press, pp. 303–312.

5.6 *Development of statistical methods for use in cancer epidemiology*

(a) *Case-control studies* (Dr N. E. Day, Professor N. E. Breslow and Mr C. Sabai)

During his stay at the Agency, Professor Breslow completed a monograph on the analysis of case-control studies, which is now in press[37].

Plans are now being made for the preparation of a second monograph, dealing with the analysis of cohort studies.

(b) *Models for the evaluation of screening programmes* (Dr N. E. Day and Mr X. Nguyen-Dinh)

Understanding of the way screening intervenes in disease processes is facilitated by the development of realistic statistical models for the disease. Such models provide a quantitative basis for predicting the changes in morbidity and mortality that could be expected from screening programmes. Collaborating on this topic are Dr J. D. F. Habbema, Erasmus University, Rotterdam, The Netherlands; and Dr S. D. Walter, Yale University, New Haven, CN, USA, who visited the Agency for a month under an ICRETT fellowship.

(c) *Clustering techniques* (Dr J. Estève)

A linear logistic model with random components has been tested by simulation and will be used in assessing familial aggregation in breast cancer. A publication reporting the results of this research is in preparation.

(d) *Statistical evaluation of carcinogenicity experiments* (Dr J. Wahrendorf)

Guidelines for simple, sensitive significance tests for carcinogenic effects in long-term animal experiments have been prepared in collaboration with Mr R. Peto, Oxford University, UK[38]. These guidelines will be reviewed on the basis of comments from their readers and users.

The preparation of a handbook on statistical methods for the analysis of animal experiments in cancer research is now planned. A small international working group will be convened in early 1981.

(e) *Models for the etiology of breast cancer*

The complex relationship of breast cancer risk to age and to reproductive history can be described in terms of a simple two-stage model of tumour development. Dr S. Moolgavkar, of the Fox Chase Institute, Philadelphia, PA, USA, visited the Agency for a month under an ICRETT fellowship to collaborate in fitting the model to the epidemiological data[39].

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[37] Breslow, N. E. & Day, N. E. (1980) *Statistical Methods for Cancer Epidemiology*, Volume I, *The Analysis of Case-Control Studies (IARC Scientific Publications No. 32)*, Lyon, International Agency for Research on Cancer.

[38] Peto, R., Pike, M. C., Day, N. E., Gray, R. G., Lee, P. N., Parish, S., Peto, J., Richards, S. & Wahrendorf, J. (1980). In: *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Supplement 2, *Long-term and Short-term Screening Assays for Carcinogens: a Critical Appraisal*, Lyon, International Agency for Research on Cancer, pp. 311–426.

[39] Moolgavkar, S. H., Day, N. E. & Stevens, R. G. (1980) *J. natl Cancer Inst.*, **65**, 559–569.

The results were presented at a meeting on the epidemiology of breast cancer held at Leeds Castle, UK, under the auspices of the UICC. As a result of that meeting, work is currently underway to examine how the model corresponds to biological models of breast cancer development[40], and how predictions based on the model might be tested epidemiologically.

5.7 *Collaboration with other Agency programmes*

(*a*) *Analytical epidemiology*

The Biostatistics Programme continued to assist with data processing and statistical analysis for the case-control study on laryngeal cancer (see section 3.1 (*d*)).

Mr G. Engholm, from 'Bygghälsan', Stockholm, spent three weeks at the Agency working with the Biostatistics Programme on the analysis of data from the man-made mineral fibre study in the Swedish building industry (see section 3.10 (*a*) (ii)); data collection from that study continues.

(*b*) *Descriptive epidemiology*

Graphic equipment will be purchased by the Agency and linked to its VAX computer in order to help in the analysis of incidence and mortality data from *Cancer Incidence in Five Continents*. It is hoped that data organization implemented by the Mathematics Department of the University of Namur, Namur, Belgium (Professor E. Schifflers, RA/78/025) will be transferred here by the end of the year.

(*c*) *SEARCH Programme*

Consultation has been given on statistical aspects of the SEARCH Programme.

(*d*) *Retrieval and coordination of carcinogenicity data*

The increasing size of the *Bulletin on the Survey of Chemicals Being Tested for Carcinogenicity* (see section 2.3 of the report of the Division of Environmental Carcinogenesis) has posed production problems. Up until now, the *Bulletin* has been printed at the Agency using photo-offset from camera-ready copy. The Biostatistics Programme was asked for advice with regard to the computerization of this publication. A feasibility study has been completed, and the purchase of a word processing system, linked to the Agency's computer, has been recommended.

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[40] Bulbrook, R. D. (1979) In: Miller, E. C., Miller, J. A., Hirono, I., Sugimura, T. & Takayama, S., eds, *Naturally Occurring Carcinogens, Mutagens and Modulators of Carcinogenesis*, Tokyo, Japan Scientific Societies Press, pp. 331–336.

6. VISITING SCIENTIST (Dr N. W. Choi)

Dr N. W. Choi, of the University of Manitoba, Canada, has been a visiting scientist at the Agency for seven months. During this period he has acted as a programme adviser to Professor R. Lambert of the CNRS Centre of Epidemiology, Lyon. In association with epidemiologists in the Division, Dr Choi has been concerned with the preparation of a cohort study on Icelandic migrants in Canada, and a study on cancer risks among employees of a Canadian copper and zinc mining and smelting company. He has also participated actively in the research programmes of the Division, with a view to integrating his department into some of these programmes on his return to Canada.

DIVISION OF ENVIRONMENTAL CARCINOGENESIS

Dr L. TOMATIS (Director)

1. INTRODUCTION

Since 1 January 1980, following the restructuration of the Agency, the Division of Environmental Carcinogenesis has incorporated those activities previously carried out by the Units of Chemical Carcinogenesis, Environmental Carcinogenesis and Biological Carcinogenesis. The activities of the newly created Division now comprise four main programmes: mechanisms of carcinogenesis; retrieval and coordination of carcinogenicity data; environmental carcinogenesis and host factors; and analysis of environmental carcinogens. The objective of the Division is to generate, collect, analyse and disseminate information useful for the primary prevention of cancer in humans. Reports on the individual programmes are presented separately below; however, the main components of the Division's activities can be summarized as follows:

(i) identification of carcinogenic chemicals and evaluation of their carcinogenic risk to humans, as well as identification of carcinogenic risks resulting from exposures to complex mixtures of chemicals, which are often encountered;

(ii) development of a network of national laboratories that collaborate in testing environmental chemicals for their possible carcinogenicity and in improving testing procedures;

(iii) development of criteria to assess the significance of experimental results for the prediction of human risks, in parallel with coordinated studies aimed at a better understanding of the mechanisms of carcinogenesis;

(iv) assessment of the possible role of interspecies and interindividual differences in response to exposure to carcinogens, with particular emphasis on the role of host factors;

(v) development and application of chemical analytical methods for the detection of carcinogens in the human environment and acquisition of data on the occurrence of such carcinogens;

(vi) continuation of studies aimed at understanding the role of the Epstein-Barr virus (EBV) as an oncogenic agent.

By October 1980, 23 volumes of the *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans* had been published and two were in press. In all, a total of 537 chemicals, groups of chemicals, or industrial processes have been evaluated or re-evaluated. For 39 chemicals or industrial processes, a positive association, or a strong suspicion of a positive association, with human cancer has been found. Whenever data on humans were inadequate to evaluate the carcinogenic risk of a compound, an evaluation was made on the basis of the available evidence from humans and from experimental animals. The fact that data from human studies were available for only 14% of the chemicals evaluated in the *Monographs* points once again to the

general scarcity of epidemiological data as compared with experimental data. Thus, for some 100 chemicals for which there is sufficient evidence of carcinogenicity in experimental animals, no human data were available.

This year, the scope of the *Monographs* was broadened to include evaluation of the carcinogenic risks resulting from exposures to complex mixtures of chemicals—situations which occur often in human populations. The first Working Group that dealt with this new area convened in June 1980 to evaluate exposures that occur in the wood and leather industries. Causal associations were found between human cancer and certain exposures in the furniture-making and footwear industries.

The survey of chemicals that are being tested for carcinogenicity has been continued. *Information Bulletin No. 8* was published and disseminated in October 1979; recently, a new survey questionnaire was dispatched, in order to prepare *Information Bulletin No. 9*, which should be ready for distribution early in 1981.

In order to establish criteria for improving experimental results of carcinogenicity tests and making them universally acceptable, the Agency, jointly with the Medizinische Hochschule Hannover and the Commission of the European Communities, convened a Working Group of experts to draft basic requirements for the conduct and reporting of long-term and short-term carcinogenicity and related tests. The results of this meeting are now available as Agency publications[1, 2]. It is hoped that the application of these guidelines by the laboratories that are participating in the Agency network of collaborating national laboratories for the testing of environmental chemicals will contribute to the improvement of experimental procedures and consequently to the general acceptance of results. At present, the network of collaborating laboratories includes 12 national laboratories in 10 countries. The collaborating laboratories either carry out carcinogenicity and/or related tests on environmental chemicals or participate in studies to develop and improve the methodologies of the tests or to validate tests currently in use. The Agency also participates directly in part of these intramural activities.

The development of better criteria to assess the significance of experimental results in terms of human risks continues in parallel with studies of the basic mechanisms of carcinogenesis. A meaningful extrapolation from experimental results to humans can only take place if progress is made in understanding the carcinogenesis process. Particular attention has been paid to studies of the role of metabolism and of DNA repair processes in organ- and species-specific responses to carcinogens and of the role of DNA repair in dose-related responses to carcinogens. Another series of investigations has been developed on the mechanisms of promotion, with emphasis on the effects of tumour promoters on the cell surface and on cell differentiation.

The programme for understanding the role of EBV as an oncogenic agent has continued by providing laboratory support to on-going field programmes on Burkitt's lymphoma and by pursuing investigations of the role of EBV gene products in the transformation process. A new project has been initiated to study cytogenetic markers and the EBV-association of non-endemic Burkitt's lymphoma.

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[1] Montesano, R., Bartsch, H. & Tomatis, L., eds (1980) *Molecular and Cellular Aspects of Carcinogen Screening Tests (IARC Scientific Publications No. 27)*, Lyon, International Agency for Research on Cancer.

[2] IARC (1980) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Supplement 2, *Long-term and short-term screening assays for carcinogens: a critical appraisal*, Lyon, International Agency for Research on Cancer.

Studies have been initiated to characterize specific antibodies against carcinogenic agents or DNA adducts of carcinogens, with the aim of developing a method to monitor human exposures to carcinogenic agents.

In the programme on the elaboration and standardization of methods to detect and analyse carcinogens present in the human environment, new methods have been developed to detect non-volatile nitrosamines, and collaborative studies have been organized to ensure the reliability of available methods for detecting volatile nitrosamines and other carcinogens. In this context, a further volume in the series *Selected Methods for Analysis of Carcinogens*[3], dealing with polycyclic aromatic hydrocarbons, has been published, and priorities for further manuals have been established.

Within a project on the destruction of carcinogenic wastes from laboratories and the safe handling of carcinogens, a monograph on the decontamination of wastes contaminated with aflatoxins has been prepared. Available data on degradation techniques and on the chemistry of carcinogens are being collected, and collaborative studies to ascertain the efficiency of destruction methods currently in use are in progress.

The study to assess interindividual differences in carcinogen metabolism has been expanded through a continuous collaboration between the Agency laboratories and numerous collaborating national laboratories. In addition, experiments are in progress to verify the correlation between antipyrine half-life and liver microsomal enzyme activity, to see if a test could be developed for estimating the capacity of individual human subjects for carcinogen metabolizing activation.

The activities of the Division are closely connected with the role the Agency will play as the leading institution for carcinogenesis within the WHO International Programme on Chemical Safety.

2. RETRIEVAL AND COORDINATION OF CARCINOGENICITY DATA[4]

2.1 *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans* (Dr L. Tomatis, Mr J. D. Wilbourn, Miss L. Haroun and Mrs C. Partensky)

The objective of the *Monographs* programme is to identify potentially carcinogenic chemicals present in the environment and to evaluate their carcinogenic risk to humans. Implementation of this programme involves three key steps: (1) the collection of data relevant to the assessment of the carcinogenic risk of chemicals to which humans are known to be exposed; (2) the critical evaluation of these data by international working groups of experts in chemical carcinogenesis, epidemiology and other related disciplines; and (3) the publication and dissemination of the data and evaluations as *Monographs*. The critical analyses of the data are intended to assist national and international authorities in formulating decisions concerning preventive measures, and to define those areas in which additional research efforts are needed.

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[3] Castegnaro, M., Bogovski, P., Kunte, H. & Walker, E. A., eds (1979) *Environmental Carcinogens Selected Methods of Analysis*, Volume 3, *Analysis of Polycyclic Aromatic Hydrocarbons in Environmental Samples (IARC Scientific Publications No. 29)*, Lyon, International Agency for Research on Cancer.

[4] The *Monograph* and *Survey* programmes are supported in part by the US National Cancer Institute under Contract No. NO1 CP 45608.

During the last year, three Working Groups convened in Lyon, resulting in Volumes 23–25 of the *Monographs*[5–7]. The monographs contained in Volume 23 re-evaluate the carcinogenicity of four metals: arsenic, beryllium, chromium and lead, which were considered first in Volumes 1 and 2[8, 9]. The Working Group concluded that several inorganic arsenic compounds are causally associated with skin and lung cancer in humans and that there is *sufficient evidence* of carcinogenic effect in men occupationally exposed to chromium compounds during the production of chromate. There was some evidence that occupational exposure to beryllium leads to an increased risk of lung cancer in humans and *sufficient evidence* that beryllium metal and several beryllium compounds are carcinogenic in experimental animals. It was concluded that the combined experimental and human data indicate that beryllium should be considered suspect of being carcinogenic to humans. Experimental and epidemiological data on metallic lead and organic lead compounds were either unavailable or inadequate, and no evaluation of the carcinogenicity of these substances was possible. There was *sufficient evidence* that lead subacetate, lead acetate and lead phosphate are carcinogens in experimental animals.

The terms *sufficient evidence* and *limited evidence* (see below) of carcinogenicity indicate the amount of evidence available and do not indicate the strength of the carcinogenic effect. They are defined in detail in the preamble to the *Monographs*[6].

Volume 24 contains monographs on 16 miscellaneous pharmaceutical drugs (see Table 1), three of which (phenacetin, phenoxybenzamine hydrochloride and reserpine) had been considered previously[9–12]. For most of the drugs there was *sufficient* or *limited evidence* of carcinogenicity in experimental animals, indicating that further experimental and epidemiological studies should be pursued. The Working Group also concluded that there is *limited evidence* that abuse of analgesic mixtures containing phenacetin causes cancer of the renal pelvis in humans, although it was not possible to specify which component(s) is responsible for this effect.

This year the scope of the *Monographs* programme was broadened to include evaluation of the carcinogenic risks resulting from exposures to complex mixtures of chemicals. Exposures that occur in the workplace were selected as the starting point for this expansion in the programme. The Working Group that met in June 1980 evaluated the risks in the following industries within the wood, leather and associated industries: the lumber and sawmill industry (including logging); the furniture and cabinet-making industry; carpentry and joinery; the pulp and paper industry; leather tanning and processing industries; boot and shoe manufacture and repair; and the leather goods manufacturing industry (other than boot and shoe manufacture and tanning). The *Monographs* include a historical overview and description of each industry, and summaries of available case reports and epidemiological studies. Data on the toxicity of wood and leather dusts to humans were also included. It was concluded that for people in certain occupations in furniture making and in boot and shoe manufacture, there is an increased risk of nasal cancer, which occurs mainly among those exposed to high levels of wood or leather dusts. The epidemiological data were not sufficient to make a definite assessment of the carcinogenic risk of employment in the other industries.

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[5] International Agency for Research on Cancer (1980) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, 23: *Some Metals and Metallic Compounds*, Lyon.

[6] Idem (1980) Ibid, 24: *Some Pharmaceutical Drugs*, Lyon.

[7] Idem (1981) Ibid, 25: *Wood, Leather and Some Associated Industries*, Lyon (in press).

[8] International Agency for Research on Cancer (1972) *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, 1: Lyon.

[9] Idem (1973) Ibid, 2: *Some Inorganic and Organometallic Compounds*, Lyon.

[10] International Agency for Research on Cancer (1975) *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, 9: *Some Aziridines, N-, S- & O-Mustards and Selenium*, Lyon.

[11] Idem (1976) Ibid, 10: *Some Naturally Occurring Substances*, Lyon.

[12] Idem (1977) Ibid, 13: *Some Miscellaneous Pharmaceutical Substances*, Lyon.

Table 1. Chemicals and industries evaluated in *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Volumes 23, 24 and 25

Chemical	Volume no.	Chemical	Volume no.
Arsanilic acid	23	Lead chloride	23
Arsenic	23	Lead chromate	23
Arsenic pentoxide	23	Lead chromate oxide	23
Arsenic sulphide	23	Lead naphthenate	23
Arsenic trioxide	23	Lead nitrate	23
Arsine	23	Lead oxide	23
Barium chromate	23	Lead phosphate	23
Basic chromic sulphate	23	Lead subacetate	23
Bertrandite	23	Lead tetroxide	23
Beryllium	23	Leather goods manufacturing industry	
Beryllium acetate	23	(other than boot and shoe manufacture	
Beryllium-aluminium alloy	23	and tanning)	25
Beryllium carbonate	23	Leather tanning and processing	
Beryllium-copper alloy	23	industries	25
Beryllium-copper-cobalt alloy	23	The lumber and sawmill industries	
Beryllium fluoride	23	(including logging)	25
Beryllium hydroxide	23	Methanearsonic acid, disodium salt	23
Beryllium-nickel alloy	23	Methanearsonic acid, monosodium salt	23
Beryllium oxide	23	Methoxsalen	24
Beryllium phosphate	23	Nafenopin	24
Beryllium silicate	23	Panfuran S	24
Beryllium sulphate and its tetrahydrate	23	Phenacetin	24
Beryl ore	23	Phenazopyridine and its hydrochloride	24
Boot and shoe manufacture and		Phenelzine and its sulphate	24
repair	25	Phenoxybenzamine and its hydrochloride	24
Calcium arsenate	23	Potassium arsenate	23
Calcium chromate	23	Potassium arsenite	23
Carpentry and joinery	25	Potassium chromate	23
Chromic acetate	23	Potassium dichromate	23
Chromic chloride	23	Proflavine, its dihydrochloride,	
Chromic oxide	23	hemisulphate and monohydrochloride	24
Chromic phosphate	23	The pulp and paper industry	25
Chromite ore	23	Reserpine	24
Chromium	23	Rifampicin	24
Chromium carbonyl	23	Sodium arsenate	23
Chromium potassium sulphate	23	Sodium arsenite	23
Chromium sulphate	23	Sodium cacodylate	23
Chromium trioxide	23	Sodium chromate	23
Clofibrate	24	Sodium dichromate	23
Cobalt-chromium alloy	23	Spironolactone	24
Dapsone	24	Strontium chromate	23
Dihydroxymethylfuratrizine	24	Sulfafurazole (sulphisoxazole)	24
Dimethylarsinic acid	23	Sulfamethoxazole	24
Ferrochromium	23	Tetraethyllead	23
The furniture and cabinet-making		Tetramethyllead	23
industry	25	Zinc beryllium silicate	23
Hydralazine and its hydrochloride	24	Zinc chromate	23
Lead	23	Zinc chromate hydroxide	23
Lead acetate and its trihydrate	23	Zinc potassium chromate	23
Lead arsenate	23	Zinc yellow	23
Lead carbonate	23		

The preamble to the *Monographs*, which gives the criteria used to evaluate the carcinogenic risk of individual chemicals to humans, was rewritten to include guidelines for the preparation of monographs on occupational exposures. The revised preamble and the monographs on the wood, leather and associated industries will be published as Volume 25 of the *Monographs*[7].

The chemicals or industries evaluated in Volumes 23, 24 and 25 of the *Monographs* are listed in Table 1. A list of the chemicals evaluated in Volumes 1–16 of the *Monographs* was published in a recent review article[13]; those compounds evaluated in Volumes 17–22 are listed in the *Annual Reports* for 1978 and 1979[14, 15].

Over 200 scientists from 25 countries participated in the preparation of the first 25 volumes of the *Monographs*. The members and observers who attended the working groups that resulted in Volumes 23, 24 and 25 are listed in Table 2. Lists of experts who participated in earlier working groups have been published previously[14–18].

In the first 25 volumes of the *Monographs*, evaluations or re-evaluations were made for 537 chemicals, groups of chemicals, industrial processes or industries. For 39 of these a positive association or a strong suspicion of an association with human cancer was found (Table 3). The list given in Table 3 reflects the conclusions of an *ad hoc* Working Group that met in Lyon in January 1979 to consider chemicals evaluated in Volumes 1–20 of the *Monographs* for which some data on carcinogenicity in humans were available (Supplement 1 to the *Monographs*)[19], and the conclusions of the working groups that prepared Volumes 21–25.

For the remaining 498 chemicals, groups of chemicals, industrial processes or industries, epidemiological data were either insufficient or unavailable to evaluate their carcinogenicity to humans. However, 493 of these were chemicals that had been tested in experimental animals, and there is *sufficient evidence* that 130 are carcinogens in animals (Table 4). There is *limited evidence* of carcinogenicity in experimental animals for a further 143 of these chemicals. The data were inadequate to evaluate the presence or absence of a carcinogenic effect in experimental animals for the remaining 220 chemicals.

2.2 *Working Group to establish basic requirements for long- and short-term tests for carcinogens*

In June 1979 a meeting was organized in Hanover jointly by the Medizinische Hochschule, Hanover, the Agency, and the Commission of the European Communities, Brussels, to define basic requirements for testing chemicals in order to establish whether or not they are cancer-producing agents (see also section 3.2 (*a*), page 70). The recommendations of that Working Group were published as Supplement 2 to the *Monographs*[2]; these guidelines will be useful to working groups evaluating the carcinogenic risk of chemicals to humans by facilitating the critical evaluation of

[13] Tomatis, L., Agthe, C., Bartsch, H., Huff, J., Montesano, R., Saracci, R., Walker, E. & Wilbourn, J. (1978) *Cancer Res.*, *38*, 877–885.
[14] International Agency for Research on Cancer (1978) *Annual Report 1978*, Lyon, pp. 73–83.
[15] International Agency for Research on Cancer (1979) *Annual Report 1979*, Lyon, pp. 89–97.
[16] International Agency for Research on Cancer (1974) *Annual Report 1974*, Lyon, pp. 70–73.
[17] International Agency for Research on Cancer (1976) *Annual Report 1976*, Lyon, pp. 86–88.
[18] International Agency for Research on Cancer (1977) *Annual Report 1977*, Lyon, pp. 90–92.
[19] International Agency for Research on Cancer (1979) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Supplement 1: *Chemicals and Industrial Processes Associated with Cancer in Humans*, Lyon.

Table 2. Experts who contributed to the preparation of monographs published in Volumes 23, 24 and 25 of the *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*

Prof. E. D. Acheson, MRC Unit of Environmental Epidemiology, Southampton General Hospital, Southampton, SO9 4XY, UK
Dr R. Althouse, University of Oxford Clinical Medical School, Radcliffe Hospital, Oxford, UK
Dr B. K. Armstrong, NH & MRC Research Unit of Epidemiology and Preventive Medicine, Department of Medicine, The Queen Elizabeth II Medical Centre, Nedlands, Western Australia, Australia
F. B. Blackwell, Head, Adhesion, Soling & Chemical Testing Department, Shoe & Allied Trades Research Association, Satra House, Rockingham Road, Kettering, NN16 9JH, UK
Dr W. J. Blot, Environmental Epidemiology Branch, Landow Building C307, National Cancer Institute, Bethesda, MD 2005, USA
Dr D. Brink, Forest Products Laboratory, University of California, 47th Street and Hoffman Boulevard, Richmond, CA 94804, USA
Dr E. Buiatti, Centre for Social Diseases and Preventive Medicine, Via Alessandro Volta 171, 50131 Florence, Italy
Prof. F. Carnevale, Institute of Occupational Medicine, Hospital and Clinical Centre of Borgo Roma, 37100 Verona, Italy
Dr P. Decoufle, Associate Professor of Biostatistics and Epidemiology, School of Health Related Professions, University of Arizona, Tucson, AZ 85724, USA
Dr G. Della Porta, Division of Experimental Oncology, National Institute for the Study and Treatment of Tumours, Milan, Italy
Prof. B. Drettner, Department of Otorhinolaryngology, Huddinge University Hospital, 14186 Huddinge, Sweden
Dr F. L. Dzhioev, N. N. Petrov Research Institute of Oncology, Leningrad, USSR
Dr A. Englund, Bygghalsan, Box 26055, 10041 Stockholm
J. Fajen, National Institute for Occupational Safety and Health, Robert A. Taft Laboratories, 4676 Columbia Parkway, Cincinnati, OH 45226, USA
Dr B. A. Fowler, Laboratory of Organ Function and Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA
Dr G. D. Friedman, Department of Medical Methods Research, Kaiser-Permanente Medical Care Program, Oakland, CA, USA
Dr A. Furst, Institute of Chemical Biology, University of San Francisco, Harney Science Center, San Francisco, CA, USA
G. Gavend, Head, Tannery Department, Centre Technique du Cuir, 181 avenue Jean-Jaurès, BP 1, 69342 Lyon Cedex 2, France
Dr J. M. Harrington, London School of Hygiene and Tropical Medicine, London
Dr P. F. Infante, Office of Carcinogen Identification and Classification, Occupational Safety and Health Administration, US Department of Labor, Washington DC
Dr C. A. Johnson, British Pharmacopoeia Commission, London
Prof. A. Jori, Institute of Pharmacological Research 'Mario Negri', Milan, Italy
Dr M. Kuratsune, Department of Public Health, Kyushu University Faculty of Medicine, Fukuoka, Japan
Dr A. G. Levis, Institute of Animal Biology, University of Padua, Padua, Italy
Dr R. Makinen, Director, Lappeenranta Regional Institute of Occupational Health, Pormestarinkatu 1, 53100 Lappeenranta 10, Finland
Dr G. Matanoski, Department of Epidemiology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, MD, USA
Dr S. Milham, Population Studies Unit, LB-15, Department of Social & Health Services, Olympia, WA 98504, USA
Prof. N. Nelson, Institute of Environmental Medicine, New York University Medical Center, New York, USA
Prof. D. Neubert, Institute for Toxicology and Embryonal-Pharmacology, Free University of Berlin, FRG
Dr G. Nordberg, Department of Community Health and Environmental Medicine, School of Medicine, Odense University, Odense, Denmark
Prof. R. Preussmann, Institute of Toxicology and Chemotherapy, German Cancer Research Centre, Heidelberg, FRG
Prof. C. Rappe, Department of Organic Chemistry, University of Umea, 90297 Umea, Sweden
Dr J. K. Reddy, Department of Pathology, Northwestern University, The Medical School, Chicago, IL, USA
Dr D. P. Rounbehler, New England Institute for Life Science, 125 Second Avenue, Waltham, MA 02154, USA
Dr J. P. Seiler, Swiss Federal Research Station for Fruit-Growing, Viticulture and Horticulture, Wadenswill, Switzerland

Table 4. Chemicals evaluated in the first 25 volumes of the *IARC Monographs* for which there is *sufficient evidence* of carcinogenicity in experimental animals[a]

	Compound	IARC *Monograph* volume and page number
1.	Actinomycins	**10**, *29*
2.	*ortho*-Aminoazotoluene	**8**, *61*
3.	2-Amino-5-(5-nitro-2-furyl)-1, 3, 4-thiadiazole	**7**, *143*
4.	Aramite®	**5**, *39*
5.	Azaserine	**10**, *73*
6.	Benz(*a*)anthracene	**3**, *45*
7.	Benzo(*b*)fluoranthene	**3**, *69*
8.	Benzo(*a*)pyrene	**3**, *91*
9.	Benzyl violet 4B	**16**, *153*
10.	Beryllium oxide	**1**, *17*; **23**, *143*
11.	Beryllium phosphate	**1**, *17*; **23**, *146*
12.	Beryllium sulphate	**1**, *17*; **23**, *146*
13.	ß-Butyrolactone	**11**, *225*
14.	Cadmium chloride	**2**, *74*; **11**, *39*
15.	Cadmium oxide	**2**, *74*; **11**, *39*
16.	Cadmium sulphate	**2**, *74*; **11**, *39*
17.	Cadmium sulphide	**2**, *74*; **11**, *39*
18.	Calcium chromate	**2**, *100*; **23**, *212*
19.	Chlordecone (Kepone)	**20**, *67*
20.	Chloroform	**20**, *401*
21.	Citrus red no. 2	**8**, *101*
22.	Cycasin	**1**, *157*; **10**, *121*
23.	Daunomycin	**10**, *145*
24.	*N,N'*-Diacetylbenzidine	**16**, *293*
25.	4,4'-Diaminodiphenyl ether	**16**, *301*
26.	2,4-Diaminotoluene	**16**, *83*
27.	Dibenz(*a,h*)acridine	**3**, *247*
28.	Dibenz(*a,j*)acridine	**3**, *254*
29.	Dibenz(*a,h*)anthracene	**3**, *178*
30.	7*H*-Dibenzo(*c,g*)carbazole	**3**, *260*
31.	Dibenzo(*a,e*)pyrene	**3**, *207*
32.	Dibenzo(*a,h*)pyrene	**3**, *207*
33.	Dibenzo(*a,i*)pyrene	**3**, *215*
34.	1,2-Dibromo-3-chloropropane	**15**, *139*; **20**, *83*
35.	3,3'-Dichlorobenzidine	**4**, *49*
36.	3,3'-Dichloro-4,4'-diaminodiphenyl ether	**16**, *309*
37.	1,2-Dichloroethane	**20**, *429*
38.	Diepoxybutane	**11**, *115*
39.	1,2-Diethylhydrazine	**4**, *153*
40.	Diethyl sulphate	**4**, *277*
41.	Dihydrosafrole	**1**, *170*; **10**, *233*
42.	3,3'-Dimethoxybenzidine (*o*-Dianisidine)	**4**, *41*
43.	*para*-Dimethylaminoazobenzene	**8**, *125*
44.	*trans*-2 [(Dimethylamino)methylimino] -5- [2-(5-nitro-2-furyl)vinyl] -1,3,4-oxadiazole	**7**, *147*
45.	3,3'-Dimethylbenzidine (*o*-Tolidine)	**1**, *87*
46.	1,1-Dimethylhydrazine	**4**, *137*
47.	1,2-Dimethylhydrazine	**4**, *145*
48.	1,4-Dioxane	**11**, *247*
49.	Ethinyloestradiol	**6**, *77*; **21**, *233*
50.	Ethylene dibromide	**15**, *195*
51.	Ethylenethiourea	**7**, *45*

[a] Excluding those chemicals associated with cancer induction in humans (see Table 3)

Table 4 *(continued)*

Compound	IARC *Monograph* volume and page number
52. Ethyl methanesulphonate	**7**, *245*
53. 2-(2-Formylhydrazino)-4-(5-nitro-2-furyl)thiazole	**7**, *151*
54. Glycidaldehyde	**11**, *175*
55. Hexachlorobenzene	**20**, *155*
56. Hexamethylphosphoramide	**15**, *211*
57. Hydrazine	**4**, *127*
58. Indeno (1,2,3-*cd*)pyrene	**3**, *229*
59. Isosafrole	**1**, *169*; **10**, *232*
60. Lasiocarpine	**10**, *281*
61. Lead acetate	**1**, *40*; **23**, *327*
62. Lead chromate	**23**, *208*
63. Lead phosphate	**1**, *40*; **23**, *327*
64. Lead subacetate	**1**, *40*; **23**, *327*
65. Merphalan	**9**, *167*
66. Mestranol	**6**, *87*; **21**, *257*
67. Methoxsalen + ultra-violet light	**24**, *101*
68. 2-Methylaziridine	**9**, *61*
69. Methylazoxymethanol and its acetate	**1**, *164*; **10**, *131*
70. 4,4'-Methylene bis (2-chloroaniline)	**4**, *65*
71. 4,4'-Methylene bis (2-methylaniline)	**4**, *73*
72. Methyl iodide	**15**, *245*
73. Methyl methanesulphonate	**7**, *253*
74. *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine	**4**, *183*
75. Methylthiouracil	**7**, *53*
76. Mirex	**5**, *203*; **20**, *283*
77. Mitomycin C	**10**, *171*
78. Monocrotaline	**10**, *291*
79. 5-(Morpholinomethyl)-3 [(5-nitrofurfurylidene)-amino] -2-oxazolidinone	**7**, *161*
80. Nafenopin	**24**, *125*
81. Nickel subsulphide	**2**, *126*; **11**, *75*
82. Niridazole	**13**, *123*
83. 5-Nitroacenaphthene	**16**, *319*
84. 1-[(5-Nitrofurfurylidene)amino] -2-imidazolidinone	**7**, *181*
85. *N*-[4-(5-Nitro-2-furyl)-2-thiazolyl] acetamide	**1**, *181*; **7**, *185*
86. Nitrogen mustard and its hydrochloride	**9**, *193*
87. Nitrogen mustard *N*-oxide and its hydrochloride	**9**, *209*
88. *N*-Nitrosodi-*n*-butylamine	**4**, *197*; **17**, *51*
89. *N*-Nitrosodiethanolamine	**17**, *77*
90. *N*-Nitrosodiethylamine	**1**, *107*; **17**, *83*
91. *N*-Nitrosodimethylamine	**1**, *95*; **17**, *125*
92. *N*-Nitrosodi-*n*-propylamine	**17**, *177*
93. *N*-Nitroso-*N*-ethylurea	**1**, *135*; **17**, *191*
94. *N*-Nitrosomethylethylamine	**17**, *221*
95. *N*-Nitroso-*N*-methylurea	**1**, *125*; **17**, *227*
96. *N*-Nitroso-*N*-methylurethane	**4**, *211*
97. *N*-Nitrosomethylvinylamine	**17**, *257*
98. *N*-Nitrosomorpholine	**17**, *263*
99. *N'*-Nitrosonornicotine	**17**, *281*
100. *N*-Nitrosopiperidine	**17**, *287*
101. *N*-Nitrosopyrrolidine	**17**, *313*
102. *N*-Nitrososarcosine	**17**, *327*
103. Oestradiol-17ß and its esters	**6**, *99*; **21**, *279*
104. Oestrone and its esters	**6**, *123*; **21**, *343*
105. Oil orange SS	**8**, *165*
106. Panfuran-S	**24**, *77*
107. Phenazopyridine and its hydrochloride	**24**, *163*

Table 4 *(continued)*

Compound	IARC *Monograph* volume and page number
108. Phenoxybenzamine and its hydrochloride	**24**, *185*
109. Ponceau MX	**8**, *189*
110. Ponceau 3R	**8**, *199*
111. 1,3-Propane sultone	**4**, *253*
112. ß-Propiolactone	**4**, *259*
113. Propylthiouracil	**7**, *67*
114. Safrole	**1**, *69*; **10**, *231*
115. Sintered calcium chromate	**23**, *302*
116. Sintered chromium trioxide	**23**, *302*
117. Sodium saccharin	**22**, *113*
118. Sterigmatocystin	**1**, *175*; **10**, *245*
119. Streptozotocin	**4**, *221*; **17**, *337*
120. Strontium chromate	**23**, *215*
121. Testosterone and its esters	**6**, *209*; **21**, *519*
122. Thioacetamide	**7**, *77*
123. Thiourea	**7**, *95*
124. Toxaphene (polychlorinated camphenes)	**20**, *327*
125. Tris (2,3-dibromopropyl) phosphate	**20**, *575*
126. Trypan blue (commercial grade)	**8**, *267*
127. Uracil mustard	**9**, *235*
128. Urethane	**7**, *111*
129. Zinc beryllium silicate	**23**, *146*
130. Zinc chromate	**23**, *215*

The methodology has been applied in two case-control studies on lung cancer—one, a small population-based study (81 cases and 111 population controls), and the other, a larger hospital-based study (150 cases and 300 controls). Age, smoking, and social class were taken into account as confounding factors. In these studies, the relative risks of occupational exposure to one or more chemicals associated with lung cancer ranged from 1.5 to 2.5.

Two large case-control studies on lung and laryngeal cancer are currently in progress, one to be completed at the beginning of 1981 and the other at the end of 1982. The cases are derived from the Varese Cancer Registry, and the results will be suitable for the evaluation of occupational risks.

2.4 *Survey of chemicals being tested for carcinogenicity* (Mrs M.-J. Ghess, Dr H. Bartsch, Mr J. Wilbourn and Dr L. Tomatis)

Due to the long duration and high costs of carcinogenicity testing, the Agency, together with the US National Cancer Institute, initiated in 1973 an international survey of institutes involved in long-term testing of chemicals for carcinogenicity.

The major aims of this project are to avoid unnecessary duplication of research, to increase communication among scientists, and to make a census of available research facilities and of the chemicals being tested. The data received from the questionnaire are collated; synonyms and Chemical Abstracts Service Registry Numbers are added; and the *Information Bulletins* are made available to participating laboratories, and are available upon request through the WHO Distribution and Sales Service. The *Bulletins* list chemicals under investigation, animal species, strains and numbers of animals, routes of exposure and dose levels, stage of experiments, principal investogator(s), and references to published reports of completed studies.

Survey results are arranged alphabetically by country, and within each country by city, and within each city by institute. For each institute, the chemicals being tested are listed in alphabetical order. Eight *Information Bulletins* have been published to date.

(a) *Information Bulletin No. 8*

Information Bulletin No. 8 was published in August 1979[20]. It contains data received from 101 institutes in 21 countries on a total of 1105 chemicals. A total of 348 published reports on 320 chemicals are also listed.

Of the compounds undergoing long-term carcinogenicity testing, 239 (21%) have already been evaluated in the first 23 volumes of the *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*. For 22 of these 239 chemicals, groups of chemicals or industrial processes, a positive association or a strong suspicion of an association with human cancer has been found, and for 61 of these 239 chemicals, *sufficient evidence* of carcinogenicity in experimental animals has been demonstrated. The survey will be a valuable guide for selecting chemicals for future monographs from among the 866 chemicals which have not yet been evaluated within the *Monographs* programme.

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[20] Ghess, M.-J., Bartsch, H. & Tomatis, L., eds (1979) *Information Bulletin on the Survey of Chemicals Being Tested for Carcinogenicity*, No. 8, Lyon.

About 67% (744/1105) of the chemicals under test are currently produced and used, or occur naturally. For 33% (361/1105), no data on use or production were found; some of these may be compounds of laboratory interest only.

The major use categories[21] of the 1105 compounds listed in *Information Bulletin No. 8* are given in Table 5.

Table 5. Major use categories for chemicals listed in *Information Bulletin No. 8* on the *Survey of Chemicals Being Tested for Carcinogenicity*

Use category	Number of chemicals[a]
No use and/or production data found[b]	361
Pharmaceutical preparations and/or veterinary drugs	233
Industrial chemicals	119
Chemical intermediates	111
Agricultural chemicals and pesticides	102
Produced but exact use unknown	85
Naturally-occurring substances	67
Food and feed additives, flavours and packaging materials	67
Dyes, pigments and printing inks	64
Used in the manufacture of plastics, rubber and/or textiles	52
Solvents and emulsifiers	44
Cosmetics and perfumes	41
Chemotherapeutic agents	28
Minerals and natural fibres	25
Contaminants and/or impurities	8

[a] Certain chemicals fit into more than one use category.
[b] The chemicals in this category comprise analogues or derivatives of known carcinogens, potential anti-cancer drugs, *N*-nitrosation products of drugs and other chemicals, and combinations of chemicals

(*b*) *Future plans*

The questionnaire for *Information Bulletin No. 9* was posted in July 1980 to all previous participants as well as to any newly identified investigators/institutes doing long-term carcinogenicity testing of chemicals.

From October 1980, all information received from the July mailing will be compiled into *Information Bulletin No. 9*, which will be finalized and distributed early in 1981.

[21] Uses of chemicals were verified using the following sources: Windholz, M., ed. (1976) *The Merck Index*, 9th ed., Rahway, N. J., Merck & Co.; Chemical Information Services, SRI International (1978) *1978 Directory of Chemical Producers, Western Europe*, Menlo Park, CA; US Environmental Protection Agency (1979) *Toxic Substances Control Act (TSCA) Chemical Substance Inventory*, Office of Toxic Substances, Washington DC.

3. PROGRAMME OF MECHANISMS OF CARCINOGENESIS (Dr R. Montesano)

3.1 *Introduction*

With the restructuration of the Agency in January 1980, a programme of mechanisms of carcinogenesis was set up, which incorporates part of the activities previously carried out by the Unit of Chemical Carcinogenesis and all those of the old Unit of Biological Carcinogenesis.

The programme has two main aims: 1) to improve the quality and efficiency of carcinogenicity tests; and 2) to coordinate studies on specific aspects of carcinogenesis.

Thus, projects previously carried out by the former Unit of Biological Carcinogenesis will be continued, and, at the same time, there will be an evolution towards new objectives integrated with the overall goals of the Agency.

In particular, the programme involves the organization and potentiation of a network of national laboratories that collaborate on the testing of environmental chemicals and on improving the methodologies for these tests; and the development of studies, carried out by strict collaboration between the Agency's laboratories and national laboratories, in the following areas: the role of DNA repair and metabolic processes in organ and species specificity of carcinogenic response; oncogenicity *in vitro*, using, in particular, epithelial cells; mammalian mutagenesis; mechanisms of tumour promotion; the preparation of specific antibodies against DNA adducts of carcinogens and against chemical carcinogens in order to monitor individual human exposure to carcinogenic agents; and the role of EBV as an oncogenic agent, with particular emphasis on non-endemic Burkitt's lymphoma (BL) and on the significance of chromosomal changes associated with BL.

A large part of the effort in the programme is aimed at developing criteria to allow a better understanding of the significance of experimental results in predicting cancer risks in humans; this task inevitably requires a deeper understanding of the mechanisms of carcinogenesis. The activities carried out within the Programme of Mechanisms of Carcinogenesis are closely integrated with those of the programmes of Environmental Carcinogenesis and Host Factors and Analysis of Environmental Chemicals, and all three together supply essential support for the Division of Epidemiology and Biostatistics.

3.2 *International network of carcinogenicity testing*

A programme has been developed over a period of years for testing environmental chemicals; it is based, to a limited extent, on intramural activities carried out in the Agency laboratories, and, to a much larger extent, on extramural activities. The latter comprise mainly the coordinated carcinogenicity tests carried out in a number of collaborating national laboratories, involving long- and short-term testing of chemicals for their possible carcinogenicity and/or mutagenicity.

At present, 12 laboratories in 10 countries are collaborating in testing environmental chemicals for carcinogenicity or in developing and validating short-term tests of carcinogenicity (see section 3.2 (*b*)). An integral component of this programme is the adoption of basic requirements for carrying out long- and short-term carcinogenicity tests and for analysing and reporting the results (see section 3.2 (*a*)).

(*a*) *Establishment of basic requirements for carrying out long- and short-term carcinogenicity and related tests*

As a first step in implementing and expanding this programme, and in order to ensure the success of the international effort, there must be agreement on the acceptability and reproducibility of results obtained in the various laboratories collaborating in the carcinogenicity testing programme.

It is with this goal in mind that the Agency, in collaboration with the Medizinische Hochschule of Hanover (F.R.G.) and the European Economic Community convened an *ad hoc* Working Group of some 100 experts from 15 countries to review the conduct of long- and short-term carcinogenicity and related tests, in order to establish the basic requirements for carrying out such tests and for reporting the results. The meeting was attended by representatives from the major national and international institutions involved in the toxicological evaluation of environmental chemicals.

In order to ensure that the review of the tests took into account the most recent advances in chemical carcinogenesis, the first part of the meeting was devoted to a discussion of the molecular and cellular bases of carcinogen screening tests. The proceedings of this part of the meeting have been published in the *IARC Scientific Publications* series[22].

A second publication[2] comprises the reports of 11 subgroups which reviewed long-term carcinogenicity bioassays and short- and medium-term assays. The reports contain basic requirements for long-term assays for carcinogenicity, for mutagenesis assays with bacteria, with mammalian cells, with yeast and moulds, with *Drosophila*, and with whole mammals, for transformation in cell culture, for DNA damage and repair in mammalian cells, and for cytogenetic damage. Two reports deal with basic requirements for metabolic activation systems in mutagenesis testing *in vitro* and with the rationale for deploying short-term assays to provide evidence of carcinogenicity. In addition, an extensive annex provides guidelines for the planning of carcinogenicity tests and for analysing the results obtained. This annex was prepared by a group of statisticians in such a way that it can by understood, and used by biologists and pathologists.

It is hoped that this, and similar efforts that have been initiated in different countries by different organizations, all of which were represented at the Hanover meeting, will further strengthen cooperation and that a continuous flow of information will be maintained among collaborating laboratories. It is only on this basis that the quality of the tests can be further improved and an international network of carcinogenicity testing can be implemented effectively.

(*b*) *Carcinogenicity studies*

The national laboratories involved and the various tests carried out within the international network of carcinogenicity testing are given below; in addition, the carcinogenicity tests carried out in the Agency laboratories are listed. Two national laboratories have been added recently, namely, the Institute of Oncology, Medical Academy, Sofia, Bulgaria (principal investigator: Dr I. Chernozemsky) and the Institute of Oncology of the University of Genoa, Genoa, Italy (principal investigator: Dr L. Rossi).

[22] Montesano, R., Bartsch, H. & Tomatis, L., eds (1980) *Molecular and Cellular Aspects of Carcinogen Screening Tests (IARC Scientific Publications No. 27)*, Lyon, International Agency for Research on Cancer.

(i) *Maleic hydrazide* (Dr R. Cabral, Dr V. Ponomarkov; in collaboration with Dr G.T. Van Esch, National Institute of Public Health, Bilthoven, The Netherlands (RA/74/006))

Maleic hydrazide (99% pure) was given to groups of C57BL mice either subcutaneously (in tricaprylin) on days 1, 7, 14 and 21 after birth at a total dose of 55 mg, or orally (in olive oil) at a weekly dose of 510 mg/kg for life. These experiments have now been completed. No significant increase in tumour incidence was observed under these experimental conditions.

Male and female Wistar rats were fed for 28 months on diets containing 1% and 2% maleic hydrazide (99% pure). There is no indication of a carcinogenic effect at present.

(ii) *Magenta,* para-*rosaniline and phenyl-ß-naphthylamine*
School of Medicine, Hanover, Federal Republic of Germany (RA/73/033)
Principal investigator: Professor U. Mohr

Groups of 40 male and 40 female Syrian golden hamsters received 400 mg/kg magenta, 300 mg/kg *para*-rosaniline or 37.5 mg/kg phenyl-ß-naphthylamine intragastrically twice weekly for life. The animals were observed for life; no differences in tumour incidences were observed among the various groups or when compared with control animals[23].

(iii) *Styrene oxide* (Dr V. Ponomarkov, Dr R. Cabral)

Female BDIV rats were treated orally with a single dose of 200 mg/kg bw styrene oxide on the 17th day of gestation. Starting from weaning, the resulting offspring were given weekly oral doses of 100 mg/kg bw for 100 weeks. All survivors were killed at 120 weeks. Histological evaluation is in progress.

(iv) *5-Bromodeoxyuridine (BUdR)* (Dr R. Cabral, Dr V. Ponomarkov)

BUdR was given intraperitoneally to female BDVI rats on the 21st day of gestation at a dose of 10 mg per animal. The experiment is still in progress.

BUdR was also administered intraperitoneally to 50 female BDVI rats on the 21st day of gestation at a dose level of 20 mg per animal, and the offspring of the treated mothers were treated with BUdR on days 1, 3, 5 and 7 after birth at a dose of 0.01 mg/g. This experiment is still in progress.

Since previous experiments have shown that BUdR induces kidney lesions, its possible interaction with another agent, ethyl methane sulfonate (EMS), known to be effective in inducing kidney tumours was examined. Thus, 177 newborn BDVI rats were treated subcutaneously with 0.1 mg/g BUdR on days 1, 3 and 5 after birth; a second group of 68 newborn BDVI rats was given 0.3 mg/g BUdR subcutaneously on day 1 after birth; groups of young BDVI rats were treated intraperitoneally with 100 mg/kg or 50 mg/kg EMS on day 10 after birth; and groups of young BDVI rats were given 0.3 mg/g BUdR subcutaneously on day 1 after birth followed by i.p. treatment with 50 mg/kg EMS on day 10. Solvent-treated and untreated controls are available. All these experiments are currently in progress.

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[23] Green, U., Holste, J. & Spikermann, A. R. (1979) *J. Cancer Res. clin. Oncol.*, **95**, 51–55.

(v) *Pesticides and drugs*

National Institute of Public Health, Budapest (RA/75/014)

Principal investigator: Dr M. Börzsönyi

Dinitrosopiperazine, the *N*-nitroso derivative of triforin, was found to be carcinogenic to adult Swiss mice and to offspring exposed perinatally and during the lactation period[24].

In vitro and *in vivo* nitrosation of trimorphamide, a pesticide under development, has been demonstrated. Infra-red and mass spectrometry showed the *N*-nitroso derivative to be *N*-nitrosomorpholine, which was strongly mutagenic for *Salmonella typhimurium* strains TA1535 and TA100[25]. Long-term carcinogenicity studies are currently in progress in mice exposed prenatally or during lactation to trimorphamide and sodium nitrite.

It has been shown that the antioxidant bis-2,2-dimethyl-4-methane sulfonic acid sodium salt-1,2-dihydroquinoline-6-6 methane (MTDQ-DA) inhibits the nitrosation of morpholine *in vivo*. Long-term animal experiments designed to study the possible inhibitory effect of the antioxidant on the hepatocarcinogenicity of *N*-nitrosomorpholine are presently underway.

(vi) *Drug and food samples*

The Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan (RA/80/001)

Principal investigator: Dr S. Riazuddin

Mutagenicity tests in bacteria and yeast have recently been established in this laboratory, and examination of the potential mutagenicity of samples of various types of local foods as well as of locally used therapeutic drugs has been initiated.

(vii) *Effects of chemicals on chromosomal rearrangements*

Laboratoire de Biophysique et Radiobiologie, Département de Biologie Moléculaire, Université Libre de Bruxelles, Rhode-Saint-Genèse, Belgium (RA/78/004)

Principal investigator: Dr M. Radman

Department of Clinical Genetics, University Hospital of Lund, Sweden (RA/78/013)

Principal investigator: Dr F. Mitelman

1st Institute of Pathology, Medical University, Budapest (RA/80/007)

Principal investigator: Professor K. Lapis

It was indicated by Kinsella & Radman[26] that promoting agents, such as 12-*O*-*n*-tetradecanoylphorbol-13-acetate, induce chromosomal rearrangements (sister chromatid exchanges) that lead to homozygosity of an initiated cell with a heterozygous latently premalignant state. Various agents have been examined for their capacity to induce mutagenesis and/or chromosomal rearrangements in mammalian cells[27].

Studies undertaken by Mitelman & Levan[28] show that chromosome aberrations in human neoplasms cluster in a few specific chromosomes of the karyotype. Studies have been initiated to examine the specificity of chromosome *observations* in relation to various etiological agents.

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[24] Börzsönyi, M., Török, G., Pintér, A., Surján, A., Nádasdi, L. & Roller, P. (1980) *Cancer Res.*, **40**, 2925–2927.

[25] Börzsönyi, M., Surján, A., Pintér, A., Török, G., Csik, M., Tamás, J., Fetter, J. & Ferencz, A. (1980) In: *N-Nitroso Compounds: Analysis, Formation, and Occurrence (IARC Scientific Publications No. 31)*, Lyon.

[26] Kinsella, A. E. & Radman, M. (1978) *Proc. natl Acad. Sci. USA*, **75**, 6149–6153.

[27] Radman, M. & Kinsella, A. E. (1979) In: Montesano, R., Bartsch, H. & Tomatis, L., eds. *Molecular and Cellular Aspects of Carcinogen Screening Tests (IARC Scientific Publications No. 27)*, Lyon, pp. 75–90.

[28] Mitelman, F. & Levan, G. (1978) *Hereditas*, **89**, 207–232.

(viii) *Study on the effects of combined exposure to radiation and to chemical carcinogens*
Deutsches Krebsforschungszentrum Heidelberg, Institut für Toxikologie und Chemotherapie, Heidelberg, FRG
Principal investigator: Professor D. Schmähl

Mice of both sexes were irradiated once with fast neutrons and treated thereafter with either carbon tetrachloride or chloroform. The experiment is in progress.

(ix) *Studies on the cocarcinogenic action of oil shale phenols on the lung carcinogenicity of asbestos dust*
Laboratory of Morphology, Institute of Experimental and Clinical Medicine, Tallinn, Estonian SSR (RA/79/004)
Principal investigator: Dr A. Küng-Vösamäe

A series of studies has been undertaken to determine the possible cocarcinogenic action of phenols extracted from Estonian oil shale generator tar on the carcinogenicity of asbestos dust and of benzo[*a*]pyrene in rat lung.

Experiments with intratracheal instillations of test substances were started in 1977 and have now been completed. The results showed that the phenols extracted from oil shale generator tar potentiate the action of intratracheally instilled benzo[*a*]pyrene but do not exert any substantial modifying effect on chrysotile-induced carcinogenesis.

An additional experiment has been started in 140 Wistar rats divided into two groups. The animals in the first group received 5 intratracheal fortnightly instillations of three test substances (5 mg benzo[*a*]pyrene, 1 mg chrysotile asbestos dust, and 5 mg phenols) in 0.5 ml polyglucin. The rats of the second group will receive the same doses of benzo[*a*]pyrene and asbestos dust in 0.5 ml of polyglucin. The combined action of the three agents will be studied by comparing these groups with previously initiated control and experimental groups. The experiment is in progress.

(x) *Investigation on the combined action of several chemical carcinogens* (in collaboration with Dr L. Griciute, Institute of Epidemiology, Microbiology and Hygiene, Vilnius, Lithuanian SSR; Dr V. Turusov, Oncological Research Centre, Moscow; Dr B. Teichmann, Central Institute for Cancer Research, Berlin-Buch; and Dr I. Chernozemsky, Institute of Oncology, Sofia) (RA/77/024)

A collaborative study in four laboratories on the combined action of several carcinogens has been continued[29], wherein mice are treated with three environmental carcinogens: benzo[*a*]pyrene, *N*-nitrosodiethylamine and aflatoxin B_1. The experiment carried out in the Agency has been completed and showed that administration of the three carcinogens shortened the latent period as compared with that in animals treated with only one of the substances. The experiments being carried out by the other collaborating laboratories are still in progress.

(xi) *N-Nitrosonornicotine and alcohol* (Dr L. Griciute, Dr R. Cabral)

An experiment designed to study the synergism of *N*-nitrosonornicotine and alcohol has been initiated, in which groups of young BDVI rats will receive intragastric instillations of *N*-nitrosonornicotine in a 40% alcoholic solution twice a week for 78 weeks.

[29] International Agency for Research on Cancer (1979) *Annual Report 1979*, Lyon, p. 73.

(*c*) *Prenatal carcinogenesis*

(i) (Dr R. Cabral, Dr A. Likhachev, Dr V. Ponomarkov)

The possibility that exposure of female rats to *N*-nitroso-*N*-ethylurea during pregnancy results in an increased cancer risk for more than one generation is being investigated.

(ii) (Dr R. Cabral, Dr A. Likhachev)

The effect on progeny of exposing male animals to a direct alkylating agent prior to mating is being investigated. For this purpose, BDVI male rats were given a single dose of 80 mg/kg *N*-nitroso-*N*-ethylurea and mated 1, 2, 3 and 4 weeks later with untreated females. This experiment is in progress.

(iii) *Cancer Research Centre, Academy of Medical Sciences of the USSR, Moscow* (RA/76/017)

Principal investigator: Dr V. S. Turusov

A series of studies have been carried out to see if treatment of rats with *N*-nitroso-*N*-methylurea during the last period of pregnancy results in an increased incidence of tumours in untreated F_1, F_2, F_3, F_4 and F_5 generations.

A dose of 60 mg/kg was administered on the 16, 18 and 20th day of pregnancy to female rats. Males and females of the F_1 generation were mated to produce the F_2 generation, and the same procedure was followed to produce the other generations. All animals surviving at 150 weeks were killed.

A more than 90% incidence of tumours of the nervous system was observed in animals of the F_1 generation; with the possible exception of rats of the F_4 generation, no significant increase in such tumours was observed in animals of the other generations. A detailed analysis of the results is underway.

(iv) *N. N. Petrov Research Institute of Oncology, Leningrad, USSR* (RA/77/022)

Principal investigator: Professor N. P. Napalkov

Investigations are being carried out to study modifying factors in transplacental carcinogenesis. Pregnant mice were treated with dimethylbenzanthracene (DMBA), benzo[*a*]pyrene or *N*-nitroso-*N*-ethylurea, and animals of the F_1 generation were treated postnatally with 12-*O*-*n*-tetradecanoylphorbol-13-acetate (TPA) applied to the skin. The postnatal effects of TPA in the F_2 generation of female mice treated with DMBA was also studied. Lifetime observation of the F_1 and F_2 generations is in progress.

The effects of thyroidectomy, methylthiouracil or thyroxine on animals of the F_1 and F_2 generations of rats from mothers treated with *N*-nitroso-*N*-methylurea (NMU) during pregnancy is also being studied. The effects of constant oestrus following reimplantation of the ovary into ovariectomized rats of F_1 and F_2 generations from mothers treated with NMU or DMBA during pregnancy is also being investigated as a modifying factor in transplacental carcinogenesis.

3.3 *Studies on DNA repair and metabolism of carcinogens*

Metabolism, DNA damage and subsequent DNA repair processes have been shown to be critical determinants in the organ and species specific carcinogenic effects of chemical carcinogens.

The biological relevance of specific DNA damage and repair processes has been investigated in particular in our laboratory, and in others, with *N*-nitroso compounds. The choice of this group of carcinogens appears particularly appropriate since it is possible to measure, both qualitatively and quantitatively, various modifications to cellular macromolecules and to assess the relevance of these modifications to the carcinogenicity of *N*-nitroso compounds[30]. More recently, the effect of chronic treatment with nitrosamines on various DNA repair processes has been examined. Results show that the efficiency of repair of specific DNA damage varies according to the dose and length of treatment; this could be relevant to the carcinogenic dose-response observed with these carcinogens. Details of the various experiments are reported below.

(*a*) *Modulation of repair of alkylated DNA in liver of rats treated chronically with* N-*nitrosodimethylamine (NDMA)* (Dr R. Montesano, Miss H. Brésil, Mrs G. Planche-Martel, in collaboration with Dr G. P. Margison, Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester, UK; Dr A. E. Pegg, The Milton S. Hershey Medical Center, Pennsylvania State University, Hershey, PA, USA)

Previous studies[31, 32] have shown that chronic treatment of rats with relatively low doses of NDMA results in an increased removal of O^6-methylguanine from liver DNA over that with a single dose of NDMA; however, no effect was observed on the other alkylated DNA bases, 7-methylguanine and 3-methyladenine. The increased removal of O^6-methylguanine was associated with induction of the enzymic DNA repair system(s). More recent studies[33, 34], aiming at a further characterization of this phenomenon, are reported here.

A series of experiments was carried out to examine the efficiency of this induced DNA repair system with regard to time and to the number of O^6-methylguanine molecules. Previous studies[32] have shown that the maximum level of induction was achieved with a dose of 2.0 mg/kg NDMA administered over a period of 3 weeks.

Figure 1 shows that in liver DNA of pretreated rats 10 min after a dose of 2 mg/kg ^{14}C-NDMA the O^6-/7-methylguanine ratio is 0.058, whereas in control rats is 0.096. When various challenge doses of ^{14}C-NDMA (0.2, 2.0 and 20 mg/kg) are administered to rats pretreated with 2 mg/kg unlabelled NDMA, an increased removal of O^6-methylguanine is observed with 2.0 and 0.2 mg/kg, but not with 20 mg/kg (Fig. 2).

These findings indicate that the increased enzymic activity induced by pretreatment with NDMA has a limited and finite capacity to cope with the removal of an increased amount of DNA damage, i.e., the removal of an increased number of O^6-methylguanine molecules. Above that level no differences are detected in liver DNA of pretreated or control rats in the rate of removal of O^6-methylguanine by the constitutive enzyme.

Although the presence of O^6-methylguanine in DNA cannot be considered in isolation, it appears to be a critical determinant in the initiation of carcinogenesis by NDMA. Thus, it appears

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[30] Montesano, R., Pegg, A. E. & Margison, G. P. (1980) *J. Toxicol. environ. Health* (in press).
[31] Montesano, R., Brésil, H. & Margison, G. P. (1979) *Cancer Res.*, **39**, 1798–1802.
[32] Montesano, R., Brésil, H., Planche-Martel, G., Margison, P. & Pegg, A. E. (1980) *Cancer Res.*, **40**, 452–458.
[33] Montesano, R., Brésil, H., Planche-Martel, G., Margison, P. & Margison, G. P. (1980) *Proc. Am. Assoc. Cancer Res.*, **21**, 5.
[34] Montesano, R. & Margison, G. P. (1980) In: Pullman, B., Ts'O, P.O.P. & Gelboin, H., eds, *Carcinogenesis: Fundamental Mechanisms and Environmental Effects*, Amsterdam, Reidel (in press).

important to examine whether the modulation of repair of this alkylation product in liver DNA following chronic treatment with NDMA at various dose schedules is reflected in the carcinogenic dose-response to NDMA in this organ.

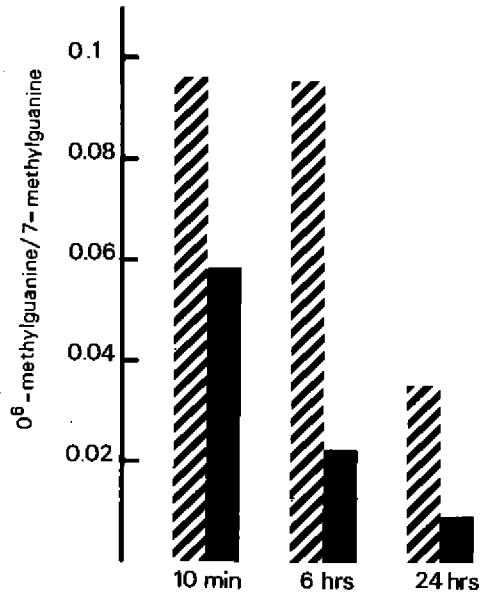

Fig. 1 O^6-/7-Methylguanine ratios in liver DNA 10 min, 6 and 24 hrs after administration of 2 mg/kg ^{14}C-*N*-nitrosodimethylamine (NDMA) to BDIV rats pretreated (■) or not (▨) with 2 mg/kg NDMA for 3 weeks

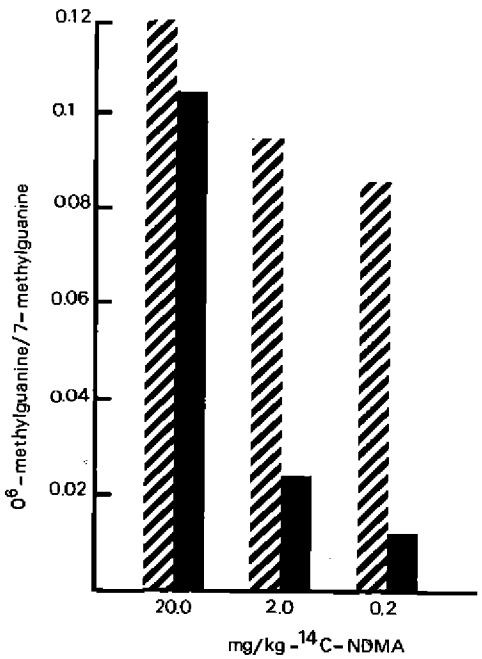

Fig. 2 O^6-/7-Methylguanine ratios in liver DNA 6 hrs after administration of various doses of ^{14}C-*N*-nitrosodimethylamine (NDMA) to BDIV rats pretreated (■) or not (▨) with 2 mg/kg NDMA for 3 weeks

(*b*) *Formation and loss of alkylated DNA adducts from chromatin fractions of liver of rats treated with* N-*nitrosodimethylamine (NDMA)* (Dr R. Montesano and Miss H. Brésil, in collaboration with Dr P. Cerutti, Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland)

These studies examine the formation and removal of various DNA adducts formed by NDMA in purified nucleosomal core DNA and in total nuclear DNA of tissues of rats that received a single or multiple doses of the nitrosamine. Groups of BDIV rats received a dose of 2.0 mg/kg ^{14}C-NDMA preceded or not by treatment with 2.0 mg/kg cold NDMA. The initial distribution and persistence of various alkylation products in nucleosomal linker and core DNA are under examination.

(*c*) *Formation and loss of DNA alkylated products in Syrian golden hamsters treated with* N-*nitrosodioethylamine (NDEA)* (Dr R. Montesano, Miss H. Brésil and Dr S. H. Lu)

The aim of these studies is to examine the role of DNA repair processes in the species-specific carcinogenicity of these alkylating agents.

Syrian golden hamsters were treated with a single dose of 160 mg/kg ^{14}C-NDEA, and the presence in the DNA of 3-ethyladenine, 7-ethylguanine and O^6-ethylguanine was measured 12, 24, 48 and 72 hours later. The results shown in Table 6 indicate that by 72 hours O^6-ethylguanine decreases to 45% of the level found at 12 hours; over the same time period a lower proportion (approximately 20%) of 3-ethyladenine and 7-ethylguanine was detected.

Table 6. Ethylation of hamster liver DNA by *N*-nitrosodiethylamine (160 mg/kg)

Time (hrs)	Ethylated purines present in DNA (μmol/mol purine)		
	3-ethyladenine	7-ethylguanine	O^6-ethylguanine
12	49	311	287
24	50	267	305
48	13	109	197
72	10	50	129

Hamster liver thus behaves quite differently from rat liver in its capacity to repair these DNA alkylated adducts. In particular, as already shown for 7-methylguanine[35, 36], the hamster liver has an efficient enzymic system capable of removing 7-ethylguanine from its DNA; O^6-ethylguanine is removed to a lesser degree. In contrast, in rat liver[37], 7-ethylguanine does not appear to be enzymically removed, whereas O^6-ethylguanine is efficiently removed.

[35] Margison, G. P., Margison, J. M. & Montesano, R. (1976) *Biochem. J.*, **157**, 627–634.
[36] Stumpf, R., Margison, J. M. & Montesano, R. (1979) *Cancer Res.*, **39**, 50–54.
[37] Pegg, A. E. & Balog, B. (1979) *Cancer Res.*, **39**, 5003–5009.

(*d*) *Alkylating and carcinogenic effects of* N-*nitroso*-N-*methylurea (NMU) and of* N-*nitroso*-N-*ethylurea (NEU) in Syrian golden hamsters* (Dr A. Likhachev, Miss H. Brésil and Dr R. Montesano, in collaboration with Dr G. P. Margison, Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester, UK; and Dr M. Ivanov, N. N. Petrov Research Institute of Oncology, Leningrad, USSR)

In this study the formation and persistence of alkylated purines in DNA of different organs of hamsters treated intraperitoneally with a single dose of NMU or NEU have been examined. In hamsters treated with 30 mg/kg NMU, the highest level of DNA methylation was found 5 hours after treatment. Up to 24 hours, the concentration of O^6-methylguanine was stable in DNA of different organs, except in the liver where a 25% loss was observed.

During 24 hours and 48 hours there was considerable loss of O^6-methylguanine in all organs studied, including the liver. During the same period, a loss of 7-methylguanine was also observed; this was more pronounced during the 24- to 48-hour period, indicating that an enzymic system is responsible for the removal of this DNA adduct. In contrast to results obtained with a dose of NDMA that produced an equivalent amount of DNA alkylation, the O^6-methylguanine produced in DNA of hamsters treated with NMU appears to be removed more efficiently. As compared with the liver, lung and kidney showed a lower capacity to remove O^6-methylguanine from their DNA.

In hamsters treated with 60 mg/kg NEU, the amounts of 7-ethylguanine and $O_6$6-ethylguanine measured in liver DNA at 48 hours were one-third of the amounts measured at 2 hours; in lung and in small intestine the disappearance of the alkylated bases was less pronounced than in the liver.

In experiments in which hamsters were treated intraperitoneally with NMU or NEU at the same doses, tumours developed predominantly in the forestomach, in addition to scattered tumours in other organs. It is not possible on the basis of these data to assess the relevance of the persistence of O^6-methylguanine in the DNA of the organ to the carcinogenic effect of these alkylating agents in Syrian golden hamsters.

(*e*) *Effect of age on formation and loss of DNA alkylated purines in rats* (Dr A. Likhachev, Miss H. Deblock and Dr R. Montesano, in collaboration with Dr V. Anisimov and Dr A. Ovsyannikov, Petrov Research Institute of Oncology, Leningrad, USSR)

Various studies indicate that the carcinogenic response may vary with the age of the animal at the time of treatment. Thus, a series of experiments has been initiated to examine the formation of various DNA adducts and the efficiency of repair of these DNA modifications in rats of different ages treated with *N*-nitroso compounds. Preliminary results in rats treated with *N*-nitrosodiethylamine and methyl-acetoxymethyl-nitrosamine show a non-uniform pattern of formation and persistence of DNA adducts among various organs. Further studies are in progress to assess the relevance of these variations in DNA repair competence to the carcinogenic response in rats of different ages.

(*f*) *Effect of ammonium molybdate on the alkylation of liver DNA of rats treated with* N-*nitrosodiethylamine (NDEA)* (Dr Shin Hsin Lu, Miss H. Brésil and Dr R. Montesano)

A deficiency in molybdenum has been observed[38] among people with a high incidence of oesophageal cancer. The effect of molybdenum on metabolism and DNA alkylation in tissues of rats treated with NDEA was therefore examined in a series of studies. The results indicate that administration of ammonium molybdenum (1.0 or 5 mg/kg × 30 days) does not significantly alter the metabolism (as measured by the amount of $^{14}CO_2$ exhaled or by the amount of radioactivity in the urine) or the degree of DNA alkylation in liver DNA after a dose of 200 mg/kg ^{14}C-NDEA.

3.4 *Chemical carcinogenesis and mutagenesis in cultured cells*

Since the majority of human cancers are of epithelial origin, it appeared relevant to establish cultures of epithelial cells and to examine whether the process of neoplastic transformation of epithelial cells *in vitro* shows similarities to that observed in mesenchymal cells. Various lines of epithelial cells originating from rat liver have been established over a number of years in our laboratory[39]. Studies on the characterization of markers of neoplastic transformation in these cell lines, carried out in collaboration with various laboratories, are reported here.

Tests of the mutagenicity of environmental chemicals in mammalian cells and the effect of multiple treatment with alkylating agents on the mutation frequency are also reported.

(*a*) *Cellular and biochemical markers of neoplastic transformation of epithelial cells in culture*
Cancer Research Centre, Academy of Medical Sciences of the USSR, Moscow (RA/79/101)
Principal investigator: Dr Y. M. Vasiliev

A series of tumorigenic and non-tumorigenic epithelial rat liver-cell lines originally established in the Agency laboratory[39] were examined for the appearance of cell culture properties associated with the acquisition of oncogenicity by the following methods: scanning electron microscopy, lactoperoxidase-catalysed iodination of cell surface proteins, immuno-morphological investigation of fibronectin and actin distribution, and histochemical analysis of γ-glutamyltranspeptidase activity.

The studies show that the non-tumorigenic cell lines spread well on the substratum and have epithelial morphology: single cells in sparse cultures had a discoid non-polarized shape, with a ring of lamellar cytoplasm at their periphery. The cells in dense cultures formed coherent sheets with firm intercellular contacts. The tumorigenic cell lines showed a number of morphological changes as compared with their parental cells: (i) single cells had reduced lamellar cytoplasm; their lamellar cytoplasm often did not form a regular ring at their periphery: as a result, the cells of certain lines often had not the discoid shape but an elongated spindle-like shape; (ii) the cells in dense cultures formed less regular monolayered sheets: large gaps between the nearby cells and areas of overlapping were seen in these cultures.

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[38] Department of Chemical Etiology and Carcinogenesis, Cancer Institute, Chinese Academy of Medical Sciences (1979) *Adv. Med. Oncol. Res. Educ.*, 3, 39–44.

[39] Montesano, R., Bannikov, G., Drevon, C., Kuroki, T., Saint Vincent, L. & Tomatis, L. (1980) *Ann. N. Y. Acad. Sci.* (in press).

Surface protein patterns of all non-tumorigenic and tumorigenic lines were similar; these patterns were different from those of fibroblastic cells. Major protein species had apparent molecular weights of about 110 to 170 kilodaltons. A protein with a molecular weight of 250 kilodaltons, identical to fibronectin, was present in all three non-tumorigenic lines and in five out of seven tumorigenic lines[40]. In lines derived from tumours obtained by inoculation into rats of cells transformed *in vitro* (IAR 6–1 RT7, its clone – IAR 6–1 RT7A, selected for growth in semi-solid agar; IAR 2–31 RT4 and IAR 2–31 RT6), a new surface protein of a non-collagenous nature and highly sensitive to trypsin was detected. It had a molecular weight of 130 kilodaltons and was a major surface protein in the clone IAR 6–1 RT7A.

The distributions of fibronectin and actin were quite different in tumorigenic and non-tumorigenic lines. In non-tumorigenic lines fibronectin was found to be localized in spots on the cell margins and as bands in the regions of cell-to-cell contacts. On neoplastic transformation the distribution of fibronectin became fibroblast-like: the bands at the intercellular contacts disapeared, and a surface filamentous network was formed.

Actin distribution was also changed characteristically in the course of transformation. Large marginal bundles of actin microfilaments were seen at the free edges of the sheets formed by non-tumorigenic lines; central parts of the sheet contained only short linear bundles.

These studies indicate that neoplastic progression of IAR cells was accompanied by morphological changes reflecting decreasing ability to form cell-to-cell and cell-substratum contacts. Loss of fibronectin and disappearance of microfilament bundles were not characteristic markers of tumorigenic IAR-lines. However, morphological changes accompanying transformation were correlated with the altered distribution of fibronectin fibres and microfilament bundles.

(*b*) *Monoclonal antibodies against human plasma fibronectin*
Institute of Oncology of the University of Genoa, Genoa, Italy (RA/80/006)
Principal investigator: Dr L. Zardi

Somatic cell hybrids between mouse plasmocytoma cells deficient in hypoxanthine phosphoribosyl-transferase (P3x63 Ag8) and spleen cells derived from mice immunized with purified human plasma fibronectin have been produced. These hybrids produce large amounts of monoclonal antibodies against different determinants of this antigen. It is planned to obtain a better characterization of the molecule and therefore a more careful comparison between plasma and cellular fibronectin and better characterization of fibronectin secreted by normal and transformed cells.

(*c*) *Metabolism of benzo*[a]*pyrene in cultured human epidermal keratinocytes*
Institute of Medical Science, University of Tokyo, Tokyo (RA/79/006)
Principal investigator: Dr T. Kuroki

The metabolism of benzo[*a*]pyrene (BP) was investigated in human epidermal keratinocytes in culture, which were isolated from discarded material used for skin-grafting in plastic surgery. The cultures consisted exclusively of keratinocytes, and all experiments were performed on primary cultures.

[40] Bannikov, G. A., Saint Vincent, L. & Montesano, R. (1980) *Br. J. Cancer* (in press).

Figure 3 shows the profiles of BP metabolites separated by high-pressure liquid chromatography (HPLC) after incubation of 20 μM ^{3}H-BP with HUSKI-1 cells, which were derived from an eight-year-old boy, for 24 and 48 hours (Fig. 3A and B, respectively). This result indicates that human epidermal keratinocytes metabolize BP significantly, forming a moderate amount (0.4 and 1.4% at 24 and 48 hours, respectively) of 7,8-dihydrodiol BP, a precursor of an ultimate metabolite, 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro BP. Conjugate formation in these cells was examined by treating the medium with β-glucuronidase and arylsulfatase. Few or no metabolites were released, as shown by HPLC (Fig. 3C) and thin-layer chromatography after treatment with these enzymes, suggesting that human epidermal keratinocytes, unlike rodent cells, have low activities of conjugate-forming enzymes, e.g., UDP-glucuronyl-transferase and sulfate transferase.

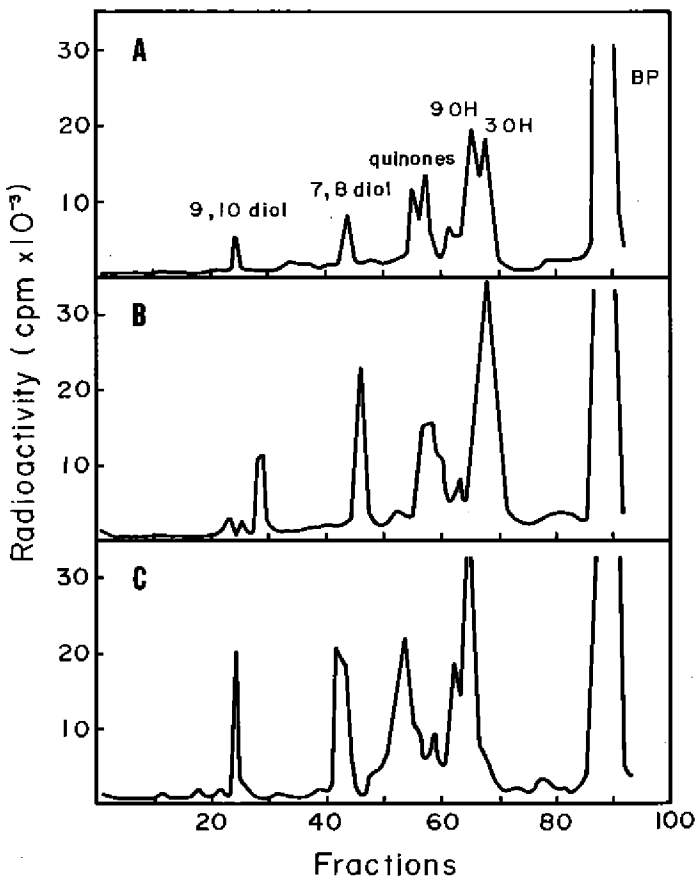

Fig. 3 High-pressure liquid chromatography (HPLC) analysis of benzo[*a*]pyrene (BP) metabolites in the medium of HUSKI-1 cells, isolated from an 8-year-old boy, after 25 days in culture. The media were sampled at 24 hrs (A) and 48 hrs (B) after treatment with 20 μM ^{3}H-BP and extracted 3 times with ethyl acetate and subjected to HPLC. An aliquot of the medium after 48 hrs incubation was treated with β-glucuronidase (1 mg/ml) at 37°C for 3 hrs before ethyl acetate extraction (C). The positions of the metabolites were determined by running authentic standards. A small peak between quinones and 9-phenol BP seems to be a contaminant in the ^{3}H-BP solution

The metabolic activity of human epidermal keratinocytes was further demonstrated by a cell-mediated assay, in which V79 Chinese hamster cells were co-cultured with epidermal keratinocytes and treated with BP for 48 hours. Mutation, measured as ouabain resistance, increased with the dose of BP: 18 and 23 ouabain-resistant colonies per 10^5 survivors were observed with 5 and 10 μM BP, respectively. This efficiency was higher than that of rat embryo fibroblasts, although the shape of the dose-response curve was different.

Cultures of human epidermal keratinocytes could be used to examine individual differences in metabolic capacity to activate environmental carcinogens.

(*d*) *Interactions of asbestos and nickel with chromosomal proteins from human tissues Institute of Oncology of the University of Genoa, Genoa, Italy* (RA/70/80/006)
Principal investigator: Dr L. Zardi

It has been suggested that neoplasia might result from a pathological disarrangement of the mechanism for the regulation of gene expression. Several studies have also implicated chromosomal proteins as likely candidates for gene regulatory functions.

Therefore, the interactions of two carcinogens, nickel and asbestos, with chromosomal proteins from human tissues have been studied. It was observed that a specific class of non-histone chromosomal proteins, with a molecular weight of about 120 kilodaltons, has a high affinity for asbestos.

The equilibrium constant of nickel with purified DNA and chromatin from human fibroblasts was also studied, using the equilibrium dialysis technique. It was observed that nickel binds to DNA with two different equilibrium constants, possibility representing a nickel-phosphate and a nickel-base binding. On the other hand, nickel binds irreversibly to chromatin, suggesting an interesting mechanism for the carcinogenicity of nickel.

A preliminary report on these data was also presented at the Fourth International Symposium on the Prevention and Detection of Cancer, London, July 26–31, 1980.

(*e*) *Mutagenicity testing of chlorinated hydrocarbons in V-79 Chinese hamster cells* (Miss C. Drevon, Miss C. Piccoli and Dr R. Montesano)

The studies examine the mutagenicity of various chlorinated hydrocarbons, including mirex, chlordane, methoxychlor and lindane, in V-79 Chinese hamster cells in the presence of freshly isolated liver cells from mice, rats or hamsters as metabolic activation systems. Many of these compounds have been found not to be mutagenic in bacteria, although they have shown to be carcinogenic in some rodent species. The studies are in progress.

(*f*) *Mutagenicity following multiple doses of* N-*nitroso*-N-*methylurea (NMU) in IAR rat liver cell lines* (Miss C. Drevon, Miss C. Piccoli and Dr M. Montesano)

In parallel to the *in vivo* studies reported in section 3.3 (*a*), in which chronic treatment with a low dose of an alkylating agent resulted in an increased removal from rat liver DNA of the miscoding lesion O^6-methylguanine, a series of studies are underway to compare the mutagenic effects of single and multiple doses of NMU. The cells used are IAR-27, an epithelial cell line derived from liver of BDIV rats. Treatment with NMU resulted in the induction of 6-thioguanine-resistant colonies, and the mutation frequencies were directly related to the dose used.

The effect of multiple doses of NMU on the mutation frequencies is under examination, as well as whether there is a parallel accumulation of O^6-methylguanine or other alkylation products in the DNA of these cells. Similar studies have been carried out using V-79 Chinese hamster cells.

3.5 *Mechanism of action of tumour promoters*

(a) Long-term effect of a tumour promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA) on induced Friend erythroleukaemia cell differentiation (Dr H. Yamasaki, Miss N. Martel and Mrs L. Saint Vincent)

It is now a well established fact that a potent mouse skin tumour promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and its congeners are potent modulators of various programmes of cell differentiation in cell culture systems[41–43]. In this study, Friend erythroleukaemia cells (FELC) are used to study the mechanism by which tumour promoters inhibit cell differentiation.

When induced to differentiate by treatment with hexamethylene bisacetamide (HMBA), FELC lose their ability to divide after a few divisions[44]. If, on the other hand, TPA is present in the culture medium FELC continue to grow, since their terminal differentiation is inhibited[45]. FELC have been maintained in the continuous presence of HMBA plus TPA for about a year by transferring the cells twice weekly to fresh medium containing these compounds. During this period, differentiation, scored by the appearance of B+ (benzidine-positive) cells, was always less than 5% and usually less than 1%, indicating that there is no significant escape of cells from the TPA blockage during this time. During the 8-month period, however, FELC underwent significant changes with respect to their ability to differentiate in response to HMBA: FELC were periodically released from the TPA blockage through washing, and their response to freshly added inducer of differentiation, HMBA, and inhibitor, TPA, was examined (Fig. 4). When cells cultured with HMBA and TPA for as long as two-and-a-half months were transferred to fresh medium containing HMBA alone, they were induced to differentiate to an extent similar (80–90%) to that observed in cells not previously cultured with TPA (Fig. 4). This confirms and extends our previous report that TPA-mediated inhibition of HMBA-induced differentiation is reversible[45, 46].

While the expression of erythroid differentiation is suppressed during this period, FELC grow and multiply normally. Thus, it appears that TPA inhibits specific functions that participate in the process of FELC differentiation, but not other, general functions related to normal cell proliferation[47].

As shown in Figure 4, if the cells are maintained in the presence of HMBA and TPA for a longer period, they tend to lose their ability to differentiate when transferred to HMBA-containing medium. After seven months' continuous growth of the cells in HMBA and TPA, only about 20%

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[41] Diamond, L., O'Brien, T. G. & Rovera, G. (1978) *Life Sci.*, **23**, 1979–1988.

[42] Weinstein, I. B., Lee, L. S., Fisher, P. B., Mufson, A. & Yamasaki, H. (1979) *J. Supramol. Struc.*, **12**, 195–208.

[43] Yamasaki, H. (1980) In: Montesano, R., Bartsch, H. & Tomatis, L., eds, *Cellular and Molecular Aspects of Carcinogen Screening Tests (IARC Scientific Publications No. 27)*, Lyon, pp. 91–111.

[44] Friend, C., Scher, W., Holland, J. G. & Sato, T. (1971) *Proc. natl Acad. Sci. USA*, **68**, 378-382.

[45] Yamasaki, H., Fibach, E., Weinstein, I. B., Nudel, U., Rifkind, R. A. & Marks, P. A. (1979) In: Ikawa, Y. & Odaka, T., eds, *Oncogenic Viruses and Host Cell Genes*, New York, Academic Press, pp. 365–376.

[46] Yamasaki, H., Fibach, E., Nudel, U., Weinstein, I. B., Rifkind, R. A. & Marks, P. A. (1977) *Proc. natl Acad. Sci. USA*, **74**, 3451–3455.

[47] Yamasaki, H., Saint Vincent, L. & Martel, N. (1980) *Cancer Res.*, **40**, 3780–3785.

were induced to differentiate by freshly added HMBA. Thus, TPA-mediated inhibition of HMBA-induced differentiation is reversible for as long as two-and-a-half months, but later becomes partially irreversible[47].

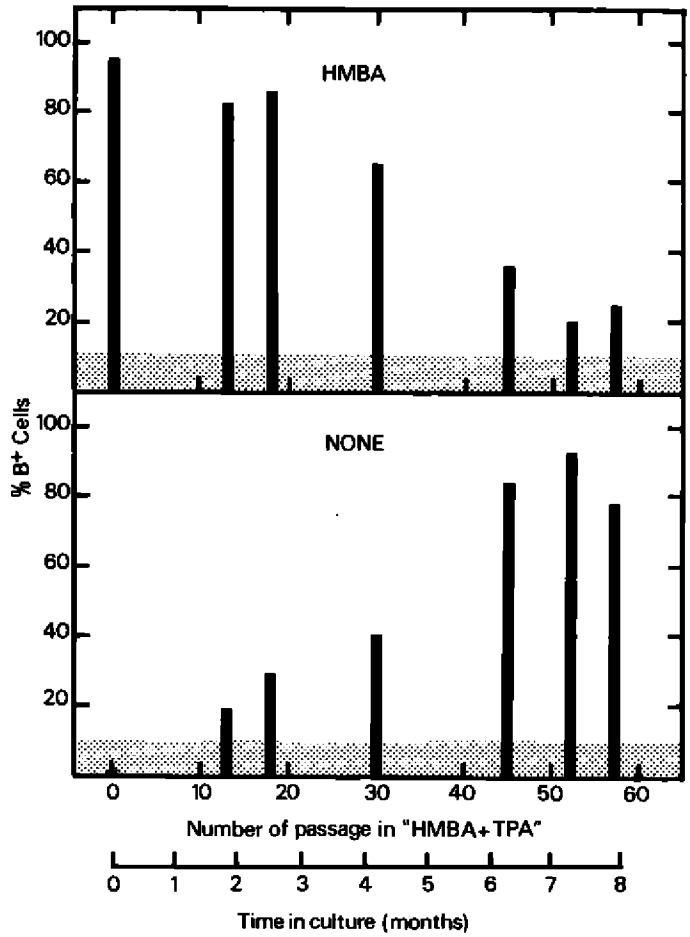

Fig. 4 Differentiation of Friend erythroleukaemia cells (FELC) clone TS19-8 after release from long-term blockage by 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Cells of clone TS19-8 were grown in the presence of 4mM hexamethylene bisacetamide (HMBA) and 100 ng/ml TPA. About 10^6 cells/ml were transferred twice a week into culture medium containing fresh HMBA and TPA. After the indicated number of passages, FELC were washed 4 times with culture medium (including 30 minutes' incubation in the medium at 37°C between the 3rd and 4th washings) and were resuspended at 10^6 cells/ml in control medium, 4 mM HMBA, 100 ng/ml TPA, or HMBA plus TPA. After 4 and 5 days in culture, the extent of differentiation was scored, and the higher percentage of benzidine-positive (B+) cells from 4th- and 5th-day cultures was recorded
A: cells were removed from HMBA and TPA at various passage levels and resuspended in the presence of HMBA alone or in the presence of HMBA and TPA (hatched area). B: cells were resuspended in control medium or in TPA-containing medium (hatched area)

Figure 4 also shows differentiation, in the absence of the inducer HMBA, of FELC periodically released from continuous growth in HMBA and TPA. When the cells were transferred to control medium after 12 passages (approximately two months' culture) in HMBA and TPA, about 20% of the cells underwent differentiation. This implies that 20% of the cells were committed to differentiate; since commitment of FELC in the absence of TPA is complete within 48 hours after the incubation of cells with HMBA[48], it appears that the majority of cells (80%) are inhibited from commitment by the action of TPA. When the cells were grown for a longer time in the presence of HMBA and TPA, the percentage of committed cells gradually increased (Fig. 4). After seven months' incubation of FELC in the presence of HMBA and TPA, for example, 90% of the cell population was committed to differentiate.

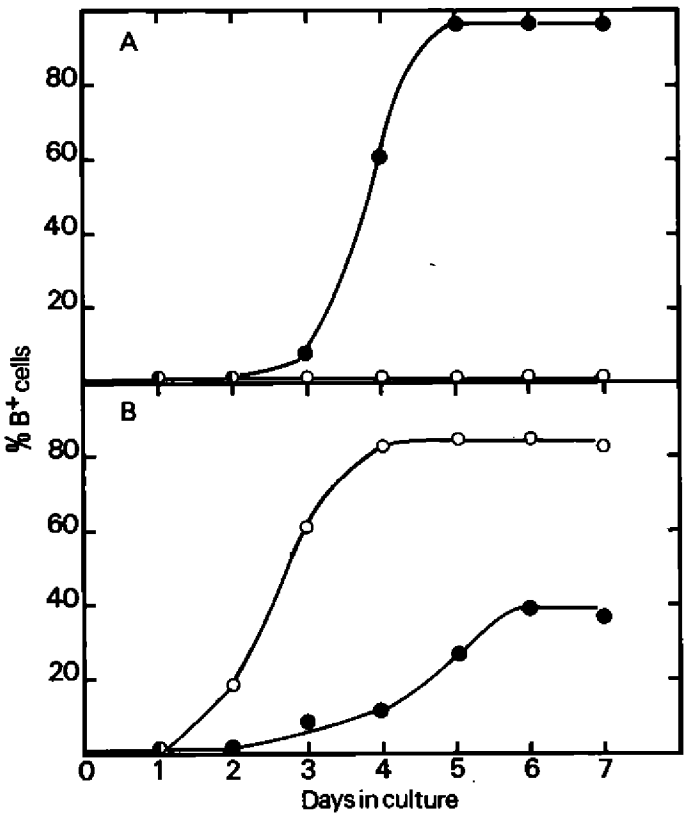

Fig. 5 Time course of differentiation of TS19-10 cells before and after long-term growth in hexamethylene bisacetamide (HMBA) plus 12-*O*-tetradecanoylphorbol-13-acetate (TPA). A: TS19-10 cells were grown in the presence of 100 ng/ml TPA for 4 passages and washed thoroughly, as described in Fig. 4, then incubated in control (○) or in 4mM HMBA-containing medium (●). B: TS19-10 cells underwent 46 serial passages in HMBA and TPA and were then washed and allowed to differentiate in control (○) and HMBA (●) medium. B+= benzidine-positive

[48] Fibach, E., Reuben, R., Rifkind, R. A. & Marks, P. A. (1977) *Cancer Res.*, 37, 440–444.

These results suggest that TPA inhibits the entry of FELC into commitment; but, because this inhibition is leaky, FELC are eventually committed to differentiate. However, TPA also inhibits the expression of commitment, so that HMBA-induced, committed cells gradually accumulate in the cell population.

Figure 5 shows the time course of differentiation of the presumptive TPA-accumulated, committed TS19-10 cells after their release from TPA blockage into control medium (Fig. 5B), as compared with the usual HMBA-induced differentiation of TS19-10 (Fig. 5A). When TS19-10 cells that had been grown in HMBA and TPA for six months were washed and grown in control medium, they started to differentiate after two days and reached a maximum on day 4 (Fig. 5B). On the other hand, induction of differentiation of TS19-10 cells by HMBA started on day 3 and was completed by day 5 (Fig. 5A). Thus, cells grown in HMBA plus TPA for a long time can differentiate at least 24 hours earlier than usual, further suggesting that FELC cultured in HMBA and TPA are blocked after their commitment to differentiate.

Although after four to five months' culture in HMBA and TPA most cells were committed and therefore underwent differentiation when washed and released from TPA into control medium, fewer cells differentiated when they were released into HMBA-containing medium (Fig. 4). Paradoxically, this indicates that HMBA, a potent inducer of FELC differentiation, inhibits the expression of commitment to differentiation.

When cells are released from TPA blockage, they lose their ability to divide (Fig. 6). Regardless of the number of cells inoculated (10^3, 10^4 or 10^5 cells/ml), they stop dividing after three to four divisions in control medium, whereas in the presence of TPA they continue to multiply. These results indicate that FELC not only become benzidine-positive, but also undergo terminal cell differentiation upon removal of TPA. When the cells were released into HMBA-containing medium, they grew more than when they were released into control medium which contained neither HMBA nor TPA (Fig. 5). This further suggests that HMBA acts as an inhibitor of terminal differentiation of FELC grown in HMBA and TPA for a few months.

In two-stage mouse skin chemical carcinogenesis, the tumour promotion step is reversible at an early stage unless the promoter is applied frequently and for a prolonged period of time; but at a later stage the tumour achieves irreversible growth[49, 50]. The finding that tumour promoter-mediated inhibition of inducible differentiation is reversible up to several months, but later becomes irreversible, may be related to the characteristics of tumour promotion *in vivo*.

(*b*) *Characterization of TPA-sensitive and TPA-resistant Friend cells* (Miss C. Drevon, Miss N. Martel and Dr H. Yamasaki)

TPA-resistant Friend cells are being characterized further. On the assumption that an early and primary site of action of tumour-promoting phorbol esters is the cell membrane[42], and on the basis of previous findings[51], research is now being concentrated on comparing the effects of TPA on the cell-surface membrane of TPA-sensitive FELC with those of TPA-resistant FELC. Preliminary studies have shown two other effects of TPA on cell membranes of FELC, which are associated with inhibition of cell differentiation[51]: when TPA-sensitive clonal FELC were incubated with TPA (30-~100 ng/ml) and examined under an interference microscope, these cells showed surface

[49] Boutwell, R. K. (1974) *CRC crit. Rev. Toxicol.*, **2**, 419–443.
[50] Van Duuren, B. L. (1969) *Prog. exp. Tumor Res.*, **11**, 31–68.
[51] Yamasaki, H. & Drevon, C. (1980) In: *Biology of Cancer Cells, Proceedings of the Fifth Meeting of the European Association for Cancer Research*, Ametelveen, Kugler Medical Publications, pp. 317–325.

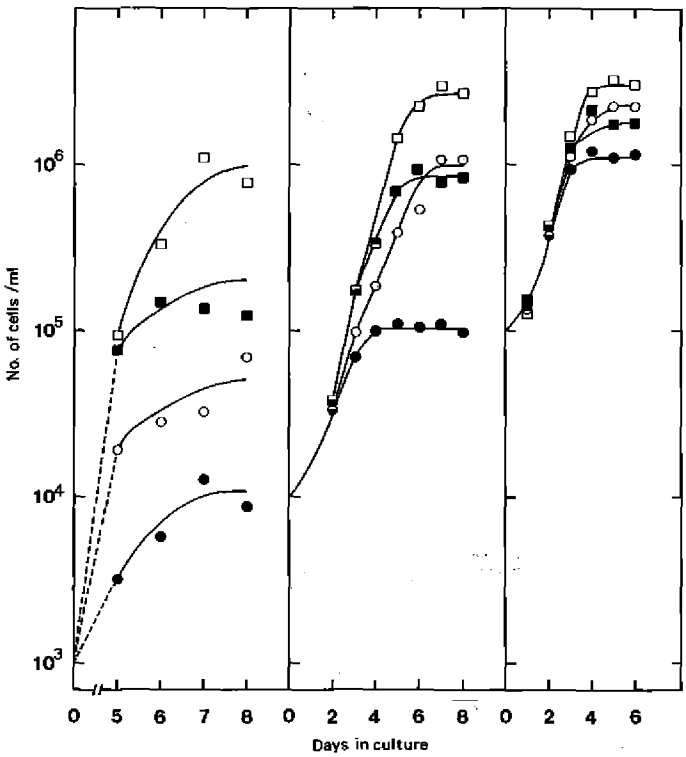

Fig. 6 Growth of Friend erythroleukaemia cells (FELC) clone TS19-8 after continuous culture in hexamethylene bisacetamide (HMBA) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) for 45 passages. Cells maintained in HMBA and TPA were washed 4 times in control medium as described in Fig. 4. The cells were then resuspended in growth medium containing none (●), 4mM HMBA (○), 100 ng/ml TPA (■) or HMBA plus TPA (□). A: 10^3 cells per ml; B: 10^4 cells per ml; and C: 10^5 cells per ml were inoculated originally, respectively

roughness and irregularity (Fig. 7). These changes can be induced within 60 minutes after the addition of TPA. When FELC are grown in TPA for several passages, they still show a rough surface and irregular shape, but the cells multiply normally; these changes are therefore not due to a toxic effect of TPA. No such morphological changes are induced by TPA when TPA-resistant clones of FELC are employed (Fig. 7).

In an attempt to demonstrate further biochemical effects of TPA on cell surface membranes, lactose peroxidase-catalysed iodination of the external surface of FELC membrane was carried out. ^{125}I-labelled proteins of FELC surface membrane were analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The surface proteins were then analysed by radioautography. When TPA-sensitive FELC (TS19-10) are incubated with TPA and the iodinated surface protein pattern is compared with those of cells incubated with solvent only, several changes can be seen. For example, when the cells have been incubated with TPA for 24 hours, a protein band of approximate molecular weight of 1×10^5 disappears, and two new proteins appear, with molecular weights of about 4×10^4 and 1×10^4. In contrast, there is essentially no qualitative change in the iodinated surface protein pattern of TPA-resistant FELC (TR19-9aR) before or after incubation of the cells with TPA.

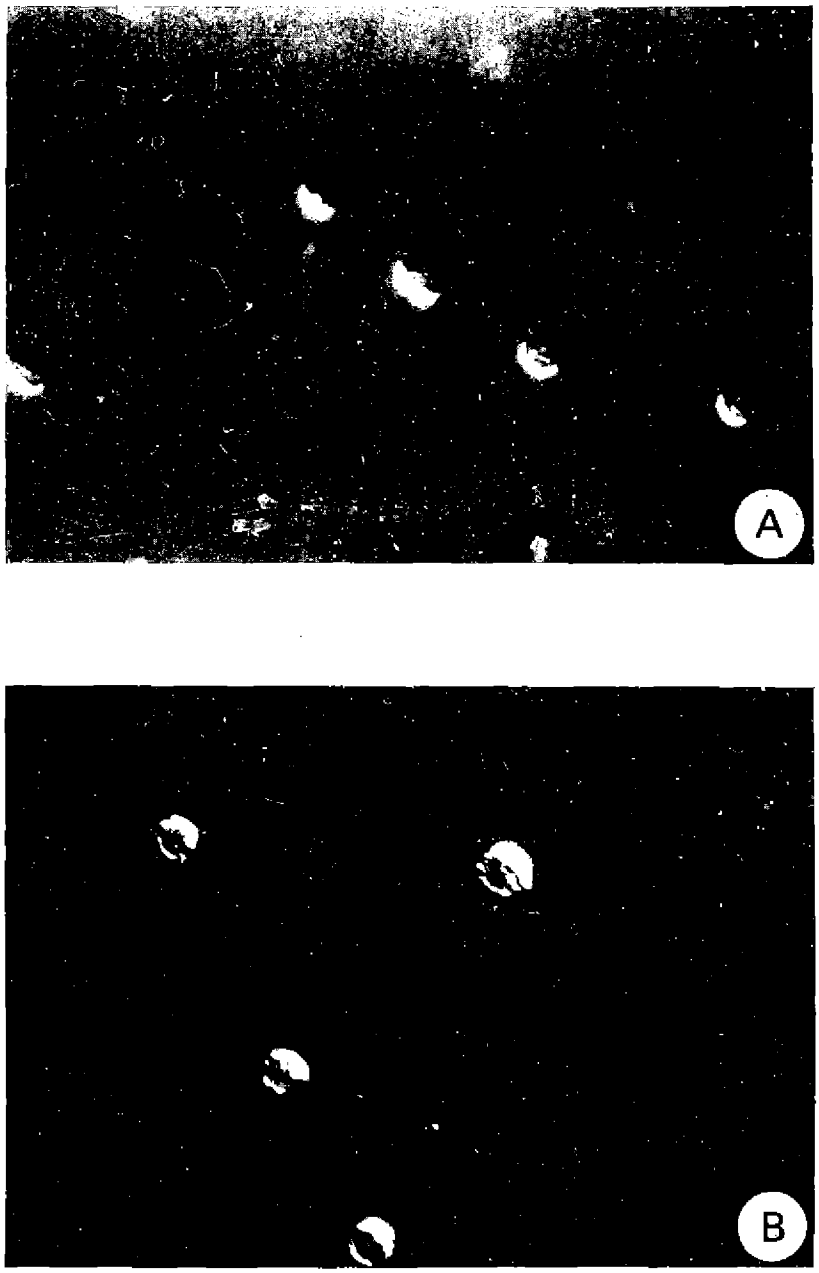

Fig. 7 Interference contrast micrograph of Friend erythroleukaemia cells (FELC) grown in the absence (A) or presence (B) of 12-*O*-tetradecanoylphorbol-13-acetate (TPA). TPA-sensitive clone of FELC (TS19-10) was exposed to TPA (30 ng/ml) for 24 hours in a bacterial Petri dish to avoid cell adhesion

(c) *Effects of tumour promoters on human mixed lymphocyte reaction* (Dr H. Yamasaki and Miss N. Martel)

The mixed lymphocyte reaction (MLR) is a stimulation of cell proliferation resulting from interactions of cell-surface antigens. This system therefore provides another model system for studying the interaction of phorbol esters with the cell surface. It has been shown previously that tumour-promoting phorbol esters inhibit bovine MLR [52]; this observation has now been extended to human MLR.

When low concentrations (10 ng/ml, 1.67×10^{-8}) of TPA were added to human lymphocytes at the beginning of MLR, the reaction (measured by ^{3}H-thymidine incorporation) was almost completely inhibited (Fig. 8A). The inhibitory effect is apparently not related to a cytotoxic action of TPA, since ^{3}H-uridine incorporation was less affected (Fig. 8B). TPA also inhibits MLR when added after the reaction has started, and this inhibition is very rapid: complete inhibition can be seen 1 hour after the addition of TPA to mixed cultures of lymphocytes (Fig. 9). Other tumour

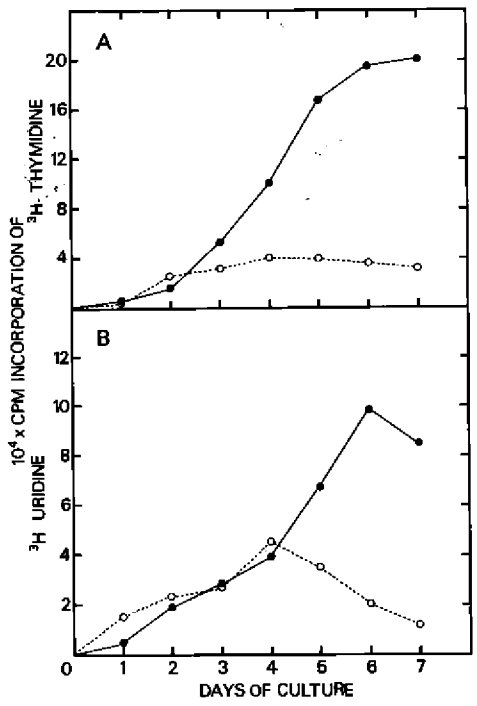

Fig. 8 Inhibition of mixed lymphocyte reaction in human cells, as measured by incorporation of ^{3}H-thymidine (A) and ^{3}H-uridine (B), after addition of 10 ng/ml 12-*O*-tetradecanoylphorbol-13-acetate

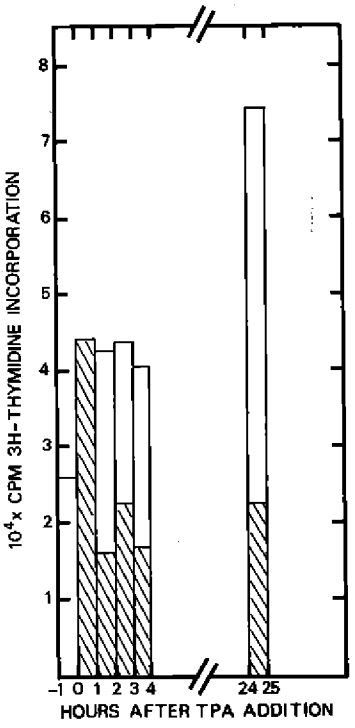

Fig. 9 Inhibition of mixed lymphocyte reaction in mixed cultures of human lymphocytes by addition of 12-*O*-tetradecanoylphorbol-13-acetate after the start of the reaction. Inhibition measured by incorporation of ^{3}H-thymidine

[52] Mastro, A., Krapa, T. A. & Smith, P. (1979) *Cancer Res.*, 39, 4078–4082.

promoters, such as phorbol-12, 13-didecanoate and mezereine, also inhibited the reaction, whereas inactive derivatives, phorbol and 4α-phorbol-12,13-didecanoate, had no effect (Table 7).

TPA had an inhibitory effect on all of 26 independent MLR from 52 donors. This finding implies that most individuals are susceptible to the action of TPA, although there may be quantitative differences among them.

Table 7. Effect of phorbol derivatives and mezereine on human mixed lymphocyte reaction

Compound (10ng/ml)	^{3}H-thymidine incorporation	
	cpm	% of controls[a]
None	161,938	100
12-*O*-*n*-Tetradecanoylphorbol-13-acetate	27,230	16.8
Phorbol-12,13-didecanoate	38,543	23.8
Mezereine	39,815	24.6
Phorbol	164,796	101.8
4α-phorbol-12,13-didecanoate	180,088	111.2

[a] Compounds were added on day 0 to mixed cultures of lymphocytes, and ^{3}H-thymidine incorporation was determined on day 6.

(*d*) *Effect of tumour promoters on rat liver epithelial cells in culture* (Dr H. Yamasaki, Miss N. Martel and Miss C. Drevon)

Peraino *et al.*[53] showed that phenobarbital administered in the diet is a promoter of rat liver carcinogenesis initiated by *N*-2-acetylaminofluorene. The effect of this compound on cultured liver cells was tested by adding 0.1–1 mg/ml phenobarbital to a rat liver epithelial cell line established by Montesano *et al.*[54]. About 10 days later, a marked difference in morphology was seen in the treated cells as compared with untreated control cells (Fig. 10). When the cells were transferred to control medium, the cell morphology returned to normal. However, when the cells were grown in the presence of phenobarbital for a long time, i.e., 30 days, they no longer regained normal morphology when transferred to control medium.

A similar effect was obtained when the cells were treated with TPA (10–100 ng/ml) (Fig. 10C), although they metabolize TPA to biologically inactive metabolites, phorbol-13-monoacetate and 12-*O*-tetradecanoylphorbol, within several days. Phenobarbital and TPA caused no significant change in either growth rate or saturation density in these liver cells.

TPA and related tumour promoters usually share no common effects with non-phorbol tumour promoters when administered to cultured cells[55]. The similar effects of TPA and phenobarbital on cultured rat liver epithelial cells seen here may indicate the importance of organ-specificity in the activity of tumour promoters.

[53] Peraino, C., Fry, R. J. M., Staffeldt, E. & Christopher, J. P. (1975) *Cancer Res.*, 35, 2884–2890.

[54] Montesano, R., Drevon, C., Kuroki, T., Saint Vincent, L., Handleman, S., Sanford, K. K., Defeo, D. & Weinstein, I. B. (1978) *J. natl Cancer Inst.*, 59, 1651–1658.

[55] Driedger, P. E. & Blumberg, P. M. (1978) *Int. J. Cancer*, 22, 63–69.

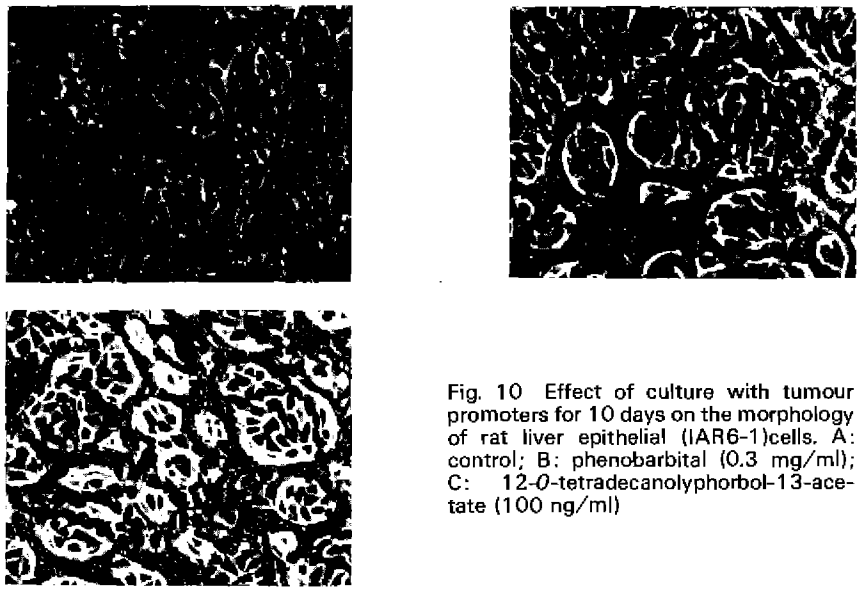

Fig. 10 Effect of culture with tumour promoters for 10 days on the morphology of rat liver epithelial (IAR6-1)cells. A: control; B: phenobarbital (0.3 mg/ml); C: 12-*O*-tetradecanolyphorbol-13-acetate (100 ng/ml)

(*e*) *Effects of tumour-promoting agents on mouse hair depigmentation* (Dr H. Yamasaki, Dr J. R. P. Cabral, Mrs D. Galendo and Dr L. Tomatis)

When TPA was injected intradermally into C57BL6 mice, an area of white hair appeared, following necrosis and depilation at the injection sites (Fig. 11). A single injection of 5 μg TPA is

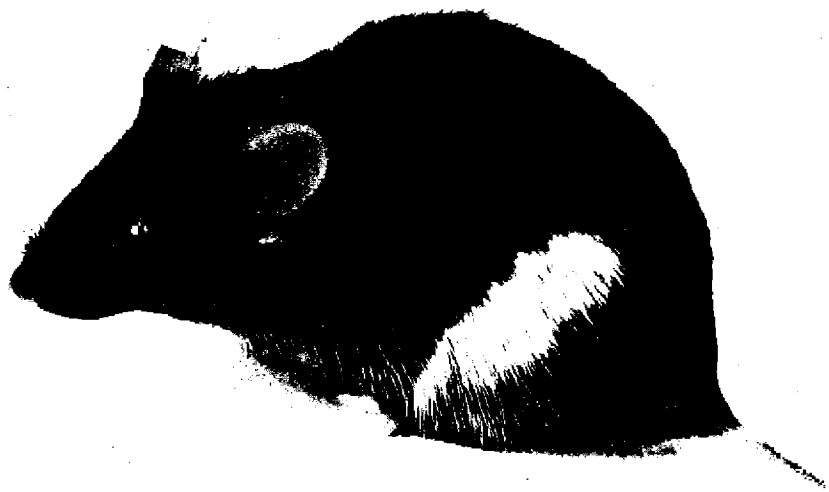

Fig. 11 Depigmentation of hair of C57BL6 mouse after intradermal injection of 10 µg 12-*O*-tetradecanoylphorbol-13-acetate (TPA) twice a week. Some depilation at the injection site was observed after 1–2 weeks; white hair was seen 3–4 weeks after injection

sufficient to produce this effect. On the other hand, ethyl phenyl propiolate, an agent which shows hyperplasminogenic and inflammatory effects on mouse skin but no tumour-promoting activity, failed to cause hair depigmentation; although 5 mg/mouse produced necrosis and depilation at the injection sites (similar to that produced by TPA). Thus, the TPA-induced hair depigmentation is not the result of a toxic effect on mouse skin; and depigmentation of mouse hair may be related more directly to promoting activity. Various tumour promoters and related compounds are currently being tested in this system.

(*f*) *Effects of tumour promoters on development and behaviour of a nematode,* Caenorhabditis elegans (Dr H. Yamasaki, in collaboration with Dr J. Miwa and Dr M. Furusawa of Osaka City University, Osaka, Japan)

Since tumour-promoting phorbol esters interfere with cell differentiation[56, 57, 58], it was proposed that they should also interfere with early development of embryos. This hypothesis was tested in the hermaphroditic soil nematode, *Caenorhabditis elegans*. The wild-type strain of *C. elegans* goes through the four larval stages (L1–L4) before it becomes an adult after 2 days at 24°C[59]. They reproduce to give a brood size of about 200 and move in a sinusoidal wave on an agar surface.

To investigate the effects of various phorbol esters, animals at various developmental stages were grown on an agar medium seeded with *Escherichia coli* in the presence of phorbol esters. Table 8 summarizes the effect of the compounds on development (growth), behaviour (movement), brood size, and hatching rate of laid eggs. For the brood size evaluation, only one animal (P) was placed on a plate, and P animals that started to lay eggs were transferred to a new plate every day to distinguish clearly between the F_1 and F_2 eggs or animals. Within 60 minutes of the addition of TPA (100 ng/ml, 1.7×10^{-7} M), the nematodes showed characteristically uncoordinated movement. When they were treated at the L1 and L3 stages with a higher concentration of TPA (1 μg/ml, 1.7×10^{-6}M), besides uncoordinated movement, their growth was completely arrested: they did not develop gonads and consequently produced no eggs. In the course of the present experiment, well over a thousand L1 animals were treated with 1 μg/ml TPA or phorbol-12,13-didecanoate, and none were observed to have produced eggs. Most of the animals, however, remained alive for several days at the arrested stage, and upon release from TPA blockage most of them underwent normal development (Table 9). Phorbol-12,13-didecanoate, another potent tumour-promoting phorbol ester, showed a similar effect, whereas non-promoting derivatives, phorbol and 4α-phorbol-12,13-didecanoate, failed to exert an effect (Table 8).

Thus, tumour-promoting phorbol esters reversibly inhibit early development of *C. elegans*, showing that tumour promoters modify not only terminal cell differentiation but also early developmental processes of embryos.

[56] Diamond, L., O'Brien, T. G. & Rovera, G. (1978) *Life Sci.*, **23**, 1979–1988.
[57] Weinstein, I. B., Lee, L. S., Fisher, P. B., Mufson, A. & Yamasaki, H. (1979) *J. Supramol. Struc.*, **12**, 195–208.
[58] Yamasaki, H. (1980) In: Montesano, R., Bartsch, H. & Tomatis, L., eds, *Cellular and Molecular Aspects of Carcinogen Screening Tests (IARC Scientific Publications No. 27)*, Lyon, pp. 91–111.
[59] Brenner, S. (1974) *Genetics*, **77**, 71–94.

Table 8. Effects of phorbol esters on development of *Caenorhabditis elegans*

Test substance[a]	Dose µg ml^{-1}	L1 Brood size[b]	L1 Phenotype[c]	L3 Brood size	L3 Phenotype	Adult Brood size	Adult Phenotype
12-*O*-tetradecanoyl-phorbol-13-acetate	1	0	arr: L2-3, unc	0	arr: L4, unc	122 ± 8 (2)	unc
	0.1	13 ± 6 (0)	unc	74 ± 9 (1)	unc	119 ± 22 (1)	unc
	0.01	152 ± 28 (1)	nml	220 ± 33 (2)	nml		—
	0.001	236 ± 28 (2)	nml	—			—
phorbol-12,13-didecanoate	1	0	arr: L2-3, unc	14 ± 6 (0)	unc	139 ± 12 (3)	unc
	0.1	0	arr: L3, unc	98 ± 11 (1)	unc	185 ± 21 (2)	unc
	0.01	232 ± 10 (3)	nml	225 ± 18 (1)	nml		—
	0.001	235 ± 14 (2)	nml	—			—
phorbol	1	210 ± 8 (1)	nml	—			—
4α-phorbol-12,13-didecanoate	1	240 ± 21 (0)	nml	—			—
dimethyl sulfoxide	0.1%	221 ± 14 (0)	nml	—			—
control	—	215 ± 19 (3)	nml	—			—

[a] Dissolved in dimethyl sulfoxide
[b] Average of 5 animals ± SD; in parentheses, the average no. of fertilized eggs/animal that did not hatch
[c] arr: L2-3: development arrested in the L2 or L3 stage; unc: uncoordinated movement; nml: apparently normal in development and behaviour

Table 9. Reversibility of inhibition of growth of *Caenorhabditis elegans* due to 12-*O*-tetradecanoylphorbol-13-acetate (TPA)

Exposure to TPA (hrs)	No. of animals	No. of survivors[a]	No. of fertile animals[b]	Brood size[c]
25	30	27	25	226 ± 31 (2)
50	70	61	37	214 ± 50 (1)

[a] Tested minus dead and missing animals
[b] Animals that grew to lay eggs
[c] No. of progeny per animal that laid eggs, given as mean ± SD; in parentheses, the average no. of fertilized eggs/animal that did not hatch

(*g*) *Tumour promoters and SARC gene expression* (Dr H. Yamasaki, in collaboration with Dr M. K. Owada and Dr K. Moelling, Max-Planck Institute for Molecular Genetics, Berlin-Dahlem, Federal Republic of Germany)

Infection of chick embryo fibroblasts (CEF) with temperature-sensitive (ts) mutants of avian sarcoma viruses (ASV) results in morphological transformation at the permissive temperature (36°C) but not at the non-permissive temperature (41°C). Addition of TPA to tsASV-CEF at 41°C induced a morphological alteration which resembled an intermediate state between ASV-transformed ans normal CEF. It also stimulated cell proliferation. The morphological alterations were reversible after removal of TPA. Addition of phorbol-12,13-didecanoate gave rise to similar responses, whereas phorbol and 4α-phorbol-12,13-didecanoate were ineffective.

Cellular transformation induced by ASV has been shown to depend on the viral-transforming gene product, pp60^{src}, which is associated with a protein kinase activity. Low levels of a homologous protein, called pp60^{sarc}, have been found in normal cells. To determine whether TPA and phorbol-12,13-didecanoate can alter the expression of pp60^{src} in correlation with the morphological changes, the level of protein kinase activity was determined by phosphate transfer from (γ^{32}–P) ATP to the IgG of tumour-bearing rabbit serum. This remained constant after addition of the phorbol esters to the cells. Recently, we succeeded in purifying pp60^{src}, and found that it phosphorylates actin *in vitro*. Addition of TPA to this reaction also had no effect on the level of protein kinase activity.

Treatment of normal and leukosis virus-infected CEF with TPA resulted in morphological changes resembling those in CEF transformed by avian erythroblastosis virus. Expression of the normal cellular pp60^{sarc} was again not affected.

Similar results have recently been obtained independently by Goldberg *et al.*[60]. TPA and other related promoters appear to enhance morphological transformation without affecting the activity of kinase, a viral gene product.

3.6 *Specific antibodies against DNA adducts of carcinogens and against carcinogens*

Studies have been initiated to prepare and characterize specific antibodies against DNA adducts formed by nitrosamines and against carcinogenic compounds such as aflatoxins. The aim is to use these antibodies for monitoring individual human exposure to carcinogenic agents and to detect in human tissues or body fluids cellular macromolecule modifications that are the result of exposure to carcinogens. These antibodies are also being used in studies on DNA repair to detect specific DNA adducts (see section 3.3). These antibodies have been formed in rabbits by injection of the appropriate immunogen; the establishment in the laboratory of the techniques for obtaining monoclonal antibodies is currently underway.

(*a*) *Detection of O^6-methylguanine in DNA by specific antibodies* (Miss C. Bordet and Dr R. Montesano)

Antisera against O^6-methylguanosine were obtained in rabbits by immunization with conjugate bovine serum albumin-O^6-methylguanosine[61]. Purification by immunoadsorption on

[60] Goldberg, A. R., Delclos, K. B. & Blumberg, P. M. (1980) *Science*, **208**, 191–192.
[61] Bordet, C., Bannikov, G. & Montesano, R. (1980) *Mutat. Res.*, **74**, 186.

epoxy-activated sepharose 6B coupled with O^6-methyl- 2′-deoxyguanosine gave antibodies with an affinity constant for O^6-methyl- 2′-deoxyguanosine of 7.8×10^8 1/mol.

In a competitive radioimmunoassay, using O^6-methyl-[8, 5′-^{3}H]- 2′-deoxyguanosine (specific activity, 9 Ci/mmol) as a tracer, 0.8 pmol O^6-methyldeoxyguanosine inhibited tracer-antibody by 50%. Gross reactivity with various natural and alkylated components of DNA is shown in Table 10.

Table 10. Radioimmunoassay for O^6-methyl-2′-deoxyguanosine: inhibition of tracer-antibody binding by various alkylated and natural nucleic acid components

Compound	Amount required for 50% inhibition of tracer-antibody binding	
	pmol	Multiples of O^6-methyl-2′-deoxyguanosine
O^6-Methyl-2′-deoxyguanosine	0.8	1
O^6-Methylguanosine	10	12.5
O^6-Ethylguanosine	12	15
O^6-Methylguanine	120	150
O^6-Ethylguanine	500	625
2′-Deoxyadenosine	5×10^5	5×10^5
2′-Deoxyguanosine	$\sim 1 \times 10^5$[a]	$\sim 1 \times 10^6$
7-Methylguanosine	$\sim 1 \times 10^5$[a]	$\sim 1 \times 10^6$
Guanosine	$\sim 5 \times 10^5$[b]	$\sim 4 \times 10^6$
Adenosine	$\sim 5 \times 10^5$[b]	$\sim 4 \times 10^6$
2′-Deoxycytidine	N.D.[c]	
Thymidine	N.D.	
Cytidine	N.D.	

[a] 15% inhibition at this concentration
[b] < 10% inhibition at this concentration
[c] No detectable inhibition at 1×10^5 pmol

A radioimmunoassay has been developed for quantifying methylation at the O^6 of guanine in methylated DNA. Use of this system will depend on its reliability and its sensitivity as compared with the classical radiochemical method.

Antibodies are being prepared against 7-methylguanosine.

(*b*) *Production of antibodies against aflatoxin B_1 and M_1* (Dr P. Sizaret, Miss A. M. Aguelon and Mr G. Toussaint)

Radioimmunoassays are being developed to determine aflatoxins B_1 and M_1 in biological samples from potentially exposed individuals. The antigen was prepared by conjugating aflatoxin B_1-oxime with bovine serum albumin according to the method of Chu *et al.*[62], and the antibodies were raised in rabbits by multiple injections of the immunogen. The antibodies are being characterized.

[62] Chu, F. S. & Ueno, I. (1977) *Appl. environ. Microbiol.*, **33**, 1125–1128.

3.7 *Virological and cytogenetic studies of tumours associated with Epstein-Barr virus*

Following the reorganization of the Agency in January 1980, the former Unit of Biological Carcinogenesis was split in two, and the laboratory group became part of the Division of Environmental Carcinogenesis, Programme of Mechanisms of Carcinogenesis. The IARC Research Centre in London will close down at the end of 1980.

The group has continued to support the field activities of the programme on African Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC), which is supervised by epidemiologists of the Division of Epidemiology and Biostatistics (see section 3.8 of the report of that Division).

Apart from that support, the major activity of the group was serological and virological studies on the Epstein-Barr virus (EBV). *In vitro* studies to understand the role of EBV as an oncogenic agent followed two approaches: 1) a biochemical one, to study further the role of EBV gene products in the transforming process; and 2) a more biological one, to study the transformation of human lymphocytes in tissue cultures.

A new virological and cytogenetic programme on European Burkitt's-type lymphomas has been developed. First results indicate that whether or not EBV is associated with some of these lymphomas, cytogenetic markers, such as translocations implicating chromosome 8, are found consistently. The important role of cytogenetic rearrangements in the etiology of lymphomas should open a new field of future research.

The group continued to prepare and distribute reference material for use in EBV studies, in the form of sera and cell lines, to various national laboratories.

(*a*) *Laboratory activities related to the field programme* (Dr G. Lenoir, Miss C. Bonnardel, Mrs M. F. Lavoué and Mrs S. Pauly)

All of the EBV serological and virological testing in 1980 for both the African BL and NPC programmes were carried out by the group (see section 3.8 of the report of the Division of Epidemiology and Biostatistics).

(i) *Prospective study of Burkitt's lymphoma in the West Nile district of Uganda*

The detection of cases of BL was terminated at the end of 1979. Two new pre-bled cases had been detected since the report published in 1978[63]: both were EBV-positive, as indicated by nucleic acid hybridization testing performed in Dr Klein's laboratory in Stockholm and in Dr Bornkamm's laboratory in Freiburg.

EBV testing of the 85 sera, comprising sera of BL cases before and after development of the tumour and of their controls and family members, was carried out in two laboratories, that of Dr Henle (Philadelphia) and the Agency. A very good correlation was found between the results from the two laboratories; statistical analysis will indicate whether they confirm those published previously[63].

(ii) *EBV serological study in Uganda*

The 3360 sera from the BL study in Uganda have been tested for the three EBV antibody reactivities, anti-viral capsid antigen (VCA), early antigen (EA) and nuclear antigen (EBNA), in

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[63] de-Thé, G., Geser, A., Day, N. E., Tukei, P. M., Williams, E. H., Beri, D. P., Smith, P. G., Dean, A. G., Bornkamm, G. W., Feorino, P. & Henle, W. (1978) *Nature*, **274**, 756–761.

order to evaluate whether variation in the EBV serological profile of this population could be related to variations in BL risk and/or to co-factors such as malaria infection. The data are being analysed in cooperation with the Biostatistics Programme of the Division of Epidemiology and Biostatistics, and preliminary results confirm that EBV serological titres are stable within individuals and that EBV infection takes place very early in life in that area.

(iii) *Malaria suppression trial in the Mara region of Tanzania*

The laboratory also carried out EBV serological and virological testing of the sera collected during the malaria suppression trial in the Mara region of Tanzania.

(iv) *EBV association of African Burkitt's lymphoma* (in collaboration with Dr G. Bornkamm, Institute for Virology, Health Centre, Freiburg, Federal Republic of Germany: Dr G. Klein, Karolinska Institute, Stockholm; and Dr D. Wright, University of Southampton, UK)

In order better to estimate the percentage of African BL cases associated with EBV, all BL cases observed since 1973 for which biological specimens were available at the Agency were studied for the presence of viral genome within the tumour cells; the EBV serological profiles were estimated and the histopathological diagnoses were reviewed by Dr D. Wright.

Biopsy material was available for molecular hybridization investigation in 51 of the 87 cases collected for this study: 49 samples were found to contain the EBV genome, i.e., 96% of African BL cases are associated with EBV. This represents the largest study ever made on this particular lymphoma.

(v) *Studies on nasopharyngeal carcinoma*

Although most of the virological programme on NPC has been phased out, the serological tests necessary to complete the immunogenetic projects in Singapore and Morocco have been carried out.

1) *Immunogenetic studies in Singapore* (see section 5.3 of the report of the Division of Epidemiology and Biostatistics)

2) *Immunogenetic studies in North Africa* (Professor R. Sohier, consultant; in collaboration with Dr H. Bétuel, Lyon Blood Transfusion Centre, Beynost, France; and Professor S. Nejmi, Director, National Virus Centre (Mohamed V Hospital), Rabat, Morocco)

Until now, genetic markers associated with NPC have been reported only in high-risk areas such as Singapore. In order to determine whether NPC is also associated with particular HLA profiles in intermediate-risk areas, a pilot study was carried out in Morocco. Sera and biopsies from more than 125 patients were collected and sent to Lyon for virological analysis. EBV testing indicated that most NPC cases from Morocco are associated with EBV; the histopathological review of these cases is still in progress.

HLA typing of patients and controls has begun. The lymphocytes are prepared in Rabat by Professor Nejmi and sent to Dr Bétuel in Lyon for testing. The cells have been shown to be viable after this long-distance shipment.

(*b*) *Host factors in East African cancer patients*

The natural history of BL and NPC suggests that host immunogenetic and immunological factors may play a part in their development and in the patient's response to treatment. Additional evidence that genetic factors may be important in determining susceptibility to malignancy comes from the finding of familial clustering of tumours in Tanzania[64].

(i) *Immunogenetic factors* (Dr A. G. Levin, Miss M. A. Jones, Mrs D. M. Kirkham and Mrs S. E. Ember, IARC Research Centre, London; Mr P. J. Hall and Dr S. Knight, Clinical Research Centre, Medical Research Council, Harrow, Middlesex, UK; Dr G. R. Brubaker and Mr Z. Siso, Shirati Mission Hospital, Shirati, Tanzania; Professor A. Wasunna and Mrs J. Safari, IARC Research Centre, Nairobi; Dr C. M. Steel, MRC Clinical & Population Cytogenetics Unit, Edinburgh, UK; Dr C. C. Entwistle, National Tissue Typing Centre, Bristol, UK)

Tissue antigens related to BL and NPC are being investigated in East Africans. Tissue typing and 'pattern testing' studies involve the reaction of cryopreserved and thawed lymphocytes against approximately 250 sera in each experiment as depicted below:

Cytotoxicity assay

147 well characterized tissue-typing anti-sera (A, B & C loci)	96 post-pregnancy African sera

Cryopreserved lymphocyte specimen

1) *Tissue typing*: Completed studies of cryopreserved lymphocyte specimens from 141 East African blacks show a relatively high frequency of several B 15-related antigens in this population, occurring in 30% of unrelated individuals[65]. This antigenic group apparently has a strong association with C locus antigens in blacks. Recognition of these B 15 variants (which are uncommon in Caucasians) has been largely responsible for reducing the proportion of unidentified or 'blank' B locus antigens in this population to 6%. This figure is considerably lower than those in previous HLA studies of African blacks, and these results have provided reasonable knowledge of the antigenic frequencies at the A, B and C loci in this population. In particular, the individual antigenic variants of the B 15 group and their association with antigens at other loci facilitates studies of HLA-disease associations in cancer patients and their families.

2) *Pattern testing of sera from parous African women in the North Mara District of Tanzania*: 96 such sera were reacted as shown above in parallel with known tissue-typing antisera. Approximately 25% of the sera showed evidence of a cytotoxic effect on cryopreserved lymphocytes; some of these reactions do not appear to be explicable on the basis of known HLA specificities. The patterns of reactivity are being subjected to a cluster analysis technique for

[64] Brubaker, G., Levin, A. G., Steel, C. M., Creasey, G., Cameron, H. M., Linsell, C. A. & Smith, P. G. (1980) *Int. J. Cancer* (in press).

[65] Hall, P. J., Levin, A. G., Entwistle, C. C., Knight, S. C., Wasunna, A. & Brubaker, G. (1980) *Tissue Antigens* (in press).

delineation of new tissue antigens and/or population groups not yet thoroughly studied. Approximately 700 additional such sera are being screened against aliquots of cryopreserved cells from East African blacks who have previously been tissue typed, and cluster analysis will be applied to results of these studies also. The sera are also being tested against tissue culture cell lines of known A, B, C and DR HLA type in Shirati and by Dr C. M. Steel. Sera with anti-tissue antibodies of potential interest will be available for testing against BL, NPC and family specimens.

3) *DR locus typing:* This locus of the HLA system appears to control somme immune responses, particularly of the cell-mediated type. Antigens at this locus, which are expressed on B lymphocytes, are of special interest, because it has recently been shown that there are receptors for the EBV on the surface of human B lymphocytes and that these receptors are closely associated with the products of the HLA D locus identifiable serologically as DR antigens.

In order to type at the DR locus as well as at the A, B and C HLA loci, a double fluorescence technique has been adopted which specifically indicates B-cell cytotoxicity. Preliminary experiments show that this technique will allow satisfactory typing with the quantities of cryopreserved cells available in individual specimens. Although DR antisera are in short supply, enough well characterized sera are available to type BL, NPC and family subjects.

(ii) *Cell-mediated immunity* (Dr A. G. Levin, Miss M. A. Jones and Mrs D. M. Kirkham, IARC Research Centre, London; Dr S. Knight and Mrs C. Doré, Clinical Research Centre, Medical Research Council, Harrow, Middlesex, UK)

A microculture technique was used to evaluate the ability of cryopreserved lymphocytes to respond to phytomitogen and allogeneic stimulators (lymphoblastoid cell lines). Under optimal conditions (determined in a first series of assays), the reactivity to these stimulants of lymphocytes from 7 BL patients, 10 NPC patients and 17 controls representative of the general East African population was measured. Using a multivariate analysis of the data, statistically significant differences were found between BL and NPC patients with disease, BL and NPC patients in remission, and controls.

(c) *Use of banked biological material*

Cryopreserved lymphocytes, tumour cells and sera have so far been supplied to 55 investigators for use either in their own research programme or for exploratory studies which might be relevant to the Agency's research programme. Among recent results are the following:

Dr R. Good and colleagues at the Sloan-Kettering Institute, New York City, USA, have found that 66% of sera from East African women with breast cancer have a factor which reacts with antigens of the mouse mammary tumour virus, whereas 4% of sera from Chinese women and 20–35% of those from American and Parsee women have this presumed antibody[66].

Recent studies by Professor A. Epstein and colleagues at the University of Bristol, UK, indicate that sera from East African NPC patients have a distinctively high incidence of antibody to human syncytial virus[67].

It has previously been noted in this laboratory that a significantly higher level of carcinoembryonic antigen (CEA) is present in East African blood donors and in East Africans

[66] Day, N. K., Witkin, S. S., Kinne, D., Sarkar, N., Levin, A., Jussawalla, D. J., Hsia, C. C. & Good, R. A. (1980) *Clin. Res.*, 28, 557A.

[67] Muller, H. K., Ball, G., Epstein, M. A., Achong, B. G., Lenoir, G. & Levin, A. (1980) *J. gen. Virol.*, 47, 399–406.

representative of the general population than in Europeans. It has now been found, in collaboration with Dr A. P. Haines of Northwick Park Hospital, UK, and Dr H. A. Fritsche of the University of Texas System Cancer Centre, Houston, TX, USA, that blacks living in London have a significantly higher CEA level than a comparable Caucasian group[68].

(*d*) *Virological studies on Epstein-Barr virus* (Dr G. Lenoir)

(i) *Serological activities* (Miss C. Bonnardel, Mrs M. F. Lavoué and Mrs S. Pauly)

During the past year, improvements have been made to current serological tests. New techniques for the induction of EBV antigen synthesis[69] have been applied to serological testing. Induction of EA by phytohaemagglutinin in the presence of iododeoxyuridine (IUdR) in the producer line RAJI was shown to be a convenient procedure for preparation of cells for EBV serology[70]. This method of induction replaces previous, more cumbersome, techniques that required production of large quantities of infectious virus (Fig. 12).

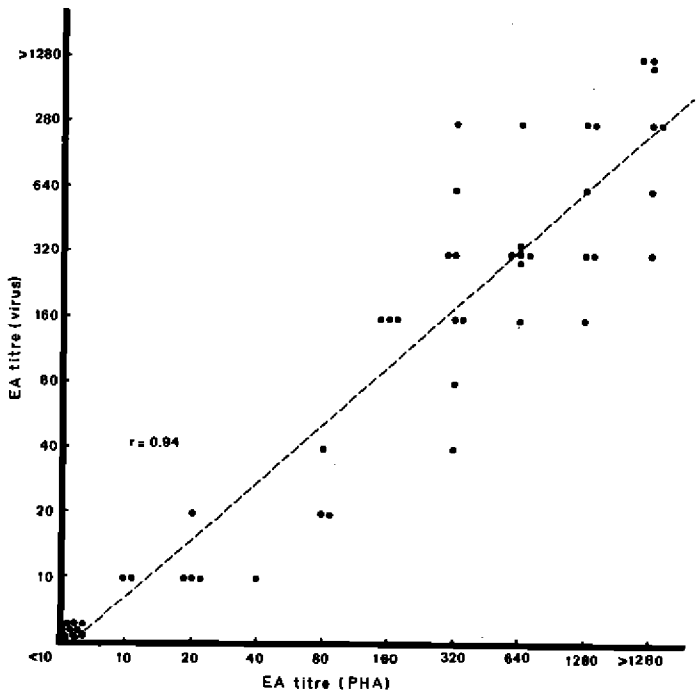

Fig. 12 Comparison of Epstein-Barr virus/early antigen(EA) serological titres of 50 human sera tested by indirect immunofluorescence on two types of smears. EA titre (virus): RAJI superinfected with P_3HR_1 virus; EA titre (phytohaemagglutinin, PHA): RAJI treated with iododeoxyuridine (10 μg) together with PHA (5 μg/ml). Titres expressed as reciprocal of serum dilution. Correlation coefficient: r = 0.94

[68] Haines, A. P., Levin, A. G. & Fritsche, H. A. (1979) *Lancet, ii*, 969.
[69] Tovey, M. G., Lenoir, G., Begon-Lours, J., Tapiero, H. & Rochette-Egly, C. (1979) *J. Immunol.*, **123**, 138–142.
[70] Lenoir, G., Tovey, M. G. & Lavoué, M. F. (1980) *J. Immunol. Meth.*, **34**, 23–29.

In order further to analyse the significance of EBV serology, anti-VCA, EA and EBNA antibody titres were estimated from sera of patients suffering from various immunodeficiencies. The results indicate that some patients, particularly those suffering from cell-mediated immunity defects, cannot mount an anti-EBNA serological response. This was found not only in ataxia telangiectasia patients, as reported previously by Berkel[71], but also in patients with Wiskott-Aldrich syndrome, suggesting that EBV infection is not well controlled in those individuals. It cannot be excluded then that EBV is one of the etiological agents of the lymphoproliferative disorders observed with a very high frequency in those individuals.

(ii) *Biochemical characterization of the EBV antigens* (in collaboration with Drs T. Ooka and A. Calender, and members of Professor J. Daillie's group, University Claude-Bernard, Lyon, France.)

The study of EBV early gene products, which are suspected to play a role in cell transformation, has been continued.

The EBV specific nuclear antigen, present in all EBV genome-containing cells, has been further characterized: EBNA, which is considered to have a molecular weight of 180 D in its native form, has a polymeric structure and is composed of 50 K subunits which carry the antigenic determinants[72]. It has also been demonstrated that EBNA, which has an affinity for double-stranded DNA, is located only within the nucleus of cells, in association with chromatin[73].

It has also been shown that the synthesis of EA can be strongly activated within latently infected cells by various agents, such as anti-immunoglobulins associated with IUdR[74] or by a combination of 12-*O*-*n*-tetradecanoylphorbol-13-acetate and sodium butyrate[75].

In an attempt to characterize the biological functions of these EBV coded molecules, it has been shown that EA includes a viral coded DNA polymerase and a thymidine kinase. The DNA polymerase has been characterized and found to be distinct from cellular enzymes[76]; the thymidine kinase is also being purified and characterized. The existence of an EBV-coded thymidine kinase is strongly supported by recent studies on the effects of arabino-furanosylthymidine on EBV replication. This compound, which can be phosphorylated by herpes virus-coded enzymes and not by cellular enzymes, was found to be a good inhibitor of the EBV cycle[77].

(iii) *Reference and banking related activities* (Professor R. Sohier, consultant)

To ensure comparability of EBV serological data obtained from various laboratories, the anti-EBV working sera have been prepared and are available in lyophilized form. They are sent on request.

Sera collected within the framework of the EBV sero-epidemiological studies, as well as lymphoid lines have also been provided regularly to various national laboratories in response to requests.

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[71] Berkel, A. I., Henle, W., Henle, G., Klein, G., Ersoy, F. & Sanal, O. (1979) *Clin. Exp. Immunol.*, **35**, 196–210.
[72] Hentzen, D., Lenoir, G. M., Berthelon, M. C. & Daillie, J. (1980) *Biochem. biophys. Res. Comm.*, **96**(1), 425–432.
[73] Daillie, J. *et al.* (in preparation).
[74] Tovey, M. G., Lenoir, G. & Begon-Lours, J. (1978) *Nature*, **276**, 270–272.
[75] International Agency for Research on Cancer (1979) *Annual Report, 1979*, Lyon, p. 83.
[76] Ooka, T., Lenoir, G. & Daillie, J. (1979) *J. Virol.*, **29**, 1–10.
[77] Ooka, T. & Calender, A. (1980) *Virology*, **104**, 219–223.

(iv) *Cytogenetic and virological studies on lymphomas: non-endemic BL* (Mrs M. Vuillaume, in collaboration with Dr T. Philip, Centre Léon-Bérard, Lyon, France; Dr G. Bornkamm, Institute for Virology, Health Centre, Freiburg, Federal Republic of Germany; and Dr R. Berger, Hôpital Saint-Louis, Paris)

A new project has been initiated in order to study the cytogenetic markers and the EBV association of non-endemic BL. Although EBV is associated with the great majority of African BL cases, it is found only very sporadically in cases of non-endemic BL. A survey is being made in Europe to estimate the percentage of such cases associated with EBV. Numerous BL cases have already been collected in France, 15 of them being EBV-associated cases[78, 79].

Cytogenetic studies were also performed on some of these cases. The usual t(8;14) translocation found in the majority of African BL so far reported has also been detected in the majority of these cases. However, variant translocations, such as t(2;8) or t(8;22), were found in six cases (Fig. 13)[80–83]. This suggests that Burkitt's lymphoma, independent of its geographic origin or EBV association, is characterized by a non-random cytogenetic change involving the long arm of chromosome 8 (in 8q23-24) and not chromosome 14, as previously thought. The significance of these consistent cytogenetic changes remains to be determined.

3.8 *Tumour-associated antigens* (Dr P. Sizaret)

Methodological problems related to the standardization of tumour-associated antigens have been investigated, and support was given to the organization of two workshops for comparing the various tumour-specific antigens described by various laboratories.

The Agency continues to act as a reference laboratory for α-fetoprotein.

(*a*) *Collaborative studies on tumour-associated antigens*

(i) *Lung tumour-associated antigens* (Dr J. F. Gennings, Charing Cross Hospital, London)

A workshop was organized by the Agency in London to compare, by double gel diffusion and rockett immunoelectrophoresis, 14 test systems described by 11 investigators for the assay of lung tumour-associated antigens. It was found that:

— four of the test systems were inactive;
— one of the test systems detected wheat-germ agglutinin, a contaminant of the antigen preparation;
— five of the test systems corresponded to antigens that are already known: carcinoembryonic antigen (CEA) in three, ferritin in one and lactoferrin in another;

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[78] Lenoir, G. & Philip, T. (1979) *Nouv. Presse med.*, **8**, 4017.
[79] Lenoir, G., Philip, T., Bornkamm, G. W., Gillet, P., Bryon, P. A., Bouvier, R., Dodat, H., Doré, J. F., Brunat-Mentigny, M. & Hermier, M. (1979) *Nouv. Presse med.*, **8**, 4031–4034.
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— four of the test systems corresponded to apparently new antigens. These were provided by Dr A. N. Ibrahim (USA), Dr S. Ikeda (Japan), Dr J. F. Gennings (UK) and Dr R. E. Nordquist (USA).

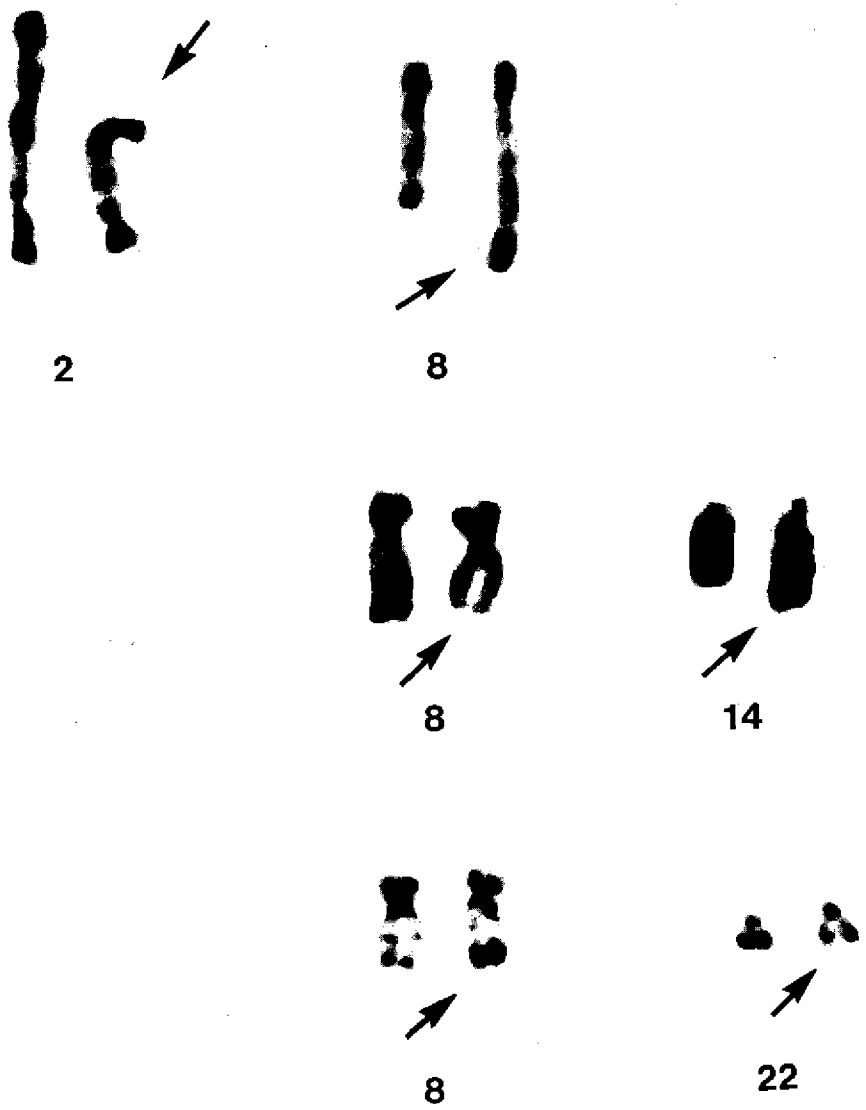

Fig. 13 Partial karyotype of Burkitt's lymphoma cells, showing the three types of translocations observed in this tumour: t (2;8) (p 12;q 23), t (8;14) (q 23;q 32) and t (8;22) (q 23;q 12) (Provided by Mrs S. Bertrand, Centre Léon-Bérard, Lyon)

4. PROGRAMMES OF ENVIRONMENTAL CARCINOGENESIS AND HOST FACTORS AND OF ANALYSIS OF ENVIRONMENTAL CARCINOGENS (Dr H. Bartsch)

4.1 *Introduction*

With the reorganization of the Agency in the beginning of 1980, parts of the previous units of Chemical Carcinogenesis and of Environmental Carcinogens were combined. This change is expected to facilitate the development and application of analytical methods that are more closely related to studies in chemical carcinogenesis and the establishment of correlations between human exposure to chemicals and increased cancer incidence.

One of the main activities of this programme is therefore to elaborate and standardize methods for the detection and analysis of carcinogens in the human environment. New methods are being developed for detecting non-volatile nitrosamines in biological specimens, collaborative studies to ensure the reliability of available methods are being organized, and a series of manuals entitled *Selected Methods for Analysis of Carcinogens* is being published.

Special emphasis is laid on developing methods for estimating the formation of *N*-nitroso compounds in human body fluids *in vivo*. These techniques should be applicable to monitoring human population groups in whom the endogenous formation of *N*-nitroso compounds has been associated with a high risk of cancer at specific sites. Because many factors are known to inhibit or catalyse the *N*-nitrosation of precursor amines by nitrite, polyphenolic compounds that occur commonly in foodstuffs and beverages are also being studied *in vitro* and *in vivo*.

Isolation of biologically active compounds from complex mixtures to which man is exposed and identification of their structures and biological properties is being pursued by a combination of appropriate analytical methods and short-term tests for the detection of carcinogens/mutagens. Such compounds include samples of pyrolysis products of opium, residues of alcoholic beverages consumed in Brittany and chewing material from India.

Research is also being devoted to the development of simple procedures for destroying carcinogenic waste from laboratories, and to ensuring that the few methods proposed in the literature do not in fact produce more toxic or harmful end products.

In the Programme of Environmental Carcinogenesis and Host Factors, studies on carcinogen metabolism both *in vitro* and *in vivo* are being continued, with emphasis on inter-species and inter-individual differences in animals and humans. Because of the limitations of currently available short-term tests for the detection of carcinogens/mutagens, research is being devoted to improving them and to developing better systems for determining the genotoxicity of pure chemicals or of environmental mixtures. Because DNA is probably a critical target in chemical carcinogenesis, studies are being made of carcinogen-DNA adducts and of their structure and biological effects (e.g., repair and mutagenesis).

The overall goal of these two programmes is to identify carcinogenic chemicals that occur in the human environment or are generated *in vivo*, and to develop better criteria for assessing the significance of experimental results in terms of predicting risks posed by carcinogens to humans.

4.2 *Methods of analysis for environmental carcinogens*

(*a*) N-*Nitrosamine analysis (collaborative studies)* (Dr M. Castegnaro and Mr E. A. Walker)

(i) *Volatile* N-*nitrosamines in pesticide formulations*

Nineteen laboratories in 10 countries participated in validating methods for the analysis of volatile *N*-nitrosamines in a herbicide formulation. Two samples containing about 10 ml of a formulation of dimethylamine salt of metachlorphenoxyacetic acid (MCPA) were distributed to the participants. These samples contained *N*-nitrosodimethylamine (NDMA), present as a contaminant, and *N*-nitrosodipropylamine (NDPA), added as an internal reference at a concentration of 0.975 mg/l. Each laboratory could choose its own method but was required to use the same one for every sample. The statistical evaluations of their results are presented in Table 11.

Table 11. Means and statistical parameters of determinations of nitrosamines in a pesticide formulation[a]

Method		NDMA	NDPA amount added 0.975 mg/l	
All methods	Mean (mg/l)	3.14	1.02	
	Standard deviation (mg/l)	1.37	0.24	
	Corresponding coefficient of variation	43.6%	23.5%	
(i) Methods using direct extraction with dichloromethane and GC/TEA	Mean (mg/l)	3.34	1.11	
	Standard deviation (mg/l)	1.2	0.31	
	Corresponding coefficient of variation	35.9%	27.9%	
(ii) Methods using distillation from mineral oil and GC/TEA	Mean (mg/l)	2.73	0.95	
	Standard deviation (mg/l)	1.24	0.21	
	Corresponding coefficient of variation	45.4%	22.1%	
(iii) Methods using adsorption of the sample on a column and GC/TEA	Mean (mg/l)	2.86	0.97	
	Standard deviation (mg/l)	0.48	0.07	
	Corresponding coefficient of variation	16.8%	7.2%	

[a] *Abbreviations: NDMA* - *N*-nitrosodimethylamine; NDPA - *N*-nitrosodipropylamine; GC/TEA - gas chromatography with thermal energy analysis

Surprisingly, considering the relatively high levels of contamination, which were about 10^3 times the levels in food, the overall interlaboratory precision was poor. The extraction techniques grouped under (i) and (ii) in the table do not appear to offer better results than the grouped methods: group (i) gives results about 20% higher than the average value obtained by the other methods. Statistical analysis of results from group (iii) show that these methods are the most suitable for this type of substrate.

(ii) *Volatile* N-*nitrosamines in malt* (in collaboration with Dr P. Scriban, National College of Agricultural and Food Industries (ENSIA), Douai, France)

A collaborative study to determine volatile nitrosamines in malt samples has been initiated, in order to produce an acceptable international standard reference method. The study will be conducted in three phases:

— phase I: comparative study of methods, from which one method should be selected for further study. If the statistical evaluation does not indicate a preferred method, the choice will be made by a suitable committee.
— phase II: collaborative study by participating laboratories of the method of choice.
— phase III: a study on bulk sample homogeneity to establish proper sampling techniques, using the method of choice.

Phase I has been completed. Each laboratory received four samples (two duplicates, A and D and B and C, of ground malt naturally contaminated with NDMA and *N*-nitrosopyrrolidine (NPYR) prepared at ENSIA. The homogeneity of each batch was checked by analysing 10 random samples from each in the Agency laboratory. The results are presented in Table 12.

Table 12. Analyses of 10 randomly chosen malt samples for nitrosamine homogeneity

Nitrosamine[a]	Samples A/D			Samples B/C		
	Mean (μg/kg)	Standard deviation (μg/kg)	Coefficient of variation (%)	Mean (μg/kg)	Standard deviation (μg/kg)	Coefficient of variation (%)
NDMA	7.04	0.7	9.9	36.45	1.74	4.8
NPYR	0.52	0.5	96	1.6	0.35	21.9

[a] NDMA – *N*-nitrosodimethylamine; NPYR – *N*-nitrosopyrrolidone

The statistical evaluations are presented in Table 13. Since only two laboratories used extraction from wort to analyse NPYR, the efficiency of the technique for that compound could not be evaluated; however, statistical evaluation of the results for NDMA suggest that those methods using extraction from wort would give the best precision. This is the method that has been selected for phase II.

(*b*) *International mycotoxin check sample programme* (Dr M. Friesen, Mr E. A. Walker, Mrs L. Garren and Miss Y. Granjard)

This programme provides an opportunity for laboratories around the world engaged in the analysis of mycotoxins in various foodstuffs to compare their results with those of other laboratories. Participants analyse identical portions of a homogeneous sample for a given mycotoxin using the method of their choice. Results are then statistically evaluated, and frequency distribution curves of the results are prepared and circulated to the participants. Three such sets of frequency distribution curves are shown in Figs 14, 15 and 16, for the concentrations of aflatoxins B_1, B_2, G_1 and G_2 found in samples of raw peanut meal, white corn meal and finished peanut butter.

Table 13. Statistical evaluation of results of analyses of *N*-nitrosodimethylamine in malt samples

Method	Samples A/D			Samples B/C		
	Mean (μg/kg)	Standard deviation (μg/kg)	Corresponding coefficient of variation (%)	Mean (μg/kg)	Standard deviation (μg/kg)	Corresponding coefficient of variation (%)
All methods	6.23	1.97	31.6	34.2	9.62	28.1
Methods using distillation from a mineral oil and GC/TEA[a]	6.87	1.93	28.1	34.76	10.37	29.8
Methods using extraction from wort and GC/TEA	6.54	1.4	21.4	37.48	8.28	22.1
Methods using extraction from wort, excluding outliers, GC/TEA	6.05	0.59	9.8	36.07	5.34	14.8
Methods using vacuum distillation and GC/TEA	6.28	1.37	21.8	35.77	9.29	25.9

[a] GC/TEA – gas chromatography with thermal energy analysis

In the current programme, samples of raw peanut meal, yellow corn meal and deoiled peanut meal are to be analysed for aflatoxins B_1, B_2, G_1 and G_2. A sample of aflatoxin M_1-contaminated cows' milk will also be included for analysis. A sub-group of results from the analysis of these four samples forms the basis for a quality assurance check of results from laboratories participating in a food and animal feed contamination monitoring programme sponsored jointly by FAO and WHO.

Lastly, in response to a large number of resquests, a series involving a mycotoxin other than aflatoxin is underway, and samples of animal feed (barley) are being distributed for analysis for ochratoxin A.

(*c*) *Manuals of selected methods for analysis of environmental carcinogens* (Dr L. Griciute, Dr M. Castegnaro and Mr E. A. Walker)

(i) *Polycyclic aromatic hydrocarbons*

A manual of methods for these compounds was published in spring 1980[91].

[91] Castegnaro, M., Bogovski, P., Kunte, H. & Walker, E. A., eds (1979) *Environmental Carcinogens—Selected Methods of Analysis*, Vol. 3, *Polycyclic Aromatic Hydrocarbons (IARC Scientific Publications No. 29)*, Lyon.

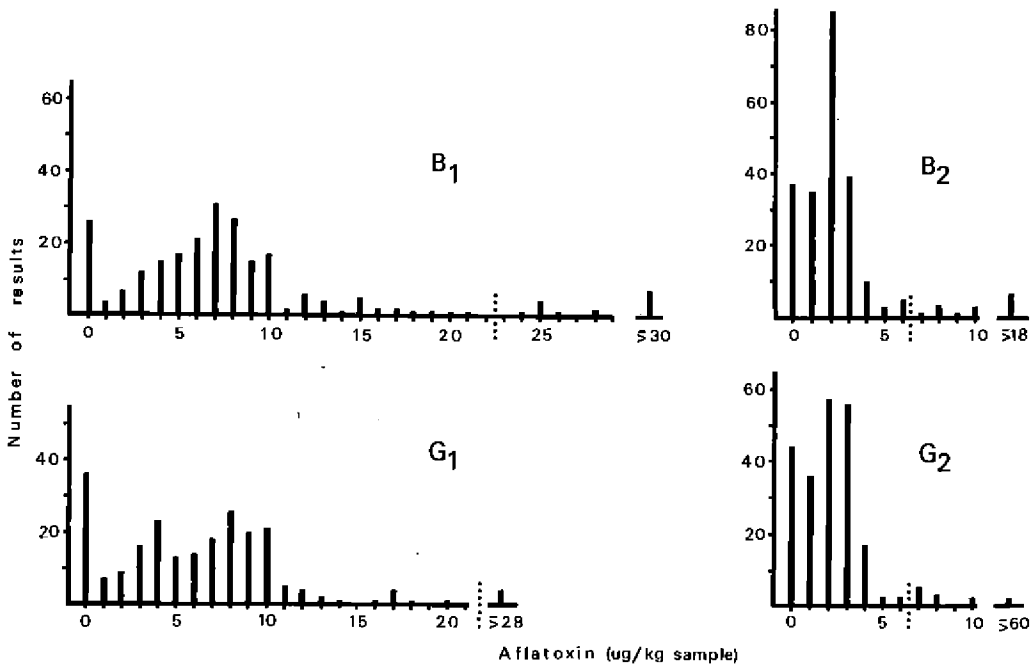

Fig. 14 Frequency distribution curves of results of analysis of aflatoxins B_1, B_2, G_1, G_2 in raw peanut meal

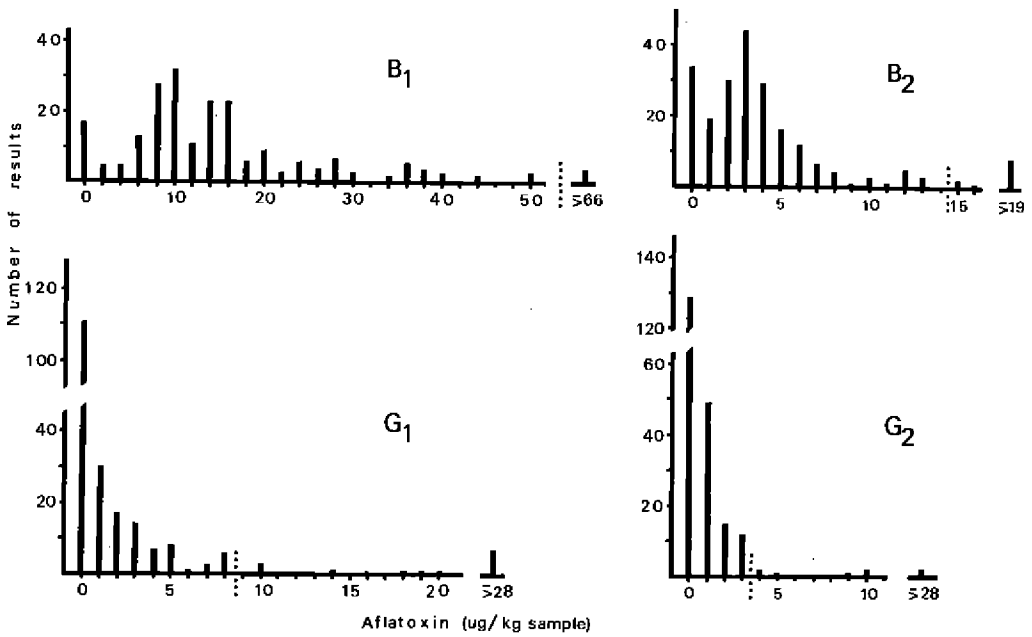

Fig. 15 Frequency distribution curves of results of analysis of aflatoxins B_1, B_2, G_1, G_2 in white corn meal

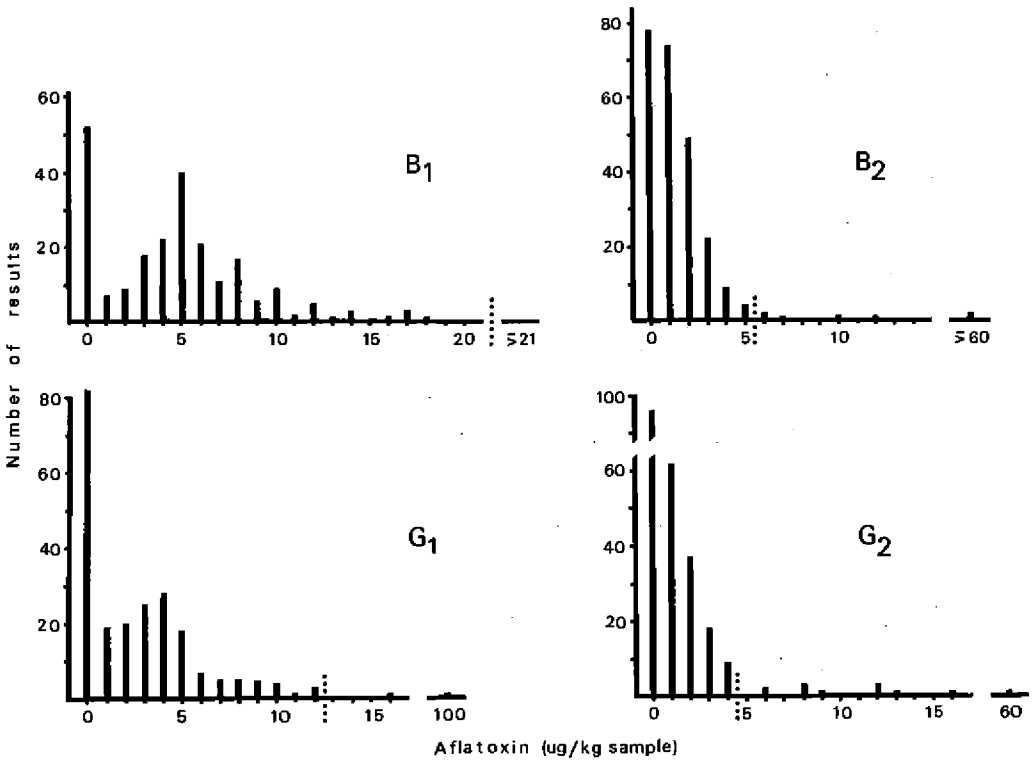

Fig. 16 Frequency distribution curves of results of analysis of aflatoxins B_1, B_2, G_1, G_2 in finished peanut butter

(ii) *Fifth meeting of the editorial board*

The fifth meeting of the editorial board was held in Lyon on 7–8 November 1979 (chairman: Professor H. Egan, Laboratory of the Government Chemist, London). Priorities for the next publication were reviewed, and it was agreed:

1) to compile a supplement to Volume 1 on nitrosamines (Volume 1, part 2), in which the range of substrates considered will be extended to include air, alcoholic beverages, cosmetics, cutting fluids, pesticides and drugs, etc., and the range of nitroso compounds to include nitrosamides and some non-volatile nitrosamines. Dr R. Preussmann was invited to chair the review board.

2) to prepare a report on the content of the manual on mycotoxins. Dr L. Stoloff was invited to chair the review committee, and to propose members including Dr P. L. Schuller and Dr N. Crosby.

3) to organize a review board, chaired by Dr L. Fishbein, for the preparation of a manual on aromatic amines. Dr N. Crosby agreed to join the review board in connection with colouring materials.

Other subjects were discussed, e.g., chlorinated dibenzodioxins, other chlorinated compounds, asbestos, diethylstilboestrol, metals and pesticides, on which scientists in the related fields will be asked to report at the next meeting.

(*d*) *Working conference on analysis and formation of* N-*nitroso compounds* (Mr E. A. Walker and Dr M. Castegnaro, in collaboration with Dr M. Börzsönyi, National Institute of Hygiene, Budapest)

A working conference on analysis and formation of *N*-nitroso compounds in the environment was held in Budapest at the Hungarian Academy of Sciences in October 1979. There were 164 participants from 21 countries; 50 papers were presented in plenary session and 21 by poster. The presented papers on *N*-nitroso compounds covered: chemistry, formation, analysis, occurrence and experimental pathology. The proceedings will be published as *IARC Scientific Publications No. 31*[92]. In several sub-committee meetings of invited members, the need was stressed for more research into field analysis of non-volatile *N*-nitroso compounds.

4.3 *Formation of* N-*nitroso compounds and their analysis in body fluids*

Rapid and sensitive methods to estimate the extent of formation of carcinogens, such as *N*-nitrosation products, in humans and experimental animals *in vivo* are lacking. One objective of this programme is to develop such assays and to use them to analyse carcinogens in human body fluids.

(*a*) *Methods for monitoring nitrosation reactions* in vivo (Mr H. Ohshima, Mr J. C. Béréziat and Miss M. C. Bourgade)

The endogenous formation of carcinogenic *N*-nitroso compounds from ingested precursors has been proposed as the largest single source of exposure to these carcinogens in the general population. The extent to which nitrosation reactions occur in humans, with typical levels of ingestion of nitrate, nitrite and nitrosatable amino compounds, is not, however, known. A study has therefore been directed to developing simple and highly sensitive methods for monitoring nitrosation *in vivo*.

N-Nitrosamino acids such as *N*-nitrosoproline (NPRO) and *N*-nitrosohydroxyproline have been reported to be non-carcinogenic and non-mutagenic[93, 94]. They do not appear to be metabolized *in vivo* and are excreted directly into the urine: more than 80% of NPRO administered orally to rats was excreted unchanged in the urine within 24 hours. On the basis of this finding, two experiments have been conducted:

1) Low doses of nitrite and proline were fed to rats, and increased levels of NPRO were detected in the 24-hour urine.

2) Red beet juice (a source of nitrate) and proline or conventional foods were administered simultaneously to a human volunteer, who showed a marked increase in urinary NPRO. In a parallel experiment with the additional ingestion of ascorbic acid, formation of NPRO was completely inhibited (Fig. 17).

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[92] Walker, E. A., Castegnaro, M., Griciute, L. & Börzsönyi, M., eds (1980) *N-Nitroso Compounds: Analysis, Formation and Occurrence (IARC Scientific Publications No. 31)*, Lyon.

[93] International Agency for Research on Cancer (1978) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 17, *Some* N-*nitroso compounds*, Lyon.

[94] Mirvish, S. S., Bulay, O., Runge, R. G. & Patil, K. (1980) *J. natl Cancer Inst.*, **64**, 1435–1442.

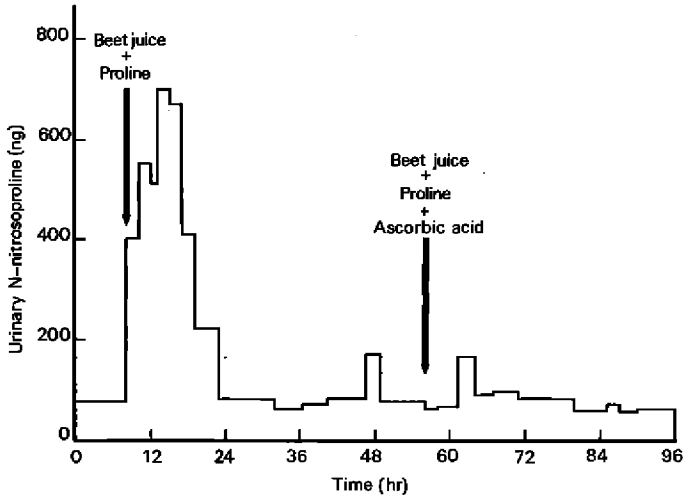

Fig. 17 *In vivo* formation and urinary excretion of *N*-nitrosoproline in man after ingestion of beet juice and proline. DL-Proline (500 mg) and beet juice (200 ml) containing 260 mg nitrate were ingested with or without ascorbic acid (1 g). *N*-Nitrosoproline was extracted from the urine with ethyl acetate and was determined by gas chromatography-thermal energy analysis after formation of its methyl ester derivative

Thus, monitoring of *N*-nitrosamino acids in human urine could provide a quantitative estimation of nitrosation *in vivo*.

(*b*) *The influence of phenolic compounds on formation of* N-*nitrosodiethylamine (NDEA)* in vivo (Mrs B. Pignatelli and Mr E. A. Walker)

In detailed experiments on *in vitro* formation of NDEA, phenol and resorcinol have been shown to exert a catalysing effect through their *C*-nitroso derivatives, *para*-nitroso-phenol[95] and 2,4-dinitrosoresorcinol[96] which are readily formed by reaction with nitrous acid. Initial rate studies showed that the catalysed reaction is first-order with respect to amine, nitrite and catalyst. Table 14 illustrates a comparison between the rates of reaction in aqueous and in aqueous/organic solvents for the two nitrosophenols. Studies involving ^{15}N-labelled nitrite undertaken to explore the role of the *C*-nitroso groups of *para*-nitrosophenol and dinitrosoresorcinol in the catalytic mechanism have indicated that nitrosation does not involve transfer of the *C*-nitroso group, and two possible mechanism have been suggested[97].

Sulfamic acid has been used to stop nitrosation at the required time by destroying excess nitrite. The inhibiting effect displayed by phenols such as catechol has been explained in terms of consumption of nitrite in an oxidation process. However, experiments have been performed using

[95] Walker, E.A., Pignatelli, B. & Castegnaro, M. (1979) *J. Agric. Food Chem.*, **27**, 393–396.

[96] Pignatelli, B., Friesen, M. & Walker, E. A. (1980) In: Walker, E. A., Castegnaro, M., Griciute, L. & Börzsönyi, M., eds, *N-Nitroso Compounds Analysis, Formation and Occurrence (IARC Scientific Publications No. 31)*, Lyon, International Agency for Research on Cancer, pp. 95–109.

[97] Walker, E. A., Pignatelli, B. & Friesen, M. (1980) (submitted for publication).

Table 14. Comparison between the rates of reaction in aqueous and in aqueous/organic solvents for two nitrosophenols:

$$v = \frac{d\,[N\text{-nitrosodiethylamine}]}{dt} = k'[\text{Nitrite}]^2\,[\text{Amine}] + k''\,[\text{Nitrite}]\,[\text{Amine}]\,[\text{Nitrosophenol}]$$

	Rate constant ($\frac{mol}{l}$)$^{-2}$ sec^{-1}]		
	Aqueous solution pH = 3.85	Aqueous solution containing 10% acetone pH 4	Aqueous solution containing 10% dimethylformamide pH 4.05
In absence of phenol (k')	$0.25 = 10^{-3}$	0.4×10^{-3}	$0.36 \times^{-3}$
In presence of *para*–nitrosophenol (k''_p)	0.018	0.025	
In presence of dinitrosoresorcinol (k''_d)	0.233		0.285

alkali to stop the reaction, and under these conditions the yield of NDEA has been considerably increased in the presence of catechol or hydroquinone and gallic, caffeic, ferulic or chlorogenic acids. This effect is illustrated with catechol in Table 15. It is evident that the amount of NDEA formed before addition of alkali is dependent on the length of time of reaction, increasing to a maximum at about 15–20 minutes and subsequently decreasing (horizontal data). The concentration of NDEA also depends on the reaction time with alkali (vertical data).

Table 15. The effect of alkali on formation of *N*-nitrosodiethylamine (NDEA) in the presence of catechol

	[NDEA] $\times 10^{-5}$ $\frac{mol}{l}$ Reaction time (min) (Reagents: amine/nitrite/catechol)				
	5	10	15	20	30
Control (catechol absent)	1	1.98	2.99	3.96	5.90
Reaction time after addition of alkali (min)					
0	0.68	1.24	1.68	2.10	—
1	14.31	25.60	31.25	28.62	13.55
3	30.19	—	53.54	41.58	14.24
20	35.20	52.98	49.81	63.35	53.27

Reaction conditions: Temperature: 37° C
Aqueous medium: pH 3.85
[DEA, HCl] 0.5 M
[$NaNO_2$] 0.016 M
Catechol 0 or 0.004 M

An experiment has been carried out in which catechol, nitrite and buffer have been mixed and the solution, at pH 4, has been sampled at intervals. Each sample has been made alkaline, and diethylamine hydrochloride has then been added to a final pH of 11. Once again, NDEA was formed in the alkaline medium. There are insufficient data at this stage to explain the formation of NDEA under alkaline conditions.

Experiments carried out with hydroquinone and quinone suggest that an intermediate formed by reaction between hydroquinone and nitrous acid can itself react with the secondary amine under alkaline conditions to form the nitrosamine; furthermore it is unlikely that this proceeds through the expected oxidation to quinone. Whatever the mechanism is, it is important to bear in mind that, in the presence of a number of naturally occurring phenols, such as ferulic, caffeic and chlorogenic acids, the addition of alkali during the course of analysis could produce artefactual formation of nitrosamines if nitrosatable precursors are present.

(*c*) N-*Nitrosamines in gastric juice, blood and urine in relation to gastric cancer and precancerous lesions* (Mr E. A. Walker, Mr H. Ohshima, Mrs B. Pignatelli, Dr M. Castegnaro and Miss M. C. Bourgade, in collaboration with Dr N. Muñoz, Division of Epidemiology and Biostatistics; Professor M. Crespi, Regina Elena Institute, Rome; and Dr C. L. Walters, British Food Manufacturing Industries Research Association, Leatherhead, UK)

A pilot study on the content of volatile nitrosamines in gastric juices of patients suffering from chronic atrophic gastritis[98] has been continued. The current evaluation of the results appears to show a higher formation of *N*-nitrosodimethylamine (NDMA) in patients than in controls (see also section 3.5 of the report of the Division of Epidemiology and Biostatistics).

No *N*-nitrosamines were detected in the blood samples, and the levels in urine were highly variable among both patients and controls.

(*d*) *Nitrosamines in urine in relation to nephropathy and bladder cancer* (Dr M. Castegnaro and Miss M. C. Bourgade, in collaboration with Dr I. N. Chernozemsky, National Centre of Oncology, Sofia)

Twenty-two urine samples from patients with nephropathy and from healthy controls were analysed for their content of volatile nitrosamines. Several nitrosamines were detected in concentrations up to 270 ng/l; however, levels were very variable in both groups, and no relationship between disease and nitrosamine levels has been established.

(*e*) *Methods for stabilization of* N-*nitroso compounds in biological fluids* (in collaboration with Dr G. Eisenbrand, Dr B. Spiegelhalder and Dr R. Preussmann, German Cancer Research Center, Institute of Toxicology and Chemotherapy, Heidelberg, Federal Republic of Germany)

Methods for the preservation of body fluids to be analysed for nitrite and *N*-nitroso compounds have been further developed. Urine samples fortified with either sodium nitrite

[98] International Agency for Research on Cancer (1979) *Annual Report, 1979*, Lyon, pp. 61–62.

(140 ppm = 2×10^{-3} M), with sodium nitrite and morpholine (90 ppm = 10^{-3} M, free of *N*-nitrosomorpholine) or with *N*-nitrosomorpholine (NMOR) alone (40 ppb) were subjected to the action of different preserving agents; ascorbic acid, amidosulfonic acid, sodium azide and sodium hydroxide. All test mixtures, except those which received sodium hydroxide, were adjusted to pH 4 in order to ensure comparable reaction conditions. All samples also received 20 mg/l merthiolate (Thiomersal, [(*ortho*-carboxylphenyl)-thiol]-ethylmercury).

The samples were analysed by standard methods (two to three parallel determinations) either immediately after mixing or after five days' storage at 37°C. Results of these investigations are summarized in Table 16. None of the urine samples fortified with nitrite alone showed any indications of nitrosamine, irrespective of how they were preserved and stored. It can be concluded that neither ascorbic acid nor amidosulfonic acid is a satisfactory preserving agent, but both sodium azide ($\geq 10^{-2}$M) and sodium hydroxide (10^{-1}M) are effective inhibitors of nitrosamine formation. The latter are also good preservatives for nitrosamines contained in urine.

Table 16. *N*-Nitrosomorpholine levels detected (μg/l) under various conditions of preservation of urine samples fortified with 140 mg/l of sodium nitrite and 90 mg/l of morpholine

Duration of experiment (days)	type of preserving agent \ Level of preserving agent (M)	0	5×10^{-3}	10^{-2}	5×10^{-2}	10^{-1}	1
0	none	4					
5	none	600					
0	ascorbic acid			5-7	5-7		26
5	ascorbic acid			118	64	33	26
0	amidosulfonic acid			319	9	15	5
5	amidosulfonic acid			809	119	54	6
0	sodium azide		4	ND[a]	ND	ND	ND
5	sodium azide		14	ND	ND	ND	ND

[a] ND – not detected

However, sodium hydroxide has the disadvantage that it decomposes nitrosamides that might be present. Sodium azide was therefore investigated for use as a preservative of nitrosamide-containing urine. Identical concentrations of nitrite were used, and 1,3-bis(2-chloroethyl) urea was added to urine and buffered to pH 4 as an easily nitrosatable urea at a concentration of 10^{-3} M (185 ppm). 1,3-Bis-(2-chloroethyl)-1-nitrosourea (BCNU) was added at 10 ppm to test the stability of this relatively unstable nitrosourea under the selected conditions. 1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) served as an internal standard. Samples were analysed after extraction with dichloromethane by high-performance liquid chromatography (with ultra-violet detection) with hexane/ethanol (98+2) on silica gel. Analyses were carried out either immediately after mixing or after five days' storage at –30°C.

In the absence of sodium azide, 700 ppb BCNU had formed after five days' storage. In the presence of sodium azide, nitrosourea formation was completely inhibited. Recoveries of added BCNU and CCNU after five days at –30°C were 81 and 95%, respectively. At 37°C, 85% of CCNU, but only 8% of BCNU was recovered after incubation for only one day.

These results show that sodium azide (10^{-2}M) can be used for preservation of body fluids to be analysed for *N*-nitroso compounds. At that concentration, it not only inhibits artefact formation but also keeps *N*-nitroso compounds, when present, stable. When storage at low temperatures is not possible, only nitrosamines are stable enough to be analysed. *N*-Nitrosoamides of the type used in this investigation can be detected only when low-temperature storage is possible.

4.4 *Analysis of carcinogens in environmental samples*

Environmental samples are being analysed for exogenous *N*-nitroso compounds to provide data for use in conjunction with epidemiological studies to identify etiological factors involved in human carcinogenesis.

(*a*) *Analysis of* N-*nitrosamines in extracts of food collected in the Transkei* (Mr E. A. Walker and Dr M. Castegnaro, in collaboration with Dr J. Nunn and Dr S. J. Van Rensburg)

Extracts of food collected in the Transkei for a study of oesophageal cancer were analysed at the Agency by gas chromatography-thermal energy analysis. Of 204 extracts analysed, 114 were found to contain nitrosamines. Those most commonly found (Table 17) were *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodipropylamine (NDPA) and one unknown compound with a retention time slightly longer than that of NDEA. The latter compound (X) was tentatively identified, on the basis of retention time and oxidation to nitramine, as *N*-nitrosomethylpropylamine. Other nitrosamines detected in a few samples were *N*-nitrosodibutylamine (NDBA), *N*-nitrosomethylpentylamine (NMPA), *N*-nitrosopiperidine (NPIP), *N*-nitrosopyrrolidine (NPYR) and a compound (Y) with a retention time slightly longer than that of NDPA. The latter compound was tentatively identified, on the basis of retention time and oxidation to nitramine, as *N*-nitrosomethylbutylamine[99].

Table 17. *N*-Nitrosamines in extracts of food samples collected in the Transkei

N-Nitrosamine detected[a]	No. of positive samples	Maximum level (ng/sample)	Minimum level (ng/sample)
NDMA	107	1075	$\leqslant$ 0.2
NDEA	78	1760	$\leqslant$ 0.2
NDPA	32	319	$\leqslant$ 0.2
NDBA	11	78	3.3
NMPA	4	12.5	1
NPIP	7	85	0.7
NPYR	1	2.9	
X	49	483[b]	$\leqslant$ 0.2
Y	16	680[c]	$\leqslant$ 0.2

[a] See text for explanation of acronyms
[b] Calculated as NDEA
[c] Calculated as NDPA

[99] Walker, E. A. & Castegnaro, M. (1980) *J. Chromatogr.*, **187**, 229–231.

These results are currently being evaluated with respect to food consumption patterns and cancer incidence.

(*b*) *Analysis of nitrosamines in animal feed* (Dr M. Castegnaro and Miss M. C. Bourgade, in collaboration with Dr B. Teichmann, Academy of Sciences of the German Democratic Republic, Berlin-Buch)

In connection with animal experiments on the combined action of low levels of chemical carcinogens, samples of animal feed, collected monthly, were analysed for their nitrosamine content and for the heterogeneity of the samples. NDMA (2.5–71 μg/kg) and NPYR (0.5–15 μg/kg) were detected in all samples, and other nitrosamines were detected at the μg/kg level. The variation of nitrosamine content, as shown by analysis of individual pellets, never exceeded a four-fold difference within a batch.

(*c*) *Analysis of nitrosamines in tobacco smoke condensate* (Dr M. Castegnaro; in collaboration with Professor R. Truhaut, Faculté de Pharmacie, Université de Paris, Paris)

Use of a clean-up method described by Walker *et al.*[100] proved satisfactory for purifying tobacco smoke condensate. However, some problems of reproducibility were encountered; and samples were therefore purified by washing with *n*-pentane prior to extraction with dichloromethane.

(*d*) *Analysis of nitrosamines in malts* (Dr M. Castegnaro and Mrs I. Brouet, in collaboration with Professor R. Scriban, National College of Agricultural and Food Industries, Douai, France, and supported by the Federation of the French Malting Industry, Paris)

The objective of this study[101] was to determine those factors that affect formation of nitrosamines in the manufacture of malt and thus to provide means for reducing the levels of nitrosamines in malt and in its products, particularly beer. The influence of the following factors has been evaluated: variety of barley; type of kiln; type of ventilation; heating mode; type of feed; type of burner; fuel consumption; geographical situation of the factory; thickness of the malt layer; humidity before kilning; starting temperature for kilning; drying temperature; drying period; roasting period; sulfuring (amount of sulfur, starting time of sulfuring, length of sulfuring period); total proteins; soluble proteins; humidity after kilning; polyphenol content; α-amino nitrogen content; and colouration of malt after kilning.

Over 400 samples for which the above parameters had been determined were analysed for their volatile nitrosamine content. Another group was investigated to analyse the effect of increased sulfuring. Statistical evaluation of the preliminary results indicated that the following factors have a significant influence:

(i) The ratio of soluble proteins to total proteins (Kolback index) is positively correlated with the NDMA content of the malt, and negatively correlated with the α-amino nitrogen content.

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[100] Walker, E. A., Castegnaro, M. & Pignatelli, B. (1975) *Analyst*, **100**, 817–821.

[101] International Agency for Research on Cancer (1979) *Annual Report, 1979*, Lyon, pp. 68–69.

(ii) Lower levels of NDMA are produced when using indirect heating than when using direct heating. This is considered to be due to direct contact between oxides of nitrogen in the flue gas and the malt. However, NDMA may be formed even with indirect heating if there is environmental pollution by nitrogen oxides.

(iii) Two-tray kilns with low loading tend to produce higher levels of nitrosamines than single-tray types with high loading. Sulfuring (i.e., introduction of sulfur dioxide into the flue gas by combustion of sulfur) drastically decreases nitrosamine formation. Optimum amounts of sulfur are between 200 and 300 g per tonne of barley.

(iv) Nitrosamine formation is directly correlated to the type of heating fuel, higher concentrations being obtained with gas. A final evaluation of the results is in progress.

A limited study has also been undertaken to evaluate the relationship between the nitrosamine content of malt and that of the beer produced from it. Although dependent on the technology used, an average ratio of nitrosamine concentration appears to be 1:10 beer:malt.

Addition of polyphenols before pasteurization of beer, and ageing have not been found to lead to significant changes in nitrosamine content.

4.5 *Destruction of carcinogenic waste from laboratories and safe handling of carcinogens* (Dr M. Castegnaro and Mr E. A. Walker)

Implementation of this programme involves:

(*a*) collection of available data relative to degradation techniques and chemistry of carcinogens;

(*b*) critical evaluation of this bibliography and production of a monograph which includes suggested methods;

(*c*) evaluation in the laboratory of the proposed methods and, when necessary, elaboration of new methods;

(*d*) initiation of collaborative studies to ascertain the efficiency of the methods; and

(*e*) critical review, by a meeting of experts, of the document before final publication by the Agency.

(*a*) *Collection of data* (Mr H. Baxter, London)

Literature has been retrieved on the following compounds: polycyclic aromatic hydrocarbons, nitrosamines, nitrosamides, chloroethers, aminoflurorene derivatives, polychlorinated biphenyls, hydrazines, vinyl chloride, aflatoxins. Over 1500 papers have been collected, mainly by hand searching as search by computer was found to be inadequate.

(*b*) *Evaluation of bibliography and preparation of individual monographs*

(i) A draft monograph on decontamination of wastes contaminated by aflatoxins has been prepared. It contains five methods, writtin in the ISO format, which have been evaluated in the laboratory. Completion of this document awaits results of a collaborative study involving seven national laboratories.

(ii) An intermediate document on decontamination of wastes which might be contaminated by seven volatile nitrosamines has been prepared. Four methods have been evaluated and are being written in the ISO format.

(iii) An intermediate document on decontamination of nitrosamide-contaminated wastes has been prepared.

(iv) A document on polycyclic aromatic hydrocarbons is under evaluation.

(c) *Evaluation and development of methods*

(i) *Aflatoxins* (Dr M. Castegnaro, Miss J. Michelon, Mr C. Malaveille, Mrs A. Hautefeuille, in collaboration with Dr P. L. Schuller and Dr H. Van Egmond, National Institute of Public Health, Bilthoven, The Netherlands)

Seven methods have been investigated for the decontamination of aflatoxin-contaminated wastes. Two previously proposed methods (i.e., treatment with caustic soda and treatment with sodium carbonate), which lead to reversible formation of aflatoxins after neutralization, were rejected. Another (i.e., treatment with sodium hypochlorite solutions), led to the formation of the carcinogenic 2,3-dichloro aflatoxin B_1; this method has therefore been modified by subsequent treatment with acetone [102]. It has been adapted to treatment of stock quantities; solutions in water, lipids and organic solvents; residues on Petri dishes; glassware; protective clothing; spills; and thin-layer chromatography plates. The four other methods, listed below, proved to be satisfactory in reducing aflatoxins by more than 99%:

— lime treatment for animal carcasses
— potassium permanganate oxidization for stock quantities of solutions in water, lipids and organic solvents
— ammoniation for animal litter
— dichromate-sulfuric acid treatment for glassware.

The efficiency of these processes is being examined in parallel by mutagenicity assays with *Salmonella typhimurium* strains.

(ii) *Nitrosamines* (Dr M. Castegnaro and Miss J. Michelon)

Treatment with dichromate-concentrated sulfuric acid, commonly used in cleaning laboratory glassware, proved to be satisfactory only when treatment was prolonged (i.e., one week). However, by rinsing the glassware five times with an adequate volume of the appropriate solvents, the contamination level is drastically decreased, and subsequent treatment with dichromate-concentrated sulfuric acid may not be required, except in laboratories performing trace analysis at the nanogram range. The rinsing solvents should be stored and treated by the following methods:

— Oxidation using potassium permanganate in acid medium has proved a potentially useful method for treatment of aqueous solutions. Low levels of nitramines which are formed as intermediate products are then further oxidized to unidentified products, which must be investigated for their biological properties and nature.

— Denitrosation by hydrobromic acid for solutions in dichloromethane has been standardized. In contrast to data in the literature, *N*-nitrosopyrrolidine required 90 minutes to be

[102] Castegnaro, M., Friesen, M., Michelon, J. & Walker, E. A. (1980) *J. Am. ind. Hyg. Assoc.* (submitted for publication).

denitrosated by more than 99.5%: 100% in 15 minutes was reported in the literature. Water and alcohol were shown to inhibit the denitrosation reaction of the seven nitrosamines investigated. The most strongly affected were nitrosopyrrolidine and nitrosodimethylamine.

— A 'suggested' treatment by sodium hypochlorite was proved to be ineffective.

— Nitrosamines are converted into relatively stable, non-volatile oxonium salts by treatment with triethyloxonium tetrafluoroborate in non-hydrolytic solvents, such as dichloromethane. This facilitates rapid removal of solvent by distillation. No nitrosamine is recovered after treatment of the residue under reflux with strong caustic soda, probably because nitrones are formed. Provided that the product is not mutagenic, this technique will offer a convenient method for handling solvent residues containing nitrosamines, and recycling of solvent could be made possible.

(iii) *Polycyclic aromatic hydrocarbons* (in collaboration with Dr P. Chambon, Faculté de Pharmacie, Lyon, France; Dr M. Coombs, Imperial Cancer Research Fund, London; and the Johnson Matthey Research Centre, Reading, UK)

Dr Chambon is currently investigating the destruction of polycyclic aromatic hydrocarbons by biodegradation and by photodegradation. However, biodegradation of benzo[*a*]pyrene by bacterial strains has so far proved unsatisfactory: degradation has been of poor reproducibility and incomplete, ranging from 15–75% over a period of a week; and photodegradation by direct exposure to sunlight of benzo[*a*]pyrene suspended in water-Tween 60 solutions has also proved unsatisfactory.

Dr M. Coombs, in collaboration with the Johnson Matthey Research Centre, has undertaken an alternative approach, using catalytic pyrolysis. Laboratory equipment has been designed for this purpose which is more compact and less expensive than incinerators and is applicable to various types of solutions.

(*d*) *Initiation of collaborative studies* (in collaboration with Dr P. L. Schuller and Dr H. Van Egmond, National Institute of Public Health, Bilthoven, The Netherlands)

A collaborative study to evaluate methods for the decontamination of aflatoxin-contaminated wastes involving seven laboratories in France, The Netherlands, the UK and the USA is in progress.

(*e*) *Safe handling of aflatoxins and their solutions* (Dr M. Castegnaro and Miss J. Michelon, in collaboration with Dr P. L. Schuller and Dr H. Van Egmond, National Institute of Public Health, Bilthoven, The Netherlands)

Rubber gloves are not a sufficient protective barrier against some carcinogens[103-106]; their efficiency in preventing aflatoxin transfer from chloroform and dimethyl sulfoxide solutions has now been investigated.

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[103] Walker, E. A., Castegnaro, M., Garren, L. & Pignatelli, B. (1978) In: Walker, E. A., Castegnaro, M., Griciute, L. & Lyle, R. E., eds. *Environmental Aspects of N-Nitroso Compounds (IARC Scientific Publications No. 19)*, Lyon, International Agency for Research on Cancer, pp. 535–543.

[104] Sansone, E. B. & Tewari, Y. B. (1978a) In: Walker, E. A., Castegnaro, M., Griciute, L. & Lyle, R. E., eds. *Environmental Aspects of N-Nitroso Compounds (IARC Scientific Publications No. 19)*, Lyon, International Agency for Research on Cancer, pp. 517–529.

[105] Sansone, E. B. & Tewari, Y. B. (1978b) *J. Am. ind. Hyg. Assoc.*, **39**, 169–174.

[106] Gough, T. A., Webbs, K. S. & McPhail, M. F. (1978) In: Walker, E. A., Castegnaro, M., Griciute, L. & Lyle, R. E., eds. *Environmental Aspects of N-Nitroso Compounds (IARC Scientific Publications No. 19)*, Lyon, International Agency for Research on Cancer, pp. 531–534.

Experiments were carried out with 9 and 4% saline solutions inside a latex glove and a 2 mg/l solution of aflatoxins in chloroform outside the glove. After 15 minutes, the saline was extracted with chloroform and the extract analysed by thin-layer chromatography. All four aflatoxins, B_1, B_2, G_1 and G_2, were detectable. The rate of transfer increased with polarity, and the rate of diffusion was in the order $G_2 > G_1 > B_2 > B_1$. In these experiments, chlorofom was observed to diffuse through the gloves at a rate dependent on their thickness: thick garden gloves ensured a much better protection than surgical gloves.

In a further experiment, a glove filled with a solution of aflatoxin B_1 in chloroform was suspended in a beaker containing chloroform only. The chloroform in the beaker was then analysed periodically. Aflatoxin was found to diffuse rapidly through the glove, until an equilibrium concentration was established.

In a similar experiment using dimethyl sulfoxide as solvent, none of the aflatoxins were found to penetrate the gloves.

Results from these experiments stress the need for care when handling aflatoxin solutions and indicate that gloves should be removed immediately after contact with the solvent.

4.6 *Carcinogen metabolism in human and experimental animal tissues: inter-individual, species and strain differences*

For most chemical carcinogens to which man is exposed, their metabolic activation by enzymes in the body is essential so that they can exert their adverse biological effects. Individual differences in the human population with regard to these enzymic processes may contribute to different susceptibilities to cancer development among individuals exposed to the same level of chemical carcinogen. Thus, studies of qualitative and quantitative inter-individual differences in the metabolic activation/detoxification of carcinogens are relevant. Inclusion of experimental animals in such comparative studies should allow better extrapolation of data from experimental animals to man.

(*a*) *Studies on oxidative benzo*[a]*pyrene (BP) metabolism in normal and tumorous surgical lung specimens from lung cancer patients* (Mrs N. Sabadie, in collaboration with Dr R. Saracci, Surveillance of Environmental Aspects Related to Cancer in Humans, Analytical Epidemiology, IARC; Dr H. B. Richter-Reichhelm and Professor U. Mohr, Hanover Medical School, Department of Experimental Pathology, Hanover, Federal Republic of Germany)

The aim of this study[107] was to assess inter-individual differences in the metabolism of carcinogens by the pulmonary microsomal mono-oxygenase system, which may be a possible host-risk factor in human carcinogenesis[108]. Biochemical investigations on surgical specimens from 105 patients have now been completed.

Oxidative BP metabolism was studied in surgical lung specimens (12 000 × *g* supernatant fractions) obtained from lung cancer patients who had undergone surgical resection. Aryl

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[107] International Agency for Research on Cancer (1979) *Annual Report, 1979*, Lyon, p. 111.

[108] Bartsch, H., Sabadie, N., Malaveille, C., Camus, A. M. & Richter-Reichhelm, H. B. (1979) In: Birch, G. M., ed., *Advances in Medical Oncology, Research and Education*, Vol. 3, Oxford, Pergamon, pp. 179–187.

hydrocarbon hydroxylase (AHH; BP-hydroxylase) activity was determined under conditions of linear production of phenolic metabolites, with respect to time of incubation, protein and substrate concentration, in both normal and tumorous lung tissue specimens from the same patient. The apparent K_M of the pulmonary enzyme ($1\text{-}2.5 \times 10^{-5}$ M) was identical in tumorous and normal tissue, and was only one-tenth of that reported for human liver. High-pressure liquid chromatographic analysis of ethyl acetate-extractable BP metabolites from such assays made it possible to identify 3- and 9-hydroxy-BP, 7,8-,9,10- and 4,5-diols of BP and three quinones of BP (Table 18). The relative proportions of these metabolites were very similar to those in lung tissues from Aroclor-treated rats; as an exception, the 9,10-diol of BP was formed in a higher proportion in the presence of human lung fractions (Table 18).

Table 18. High-pressure liquid chromatographic analysis of benzo[*a*]pyrene (BP) metabolites in normal lung tissue from six lung cancer patients and in rat lung

	$OD_{254} \times 10^2$			
	Human lung		Rat lung	
BP metabolite	Range	Mean	Induced[a]	Non-induced
3 HO-BP	4-30.8	13.9	67.5	12.5
9 HO-BP	0-15.4	7.2	40.5	9.6
Quinones	2.4-48.4	20.0	81.0	35.0
7,8-Diol	2-6.8	4.3	18.2	2.5
4,5-Diol	0-3.6	2.1	9.0	1.8
9,10-Diol	2.7-34	11.9	38.7	2.6
Polar metabolites	3.6-23	10.0	ND[b]	ND

[a] with Aroclor 1254
[b] ND, not detected

A more than 20-fold inter-individual variation in AHH activity was found in the normal lung tissue preparations from these 105 patients; the formation of 3-hydroxy-BP and 9,10-diol in lung tissue from six patients varied by seven- and 13-fold, respectively (Table 18). The total amount of 3-hydroxy-BP and 9-hydroxy-BP formed correlated significantly with the total amount of 7,8-, 9,10- and 4,5-diols of BP formed in the same lung tissue specimen ($r = 0.83$; $P < 0.05$; $n = 6$) (Table 19). The frequency distribution of pulmonary AHH activity in 95 male and 10 female lung cancer patients, in both normal and tumorous tissue, was compatible with a unimodal distribution

Table 19. Correlation studies on amounts of benzo[*a*]pyrene (BP) metabolites formed in normal lung tissue fractions from six lung cancer patients, as measured by high-pressure liquid chromatographic separation techniques

3-HO-BP *vs* BP-7,8-diol	$r = 0.81$; $P < 0.05$
3-HO-BP *vs* BP-9,10-diols	$r = 0.80$; $P < 0.1$
3-HO-BP *vs* BP-7,8-, 9,10- & 4,5-diols	$r = 0.87$, $P < 0.05$
3- & 9 HO-BP *vs* 7,8-, 9,10- & 4,5-diols	$r = 0.83$; $P < 0.05$

of the enzyme activity. A similar plot for 43 patients with squamous-cell carcinoma was also unimodal. In the latter patients, AHH activity in tumorous tissue was correlated negatively with the number of cigarettes smoked per day prior to surgery:

$$\log \frac{\text{AHH (tumour)}}{\text{AHH (normal tissue)}} \quad \textit{vs.}\ \text{cigarette consumption: } (r = 0.18;\ P < 0.10;\ n = 43).$$

(*b*) *Study on AHH inducibility and individual susceptibility to toxic effects of cigarette smoke Department of Pharmacology, University of Oulu, Oulu, Finland* (RA/79/002)
Principal investigator: Dr O. Pelkonen

Cigarette smoking is a major determinant in many human diseases. A considerable proportion of the population is exposed to smoke in their mothers' womb; however, assessment of this exposure is unreliable. Genetically determined susceptibility has also so far remained unexplored. The purposes of the present project are two-fold [109]: 1) by measuring placental enzymic parameters (such as AHH activity) to find a reliable indication of actual exposure of the fetus to cigarette smoke; and 2) by measuring AHH inducibility in peripheral lymphocytes from cord blood, maternal blood and placental cells to determine an individual's genetically determined responsiveness to constituents of cigarette smoke.

In a completed study, pregnant mice of polycyclic aromatic hydrocarbon-responsive (C57BL/6) and non-responsive (DBA/2) strains were exposed to cigarette smoke, and the offspring were studied. Fetuses of cigarette smoke-exposed mothers were smaller than those of control mothers. The decrease in birth weight was greater (although not statistically significant) in responsive mice than in non-responsive mice. In back-cross experiments (B6D2 × D2), it was demonstrated that measurement of placental AHH activity made it possible to discriminate between responsive and non-responsive individuals. These experiments also demonstrated that the A*h*-locus-controlled inducibility of AHH activity was not a factor in the susceptibility of fetuses to cigarette smoke. Nevertheless, placental AHH activity was induced in responsive animals by cigarette smoke [110].

In another study in humans, AHH activity in the placentas of about 250 mothers, both smokers and non-smokers, was studied [111]. The offspring from pregnancies in which placental AHH activity was elevated weighed about 400 g less and were over 1 cm shorter than those from non-smoking mothers, whereas those from AHH-negative pregnancies (despite the similar number of cigarettes smoked daily) were of the same size as those from non-smoking mothers. Thus, placental AHH activity seems to be a sort of index for susceptibility to cigarette smoke-induced effects; but whether it is related to environment (actual exposure) or to genetic background is not known.

In another study in smoking mothers, AHH inducibility in cord blood and in maternal lymphocytes was compared with placental AHH activity. Preliminary results indicate a statistically significant correlection between cord-blood lymphocyte AHH inducibility and

[109] International Agency for Research on Cancer (1979) *Annual Report, 1979*, Lyon, p. 113.
[110] Vahakangas, H., Hirn, M. & Pelkonen, O. (1979) In: *Abstracts of the XVI Scandinavian Congress of Physiology and Pharmacology*, p. 83.
[111] Pelkonen, O., Karki, N. T., Koivisto, M., Tuimala, R. & Kauppila, A. (1979) *Toxicol. Lett.*, 3, 331–335.

placental AHH activity, but no correlation between maternal lymphocyte AHH inducibility and placental AHH activity. This finding seems to support the hypothesis that placenta and cord blood (i.e., fetus) share a common genetic background, with common regulation of AHH inducibility[112].

True exposure to cigarette smoke may be determined in two assays measuring: 1) serum and/or urinary thiocyanate concentration; and 2) urinary excretion of nicotine/cotinine.

Preliminary results indicate that assay 1 is very useful in discriminating between smokers and non-smokers, whereas assay 2 can be used to measure extent of exposure. Studies on the correlation between placental AHH activity and thiocyanate levels in smokers are in progress.

(*c*) *Interrelationships between antipyrine half-life, liver mixed-function oxidase activities and liver microsome-mediated mutagenicity* (Mr C. Malaveille, Mrs A. Hautefeuille and Mrs G. Brun; in collaboration with Professor M. R. Roberfroid, Laboratory of Biotoxicology, Université Catholique de Louvain, Brussels 1200, Belgium) (RA/78/002)

Antipyrine half-life in serum has been correlated negatively with the activity of liver AHH, a key enzyme in the metabolism of polycyclic aromatic hydrocarbons and other carcinogens. Measurement of this parameter *in vivo* could thus test the metabolic activating capacities of individual human subjects.

The aim of the present research was to correlate antipyrine half-life in mouse serum by radioimmunoassay according to Chang *et al.*[113] with the liver microsomal activity of either benzo[*a*]pyrene hydroxylase by the spectrofluorimetric assay of Dehnen *et al.*[114] or arylamide *N*-hydroxylase by the gas-liquid chromatographic method of Razzouk[115] and with the liver microsome-mediated mutagenicity of aflatoxins B_1, benzo[*a*]pyrene-7,8-dihydrodiol, *N*-nitrosomorpholine and 2-acetylaminofluorence in the Salmonella microsome test. B6 and D2 mice were treated with inducers (phenobarbital, pregnenolone 16α-carbonitrile, 5,6-benzoflavone, 3-methylcholanthrene) or inhibitors (7,8-benzoflavone, aminoacetonitrile, disulfiram) of mixed-function oxidases. Groups of mice were also given ethanol (3% in drinking-water) for 12 or 20 days. The results show a good correlation between antipyrine half-life and liver microsomal enzyme activity; but no such simple correlation existed for all substances assayed in enzyme-mediated mutagenicity tests.

4.7 *Enzymic formation of reactive intermediates, structure and biological effects of their DNA adducts*

The various adverse biological effects of many carcinogens, including genetic and toxic damage or carcinogenesis, have been associated with the generation of ultimate reactive metabolites and their subsequent covalent binding to information-controlling cellular macromolecules. The chemico-physical properties of the ultimate metabolites, their electrophilicity, their half-life and

[112] Pelkonen, O. & Karki, N. T. (1980) (in preparation).
[113] Chang, R. L, Wood, A. W., Dixon, W. R., Conney, A. H., Anderson, K. E., Eiseman, J. & Alvares, A. P. (1976) *Clin. Pharmacol. Ther.*, **20**, 219–226.
[114] Dehnen, W., Tomingas, R. & Roos, J. (1973) *Anal. Biochem.*, **53**, 373–383.
[115] Razzouk, C., Lhoest, G., Roberfroid, M. & Mercier, M. (1977) *Biochem. J.*, **83**, 194–203.

the cell compartment in which they are generated, are therefore parameters that influence reactions with cellular constituents and thus alter the biological effects of the parent compound. The objectives of this programme are to characterize the ultimate reactive metabolites of mutagenic and carcinogenic compounds that have not hitherto been investigated in detail, to characterize their DNA binding products and to study their biological consequences, such as mutagenesis and DNA repair.

(*a*) *Studies on the miscoding properties of 1,*N^6*-ethenoadenine (εA) and 3,*N^4*-ethenocytosine (εC), two DNA adducts formed by vinyl-chloride metabolites* (Mr A. Barbin; in collaboration with Dr M. Radman and Dr P. Leconte, Laboratory of Biophysics and Radiobiology, Free University of Brussels, Rhode St Genèse, Belgium; Dr E. Huberman, Oak Ridge National Laboratory, Oak Ridge, TN, USA; and Dr A. Croisy, Institute of Chemistry of Natural Substances, Gif-sur-Yvette, France)

1,N^6-Ethenoadenine (εA), 3,N^4-ethenocytosine (εC) and 1,N^2-ethenoguanine are modified nucleic acid bases with an additional imidazole ring between the exo nitrogen and an adjacent endo nitrogen. This type of lesion is caused by vinyl chloride[116–118] and by vinyl bromide[119] metabolites. Structurally related derivatives are formed by glycidaldehyde[120] and haloethylnitrosoureas[121, 122].

The biological effects of vinyl chloride, a recognized carcinogen in animals and humans[123] appear to depend on its conversion by microsomal cytochrome-P450-dependent mono-oxygenases into chloroethylene oxide[116] and into carcinogenic[124] metabolites that can rearrange to chloroacetaldehyde. Chloroethylene oxide and chloroacetaldehyde both react with DNA bases to yield εA[116, 117] and εC[117]. Metabolically activated vinyl chloride, chloroethylene oxide or chloroacetaldehyde specifically induce base-pair substitution mutations in *Salmonella typhimurium*[125–127]. To investigate whether these mutagenic effects could result from the formation of miscoding DNA adducts, the synthetic templates poly(dA) and poly(dC) were reacted with increasing amounts of chloroethylene oxide or chloroacetaldehyde. The modified polynucleotides were then assayed with *Escherichia coli* DNA polymerase I for template activity and for misincorporation (incorporation of non-complementary nucleotides). A similar approach has been used to demonstrate the miscoding properties of O^6-methylguanine[128] which is thought to be involved in the induction of mutagenesis and carcinogenesis by methylating agents[129]. Our experiments have revealed that εA and εC miscode for G (Fig. 18A) and T (Fig. 18B), respectively; εA and εC are thus premutagenic

[116] Barbin, A., Brésil, H., Croisy, A., Jacquignon, P., Malaveille, C., Montesano, R. & Bartsch, H. (1975) *Biochem. biophys. Res. Commun.*, **67**, 596–603.
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[123] International Agency for Research on Cancer (1979) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 19, *Some Monomers, Plastics and Synthetic Elastomers and Acrolein*, pp. 377–438.
[124] Zajdela, F., Croisy, A., Barbin, A., Malaveille, C., Tomatis, L. & Bartsch, H. (1980) *Cancer Res.*, **40**, 352–356.
[125] Malaveille, C., Bartsch, H., Barbin, A., Camus, A. M., Montesano, R., Croisy, A. & Jacquignon, P. (1975) *Biochem. biophys. Res. Commun.*, **63**, 363–370.
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[128] Abbott, P. J. & Saffhill, R. (1979) *Biochem. biophys. Acta*, **562**, 51–61.
[129] Pegg, A. E. (1977) *Adv. Cancer Res.*, **25**, 195–269.

lesions which are expected to lead to A·T → C·G and C·G → A·T transversions [130]. The formation of such miscoding lesions in DNA may well represent a critical step in vinyl chloride- or chloroethylene oxide-induced carcinogenesis. The miscoding potencies of εA and εC in poly(dA) and poly(dC) will be measured using ^{14}C-labelled chloroacetaldehyde and chloroethylene oxide. The fidelity of DNA polymerase α from Chinese hamster V79 cells is being studied in the presence of synthetic templates containing εA and εC, which are the alternating copolymers poly(dA·dT) and poly(dC·dG) modified to various extents by chloroacetaldehyde. The types of base-pair substitutions induced by chloroethylene oxide and chloroacetaldehyde will be investigated in specific *Escherichia coli* and phage λ Trp⁻ strains.

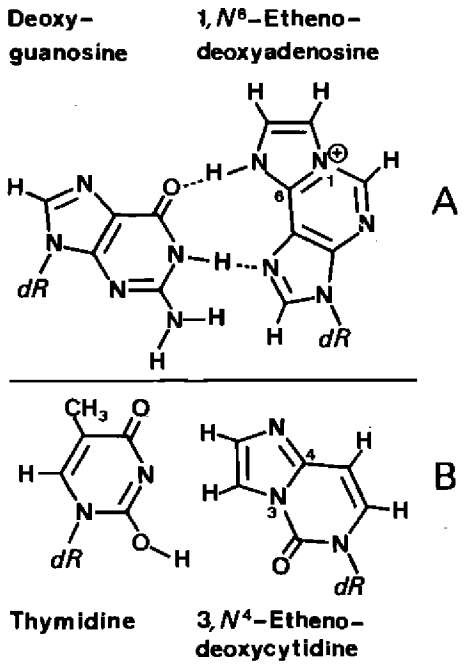

Fig. 18 Base-pairing schemes for the miscoding bases 1,N^6-ethenoadenine (εA) (A) and 3,N^4-ethenocytosine (εC) (B)

(*b*) *Metabolic activation of some aliphatic and heterocyclic* N-*nitramines and* N-*nitrosamines and inhibition of bacterial mutagenesis by ascorbic acid and disulfiram* (Dr V. Khudoley and Mr C. Malaveille)

N-Nitrosamines, because of their wide environmental occurrence, have been studied in detail with regard to their mechanism of action in carcinogenesis and mutagenesis. *N*-Nitramines, a related class of compounds in which the nitroso group is replaced by a nitro group, have not yet

[130] Barbin, A., Bartsch, H., Leconte, P. & Radman, M. (1980) In: Seeberg, E., ed., *Proceedings of the NATO/EMBO Lecture Course on Chromosome Damage and Repair, Godøysund, Norway* (in press).

been investigated, although they may be generated as atmospheric pollutants [131] and some are used in industry [132]. Furthermore, dimethylnitramine [133–135] and diethylnitramine [136] have been reported to be carcinogenic: mainly liver tumours (in rats and mice) and kidney tumours (in mice) were produced, as with *N*-nitrosodimethylamine.

Mutagenesis tests *in vitro* and *in vivo* have been used to detect electrophilic intermediates formed in the presence of rat liver enzymes from three *N*-nitramines, *N*-nitrodimethylamine (DMNO), *N*-nitrodiethylamine (DENO) and *N*-nitromorpholine (NMO). These nitramines and their *N*-nitroso analogues, *N*-nitrosodimethylamine, *N*-nitrosodiethylamine and *N*-nitrosomorpholine, were tested in *Salmonella typhimurium* strains TA100 and TA1530 in a liquid incubation assay. All compounds except DENO were shown to be mutagenic in at least one of the tester strains, and all required the presence of a postmitochondrial supernatant from the liver of Aroclor-treated rats, a NADPH generating system and oxygen. When compared on a molar basis with their *N*-nitroso analogues, NMO was about 10 times and DMNO about 70 times less mutagenic. Addition of disulfiram to the assays at a final concentration of 0.1 mM efficiently inhibited the mutagenesis of all compounds; ascorbic acid at a 7.4 mM concentration produced less inhibition.

When *N*-nitrodimethylamine was used as substrate, oxidative *N*-demethylation by rat liver microsomes paralleled the rate of formation of mutagens. After incubation of DMNO in the presence of rat liver postmitochondrial supernatant, an NADPH-generating system and 3,4-dichlorothiophenol, the 3,4-dichlorophenyl methylthioether was identified by gas chromatography. The isolation of this *S*-methyl derivative indicate the formation of methylating agents from DMNO in the presence of rat liver enzymes. On the basis of these data, and others which show the similarity of the inhibition and strain specificity of *N*-nitro and *N*-nitroso compounds, a tentative metabolic scheme for the formation of mutagenic intermediates from the *N*-nitramines has been proposed [137]. Mutagenic activity of DMNO, DENO and NMO was also determined in a host-mediated assay in female BDVI rats, where cells of *S. typhimurium* strain TA1530 or TA100 were injected intraperitoneally into animals. All three nitramines were mutagenic to strain TA1530 but not TA100. Mutation frequencies, expressed as number of *his*$^+$ revertants per 10^6 survivors, were in the descending order: NMO > DMNO > DENO. One, three and six hours after an oral dose of DENO to rats, following inoculation of cells of TA1530 strain, bacteria were also isolated from liver, lung and kidney: mutation frequency was higher in bacteria obtained from liver tissue but was not increased in those from lung and kidney. These data suggest a liver-specific metabolic activation of the hepatocarcinogen DENO [138].

(*c*) *Mutagenicity studies on the metabolic activation of polynuclear aromatic hydrocarbons* (Mr C. Malaveille, Miss A.-M. Camus, Mrs G. Brun and Mrs A. Hautefeuille; in collaboration with Dr P. L. Grover and Dr P. Sims, Chester Beatty Research Institute:

[131] Pitts, J. N., Grosjean, D., Cauwenberghe, K. V. & Fitz, D. R. (1978) *Environ. Sci. Technol.*, **12**, 946–953.
[132] Dushutin, K. K. (1977) *Methody Anal. Kontolya Proizvod. Khim. Prom-sti.*, **3**, 33–34.
[133] Druckrey, H., Preussmann, R., Ivanovic, S. & Schmähl, D. (1967) *Z. Krebsforsch.*, **69**, 103–201.
[134] Goodall, M. & Kennedy, T. H. (1976) *Cancer Lett.*, **1**, 295–298.
[135] Mirvish, S. S., Bulay, O., Runge, R. G. & Patel, K. (1980) *J. natl Cancer Inst.*, **64**, 1435–1442.
[136] Pliss, G. B., Zabezhinsky, M. A., Khudoley, V. V., Sysenko, O. A. & Petrov, A. S. (1980) In: *Proceedings of the IV Symposium on N-Nitrosamines, the Action and Determination, Leningrad* (in press).
[137] Khudoley, V., Malaveille, C. & Bartsch, H. (1980) (submitted for publication).
[138] Khudoley, V. & Bartsch, H. (1980) (submitted for publication).

Institute of Cancer Research, The Royal Cancer Hospital, London; and Dr W. G. Pyerin, German Cancer Research Centre, Institute of Experimental Pathology, Heidelberg, Federal Republic of Germany)

Tissue-mediated mutagenicity tests have been useful in studying the metabolic activation of a variety of chemical carcinogens. With the polycyclic hydrocarbons, these tests helped to pinpoint which dihydrodiols are precursors of the biologically-active vicinal diol epoxides. In an extension of earlier studies [139] several dihydrodiols derived from polycyclic aromatic hydrocarbons were tested for mutagenicity towards *S. typhimurium* in the presence of a postmitochondrial supernatant from rat liver or from mouse skin.

(i) *Metabolic activation of dibenz*[a,c]*anthracene dihydrodiols to 'bay region' and 'non-bay region' vicinal diol-epoxides* [140]

Dibenz[*a,c*]anthracene and three related dihydrodiols have been tested for microsome-mediated mutagenicity towards *S. typhimurium* strain TA100. The parent hydrocarbon and the 10,11-diol were the most mutagenic compounds, the 1,2- and 3,4-diols both being significantly less active. This result is interesting, since both the 1,2- and 3,4-diols would, in theory, give rise to *vicinal* diol-epoxides of the 'bay-region' type on further metabolism, while the 10,11-diol would not.

A possible explanation for the reduced activity of the 1,2- and 3,4-diols may be their conformation: these diols are known to adopt a quasi-diaxial conformation. Such diols are not readily converted by metabolism into the related diol-epoxides, whereas diols that exist in the preferred quasi-equatorial conformation are.

(ii) *Mutagenicity of benzo*[a]*pyrene 7,8-dihydrodiol in* S. typhimurium *mediated by microsomes from rat liver and mouse skin* [141]

Previous studies in our laboratory [142] have shown positive association between the microsome-mediated mutagenicities of the dihydrodiols of five hydrocarbons, including benzo[*a*]pyrene (BP), that could yield 'bay region' diol-epoxides and (1) the extent of reaction with DNA in hydrocarbon-treated mouse skin and (2) the carcinogenic potencies of the parent hydrocarbons. In view of the apparent role that AHH activity plays as one determinant of the organ-specific action of these polycyclic aromatic hydrocarbons, the mutagenicity of BP 7,8-diol in the presence of microsomal preparations from mouse skin, a target tissue, was measured and compared with those obtained in the presence of microsomal preparations from rat liver, an organ normally refractory towards the carcinogenic effects of these compounds. Table 20 shows the specific mutagenicity in *S. typhimurium* TA100 of BP 7,8-diol in the presence of microsomes from rat liver or from mouse skin, compared with the respective values for AHH activity in those two tissues. Topical application of benz[*a*]anthracene (BA) or 7,12-dimethylbenz[*a*]anthracene (DMBA) to mice increased epidermal microsome-mediated mutagenesis by a factor of two; AHH activity was enhanced maximally by 4.7-fold when BA was applied. Thus, there appears to be a fair, but not perfect, parallelism between epidermal AHH activity in the groups of mice pretreated with different

[139] International Agency for Research on Cancer (1979) *Annual Report, 1979*, Lyon, p. 117.
[140] Malaveille, C., Hautefeuille, A., Bartsch, H., MacNicoll, A. D., Grover, P. L. & Sims, P. (1980) *Carcinogenesis*, **1**, 287–289.
[141] Camus, A. M., Pyerin, W. G., Grover, P. L., Sims, P., Malaveille, C. & Bartsch, H. (1980) *Chem.-biol. Interactions* (in press).
[142] International Agency for Research on Cancer (1979) *Annual Report, 1979*, Lyon, p. 117.

polycyclic aromatic hydrocarbons and the specific mutagenicities observed when BP 7,8-diol was metabolized by the respective epidermal microsomal preparations. This correlation was not apparent when values for liver and skin microsomal preparations from untreated rats and mice were compared (Table 20).

Table 20. Microsome-mediated mutagenicity of benzo[*a*]pyrene (BP) 7,8-diol in the presence of microsomes from rat liver or mouse skin towards *S. typhimurium* TA100, and comparative aryl hydrocarbon (BP) hydroxylase (AHH) activities

Source of microsomes	Hydrocarbon treatment (nmol/animal)[a]	AHH activity[b]	Microsome-mediated mutagenicity[c]
Rat liver	None	3730	2160
Mouse skin	None	90	1700
	DMBA (50)	250	2830
	(100)	270	2860
	BA (50)	180	3000
	(100)	430	3480

[a] DMBA – 7,12-dimethylbenz[*a*]anthracene; BA-benz[*a*]anthracene
[b] pmol of 3-hydroxy-BP per mg microsomal protein per 10 min
[c] *his*+ revertants per μM concentration of BP 7,8-diol per mg microsomal protein

The data further confirm that mouse skin possesses the microsomal enzymes necessary to convert BP 7,8-diol into reactive mutagenic derivatives, which are probably the related 'bay-region' diol-epoxides. The capacity of mouse epidermal microsomal preparations to convert BP 7,8-diol into mutagens was similar to that of rat liver, even though the AHH activity of the rat liver preparations was 40-fold higher than that of similar mouse epidermal fractions (Table 20). Possible variations in the relative amounts of cytochromes P450 and P448 present in liver and in epidermis may be related to the susceptibility of mouse skin and the comparative resistance of rat liver to the carcinogenic effects of polycyclic hydrocarbons.

(iii) *Identification of mutagenic metabolites in the urine of phenacetin-treated hamsters and rats* (Miss A.-M. Camus, Dr M. Friesen and Mrs L. Garren; in collaboration with Dr A. Croisy, Institute of Chemistry of Natural Substances, Gif-sur-Yvette, France)

Phenacetin, a drug that occurs in analgesic and antipyretic formulations, has been classified as a possible human carcinogen [143].

Although it was not initially found to be mutagenic in *S. typhimurium* in the presence of rat liver fractions [144], further independent studies showed it has a mutagenic effect in *S. typhimu-*

[143] International Agency for Research on Cancer (1980) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 24, *Some Pharmaceutical Compounds* (in press).

[144] Sugimura, T., Sato, S., Nagao, M., Yahagi, T., Matsushima, T., Seino, Y., Takeuchi, M. & Kawachi, T. (1976) In: Magee, P. N., Takayama, S., Sugimura, T. & Matsushima, T., eds, *Fundamentals in Cancer Prevention*, Baltimore, University Park Press, pp. 191–215.

rium TA100 when liver fractions from Aroclor-pretreated hamsters, instead of rats, were used[145, 146].

Of the known metabolites or putative synthetic derivatives of phenacetin, *N*-hydroxyphenacetin and *N*-acetoxyphenacetin were found to be mutagenic in *S. typhimurium* TA100 in liquid assays, as reported previously[147]. Both required activation by liver fractions from Aroclor-pretreated hamsters. 2-Hydroxyphenacetin and 2-acetoxyphenacetin were inactive.

Phenacetin was also administered to BDVI rats and male Syrian golden hamsters and the urinary metabolites concentrated on XAD-2 resin[148]; the concentrate was then deconjugated with ß-glucuronidase/arylsulfatase and reactivated by hamster liver fractions. Mutagenicity was demonstrated in *S. typhimurium* TA100 with urine from phenacetin-treated hamsters but not rats. *N*-Hydroxyphenacetin was isolated by thin-layer and high-performance liquid chromatography from hamster urine after treatment by deconjugating enzymes, and was identified by mass spectral analysis. The data suggest that *N*-hydroxyphenacetin is a proximate mutagenic metabolite of phenacetin, which is possibly responsible for the mutagenicity observed *in vitro* and in the urine.

4.8 *Validation, improvement and development of short-term tests for carcinogens/mutagens*

One major goal of this research is to contribute to a long-term programme for primary cancer prevention, identifying and minimizing human exposure to environmental or industrial carcinogens or mutagens (either pure chemicals or complex mixtures), by systematic screening in a series of short-term tests. The chemicals studied are selected from among those for which some suspicion of carcinogenecity exists, and for which there is evidence of their use, production and social importance, implying human exposure. In view of the large number of environmental and industrial chemicals for which long-term animal tests cannot at present be done, it is hoped that the results of a battery of short-term tests will enable selection of those chemicals that should be given priority in further testing in animals.

(*a*) *Validation and comparative studies on 180 chemicals using* S. typhimurium *strains and V-79 Chinese hamster cells in the presence of various metabolizing systems* (Mr C. Malaveille, Miss A.-M. Camus, Mrs G. Martel-Planche, Mrs G. Brun, Mrs A. Hautefeuille, Mrs N. Sabadie, Mr A. Barbin, Dr T. Kuroki, Miss C. Drevon, Mrs C. Piccoli and Dr R. Montesano)

Results of testing 180 compounds in the *Salmonella*/microsome assay and adapted procedures have been summarized, and the full data have been published[149]. The following aspects were analysed: the *predictive value* of the test; frequency distribution of chemicals (classes) according to

[145] Matsushima, T., Yahagi, T., Takamoto, Y., Nagao, M. & Sugimura, T. (1980) In: *Microsomes, Drug Oxidations and Chemical Carcinogenesis*, New York, Academic Press (in press).

[146] Bartsch, H., Malaveille, C., Camus, A.-M., Martel-Planche, G., Brun, G., Hautefeuille, A., Sabadie, N., Barbin, A., Kuroki, T., Drevon, C., Piccoli, C. & Montesano, R. (1980) *Mutat. Res.*, **76**, 1–50.

[147] Shudo, K., Ohta, T., Orihara, Y., Okamoto, T., Nagao, M., Takahashi, Y. & Sugimura, T. (1978) *Mutat. Res.*, **58**, 367–370.

[148] Yamasaki, E. & Ames, B. N. (1977) *Proc. natl Acad. Sci. USA*, **74**, 3555–3559.

[149] Bartsch, H., Malaveille, C., Camus, A. M., Martel-Planche, G., Brun, G., Hautefeuille, A., Sabadie, N., Barbin, A., Kuroki, T., Drevon, C., Piccoli, C. & Montesano, R. (1980) *Mutat. Res.*, **76**, 1–50.

their mutagenic activity; quantitative relationship between mutagenicity, electrophilicity and carcinogenicity for some selected carcinogens; chemicals that are activated into mutagens by human liver enzymes; compounds that have been tested in the presence of rodent hepatic and extra-hepatic tissue fractions; and some factors necessary for the efficient detection of mutagens *in vitro*, i.e., the source and concentration of liver microsomal protein required for maximal mutagenic activity. Since 34 chemicals have also been tested in microsome- or cell-mediated mutagenicity assays using V79 Chinese hamster cells, comparison of test results obtained in bacterial and mammalian assays has also been made.

Figure 19 shows the frequency distribution and range of mutagenic activity for 101 mutagenic chemicals that were tested in plate assays. The mutagenic activity is expressed on the abscissa as the logarithm of the number of revertants in the most sensitive *Salmonella* strain per μmol of the test compound. The mutagenic activities for these compounds varied over a 100 million-fold. The same finding was reported previously by McCann & Ames[150] and by Nagao *et al.*[151], on the basis of

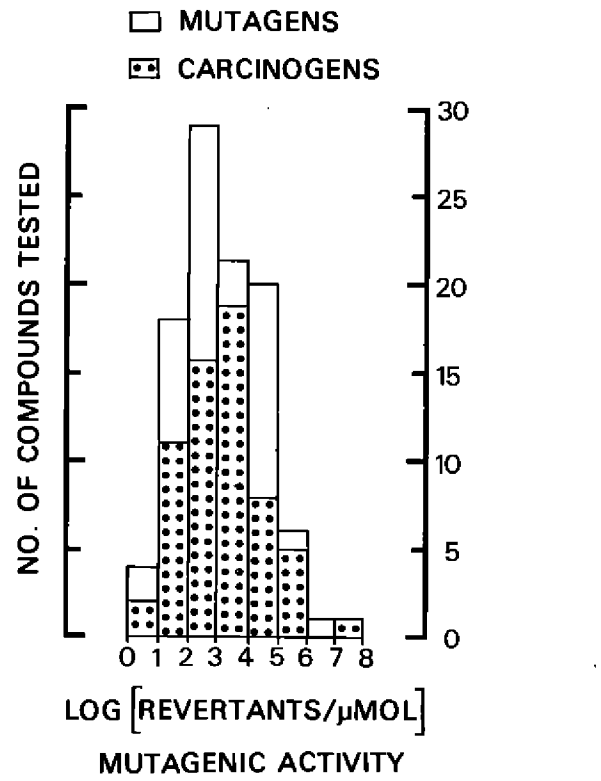

Fig. 19 Frequency distribution of 101 chemicals tested in *Salmonella typhimurium* by plate assays according to their mutagenic activity (revertants/μmol) in a semi-logarithmic plot

[150] McCann, J. & Ames, B. N. (1977) In: Hiatt, H., Watson, J. D. & Winsten, J. A., eds, *Origins of Human Cancer*, Cold Spring Harbor, NY, Cold Spring Harbor Laboratory, pp. 1431–1450.

[151] Nagao, M., Sugimura, T. & Matsushima, T. (1978) *Ann. Rev. Genet.*, **12**, 117–159.

lists of different chemicals. In the (nearly) Gaussian distribution curve, one-third of the chemicals (29/101) displayed mutagenic activity ranging from 100-1000 revertants per μmol of test compound; and about nine-tenths of the chemicals (89/101) showed mutagenicity varying only by four orders or magnitude ($10-10^5$ revertants per μmol).

(*b*) *Effect of glutathione and uridine 5'-diphosphoglucuronic acid on benzo*[a]*pyrene and aflatoxin B_1 mutagenesis in the* Salmonella*/microsome assay*[152]

NADPH (or an NADPH generating system), required for the activity of the mixed-function oxygenase system, is the only cofactor added to the *Salmonella*/microsome assay. It is well known that mutagenic epoxides of benzo[*a*]pyrene (BP) and of aflatoxin B_1 (AFB_1) are inactivated by enzymatic conjugation with glutathione, while various phenols and diols of BP and hydroxylated metabolites of AFB_1 are conjugated enzymatically with uridine 5'-diphosphoglucuronic acid (UDPGA). The action of some conjugating enzymes, GSH- and UDPGA-transferases on rat liver microsome-mediated mutagenesis of BP and AFB_1 was thus examined in *Salmonella typhimurium* TA100 strain, using plate and liquid incubation assays. By selective conjugating activities, both enzymes may alter the pattern and the concentration of mutagenic BP and AFB_1 metabolites. This could be expected to lead to changes in the overall mutagenic activity of the parent compounds.

The results of this study are summarized in Table 21. Addition of GSH and UDPGA altered rat liver microsome-mediated mutagenesis of BP and AFB_1. With both carcinogens, an increased, unchanged or decreased number of revertant colonies of *S. typhimurium* was observed, depending on the substrate concentration, the source of liver 9000 × *g* supernatant, the time of incubation and the type of mutagenicity test (liquid or plate assay).

Table 21. Effect of glutathione (GSH) and uridine 5'-diphosphoglucuronic acid (UDPGA) on the microsome-mediated mutagenicity of benzo[*a*]pyrene and aflatoxin B_1

Cofactors added (conc. in assay medium)	Type of assay	Rat liver microsome-mediated mutagenicity: increase (+); unchanged (0); decrease (–)	
		Benzo[*a*]pyrene	Aflatoxin B_1
UDPGA (2.5 mM)	plate	(0) (–) [a]	not tested
	liquid	(+) (0) (–) [b]	(0)
GSH (10 mM)	plate	(–)	(0) (–)[b]
	liquid	(+) (0) (–)[bc]	(+) (0)

Depending on [a] : concentration of liver 9000 × *g* supernatant (S-9); [b] : concentration of test compound or incubation time; [c] : liver S-9 pool

[152] Malaveille, C., Brun, G., Hautefeuille, A. & Bartsch, H. (1980) In: *Proceedings of the Second International Congress on Toxicology, Brussels*, Amsterdam, Elsevier/North Holland Biomedical Press (in press).

The following factors appear to determine quantitative changes in the distribution of BP and AFB_1 metabolites under various assay conditions *in vitro*, thus leading to changes in the overall mutagenic activity of the parent compound:

(i) increase in the formation of BP 7,8-dihydrodiol 9,10-epoxide and in the rate of oxidative BP metabolism in the presence of UDPGA;

(ii) increase in the formation of BP 7,8-dihydrodiol 9,10-epoxide in the presence of GSH, depending on the ratio of enzyme activity of epoxide hydrase and GSH-transferases and on the rates of formation of both primary and secondary mutagenic BP epoxides;

(iii) increase in the rate of oxidative AFB_1 metabolism subsequent to the trapping by GSH of non-mutagenic metabolites (possibly AFB_1 2,3-diol), which may be partly responsible for inactivation of microsomal enzyme through covalent reactions.

These data illustrate the difficulty of extrapolating results obtained in one type mutagenicity assay to reactions that may occur in the intact mammalian organism.

(c) *Quantitative comparisons between mutagenicity, electrophilicity and carcinogenic activity of some direct-acting agents* (Mr C. Malaveille, Mrs G. Brun, Dr L. Tomatis and Mrs B. Dodet; in collaboration with Professor B. Terracini, University of Turin, Turin, Italy; and Dr N. Breslow, University of Washington, Seattle, WA, USA)

Attempts to obtain good correlations between carcinogenicity and mutagenic activity are more successful when the pathways involved in the metabolic activation of carcinogens *in vivo* and *in vitro* (and thus the molecular binding products) are both qualitatively and quantitatively similar. Since it has become apparent that for certain carcinogens, such as polycyclic hydrocarbons, metabolic activation reactions *in vitro* and *in vivo* differ[153], probably attributable to activation processes, some direct-acting alkylating agents were chosen for comparison of their biological activities.

Ten known carcinogenic alkylating agents (see legend to Fig. 20), including several *N*-nitrosamides, methane sulfonates, epoxides, propiolactone and 1,3-propane sultone, were assayed for mutagenicity, *firstly* in two *Salmonella* strains, TA1535 and TA100, and *secondly*, in two test procedures, plate and liquid assays; *thirdly*, mutagenicity observed with strain TA100 in plate assays was compared either with the alkylating activity, using a colour reaction with 4-(4-nitrobenzyl)pyridine, or, *fourthly*, with the half-life of those compounds in an aqueous medium at pH 7.4; *finally*, attempts were made to compare carcinogenic activity in rodents with mutagenicity in *S. typhimurium* TA100 (plate assays)[154].

Figure 20 gives a comparison of the mutagenic effects of the 10 compounds in strains TA100 and TA1535. When the value for methylmethane sulfonate, which was not detected as a mutagen in strain TA1535, was omitted from the calculations, the interstrain comparison of mutagenicity yielded a statistically significant positive correlation ($r = 0.98$; $P < 0.001$). These data suggest that the relative mutagenicities of the remaining nine alkylating agents are not affected by the presence of the R-factor plasmid (ampicillin resistance) in strain TA100. Comparison of the mutagenic effect in *S. typhimurium* TA100 in plate assays *versus* that in liquid incubation assays (incubation time,

[153] Bartsch, H. (1978) *Staub. Reinhalt. Luft.*, **38**, 240–243.

[154] Bartsch, H., Malaveille, C., Camus, A. M., Martel-Planche, G., Brun, G., Hautefeuille, A., Sabadie, N., Barbin, A., Kuroki, T., Drevon, C., Piccoli, C. & Montesano, R. (1980) *Mutat. Res.*, **76**, 1–50.

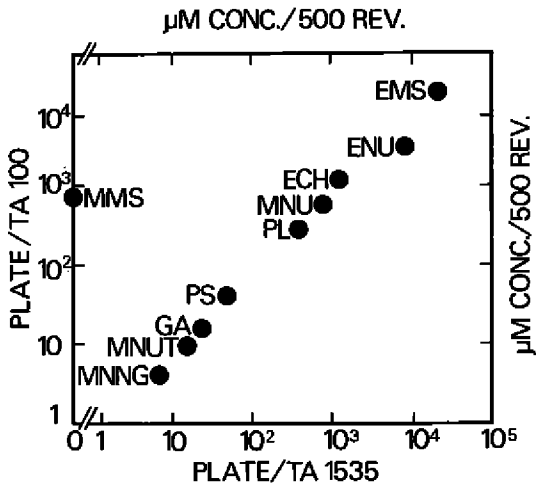

Fig. 20 Comparative mutagenicities of 10 alkylating agents in *Salmonella typhimurium* TA100 (ordinate) *versus* that in TA1535 (abscissa). Measured in plate assays, mutagenic activity is expressed as the µM concentration of the test compound required to induce 500 revertants/plate, taken in most cases from non-linear dose-response curves and plotted on a double logarithmic scale; the number of spontaneous mutants was subtracted. ENU, *N*-nitroso-*N*-ethylurea; NMU, *N*-nitroso-*N*-methylurea; MNUT, *N*-nitroso-*N*-methylurethane; MNNG, *N*-nitroso-*N'*-nitro-*N*-methylguanidine; ECH, epichlorohydrin; GA, glycidaldehyde; EMS, ethylmethane sulfonate; MMS, methylmethane sulfonate; PL, β-propiolactone; PS, 1,3-propane sultone. When the value for MMS was omitted, interstrain comparison of the mutagenicity yielded $r = 0.98$; $P > 0.001$

up to 2 hrs) showed the latter procedure to be inferior: three of the 10 compounds, *N*-nitroso-*N*-ethylurea, epichlorohydrin and ethylmethane sulfonate were not detected as mutagens. When comparing the mutagenicity of the seven remaining compounds in plate and liquid assays, positive correlations were again obtained ($r = 0.95$; $P < 0.001$).

Comparison of the mutagenicities (in TA100 strain and in plate assays) of 10 alkylating agents with their alkylating activity or with their half-lives in an aqueous medium at pH 7.4 and 37°C revealed no significant positive association among the three variables.

A comparison was attempted of the mutagenic activities of seven alkylating agents in TA100 (plate assays) with their carcinogenic potencies. The compounds have all been evaluated in *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, and carcinogenicity studies permitted calculation of TD_{50} values (the daily dose of carcinogen in mg/kg bw required to reduce by one-half the probability of animals being tumour-free when administered over a standard life time) (Fig. 21). The following conclusions may be drawn from the limited data presented: The TD_{50} values for a given compound, for example, 1,3-propane sultone, varied considerably, depending on route and mode of administration and animal species. For several compounds, there was a rough proportionality between carcinogenicity in rodents and mutagenicity in *Salmonella;* but there were exceptions, e.g., *N*-nitroso-*N*-ethylurea, which appeared to be a potent carcinogen in rats but was only weakly active as a mutagen; in contrast, glycidaldehyde was highly mutagenic but only weakly carcinogenic.

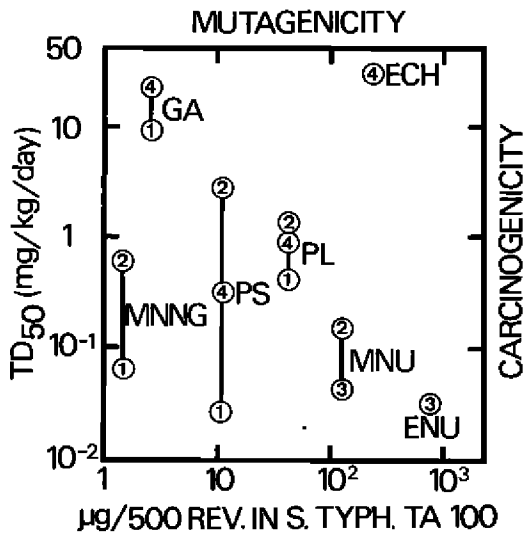

Fig. 21 Comparison of mutagenic activity and carcinogenic potency of seven alkylating agents. The TD_{50} values (the daily dose of carcinogen in mg/kg bw required to reduce by one-half the probability of animals being tumour-free when administered over a standard life time) were plotted (ordinate) against mutagenic activity, expressed as µg of compound necessary to induce 500 revertants in *Salmonella typhimurium* TA100 in plate assays (abscissa). The numbers 1–4 in open circles refer to different types of experiments: (1) subcutaneous, repeated, rats; (2) oral, continuous, rats; (3) intravenous, repeated, rats; (4) subcutaneous, repeated, mice. ENU, *N*-nitroso-*N*-ethylurea; MNU, *N*-nitroso-*N*-methylurea; MNNG, *N*-nitroso-*N'*-nitro-*N*-methylguanidine; ECH, epichlorohydrin; GA, glycidaldehyde; PL, β-propiolactone; PS, 1,3-propane sultone

It would appear that, for the carcinogens studied no quantitative relationship between carcinogenesis and mutagenesis in *Salmonella* can be sufficiently established such as to allow confident prediction of the carcinogenic potency of new compounds of this class. However, an increasing demand for quantitative data on carcinogenesis poses the necessity for further examination of whether there is a correlation between the potency of a carcinogen in experimental animals (or humans) and its activity in short-term tests.

(*d*) *Studies on mutagenic metabolites in the urine of carcinogen-treated rodents* (Miss A.-M. Camus, Miss B. Tudek, Mrs N. Sabadie and Mrs A. Hautefeuille)

Detection of mutagens in urine has received increasing attention as a means for monitoring exposure of humans to mutagenic/carcinogenic chemicals. Mutagens have been detected in the urine of cigarette smokers [155], of patients treated with various drugs and of anaesthesiologists [156], as well as in that of nurses who administered various cytostatic agents [157]. However, no detailed studies

[155] Yamasaki, E. & Ames, B. N. (1977) *Proc. natl Acad. Sci. USA*, **74**, 3555–3559.
[156] McCoy, E. C., Hankel, R., Rosenkranz, H. S., Giuffrida, J. G. & Bizzari, D. V. (1977) *Environ. Health Perspect.*, **21**, 221–223.
[157] Falck, K., Grohn, P., Sorsa, H., Vainio, H., Heinonen, E. & Holsti, L. R. (1979) *Lancet, ii*, 1250–1251.

of the kinetics of excretion of mutagenic metabolites following administration of carcinogenic compounds to animals or humans or of their structural characterization have been reported. In this project, data were obtained on the kinetics of excretion of mutagenic 2-acetylaminofluorene (AAF) and BP metabolites in rat urine under various experimental conditions.

Using an XAD-2 adsorption-elution technique for the isolation of urinary metabolites, mutagenicity for *S. typhimurium* strains was detected in the urine of AAF-, *N*-acetoxy-AAF- and BP-treated rats (strain TA100). Mutagenicity could be demonstrated following deconjugation of the urinary metabolites with β-glucuronidase and subsequent activation by liver postmitochondrial fractions from Aroclor-treated rats. The kinetics of excretion up to 72 and 96 hours after administration of aromatic amines and BP, respectively, were also studied. Aroclor-pretreatment of animals resulted in an accelerated excretion of higher amounts of mutagenic BP metabolites in the urine. Following incubation with β-glucuronidase/arylsulfatase, high-pressure liquid chromatographic analysis of ethyl acetate-extractable metabolites from such assays demonstrated the presence of 3- and 9-hydroxy BP, 7,8-, 9,10- and 4,5-dihydrodiols and quinones of BP. A similar pattern of metabolites was detected when BP was incubated with liver microsomes from Aroclor-treated rats, an NADPH-generating system and oxygen.

Thus 3- and 9-hydroxy BP and the 7,8- and 9,10-dihydrodiols of BP are largely responsible for the mutagenicity observed in the urine of BP-treated rats after deconjugation and subsequent activation by rat liver microsome fractions[158].

(*e*) *Detection of potential chemical carcinogens in* Salmonella/*hepatocyte assays* (Mr C. Malaveille and Mrs G. Brun; with the collaboration of Mrs L. Saint Vincent)

Validation studies of the *Salmonella*/microsome assays for the detection of chemical carcinogens have shown that certain carcinogens are not revealed as mutagens (false-negative compounds), e.g., DDT, urethane, safrole, 1,2-dimethylhydrazine. Such lack of reliability in the prediction of carcinogenic potential may arise from the use of liver 9000 × *g* supernatant as the metabolic activation system: this system may be inefficient in producing mutagenic metabolites from precarcinogens that are activated through a multistep metabolic process or in the presence of specific cofactors. As an alternative to the liver cell-free extract, freshly isolated hepatocytes are being tested for use as a source of metabolic activation.

Freshly isolated rat hepatocytes are obtained by perfusion of liver with collagenase[159]; and 10^5–10^6 viable hepatocytes, as judged by the trypan blue exclusion test, and 5–15 × 10^8 bacteria (*S. typhimurium his*⁻strains), are suspended in 4 ml Williams E or Hanks' medium buffered with 20 mM HEPES to pH 7.4. Hepatocytes and bacteria are coincubated in suspension at 37°C for up to three hours in the absence or presence of varying concentrations of the test compound. At the end of incubation, bacteria are recovered by centrifugation and plated on a minimal agar plate. In this mutagenicity assay, electrophilic species derived from the procarcinogen/promutagen must escape the detoxification action of the hepatocytes in order to reach and mutate bacteria.

Benzo[*a*]pyrene, aflatoxin B_1 and *N*-nitrosomorpholine were found to be mutagenic with the hepatocyte-mediated assay. Preliminary results indicate that procarbazine and 1,2-dimethylhy-

[158] Camus, A. M., Pyerin, W. G., Grover, P. L., Sims, P., Malaveille, C. & Bartsch, H. (1980) *Chem -biol. Interactions* (in press).

[159] Seglen, P. O. (1976) In: Prescott, D. M., ed., *Methods in Cell Biology*, Vol. 13, New York, Academic Press, pp. 29–59.

drazine, carcinogens that are not shown to be mutagens in the liver microsome-mediated assay, are mutagens in the *Salmonella*/hepatocyte assay. These data demonstrate the usefulness of this assay as an alternative to the microsome-mediated assay for the detection of both non- and hepatocarcinogens.

(*f*) *DNA damage/repair as a short-term test for the detection of potential carcinogens* (Mr A. Barbin and Mr J. C. Béréziat)

The measurement of unscheduled DNA synthesis in various organs of rodents exposed to carcinogens *in vivo* and *in vitro* is being continued[160]. The alkaline solution assay provides another method for studying the organ-specificity of carcinogens[161, 162]; this technique, which allows for the detection of DNA single-strand breaks and alkali-labile sites, is being set up. Cells or nuclei are prepared from various organs of rats treated with a carcinogen; they are lysed on Millipore filters, and DNA is diluted from the filters with an alkaline solution. The rate of DNA dilution in the initial phase follows first-order kinetics and is directly dependent on the dose of the DNA-damaging agent administered[163]. Measurement of DNA in the elution fractions is done fluorimetrically[164].

(*g*) *Sister chromatid exchanges and chromosome aberration tests in Chinese hamster cells, using DDT derivatives* (in collaboration with Dr A. T. Natarajan, Department of Radiation, Genetics and Chemical Mutagenesis, State University of Leiden, Leiden, The Netherlands)

Previous studies at the Agency revealed (or strongly implied) the enzymic formation of chemically reactive DDT intermediates which can bind covalently to nucleophilic sites and are also mutagenic in *S. typhimurium* strains[165]. To further characterize the biological properties of known mammalian DDT metabolites and synthetic model compounds, a series of DDT derivatives is being tested for the induction of sister chromatid exchanges and chromosome aberrations in Chinese hamster cells in the presence or absence of mouse liver fractions from OF- 1 mice[166]. Statistical evaluation of the final results is in progress.

(*h*) *Testing of selected chemicals in multiple short-term tests for the detection of carcinogens/mutagens*
(Genetics Laboratory, Institute of Anthropology and Human Paleontology, University of Pisa, Italy
Principal investigator: Professor N. Loprieno

The value of short-term tests as predictors of the potential carcinogenic hazard of chemicals is increased when several test systems are used in combination. Those assays in which mammalian metabolism is taken into account appear to be the most promising at the present time. These

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[160] International Agency for Research on Cancer (1979) *Annual Report, 1979*, Lyon, p. 127.
[161] Petzold, G. L. & Swenberg, J. A. (1978) *Cancer Res.*, **38**, 1589–1594.
[162] Parodi, S., Taningher, M., Santi, L., Cavanna, M., Sciaba, L., Maura, A. A. & Brambilla, G. (1978) *Mutat. Res.*, **54**, 39–46.
[163] Kohn, K. W., Erickson, L. C., Ewig, R. A. G. & Friedman, C. A. (1976) *Biochemistry*, **15**, 4629–4637.
[164] Kissane, J. M. & Robins, E. (1958) *J. biol. Chem.*, **233**, 184–188.
[165] Planche, G., Croisy, A., Malaveille, C., Tomatis, L. & Bartsch, H. (1979) *Chem. -biol. Interactions*, **25**, 157–175.
[166] Natarajan, A. T., Tates, A. D., van Buul, P. P. W., Meijers, M. & Vogel, E. (1976) *Mutat. Res.*, **37**, 83–90.

concepts guide a collaborative study that is based on the use of simultaneous short-term tests with different indicator organisms.

Mutagenicity experiments were carried out on four fractions of dichloromethane extracts of particulates obtained from a European diesel engine in five *Salmonella* strains (TA1535, TA1537, TA1538, TA98 and TA100) and two yeast species, *Schizosaccharomyces pombe* (P1 strain) and *Saccharomyces cerevisiae* (D4 strain). A direct mutagenic activity is seen when the fractions are tested *in vitro* in strain TA98. One fraction produced forward mutations and mitotic gene conversions in the two yeast systems. One sample of diesel extract was also tested for induction of unscheduled DNA synthesis in human (EUE) cell lines: it was negative. In preliminary host-mediated assays in mice, in which diesel particles were injected, negative results were obtained in yeast cells[167].

Cytogenetic analyses of lymphocytes from workers exposed occupationally to styrene vapours at an ambient air concentration of 200–300 ppm indicated a possible correlation between chromosomal damage and exposure to this agent[168]. Cytogenetic analyses of bone-marrow cells from mice treated orally with a single dose of styrene (500 or 1000 mg/kg) showed no chromosomal damage[169]. In further studies, bone-marrow cells from CD-1 mice treated for 3 and 4 days with 500 mg/kg and for 10 weeks (5 days/week) with 300 mg/kg styrene had no increase in chromosome aberrations: however, single intraperitoneal injections of cyclophosphamide (10 to 40 mg/kg) did induce chromosome aberrations[170].

In another study, a mutagenicity test system for the estimation of *N*-nitrosodimethylamine *in vivo* was developed. Yeast cells are injected into mice by the tail vein and isolated from the liver at different times following administration of *N*-nitrosodimethylamine (1 mg/kg bw). This method has been used to study catalysts and inhibitors of the nitrosation reaction, such as thiocyanate, ascorbic acid and tannic acid[171] by feeding mice with aminopyrine and nitrite in the presence of these agents. Aminopyrine, an analgesic drug, is a tertiary amine and is rapidly nitrosated to give *N*-nitrosodimethylamine.

4.9 *Screening for environmental mutagens/carcinogens and isolation of biologically active compounds from complex mixtures*

The combination of short-term tests for the detection of carcinogens/mutagens with modern analytical separation procedures appears to be the most promising means of identifying new carcinogens in the environment, of isolating the active constituents and of elucidating their structures. Work has been concentrated on samples collected in areas with a high incidence of oesophageal cancer, i.e., Linhsien county in Northern China, north-eastern Iran, and Brittany, France. Mutagenic compounds have been detected in samples of materials that are ingested by the human populations in the countries concerned, and characterization of some of their constituents is in progress.

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[167] Loprieno, N., Barale, R., Zaccaro, L., Zucconi, D., De Lorenzo, F., Belisario, A., Buonocore, V., Quinto, J., Vricella, G. & Cornetti, G. M. (1980) (submitted for publication).

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[169] Loprieno, N., Presciuttini, S., Sbrana, I., Stretti, G., Zaccaro, L., Abbondandolo, A., Bonatti, S., Fiorio, R. & Mazzaccaro, A. (1978) *Scand. J. Work. Environ. Health*, **4**, *Suppl. 2*, 169–178.

[170] Sbrana, I., Bonatti, S. & Loprieno, N. (1980) (submitted for publication).

[171] Barale, R., Zucconi, D. & Loprieno, N. (1980) (submitted for publication).

(*a*) *Mutagenicity studies on environmentally occurring* N-*nitrosamines and on extracts of pickled vegetables collected in Linhsien County, a high incidence area for oesophageal cancer in Northern China* (Dr S.-H. Lu and Miss A.-M. Camus)

Extracts of pickled vegetables commonly eaten in Linhsien county, a high-incidence area for oesophageal cancer in Northern China [172], were studied for mutagenicity. The liquid residue from ethereal extracts produced a dose-dependent increase of mutants in *S. typhimurium* TA98 and TA100 strains; mutagenicity required the presence of a fortified liver microsomal activation system from Aroclor-induced rats. An amount of extract equivalent to 2.8 g of fresh pickled vegetables produced six-fold (75 revertants per g) and two-fold (45 revertants per g) increases in revertant frequencies in strains TA98 and TA100, respectively. Roussin's red methyl ester, a tetranitroso compound, $[(NO)_2Fe(CH_3S)]_2$, that has not previously been reported to occur in nature, was isolated and identified from the ethereal extracts. The synthetic compound was mutagenic in strain TA100 in the presence of a rat liver activation system, producing 25 revertants per μmol [173].

Two synthetic *N*-nitrosamines (*N*-3-methylbutyl-*N*-1-methyl-*N*-acetonylnitrosamine and *N*-methyl-*N*-benzylnitrosamine), also isolated from corn-bread which had been inoculated with moulds occurring in Linshien county, Northern China and subsequently nitrosated with sodium nitrite [174, 175], were tested in *S. typhimurium* strains TA1535 and TA100 in the presence of a liver postmitochondrial supernatant from Aroclor-treated rats. The two nitrosamines produced a concentration-dependent increase in the number of mutant colonies in both bacterial strains when assayed in liquid suspension and in plate incorporation assays [176]. Experimental findings from a combination of short-term tests with appropriate analytical procedures support the hypothesis that there are mutagenic, and thus potentially carcinogenic, substances in food commonly eaten in Linshien county, the possible role of which should be investigated further.

(*b*) *Isolation of mutagenic compounds from opium dross (sukhteh) and pyrolysis products of opium, or opium alkaloids* (Dr M. Friesen, Miss A.-M. Camus, Mrs A. Hautefeuille and Mr C. Malaveille; in collaboration with Dr N. Day, Division of Epidemiology and Biostatistics, IARC; and Dr K. Szendrei, University Medical School, Department of Pharmacognosy, Szedged, Hungary)

To study possible etiological factors involved in cancer of the oesophagus in areas of north-eastern Iran, crude opium and opium pyrolysates, which are consumed in considerable quantities, were investigated for their mutagenic activity. It was shown previously that samples of sukhteh (residues formed inside opium smokers' pipes) collected from villages in north-eastern Iran and smoke condensate obtained by pyrolysis of crude opium at 450°C in a glass apparatus are mutagenic in *S. typhimurium* TA100 and TA98 strains [177, 178].

As a first step in the isolation and characterization of the mutagenic compounds formed during opium pyrolysis, smoke condensates from pyrolysis of the major alkaloid constituents of opium

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[175] Li, M. H., Lu, S. H., Ji, C., Wang, M. Y., Cheng, S. J. & Jin, C. L. (1979) *Sci. Sin.*, **22**, 471–477.
[176] Lu, S. H., Camus, A. M., Ji, C., Wang, Y. L., Wang, H. Y. & Bartsch, H. (1980) (submitted for publication).
[177] International Agency for Research on Cancer (1979) *Annual Report, 1979*, Lyon, p. 126.
[178] Hewer, T., Rose, E., Ghadirian, P., Castegnaro, M., Bartsch, H., Malaveille, C. & Day, N. (1978) *Lancet, ii*, 494–496.

were investigated (Table 22). Pyrolysis products of morphine and thebaine showed higher mutagenic activity (expressed as revertants per mg pyrolysate) than did the four opium samples tested.

Table 22. Mutagenic activity of pyrolysis products obtained from opium and its major constituents

Pyrolysed product	Mutagenic activity in the presence or absence of S-9 mix[a]	
	(+)	(-)
Opium (India)	1 620	290
Opium (Iran)	2 320	310
Opium (Turkey)	5 910	680
Opium (Afghanistan)	3 730	550
Morphine	32 170	7 390
Thebaine	10 250	670
Sanguinarine	4 500	1 560
Codeine	1 230	980
Noscapine	140	100
Narceine	410	60
Papaverine	120	0
Plant sterols	200	0

[a] Mutagenic activity in *S. typhimurium* TA100 strain determined in plate assays in the presence (+) or absence (-) of 50 µl liver postmitochondrial fraction from Aroclor-treated rats and an NADPH generating system (S-9 mix). Mutagenicity is expressed as revertants/mg of pyrolysis product

A sample of Indian opium contained the followings percentages of the major alkaloids: 9% morphine, 4% codeine, 2% thebaine, 12% noscapine and 1% papaverine. A major fraction of the mutagenic activity of pyrolysed opium can thus be accounted for by that of its alkaloids.

Pyrolysis products from opium and morphine were fractionated by high-performance liquid chromatography. The mutagenic activities of seven fractions and of the crude pyrolysis mixture are shown in Table 23: fractions 1, 2 and 3 of both opium and morphine showed the highest specific mutagenic activities, and about 90% of the mutagenic activity was located in these first three fractions. Work is in progress to identify and structurally characterize the mutagenic principles in these fractions.

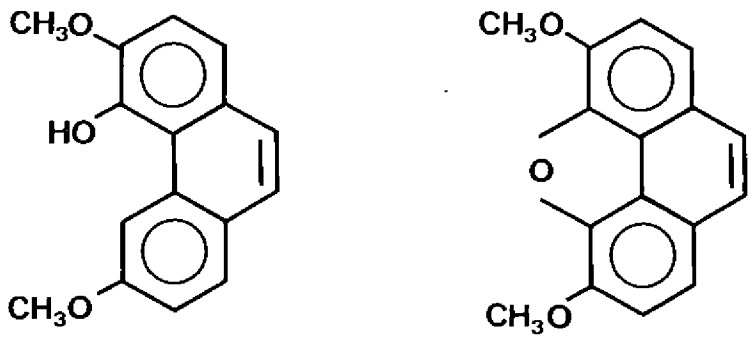
Sukhteh samples were analysed for polycyclic aromatic hydrocarbons by gas chromatography-mass spectrometry. The profile was found to be relatively simple. In contrast, a relatively complex mixture of nitrogen-containing (basic) polycyclic compounds was detected. In order to facilitate the characterization of each active compound of sukhteh, a large sample (1 kg) was fractionated by two methods: (1) by the method established earlier in pilot studies; and (2) using Grimmer's method for the purification of neutral and basic polycyclic compounds. The fractions obtained will be analysed further by gas chromatography-mass spectrometry and by short-term tests.

Table 23. Mutagenic activity of high-pressure liquid chromatographic fractions of pyrolysis products and of the crude pyrolysis mixture from opium and morphine

Pyrolysed material	Pyrolysed Indian opium		Pyrolysed morphine	
	Weight (mg)	Mutagenicity[a]	Weight (mg)	Mutagenicity
Mixture		1 820		42 500
Fraction no.				
1	0.79	23 980	0.43	137 500
2	0.76	3 820	0.31	92 500
3	0.33	13 080	0.23	75 000
4	0.43	1 680	0.18	36 500
5	0.61	1 520	0.14	42 500
6	0.11	0	0.09	53 050
7	0.36	0	1.30	1 750
Total fractions	3.39	8 200	2.68	46 500

[a] Mutagenic activity in *S. typhimurium* TA98 strain determined in plate assays in the presence of 50 µl liver postmitochondrial fraction from Aroclor-treated rats and an NADPH generating system. Mutagenicity is expressed as revertants/mg of pyrolysed product and as revertants/fraction, respectively.

Two pure substances, possible pyrolysis products of opium components, were characterized chemically and tested for mutagenicity. These were (1) thebaol, a decomposition (and pyrolysis?) product of thebaine, and (2) 3,5-dimethoxy-phenanthreno(4,6-*bcd*)furan, a degradation product of thebaine (Fig. 22). These two compounds were assayed for mutagenicity in *S. typhimurium* TA100 and TA98 strains. Compound 1 was inactive; compound 2 was mutagenic only in TA100 strain in the presence of a rat liver microsomal preparation, revealing a specific mutagenicity of 7300 revertants/mg of pure substance, about 46-fold higher than that of a dichloromethane extract of sukhteh.



1 2

Fig. 22 Chemical structures of (1) thebaol and (2) 3,5-dimethoxy-phenanthreno-(4,6-*bcd*)-furan

Crude opium contains substantial amounts of sterols, such as β-sitosterol, cycloartenol and cyclolaudenol, which are possible sources of the mutagenic polycyclic hydrocarbons formed during pyrolysis. The pyrolysis products of each sterol will thus be tested for mutagenecity. Pyrolysed plant sterols (khat) showed relatively little activity as compared with some pyrolysed opium alkaloids (see Table 22).

(*c*) *Characterization of a biologically active fraction from distilled apple brandies* (Mr G. Toussaint, Miss C. Loquet, Dr M. Friesen, Mr C. Malaveille and Mrs A. Hautefeuille; in collaboration with Professor J. Y. Le Talaer, Regional Centre François-Baclesse, Caen, France)

The isolation of mutagens from home-distilled apple brandies, collected in Britanny, France[179] has been completed. One sample was selected because of its mutagenicity in the *Salmonella*/microsome assay: a non-volatile fraction was obtained by distillation under reduced pressure, followed by fractionation by high-pressure liquid chromatography; and six fractions were collected, concentrated and assayed for mutagenicity in *S. typhimurium* TA98 strain in the presence of a rat liver activation system.

One fraction was found to be mutagenic, so some of the compounds present in that fraction have been identified, after chemical derivatization (silylation, methylation, dansylation and hydrazone formation) to convert them into volatile or fluorescent products. Among the compounds identified (3-cyclohexane-1-carboxylic acid, phenol, oleoamide, aliphatic ketones), only a vicinal diol seemed of interest, because of the suspected biological activity of a possible precursor epoxide. The *n*-octane 2,3-diol was thus synthesized, and the mass and nuclear magnetic resonance spectra of the natural and the synthetic diol were compared. The spectral data suggest that the naturally occurring diol may have a branched carbon chain. As the synthetic and the isolated octane diol showed no mutagenic activity in *S. typhimurium* TA98 and TA100, the corresponding epoxide was synthesized: no mutagenic activity was found with or without a rat liver metabolic activation system. Although previous studies have confirmed the presence of mutagens in alcoholic beverages[180], isolation and structural elucidation of the mutagens remains to be carried out.

(*d*) *Mutagenicity tests on chewing material 'masala supari' collected in India* (Mr C. Malaveille and Mrs A. Hautefeuille; in collaboration with Dr M. Thangavelu, WHO, New Delhi, India)

Upon request from the WHO Regional Office in south-east Asia, in relation to an increasing trend of chewing habits and their possible association with the rising incidence of buccal cancer in certain areas of India, four samples of chewing material of variable composition were tested for their mutagenic content. The four samples (A-D) all contained 'supari', slaked lime, and either plain tobacco (A), tobacco No. 32 (B), tobacco No. 120 (C) or tobacco No. 300 (D). Sample D showed a mutagenic effect in *S. typhimurium* strain TA100 (7000 revertants per g of starting material) and in TA98 (2000 revertants/g), in the presence of a rat liver activation system. The other three samples were negative.

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[179] International Agency for Research on Cancer (1979) *Annual Report, 1979*, Lyon, p. 64.
[180] Loquet, C., Toussaint, G. & Le Talaer, J. Y. (1980) *Mutation Res.* (in press).

4.10 *Research training and visiting fellows*

Dr S.-H. Lu, from the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, spent one year at the Agency under a WHO fellowship and received training in carrying out a number of short-term tests for the detection of carcinogens. He was involved in testing several compounds that occur environmentally in China for their mutagenic and DNA damaging potential.

Dr V. V. Khudoley, from the Professor N. N. Petrov Research Institute for Oncology, Leningrad, USSR, spent one year at the Agency to gain experience in carrying out short-term carcinogenicity tests and to assist in the programme on long-term carcinogenicity testing of chemicals.

Mr Nguyen Van Trung, Laboratory of Toxicology and Industrial Hygiene, Faculty of Pharmaceutical and Biological Sciences, René Descartes University, Paris, received training in methods for the determination of volatile nitrosamines in environmental samples.

PROGRAMME OF RESEARCH TRAINING AND LIAISON

Dr W. DAVIS (Head)

1. INTRODUCTION

In this present year, twelve research training fellowships have been awarded, of which ten were considered as closely related to the Agency's own research interest. Three specialized courses have been held and two more will take place before the end of 1980. In collaboration with the French National Institute of Health and Medical Research (INSERM), and the Medical Research Council Pneumoconiosis Unit, UK, a symposium was organized on the biological effects of mineral fibres, and preparations are already advanced for a symposium to be held in 1981 on host factors in human carcinogenesis. Five new titles have been added to the *IARC Scientific Publications* series, and there have been two non-serial publications. The computerized bibliographic service has seen a marked increase in utilization by the scientific staff this year.

Plans are being prepared to maintain the level of educational activity by IARC in the six regions of WHO, by organizing cancer epidemiology courses in each region every other year with the co-operation of the Regional Offices. In addition, an advanced course or workshop, related to any selected aspect of the Agency's research programme, will be given in Lyon. The scientific staff of the two divisions will continue to contribute both to the planning and teaching of the courses, and will be joined by internationally-recognized teachers from the universities of many countries.

Multidisciplinary symposia will be organized, normally on alternate years; the programme of research training and liaison will be responsible for all the administrative arrangements and will assist in the preparation of the scientific programme with designated staff from the two divisions. Emphasis will be placed on the educational aspects of such symposia to which a small number of less experienced, but promising young scientists should be invited.

The programme will continue to be responsible for the *IARC Scientific Publications*, and will ensure that, wherever relevant, the proceedings of symposia and workshops are published in the series.

2. RESEARCH TRAINING FELLOWSHIPS

2.1 *The Fellowships Selection Committee*

The Fellowships Selection Committee met in Lyon 24–25 April 1980, to review applications for Research Training Fellowships and for the Isabelle Decazes de Noüe Prize.

The members of the Committee were:

Dr N. Gray	Director, Anti-Cancer Council of Victoria, Melbourne, Australia *(Epidemiology)*
Dr E. Klein	Department of Tumor Biology, Karolinska Institute, Stockholm; representing the Chairman of the Commission on Fellowships and Personnel Exchange of the UICC *(Virology-Immunology)*
Dr R. Kroes	Central Institute for Nutrition and Food Research, Zeist, The Netherlands *(Chemical Carcinogenesis)*
Dr T. Kuroki	Department of Pathobiochemical Cell Research, Institute of Medical Sciences, University of Tokyo, Tokyo *(Chemical Carcinogenesis)*
Dr V. B. Okulov	Laboratory of Clinical Immunology, N. N. Petrov Research Institute of Oncology, Leningrad, USSR *(Immunology)*

Drs Gray and Kuroki were attending for the first time, and Dr B. G. Mansourian, Office of Research Promotion and Development, WHO, Geneva, again participated in the work of the meeting.

2.2 *Fellowships awarded*

Seventy-eight applications had been received of which 18 were inadequate or invalid. The committee recommended the award of 12 fellowships of which the distribution by discipline is given in Table 1. The two molecular biologists eventually decided to accept other fellowships that they had been offered, and so an alternate fellowship was activated in experimental carcinogenesis.

Table 1. Distribution of research training fellowships by scientific discipline, 1980

Scientific discipline	No. of fellowships
Biostatistics	1
Cell biology	1
Environmental/chemical carcinogenesis	1
Epidemiology	3
Experimental carcinogenesis	3
Molecular biology	2
Physiology	1

2.3 *Isabelle Decazes de Noüe prize*

The Isabelle Decazes de Noüe Foundation has again provided prizes to be awarded to former Research Training Fellows to enable them to acquire equipment and laboratory reagents, required for their continuing research in their laboratories.

On the recommendation of the Fellowships Selection Committee, two prizes, each of US$ 2500 have been awarded to:

Dr R. Lotan, The Weizmann Institute of Science, Rehovot, Israel, for research on the effects of vitamin A analogues (retinoids) on the growth, differentiation and cell surface components of malignant human melanomas and breast carcinomas.
Dr M. S. S. Murthy, Bhabha Atomic Research Centre, Bombay, India, for research on the correlation of the induction or mutation and recombination by some alkylating agents with the degree of alkylation at certain positions in the DNA.

3. SPECIALIZED COURSES

3.1 *Epidemiology of Cancer, Beijing, 5 November – 1 December 1979*

With the support of the Western Pacific Regional Office, WHO, the Agency organized an epidemiology course in Beijing. The Cancer Research Institute of the Chinese Academy of Medical Science provided the local organization. 34 Chinese participants came from most of the provinces of China and there were also 7 non-Chinese participants from Japan, the Philippines, Malaysia, Singapore and Sri Lanka (Fig. 1). Major teaching responsibility was taken by Professor Bruce Armstrong (NH & MRC Research Unit in Epidemiology & Preventive Medicine, University of Western Australia), and Dr John Osborn (Department of Demography, London School of Hygiene

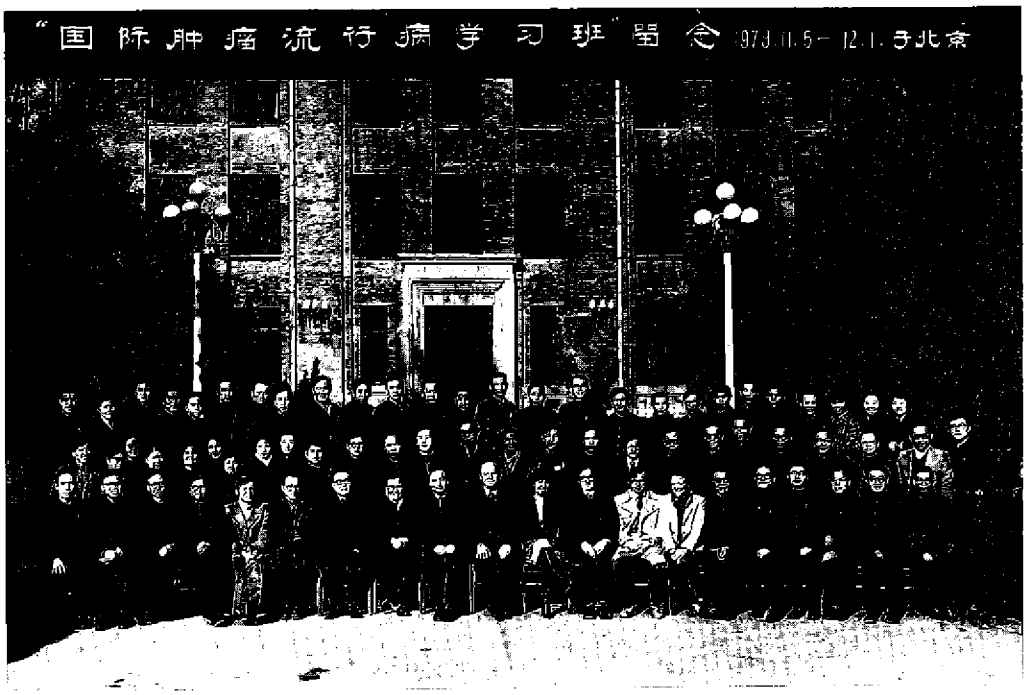

Fig. 1 The lecturers and participants at the international course on the epidemiology of cancer, Beijing, 5 November-1 December 1979

and Tropical Medicine). Professor T. Hirayama (National Cancer Centre Research Institute, Tokyo) joined the faculty for the second half of the course and Professor Marvin Zelen (Harvard University, Boston, USA) gave a three-day course at the end of the period on the statistical approach to clinical trials. Chinese lecturers were Drs Gao Run-Guan, Gao Yu-Tang, Gu Xin-Yuan, Hsui Hai-Hsio, Hu Meng-Xuan, Li Chun-Yao, Li Ping, Tu Qi-Tao, Zhou Yu-Shang and Dai Hui-Dong. Drs C. S. Muir, Nubia Muñoz, O. M. Jensen and W. Davis from the Agency completed the overseas members of the faculty.

Translation throughout the course was provided by Dr Gao Yu-Tang, Shanghai Cancer Institute and Dr Hu Meng-Xuan, Kwangzhow (Canton) Cancer Institute.

3.2 *Epidemiological Approach to Occupational Cancer, Lyon, 17–22 March 1980*

Professor Donald Acheson, head of the Medical Research Council's Environmental Epidemiology Unit, University of Southampton, UK, was the programme coordinator for a course designed principally for works medical officers and physicians and statisticians working in various regulatory agencies and labour health authorities. The teaching faculty included Dr A. Englund (The Construction Industry's Organization for Working Environment, Safety & Health, Stockholm), Dr A. J. Fox (Office of Population Censuses and Surveys, London), Dr P. Lazar, (Unit for Epidemiological and Statistical Research, INSERM-U 170, Villejuif, France), Professor F. D. K. Liddell (Department of Epidemiology and Health, McGill University, Montreal, Canada), Dr R. Murray (consultant in occupational health, London), Mr J. T. Sanderson (Industrial Hygiene Adviser, Esso Europe Inc., London) and Drs R. Saracci, C. S. Muir, L. Tomatis and N. E. Day from the Agency's staff.

50 participants from 21 countries were selected from among more than 120 applicants. In view of the very large demand, it was decided to repeat the course later in 1980.

3.3 *Utilization of Non-human Primates in Cancer Research, Sukhumi, Abkhazian SSR, May 11–20, 1980*

Academician Boris Lapin and his staff of the Institute of Experimental Pathology and Therapy in Sukhumi provided the local organization and Professor Friedrich Deinhardt (Max von Pettenkofer Institute, Munich, FRG) prepared the scientific programme for a course that covered primate pathology and husbandry, and also relevant aspects of research in viral, chemical and radiation carcinogenesis. The foreign faculty included Professor H. Balner (Primate Center TNO, Rijswijk, The Netherlands), Dr R. Bronson (New England Regional Primate Research Center, Southboro, USA), Dr J. B. Deinhardt (Max von Pettenkofer Institute, Munich, FRG), Professor C. F. Hollander, (Institute for Experimental Gerontology TNO, Rijswijk, The Netherlands), Dr S. S. Kalter (Department of Microbiology and Infectious Diseases, Southwest Foundation for Research and Education, San Antonio, USA), Dr J. Moor-Jankowski (Laboratory for Experimental Medicine and Surgery in Primates, New York, USA), Dr R. Scheid (Max von Pettenkofer Institute, Munich, FRG), Dr R. A. Whitney Jr (Veterinary Research Branch, National Institute of Health, Bethesda, USA), Dr H. Wolf (Max von Pettenkofer Institute, Munich, FRG) and Drs J.-F. Duplan and W. Davis from the Agency. Lecturers from the USSR were Professor B. A. Lapin, Dr Z. N. Dzhelieva, Dr E. K. Dzhikidze, Dr L. A. Yakovleva, Dr G. M. Cherkovich, Dr V. G.

Chaljan, Professor N. P. Napalkov, Dr Aleksandrov, Dr V. S. Turusov, Dr F. L. Kisiljev, Dr M. A. Shljankevich, Dr R. A. Dreizen and Professor L. M. Shabad.

There were 36 participants coming from the USSR, Federal Republic of Germany, German Democratic Republic, Greece, The Netherlands, Japan, Hungary, Poland, Czechoslovakia and Bulgaria (Fig. 2).

Together with an intensive programme of lectures, the participants were able to visit the extensive primate colony developed by Professor Lapin.

Fig. 2 The lecturers and participants at the international course on the utilization of non-human primates in cancer research, Sukhumi, Abkhazian SSR, May 11-20, 1980

3.4 *Future courses*

A repeat of the course on epidemiological aspects of occupational cancer is scheduled for Lyon, 28 July–1 August 1980; aspects of chemical carcinogenesis, Lyon, 3–8 November 1980; cancer epidemiology, Limassol, Cyprus, 17–28 November 1980 with the Regional Office for the Eastern Mediterranean; cancer epidemiology, Ndola, Zambia, 11–29 May 1981 with the Regional Office for Africa; cancer epidemiology, Bogota, November 1981; and cancer epidemiology, Bombay, January 1982.

4. SYMPOSIA

4.1 *Biological Effects of Mineral Fibres, Lyon 25–27 September 1979*

The symposium, the third to be organized jointly with the French National Institute of Health and Medical Research (INSERM), was also co-sponsered by the Medical Research Council Pneumoconiosis Unit, UK.

Two-hundred-and-thirty-five scientists from 21 countries participated in the meeting, which reviewed the developments that had taken place since the meeting devoted to the biological effects of asbestos that took place at the Agency in November 1972.

The proceedings will be published in the *IARC Scientific Publications* series.

4.2 *Future symposia and workshops*

Preparations are well advanced for a symposium on 'Host Factors in Human Carcinogenesis' to be held at Cape Sounion, Greece, 8–11 June 1981.

An international committee met in Lyon in February 1980 to assist in the preparation of a detailed programme and a complete panel of invited speakers. The programme and list of speakers are now complete, and further invitations are to be distributed.

Support to date has been promised by the Ministry of Welfare and Science, Greece, and the Directorate General for Research, Science and Education, Commission of the European Communities.

Preliminary plans have been made for an applied statistics workshop to be held at the Agency in the summer of 1981.

5. PUBLICATIONS

The list of new titles appearing in the *IARC Scientific Publications* series this year includes:

Molecular and Cellular Aspects of Carcinogen Screening Tests, 1980

Directory of On-going Research in Cancer Epidemiology 1979, 1979

Environmental Carcinogens – Selected Methods of Analysis – Volume 3, *Analysis of Polycyclic Aromatic Hydrocarbons*, 1979

Handling Chemical Carcinogens in the Laboratory – Problems of Safety, 1980

and two non-serial publications:

Information Bulletin on the Survey of Chemicals Being Tested for Carcinogenicity, 1979

Cancer Morbidity and Causes of Death among Danish Brewery Workers, 1980

There are four titles in press:

Biological Effects of Mineral Fibres, 1980

N-*Nitroso Compounds: Analysis, Formation and Occurrence*, 1980

Statistical Methods in Cancer Research, 1980

Directory of On-going Research in Cancer Epidemiology 1980, 1980

and in preparation:

Pathology of Tumours in Laboratory Animals, Volume III, *Tumours of the Hamster*, 1981

The complete list of publications is given in Table 2.

The figures for the distribution and sales of scientific publications and monographs are given in Table 3.

6. LIBRARY (Mrs A. Nagy-Tiborcz)

The library provided a service to the scientific staff of the Agency, and to the local medical and scientific community. Close liaison is maintained with the library of the medical faculty of Lyon.

At present, there are 240 subscriptions to journals and annuals. Thanks to a large anonymous donation, which has been devoted to the purchase of books for the library, the book stock is now approximately 4200.

7. INTERDISCIPLINARY SUPPORT SERVICES

7.1 *Computerized bibliographic service* (Mrs M. Soulat)

The use of the terminal, giving access to the computerized bibliographic files of the National Library of Medicine, USA, has increased during the year. A total of 322 searches were made, taking 128 hours of search-time. There were 152 off-line searches requested and 40 regular monthly up-datings are made.

The use of the terminal is supported by contracts with the National Cancer Institute (Bethesda, MD, USA) to the *Clearing-house for On-going Research in Cancer Epidemiology* and to the *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans.*

7.2 *Scientific illustrations group*

The photographic and drawing services have been brought together to provide a more rational and efficient service for the preparation of illustrations for publications, and slides for lectures; the other aspects of the photographer's work for the laboratories continues.

8. COORDINATING COMMITTEE FOR HUMAN TUMOUR INVESTIGATIONS

Plans are underway for the Ninth International Symposium on the Biological Characterization of Human Tumours to be held in Bologna, 23–27 September 1981.

Table 2. List of *IARC Scientific Publications*

No.	Title	Year of Publication
1	*Liver Cancer*	1971
2	*Oncogenesis and Herpesviruses*	1972
3	*N-Nitroso Compounds – Analysis and Formation*	1972
4	*Transplacental Carcinogenesis*	1973
5	*Pathology of Tumours in Laboratory Animals*, Vol. 1: *The Rat*, Part 1	1973
6	*Pathology of Tumours in Laboratory Animals*, Vol. 2: *The Rat*, Part 2	1976
7	*Host-Environment Interactions in the Etiology of Cancer in Man*	1973
8	*Biological Effects of Asbestos*	1973
9	*N-Nitroso Compounds in the Environment*	1974
10	*Chemical Carcinogenesis Essays*	1974
11	*Oncogenesis and Herpesviruses II*, Parts 1 and 2	1975
12	*Screening Tests in Chemical Carcinogenesis*	1976
13	*Environmental Pollution and Carcinogenic Risks*	1976[a]
14	*Environmental N-Nitroso Compounds — Analysis and Formation*	1976
15	*Cancer Incidence in Five Continents*, Vol. III	1976
16	*Air Pollution and Cancer in Man*	1977
17	*Directory of On-Going Research in Cancer Epidemiology, 1977*	1977[b]
18	*Environmental Carcinogens — Selected Methods of Analysis*, Vol. I: *Nitrosamines*	1978
19	*Environmental Aspects of N-Nitroso Compounds*	1978
20	*Nasopharyngeal Carcinoma: Etiology and Control*	1978
21	*Cancer Registration and its Techniques*	1978
22	*Environmental Carcinogens — Selected Methods of Analysis*, Vol. II: *Vinyl Chloride*	1978
23	*Pathology of Tumours in Laboratory Animals*, Vol. 2: *The Mouse*	1978
24	*Oncogenesis and Herpesviruses III*, Parts 1 and 2	1978
25	*Carcinogenic Risks — Strategies for Intervention*	1978[a]
26	*Directory of On-Going Research in Cancer Epidemiology, 1978*	1978[b]
27	*Carcinogen Screening Tests — Molecular and Cellular Aspects*	1979
28	*Directory of On-Going Research in Cancer Epidemiology 1979*	1979[b]
29	*Environmental Carcinogens — Selected Methods of Analysis*, Vol. II: *Polycyclic Aromatic Hydrocarbons*	1979
30	*Biological Effects of Mineral Fibres, Parts 1 and 2*	1980[a, c]
31	*N-Nitroso Compounds: Analysis, Formation and Occurrence*	1980[c]
32	*Statistical Methods for Cancer Epidemiology*, Vol. I: *The Analysis of Case-Control Studies*	1980[c]
33	*Handling Chemical Carcinogens in the Laboratory: Problems of Safety*	1980
34	*Pathology of Tumours in Laboratory Animals*, Vol. III: *Tumours of the Hamster*	1981[d]
35	*Directory of On-Going Research in Cancer Epidemiology 1980*	1980[b]
	Non-serial Publications	
	Alcool et Cancer	1978
	Information Bulletin on the Survey of Chemicals Being Tested for Carcinogenicity No. 8	1979
	Cancer Morbidity and Causes of Death among Danish Brewery Workers	1980

[a] joint publication with INSERM
[b] joint publication with DKFZ
[c] in press
[d] in preparation

Table 3. Distribution of *IARC Scientific Publications* and *Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*

	Official distribution	Sales
Scientific Publications		
No. 1	714	851
2	822	1378
3	982	945
4	943	881
5	1059	1413
6	924	1210
7	1069	752
8	1062	1114
9	1014	924
10	1043	1090
11 — Part 1	1107	621
11 — Part 2	1114	634
12	1284	1224
13	963	866
14	989	890
15	973	1022
16	1035	865
17	1025	575
18	972	619
19	1126	639
20	930	556
21	1023	667
22	941	523
23	1039	590
24 — Part 1	873	500
24 — Part 2	873	512
25	1050	556
26	1167	528
27	1084	454
28	971	470
29	915	488
33	989	576
Alcool et Cancer	660	141
Monograph Series		
No. 1	2638	2099
2	1952	2295
3	2011	2286
4	1769	2171
5	2006	1882
6	1797	1881
7	2087	1677
8	1993	1664
9	1990	1524
10	2056	1690
11	2173	1330
12	2072	1511
13	2022	1360
14	2207	1981
15	2109	1478
16	2070	1407
17	2212	1270
18	2112	1218
19	2047	1247
20	2095	1020
21	2039	837
22	2023	743
Supplement No. 1	2202	1037

Annex 1

PARTICIPATING STATES AND REPRESENTATIVES AT THE NINETEENTH SESSION OF THE IARC GOVERNING COUNCIL, 2–3 MAY 1980

Australia

Dr D. F. BOOTH
Assistant Director-General
International Health
Australian Department of Health
Woden, Australia

Mr B. FRIEND *(Rapporteur)*
Director
Accounting Office
Australian Department of Finance
Australian Consulate
Geneva, Switzerland

Belgium

Professor S. HALTER
Secretary-General
Ministry of Public Health and the Family
Brussels

Dr J. FRANÇOIS
Director-General
Ministry of Public Health and the Family
Brussels

France

Professor E. J. AUJALEU
Honorary Director-General
National Institute of Health and Medical Research
Counsellor of State
Paris

Federal Republic of Germany

Mr H. VOIGTLÄNDER
International Health Relations Section
Federal Ministry for Youth, Family Affairs and Health
Bonn

Italy

Professor R. VANNUGLI *(Chairman)*
Director
Bureau of International Relations
Ministry of Health
Rome

Professor L. SANTI
Director
Institute of Oncology
University of Genoa
Genoa, Italy

Japan

Dr S. YOSHIZAKI
Director-General
Statistics and Information Department
Ministry of Health and Welfare
Tokyo

Dr Y. KAWAGUCHI
Deputy-Director
International Affairs Division
Minister's Secretariat
Ministry of Health and Welfare
Tokyo

Sweden

Professor S. T. LINDSTEDT
Department of Clinical Chemistry
University of Gothenburg at Sahlgren's Hospital
Göteborg, Sweden

The Netherlands

Dr J. SPAANDER *(Vice-Chairman)*
Late Director-General of the National Institute of Public Health
Bilthoven, The Netherlands

Mr W. J. KAKEBEEKE
Deputy-Director for International Affairs
Ministry of Public Health and Environmental Protection
Leidschendam, The Netherlands

Union of Soviet Socialist Republics

Dr A. M. GARIN
Deputy Director-General
Cancer Research Center
Academy of Medical Sciences
Moscow

Professor V. P. DEMIDOV
Chief, Cancer Department
Ministry of Public Health of the USSR
Moscow

Dr S. LITVINOV
Deputy Chief
External Relations Board
Ministry of Public Health of the USSR
Moscow

United Kingdom

Dr J. L. GOWANS
Secretary
Medical Research Council
London

Dr R. J. WRIGHTON
Senior Medical Officer
Department of Health and Social Security
London

United States of America

Dr J. M. SONTAG
Assistant Director for Interagency Affairs
Office of the Director
National Cancer Institute
National Institutes of Health
Bethesda, USA

Mr N. A. BOYER
Director, Health and Narcotic Programs
Bureau of International Organization Affairs
Department of State
Washington DC

Dr V. DeVITA Jr
Director
National Cancer Institute
National Institutes of Health
Bethesda, USA

World Health Organization

Dr Ch'en WEN-CHIEH
Assistant Director-General

Mr A. GROENENDIJK
Director, Division of Budget and Finance

Dr L. SOBIN
Cancer Unit

Dr M. S. TSECHKOVSKI
Cancer Unit

Dr C.-H. VIGNES
Director, Legal Division

Observer

Professor J. MILLER
Outgoing Chairman of the IARC Scientific Council

Annex 2

MEMBERS OF THE SCIENTIFIC COUNCIL AT ITS SIXTEENTH SESSION, 12–14 FEBRUARY 1980

Professor P. BOGOVSKI
Director
Institute of Experimental and Clinical Medicine
Tallinn, Estonian SSR

Professor J. CAIRNS
Department of Microbiology
Harvard School of Public Health
Boston, USA

Professor G. DELLA PORTA
Director
Division of Experimental Oncology A
Istituto Nazionale per lo Studio
e la Cura dei Tumori
Milan, Italy

Professor P. EMMELOT *(Rapporteur)*
Acting Scientific Director
Department of Biochemistry
The Netherlands Cancer Institute
Slotevaart-Amsterdam

Professor A. GEORGII
Secretary-General, German Cancer Society
Director, Institute of Pathology
Medical School
Hanover, Federal Republic of Germany

Professor B. GUSTAFSSON
Professor and Chairman
Department of Germfree Research
Karolinska Institute
Stockholm

Professor A. R. M. LAFONTAINE
Director
Institute of Hygiene and Epidemiology
Ministry of Public Health and the Family
Brussels

Professor J. MILLER *(Chairman)*
Head, Experimental Pathology Unit
Walter and Eliza Hall Institute of Medical Research
Melbourne, Australia

Professor E. R. SAXEN
Director
Department of Pathology
University of Helsinki
Helsinki

Professor T. SUGIMURA *(unable to attend)*
Director
National Cancer Center Research Institute
Tokyo

Professor M. TUBIANA *(Vice-Chairman)*
Head, Radiation Department
Institut Gustave Roussy
Villejuif, France

Professor I. B. WEINSTEIN
Director
Division of Environmental Sciences
College of Physicians and Surgeons of Columbia University
Cancer Center Institute of Cancer Research
New York, USA

Annex 3

RESEARCH AGREEMENTS IN OPERATION BETWEEN IARC AND VARIOUS INSTITUTIONS July 1979–June 1980

Support of IARC Research Centres

RA/68/002	University of Singapore (Contribution to the maintenance of an IARC Research Centre at the University of Singapore)
RA/75/020	University of Nairobi (Contribution to the maintenance of an IARC Research Centre at the University of Nairobi)

Reference centres/Serum banks

RA/73/029	Institute of Experimental Oncology, University of Genoa, Genoa, Italy (IARC Reference Centre for environmental carcinogenesis)
RA/73/033	Medical College, Hanover, Federal Republic of Germany (Creation of an IARC Reference Centre for environmental carcinogenesis)
RA/74/003	Institute for Documentation, Information and Statistics, German Cancer Research Centre, Heidelberg, Federal Republic of Germany (Clearing-house for on-going research in cancer epidemiology)
RA/74/006	National Institute for Public Health, Antonie van Leeuwenhoeklan, 9, Bilthoven, The Netherlands (Creation of an IARC Reference Centre for environmental carcinogenesis)
RA/75/014	National Institute of Hygiene, Budapest (Creation of an IARC Reference Centre for environmental carcinogenesis)
RA/76/019	Angel H. Roffo Oncological Institute, Buenos Aires (Creation of an IARC Reference Centre for environmental carcinogenesis)
RA/78/002	School of Pharmacy, Catholic University of Louvain, Brussels (Creation of an IARC Reference Centre for the *in vivo* monitoring of drug metabolizing enzymes)
RA/78/006	Laboratory of Genetics, University of Pisa, Pisa, Italy (Creation of an IARC Reference Centre for environmental carcinogenesis)

RA/75/015 National Institute of Health and Medical Research, Division of Medico-Social Research, Le Vésinet, France
(Study of cases of oesophageal cancer and their controls in the Calvados region of France)

RA/79/016 Hospital Centre for Physiotherapy, Regina Elena Institute, Rome
(Feasibility study on the sampling of gastric juice and blood for *N*-nitroso compounds)

Studies on cancers linked with herpesviruses

RA/70/017 Department of Pathology, University of Singapore, Singapore
(Studies on the relationship between herpes-type infection and nasopharangeal carcinoma)

RA/71/007 Shirati Mission Hospital, Tarime, Tanzania
(Study of the epidemiology of Burkitt's lymphoma in the North Mara District, Tanzania)

RA/75/002 Ross Institute, London School of Hygiene and Tropical Medicine, London
(Malaria antibody testing to be carried out by the Institute on sera from Burkitt's lymphoma studies in the West Nile District of Uganda and the Mara Region of Tanzania)

RA/77/008 Shirati Mission Hospital, Musoma, Tanzania
(Studies on the effect of partial malaria suppression on incidence of Burkitt's lymphoma in North Mara)

RA/77/015 Department of General and Applied Biology, Claude Bernard University, Villeurbanne, France
(Characterization and purification of viral antigens for the development of serological tests)

RA/78/027 Immunogenetics Laboratory for Organ Transplants, Saint-Louis Hospital, Paris
(Characterization of the European Burkitt's lymphoma line 'CHE')

RA/79/011 & RA/80/008 Microbiology Laboratory, Mohamed V Hospital, Rabat
(Collaboration with the research programme on nasopharyngeal carcinoma in Morocco)

RA/79/014 Department of Clinical Genetics, University Hospital, Lund, Sweden
(Cytogenetic study of 8–14 translocation in non-tumoral cells to be carried out by the Department in children from Mara Region of Tanzania)

RA/80/009 Cytogenetics Laboratory, Research Institute on Leukemias and Blood Diseases, Saint-Louis Hospital, Paris
(Characterization of cytogenetic abnormalities observed in cells of a Burkitt-type lymphoma)

Liver cancer studies

RA/76/012 Geneva Tumour Registry, Geneva, Switzerland
(Study of liver disease, including primary liver cancer, in the Canton of Geneva)

RA/79/021 Department of Social Medicine and Public Health of the University of Singapore, Singapore
(Cohort study on hepatitis B carriers and liver cancer)

Studies on chemical carcinegenesis

RA/70/003 Institute of Pathology, Medical University, Budapest
(Investigation of the effects of minute doses of chemical carcinogens on cells cultured *in vitro*)

RA/76/017 Cancer Research Center, Academy of Medical Sciences of the USSR, Moscow
(Investigation on the effect of prenatal exposure to a chemical on successive untreated generations)

RA/76/027 Institute for Experimental Toxicology and Chemotherapy, German Cancer Research Centre, Heidelberg, Federal Republic of Germany
(Study of elaboration of analytical methods for identification and quantification of *N*-nitroso compounds in various environmental media)

RA/77/022 N. N. Petrov Research Institute of Oncology, Leningrad, USSR
(Multi-generation studies of modifying factors in transplacental carcinogenesis)

RA/77/024 Institute of Oncology, Sofia
(Investigation on the combined action of several chemical carcinogens)

RA/77/025 Cancer Research Center, Academy of Medical Sciences of the USSR, Moscow
(Investigation on the combined action of several chemical carcinogens)

RA/77/026 Central Institute for Cancer Research, Academy of Sciences of the German Democratic Republic, Berlin-Buch
(Investigation on the combined action of several chemical carcinogens)

RA/78/004 Laboratory of Biophysics and Radiobiology, Free University of Brussels, Rhode-Saint-Genèse, Belgium
(Investigation of an *in vitro* biochemical assay for somatic mutagenesis by chemical mutagens/carcinogens and the effects of promoters in chemical carcinogenesis)

RA/78/005 Department of Pharmacognosy, Medical School, University of Szeged, Szeged, Hungary
(Study to identify the mutagenic components of crude opium and opium dross)

RA/78/009 National Institute for Research and Treatment of Tumours, Milan, Italy
(Study on possible role of environmental factors in the origin of human cancer)

RA/79/002 Department of Pharmacology, University of Oulu, Oulu, Finland
(Study on the AHH-inducibility and individual susceptibility to toxic effects of cigarette smoke)

RA/79/003 The Oncological Institute of the Lithuanian SSR, Vilnius, Lithuanian SSR
(Investigation on the combined action of several chemical carcinogens)

RA/79/004 Institute of Experimental and Clinical Medicine, Tallinn, Estonian SSR
(Studies on co-carcinogenic action of shale phenols on lung carcinogenicity of asbestos dust)

RA/79/006 Institute of Medical Sciences, University of Tokyo, Tokyo
(Mutagenesis and neoplastic transformation *in vitro* of cultured cells by environmental chemicals)

RA/79/007 Confederation of the French Malting Industry, Paris
(Study of volatile *N*-nitroso compounds in samples of malt or in the treatment process)

RA/79/010 Cancer Research Centre, Academy of Medical Sciences of the USSR, Moscow
(Investigation on the development of cellular and biochemical markers of *in vitro* transformation of epithelial cells in culture)

RA/79/012 Department of Environmental Health, National Institute of Health of Colombia, Avenida el dorado con Carrera 50, Bogota
(Feasibility study on long-term effects of pesticides on human health)

RA/80/001 Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan
(Investigation of mutagenicity testing in bacteria and yeast by environmental chemicals within an international network of carcinogenicity testing)

Studies on carcinogens other than chemicals

RA/76/026 Institute of Oncology of the University of Genoa, Genoa, Italy
(Study on interaction between asbestos and chromosomal proteins from different human tissues)

RA/78/007 Medical Research Council of the United Kingdom, London
(Study of mineral fibres, natural and man-made, including asbestos, erionite and others causing airborne pollution)

RA/78/011 Medical Research Council Pneumoconiosis Unit, Llandough Hospital, Penarth, UK
(Study of mesothelioma in Central Turkey)

RA/78/012 Department of Chest Diseases, Hacettepe University, Ankara
(Study of mesothelioma in Central Turkey)

Studies of various other cancer forms

RA/77/016 Institute of Oncology and Radiobiology, Havana
(Study of lung cancer in Cuban women)

RA/78/013 Department of Clinical Genetics, University Hospital of Lund, Lund, Sweden
(Study on the possibility of correlating karyotypes of cancer cells to specific etiological factors)

RA/80/005 Swedish Cancer Registry, Stockholm
(Cohort study to evaluate the risk of second tumours in patients originally diagnosed for cervical cancer)

Support of meetings

RA/79/009 MH Advertising Service, Budapest
(In respect of work involved in the Sixth Meeting on the analysis and formation of *N*-nitroso compounds, Budapest, October 1979)

RA/80/003 Cancer Registry, Viana do Castelo District Hospital, Viana do Castelo, Portugal
(Support for meeting of Latin-tongued cancer registries)

RA/80/004 German Cancer Research Centre, Heidelberg, Federal Republic of Germany
(Support for part cost of symposium on co-carcinogenesis and biological effects of tumour promoters)

RA/80/010 Dr J. Burchenal, Organizing Committee 1980 International Symposium on Cancer, c/o Memorial Sloan-Kettering Cancer Center, New York, USA
(Part cost of Symposium from 13–18 September 1980)

Annex 4

SCIENTISTS COLLABORATING WITH THE AGENCY [1]

Professor E. D. ACHESON
MRC Environmental Epidemiology Unit, University of Southampton, UK

Dr C. AMIRI
Cancer Institute, Teheran

Dr V. ANISIMOV
N. N. Petrov Research Institute of Oncology, Leningrad, USSR

Dr B. ARAMESH
Institute of Public Health Research, Teheran

Professor B. ARMSTRONG
NH & MRC Research Unit in Epidemiology and Preventive Medicine, University of Western Australia, Nedlands, W. Australia

Dr N. AXELSEN
State Serum Institute, Copenhagen

Professor H. BALNER
Primate Centre, TNO, Rijswijk, The Netherlands

Dr J. BARA
Laboratory of Immunology, INSERM, Villejuif, France

Dr Y. I. BARIS
Department of Chest Diseases, Hacettepe University, Ankara

Dr H. BENHAMOU
Gustave Roussy Institute, Villejuif, France

Mrs E. V. BENJAMIN
Liberian Cancer Registry, Liberia

Dr R. BERGER
Saint-Louis Hospital, Paris

Dr F. BERRINO
National Institute for the Study and Treatment of Tumours, Milan, Italy

Dr P. A. BERTAZZI
Work Clinic, Milan, Italy

Dr H. BÉTUEL
Lyon Blood Transfusion Centre, Beynost, France

Dr M. BHATTACHARYA
Roswell Park Memorial Institute, Department of Health, Buffalo, NY, USA

Dr A. M. BOLLANDER
Swedish Death Registry, Stockholm

Dr G. BORNKAMM
Institute for Virology, Health Centre, Freiburg, Federal Republic of Germany

Dr M. BÖRZSÖNYI
National Institute of Public Health, Budapest

Dr R. BRONSON
New England Regional Primate Research Center, Southboro, USA

Dr G. R. BRUBAKER
Shirati Mission Hospital, Tanzania

Dr CAI HAI YING
Cancer Institute, Chinese Academy of Medical Sciences, Beijing

Dr A. CALENDER
Claude-Bernard University, Lyon, France

Dr M. CARABALLOSO
Institute of Oncology and Radiobiology, Havana

[1] Members of the Working Groups for the Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans are listed separately on page 61.

Dr R. H. CASELLETTO
National University of La Plata, Argentina

Dr P. CERUTTI
Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland

Professor P. CHAMBON
Faculty of Pharmacy, Lyon, France

Professor S. H. CHAN
University of Singapore, Singapore

Mrs C. CHAPUIS-CELLIER
Edouard Herriot Hospital, Lyon, France

Dr I. CHERNOZEMSKY
Institute of Oncology, Medical Academy, Sofia

Dr CHU CHUAN YEN
Cancer Institute, Chinese Academy of Medical Sciences, Beijing

Dr J. CLARK
Scottish Health Service, Edinburgh, UK

Dr A. CLARKE
Department of Preventive Medicine & Biostatistics, University of Toronto, Toronto, Canada

Professor M. CLERC
Abidjan University Medical Centre, Abidjan

Dr A. H. CONNEY
Hoffmann La Roche Inc., Nutley, NJ, USA

Dr M. COOMBS
Imperial Cancer Research Fund, London

Professor M. CRESPI
Regina Elena Institute, Rome

Dr A. CROISY
Institute of Chemistry of Natural Substances, CNRS, Gif-sur-Yvette, France

Dr C. CUELLO
Valle University, Cali, Colombia

Professor J. DAILLIE
Claude-Bernard University, Lyon, France

Dr J. DAVIES
Department of Epidemiology and Public Health, University of Miami, FL, USA

Dr J. R. DAWSON
Duke University Medical Center, Durham, NC, USA

Dr J. M. DE CARVALHO
Viana do Castelo Cancer Registry, Portugal

Professor F. DEINHARDT
Max von Pettenkofer Institute, Munich, Federal Republic of Germany

Dr J. B. DEINHARDT
Max von Pettenkofer Institute, Munich, Federal Republic of Germany

Dr N. DEKKAR
National Commission for Information, Alger

Dr A. DEL MORAL ALDAZ
Health Department of Navarra, Pamplona, Spain

Mr J. DODGSON
Institute of Occupational Medicine, Edinburgh, UK

Dr E. DOMINGO
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Annex 5

MEETINGS AND WORKSHOPS ORGANIZED BY IARC, 1979–1980

Symposium on the biological effects of mineral fibres	Lyon, 25–27 September 1979
First study group meeting of the International Radiation Study of Cervical Cancer	Lyon, 4–5 October 1979
VIth international symposium on *N*-nitroso compounds: analysis, formation and occurrence	Budapest, 16–20 October 1979
Working group on laryngeal cancer	Geneva, 17–18 October 1979
Cancer epidemiology course	Beijing, 5 November–1 December 1979
Editorial board meeting for the manual of selected methods of analysis	Lyon, 7–8 November 1979
Joint EURO/IARC study group on cervical cancer screening programmes	Copenhagen, 3–5 December 1979
Working group on large bowel cancer	Cambridge, United Kingdom, 11–13 December 1979
Working group on laryngeal cancer	Milan, Italy, 7–8 February 1980
Scientific Council	Lyon, 12–14 February 1980
Organizing committee meeting on host factors in human carcinogenesis	Lyon, 15 February 1980
Review board meeting for the manual of selected methods of analysis	Lyon, 18 February 1980
Working group on the evaluation of the carcinogenic risk of chemicals to humans: some pharmaceutical drugs	Lyon, 19–26 February 1980
International course on the epidemiological approach to occupational cancer	Lyon, 17–21 March 1980
Annual meeting of investigators of alcohol-cancer projects	Lyon, 29–30 March 1980
Fellowships selection committee meeting	Lyon, 24–26 April 1980

Governing Council	Lyon, 2–3 May 1980
International course on the utilization of non-human primates in cancer research	Sukhumi, USSR, 10–20 May 1980
Working group on laryngeal cancer	Oporto, Portugal, 12–14 May 1980
Second study group meeting of the International Radiation Study of Cervical Cancer	Lyon, 14–15 May 1980
Fifth meeting of the Latin-tongued cancer registries	Viano do Castelo, Portugal, 15–16 May 1980
IUPAC/IARC workshop on nitrite analysis and protein-bound nitrite	Lyon, 19–21 May 1980
Working group on the evaluation of the carcinogenic risk of chemicals to humans: wood and leather industries	Lyon, 3–10 June 1980

Annex 6

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VISITING LECTURERS TO IARC – JULY 1979 TO JUNE 1980

Dr R. Van Zonneveld	'Cancer control and research in the Netherlands'
Dr T. Hirayama	'Prospective studies on cancer in Japan'
Dr G. H. Kellermann	'Host factors in human carcinogenesis'
Dr C. Quenum	'Difficulties in measuring cancer incidence in Africa'
Prof. J. J. Picard	'Normal and transformed cell lines of the amphibions Xenopus and their interaction with embryonic tissues'
Dr P. D. Lawley	'The alkylation of DNA: mutagenicity and carcinogenicity'
Dr H. Hoellinger	'Les pyréthrinoïdes – Nouvelle classe d'insecticides'
Prof. M. Furusawa	'A new simple microinjection technique: method and application'
Dr K. Hooper	'A quantitative approach to estimate the carcinogenic potency of chemicals'
Dr J. Hoey	'Pulmonary emboli – evaluation of diagnostic manoeuvres'
Prof. E. Hecker	'Co-carcinogenesis and the environmental significance of promoters of the diterpene ester type'
Dr Gao Yu-Tang	'Lung cancer in Hong Kong Chinese'
Dr S. Moolgavkar	'Two-stage model for carcinogenesis'
Dr R. Spirtas	'Some aspects of the work of NIOSH'
Dr G. E. Neal	'Aflatoxin activation *in vivo* and *in vitro*'
Dr A. O. Sobo	'Cancer in Liberia'
Dr H. J. Evans	'Carcinogen-induced chromosome damage in man – *in vivo* and *in vitro* studies'
Prof. A. J. Zuckerman	'Hepatitis B infection and primary liver cancer'
Dr S. Vaidya	'Cancer in Goa'
Dr O. Pelkonen	'Carcinogen metabolism in human tissues *in vitro* and in human cell cultures'

Dr P. A. Jeggo	'Is recombination a step in carcinogenesis'
Dr D. I. Thurnham	'Riboflavin deficiency in the elderly'
Dr J. R. Hass	'Applications of mass spectrometry in the environmental health sciences'
Dr T. J. Slaga	'Multi-stage chemical carcinogenesis'
Dr G. van Oortmarssen	'Models of screening for cancer: validity and usefulness'
Prof. Shen K. Yang	'Structural requirements for the carcinogenicity of polycyclic hydrocarbon metabolites'
Dr R. Wilson	'Interspecies comparison of carcinogenic potency'
Dr L. Brinton	'Oestrogens and breast cancer'
Dr A. Aitio	'Effect of tetrachlorodibenzodioxin (TCDD) on drug metabolizing enzymes'
Dr B. Henderson	'Recent developments in breast cancer with emphasis on oral contraceptives'
Dr I. Chouroulinkov	'Effets biologiques du phorbol ester: effet promotionnel *in vitro* et effet cancérogène propre'
Dr E. van der Esch	'Stage I of melanoma of the skin: evaluation of prognosis according to histological characteristics'
Dr S. D. Walter	'Estimating the effects of preventive intervention for multifactorial diseases'
Dr B. Singer	'Effects of chemical modifications of bases on transcription'

Annex 8

INTERNAL TECHNICAL REPORTS, 1979–80

IARC Internal Technical Report No.	
79/003	Report of an IARC Working Group on the Criteria to Select Chemicals for IARC Monographs (Lyon, 3–10 June 1979)
79/004	Cancer Cumulative Risks – based on the three volumes of Cancer Incidence in Five Continents, Lyon, IARC, 1979 (Dr M. Stukonis)

Annex 9

PAPERS PUBLISHED OR SUBMITTED FOR PUBLICATION BY IARC STAFF AND FELLOWS

Adelstein, A. M., Staszewski, J. & Muir, C. S. (1979) Cancer mortality in 1970–1972 among Polish-born migrants to England and Wales. *Br. J. Cancer,* **40**, 464–475

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Barbin, A., Bartsch, H., Leconte, P. & Radman, M. (1980) *On the possible role of the miscoding DNA-lesions, 1,N⁶-ethenoadenine and 3,N⁴-ethenocytosine, in vinyl chloride-induced mutagenesis and carcinogenesis.* In: Seeberg, E., ed., *Proceedings of the NATO/EMBO Lecture Course on Chromosome Damage and Repair, Godøysund, Norway* (in press)

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Bartsch, H. & Tomatis, L. (1980) Letter to the editor. (submitted for publication)

Bertrand, S., Berger, R., Philip, T., Bernheim, A., Bryon, P. A., Bertoglio, J., Doré, J.-F., Brunat-Mentigny, M. & Lenoir, G. M. (1980) Variant translocation in a non-endemic case of Burkitt's lymphoma: t(8; 22) in an Epstein-Barr virus negative tumour and in a derived cell line. (submitted for publication)

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